

**Komoditas : Tembakau
Tahun 2004-2008 (583 judul)**

Agnieszka Piotrowska-Cyplik, Anna Olejnik, Pawel Cyplik, Jacek Dach, Zbigniew Czarnecki, The kinetics of nicotine degradation, enzyme activities and genotoxic potential in the characterization of tobacco waste composting, *Bioresource Technology*, Volume 100, Issue 21, November 2009, Pages 5037-5044, ISSN 0960-8524, DOI: 10.1016/j.biortech.2009.05.053.

(<http://www.sciencedirect.com/science/article/B6V24-4WR2BYV-1/2/dd5034b59b14ebdb8da4405c78609cbd>)

Abstract:

This study aimed to determine nicotine biodegradation and the genotoxic potential of nicotine and its degradation products during the process of tobacco waste composting. Composting was carried out using two methods, i.e. the addition of 20% (bioreactor A) or 40% tobacco wastes to sewage sludge (bioreactor B) and control - sewage sludge (bioreactor C). Wheat straw was used as a structure-forming material. As a result of composting the contents of C and N in the bioreactors changed, the C:N ratio in bioreactor A changed from 22.8 to 13.00, and that in bioreactor B changed from 23.5 to 12.00. After composting, the biodegradation rate of nicotine was 78% in bioreactor A and 80% in bioreactor B, respectively. Using the Ames test it was shown that the composts produced did not exhibit mutagenicity.

Keywords: Composting; Enzyme activities; Genotoxic potential; Nicotine; Tobacco waste

Helmut J. Geist, Kang-tsung Chang, Virginia Etges, Jumanne M. Abdallah, Tobacco growers at the crossroads: Towards a comparison of diversification and ecosystem impacts, *Land Use Policy*, Volume 26, Issue 4, October 2009, Pages 1066-1079, ISSN 0264-8377, DOI: 10.1016/j.landusepol.2009.01.003.

(<http://www.sciencedirect.com/science/article/B6VB0-4VMX83R-1/2/b7dd5d95337d5ea76b7ec46443676b2f>)

Abstract:

An international Framework Convention on Tobacco Control has been in force since 2005, also aimed at regulating tobacco farming: FCTC article 17 on diversification, and FCTC article 18 on socio-ecological issues. Relating to the FCTC, information was gained and evaluated from tobacco farmers of growing areas sampled from major world regions (Rio Grande do Sul/Brazil, Tabora/Tanzania, Meinung/Taiwan, and Germany/Europe). A local farming survey was carried out in 2007, using a common data protocol, which covered, among others, questions on area and production development, energy used in curing, workforce, economic livelihood situation, and diversification opportunities. In addition to the survey, secondary (national-scale) statistics, public testimonies and other published data were explored. We analyzed these data using a portfolio approach, which combined statistical analysis, meta-analytical study and descriptive narratives. The projected trend of a global shift of tobacco cultivation into the developing world is confirmed, but also refined. Wood is used in Brazil and Tanzania for curing Virginia green leaf, thus contradicting the projected continuous reduction of this energy source. Child labour remains a major component of family farm tobacco operations in Brazil and Tanzania, while the cost and availability of seasonal labour turns into a bottleneck of production in Germany. More diversification opportunities exist than generally claimed, but no efforts are seen to address poor and vulnerable growers, in particular. German and Taiwanese tobacco growers can reasonably be predicted to discontinue farming in the near future, while tobacco cultivation in Brazil and Tanzania is seen to expand, mainly due to the political economy of low-cost production. Conclusions are drawn with respect to the work of the UN Study Group on Economically Sustainable Alternatives to Tobacco Growing (ESATG), effective since 2007.

Keywords: Land use transition; Tobacco transition; Agricultural alternatives; Crop substitution; Rural livelihood; Framework convention on tobacco control; Wood use; Deforestation

K.C. Allen, R.G. Luttrell, Spatial and temporal distribution of heliothines and tarnished plant bugs across the landscape of an Arkansas farm, *Crop Protection*, Volume 28, Issue 9, September 2009, Pages 722-727, ISSN 0261-2194, DOI: 10.1016/j.cropro.2009.04.007.

(<http://www.sciencedirect.com/science/article/B6T5T-4WK3YBS-1/2/b05847fd56a9230bdeaf72988d77d647>)

Abstract:

Farm records were used to study the temporal and spatial distribution of bollworm, *Helicoverpa zea* (Boddie), tobacco budworm, *Heliothis virescens* (F.), (collectively heliothines) and tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), on cotton, *Gossypium hirsutum* L., across a 4000 ha farm in southeastern Arkansas. The influence of the percentage of corn, *Z. mays* L., cotton, rice, *Oryza sativa* L., soybean, *Glycine max* L., and non-crop land within a 0.4 km buffer surrounding a cotton field and populations of heliothine eggs and tarnished plant bugs in cotton were examined over a three-year period. There was a positive relationship between the area in corn, *Zea mays* L., within 0.4 km of cotton fields and numbers of heliothine eggs in cotton in June 2004 and 2005. Positive relationships were observed between numbers of tarnished plant bugs in cotton and the surrounding area planted to corn, while negative relationships were observed for the area planted to cotton. Cotton fields with earlier dates of first flower had greater overall populations of tarnished plant bugs. Distributions of all three pests in cotton were at least partially explained by the time of year and the type of crop within the local environment. This indicates that more detailed spatial information and historical records may have value for managing cotton insects across large farms or communities.

Keywords: *Helicoverpa zea*; *Heliothis virescens*; *Lygus lineolaris*; IPM; Historical data

Demirhan Citak, Mustafa Tuzen, Mustafa Soylak, Simultaneous coprecipitation of lead, cobalt, copper, cadmium, iron and nickel in food samples with zirconium(IV) hydroxide prior to their flame atomic absorption spectrometric determination, *Food and Chemical Toxicology*, Volume 47, Issue 9, September 2009, Pages 2302-2307, ISSN 0278-6915, DOI: 10.1016/j.fct.2009.06.021.

(<http://www.sciencedirect.com/science/article/B6T6P-4WJ3DXY-1/2/2e7c8968e9dc3cc6d807d6516cf45bee>)

Abstract:

A simple and new coprecipitation procedure is developed for the determination of trace quantities of heavy metals (lead, cobalt, copper, cadmium, iron and nickel) in natural water and food samples. Analyte ions were coprecipitated by using zirconium(IV) hydroxide. The determination of metal levels was performed by flame atomic absorption spectrometry (FAAS). The influences of analytical parameters including pH, amount of zirconium(IV), sample volume, etc. were investigated on the recoveries of analyte ions. The effects of possible matrix ions were also examined. The recoveries of the analyte ions were in the range of 95-100%. Preconcentration factor was calculated as 25. The detection limits for the analyte ions based on 3 sigma ($n = 21$) were in the range of 0.27-2.50 $[\mu\text{g L}^{-1}]$. Relative standard deviation was found to be lower than 8%. The validation of the presented coprecipitation procedure was performed by the analysis certified reference materials (GBW 07605 Tea and LGC 6010 Hard drinking water). The procedure was successfully applied to natural waters and food samples like coffee, fish, tobacco, black and green tea.

Keywords: Heavy metals; Zirconium(IV) hydroxide; Preconcentration; Coprecipitation; Atomic absorption spectrometry

Neal T. Dittmer, Maureen J. Gorman, Michael R. Kanost, Characterization of endogenous and recombinant forms of laccase-2, a multicopper oxidase from the tobacco hornworm, *Manduca*

sexta, *Insect Biochemistry and Molecular Biology*, Volume 39, Issue 9, September 2009, Pages 596-606, ISSN 0965-1748, DOI: 10.1016/j.ibmb.2009.06.006.

(<http://www.sciencedirect.com/science/article/B6T79-4WNGW65-1/2/af94d7185d928316975ee3a985354ecc>)

Abstract:

Laccases belong to the group of multicopper oxidases that exhibit wide substrate specificity for polyphenols and aromatic amines. They are found in plants, fungi, bacteria, and insects. In insects the only known role for laccase is in cuticle sclerotization. However, extracting laccase from the insect's cuticle requires proteolysis, resulting in an enzyme that is missing its amino-terminus. To circumvent this problem, we expressed and purified full-length and amino-terminally truncated recombinant forms of laccase-2 from the tobacco hornworm, *Manduca sexta*. We also purified the endogenous enzyme from the pharate pupal cuticle and used peptide mass fingerprinting analysis to confirm that it is laccase-2. All three enzymes had pH optima between 5 and 5.5 when using N-acetyldopamine (NADA) or N-[beta]-alanyldopamine-alanyldopamine (NBAD) as substrates. The laccases exhibited typical Michaelis-Menten kinetics when NADA was used as a substrate, with K_m values of 0.46 mM, 0.43 mM, and 0.63 mM, respectively, for the full-length recombinant, truncated recombinant, and cuticular laccases; the apparent k_{cat} values were 100 min⁻¹, 80 min⁻¹, and 290 min⁻¹. The similarity in activity of the two recombinant laccases suggests that laccase-2 is expressed in an active form rather than as a zymogen, as had been previously proposed. This conclusion is consistent with the detection of activity in untanned pupal wing cuticle using the laccase substrate 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Immunoblot analysis of proteins extracted from both tanned and untanned cuticle detected only a single protein of 84 kDa, consistent with the full-length enzyme. With NBAD as substrate, the full-length recombinant and cuticular laccases showed kinetics indicative of substrate inhibition, with K_m values of 1.9 mM and 0.47 mM, respectively, and apparent k_{cat} values of 200 min⁻¹ and 180 min⁻¹. These results enhance our understanding of cuticle sclerotization, and may aid in the design of insecticides targeting insect laccases.

Keywords: Cuticle; Insect; Multicopper oxidase; Laccase; Polyphenol oxidase; Sclerotization; Substrate inhibition

Lars Maue, Derek Meissner, Hans Merzendorfer, Purification of an active, oligomeric chitin synthase complex from the midgut of the tobacco hornworm, *Insect Biochemistry and Molecular Biology*, Volume 39, Issue 9, September 2009, Pages 654-659, ISSN 0965-1748, DOI: 10.1016/j.ibmb.2009.06.005.

(<http://www.sciencedirect.com/science/article/B6T79-4WNGW65-2/2/7ccc90984e6962b193a6302cd4a00b1b>)

Abstract:

Chitin formation depends on the activity of a family II glycosyltransferase known as chitin synthase, whose biochemical and structural properties are largely unknown. Previously, we have demonstrated that the chitin portion of the peritrophic matrix in the midgut of the tobacco hornworm, *Manduca sexta*, is produced by chitin synthase 2 (CHS-2), one of two isoenzymes encoded by the *Chs-1* and *Chs-2* genes (also named *Chs-A* and *Chs-B*), and that CHS-2 is located at the apical tips of the brush border microvilli. Here we report the purification of the chitin synthase from the *Manduca* midgut as monitored by its activity and immuno-reactivity with antibodies to the chitin synthase. After gel permeation chromatography, the final step of the developed purification protocol, the active enzyme eluted in a fraction corresponding to a molecular mass between 440 and 670 kDa. Native PAGE revealed a single, immuno-reactive band of about 520 kDa, thrice the molecular mass of the chitin synthase monomer. SDS-PAGE and immunoblotting indicated finally that an active, oligomeric complex of the chitin synthase was purified. In summary, the chitin synthase from the midgut of *Manduca* may prove to be a good model for investigating the enzymes' mode of action.

Keywords: Chitin; Chitin synthase; *Manduca sexta*; Midgut; Peritrophic matrix; Oligomerization

Sunghoon Baek, Kijong Cho, Yoo Han Song, Joon-Ho Lee, Sampling plans for estimating pepper fruit damage levels by Oriental tobacco budworm, *Helicoverpa assulta* (Guenee), in hot pepper fields, *Journal of Asia-Pacific Entomology*, Volume 12, Issue 3, September 2009, Pages 175-178, ISSN 1226-8615, DOI: 10.1016/j.aspen.2009.03.003.

(<http://www.sciencedirect.com/science/article/B8JJN-4VYP9FR-1/2/2693ecc33ec00051b86795a88e519127>)

Abstract:

Sequential sampling programs for the management of Oriental tobacco budworm, *Helicoverpa assulta* (Guenee), on red hot peppers were developed using the data of damaged pepper fruits by *H. assulta*. Taylor's power law indicated that the damaged pepper fruits were distributed randomly in hot pepper fields. A fixed-precision-level sequential sampling plan for classifying fruit damage density levels at a critical density of 2 damaged fruits per plant was developed to assist in decision making. The sequential classification sampling plan was evaluated using the operating characteristic (OC) and the average sample size (ASN) curves. The OC and ASN curves indicated that this sampling plan was robust and properly classified the population density. A resampling simulation demonstrated that average actual sampling precision value at $D = 0.25$ was ≤ 0.25 . With sequential sampling for classifying the damaged fruit levels in terms of a critical density, sample size was fixed to 18 plants. The fixed-precision-level sequential sampling plan developed in this study should greatly enhance the monitoring efficacy and provide practical solutions suitable for reliable decision-making process in the management of *H. assulta*.

Keywords: Taylor power law; Spatial distribution pattern; Fixed-precision-level sampling; Hot pepper

Osmany Chacon, Ingrid Hernandez, Roxana Portieles, Yuniur Lopez, Merardo Pujol, Orlando Borrás-Hidalgo, Identification of defense-related genes in tobacco responding to black shank disease, *Plant Science*, Volume 177, Issue 3, September 2009, Pages 175-180, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2009.05.009.

(<http://www.sciencedirect.com/science/article/B6TBH-4WBK762-1/2/95c641da52bc6699dc9b8cfb4313c6d4>)

Abstract:

In order to identify tobacco (*Nicotiana megalosiphon*) genes involved in broad-spectrum resistance to tobacco black shank (*Phytophthora parasitica* var. *nicotianae*), suppression subtractive hybridization (SSH) was used to generate a cDNA from transcripts that are differentially expressed during an incompatible interaction. Forty-eight differentially expressed genes were selected, sequenced and analyzed. The cDNA collection comprised a repertoire of genes associated with various processes. Real-time PCR analysis of a subset of these genes confirmed the differential expression patterns between the compatible and incompatible interaction. The experiments demonstrated for the first time that *hrs203J* gene and RING finger protein gene exhibited strong induction during several incompatible interactions. Also, these genes were found not to be induced in compatible interactions. The set of differentially expressed tobacco genes associated to resistance could be exploited in strategies to develop durable resistance in cultivated tobacco plants.

Keywords: *Nicotiana megalosiphon*; *Phytophthora parasitica* var. *nicotianae*; Suppression subtractive hybridization; Plant resistance

Min Wang, Gang Wang, Jing Ji, Jiehua Wang, The effect of *pds* gene silencing on chloroplast pigment composition, thylakoid membrane structure and photosynthesis efficiency in tobacco plants, *Plant Science*, Volume 177, Issue 3, September 2009, Pages 222-226, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2009.04.006.

(<http://www.sciencedirect.com/science/article/B6TBH-4W3PT3D-3/2/0eb61404640af5f777fbf4a1e3966d0d>)

Abstract:

Nicotiana tabacum plants that are silenced in *pds* gene have been constructed by RNAi technology. Transgenics showed different degrees of albinism and PSII efficiency was decreased significantly compared to wild type plants. The total carotenoid contents decreased. The ratio of chlorophyll (Chl) a to b was unchanged while the content of both declined. The grana stacking of thylakoid in variegation part was as normal as in the control and etioplast occurred in the white sector of leaf. The concentration of thylakoid membrane protein of molecular weight about 20 kDa was significantly increased compared to wild type plants according to the same contents of Chl. These observations suggest the exact matching of pigments and proteins is important for energy absorption and transfer in photosynthesis and mismatching of them could cause malfunction of PSII.

Keywords: RNAi; *pds* gene; Photosynthesis pigment; Chloroplast

Fumiko Taguchi, Tomoko Suzuki, Kasumi Takeuchi, Yoshishige Inagaki, Kazuhiro Toyoda, Tomonori Shiraishi, Yuki Ichinose, Glycosylation of flagellin from *Pseudomonas syringae* pv. *tabaci* 6605 contributes to evasion of host tobacco plant surveillance system, *Physiological and Molecular Plant Pathology*, In Press, Accepted Manuscript, Available online 21 August 2009, ISSN 0885-5765, DOI: 10.1016/j.pmpp.2009.08.001.

(<http://www.sciencedirect.com/science/article/B6WPC-4X24VMK-1/2/a2e33c70c333c666c1f84ba8e7587148>)

Abstract:

Pseudomonas syringae pv. *tabaci* (Pta) possesses a genetic region composed of two open reading frames (ORFs), *fgt1* and *fgt2*, that are involved in glycosylation of flagellin. The deletion mutant [increment] *fgt1* produced non-glycosylated flagellin, and exhibited reduced ability to cause disease in the host tobacco plant. Flagellin is known to induce plant defense responses, and the recognition of flagellin by *Arabidopsis thaliana* is mediated by a conserved N-terminal region, *flg22*, in flagellin and a leucine-rich repeat domain in the FLS2 receptor. Because *flg22* localizes inside the flagellum, polymerized flagellum needs to be dissociated to be recognized. Therefore, the effect of glycosylation on flagella stability was investigated. The polymerized flagella from glycosylated flagellins were more resistant to heat treatment than those from non-glycosylated flagellins, suggesting that the glycosylation of flagellin contributes to the structural stability of flagella and prevents exposure of the *flg22* region. Polymerized flagella from Pta [increment] *fgt1* flagellin and depolymerized and glycosylated flagellin from Pta wild type induced cell death and callose deposition, and inhibited seedling growth in tobacco more effectively, whereas polymerized flagella from Pta wild-type flagellin caused a low level of these responses. These results suggest Pta might have evolved the flagellin glycosylation system to evade detection and defense response of a host by increasing flagella stability and suppressing their dissociation.

Keywords: Defense Response; Flagellar Stability; Flagellin; Glycosylation; Host Specificity; MAMP

Masaru Sakamoto, Reiko Tomita, Kappei Kobayashi, A protein containing an XYPPX repeat and a C2 domain is associated with virally induced hypersensitive cell death in plants, *FEBS Letters*, Volume 583, Issue 15, 6 August 2009, Pages 2552-2556, ISSN 0014-5793, DOI: 10.1016/j.febslet.2009.07.020.

(<http://www.sciencedirect.com/science/article/B6T36-4WSY4GH-3/2/02245748997b4a52df2f89d1ca3394>)

Abstract:

In this study, we characterized a *Capsicum* hypersensitive response (HR)-associated gene, *SS52*, which encodes a protein that contains an N-terminal C2 domain and a C-terminal XYPPX repeat. Expression analyses revealed that *SS52* and its homologue in *Arabidopsis* were induced by

infection with incompatible viruses, indicating the conserved function of this gene. SS52 was not induced by treatment with defense-related hormones, but was induced by abiotic stresses, including wounding. Overexpression of SS52 in tobacco plants suppressed the spread of HR cell death and restricted the spread of an incompatible virus from local lesions. Collectively, the results suggest that SS52 negatively regulates plant HR cell death.

Keywords: Abiotic stress; C2 domain; HR cell death; XYPPX repeat; Capsicum

Alessandra F.M. Viu, Marco A.O. Viu, Armando R. Tavares, Fabio Vianello, Giuseppina P.P. Lima, Endogenous and exogenous polyamines in the organogenesis in *Curcuma longa* L., *Scientia Horticulturae*, Volume 121, Issue 4, 4 August 2009, Pages 501-504, ISSN 0304-4238, DOI: 10.1016/j.scienta.2009.03.003.

(<http://www.sciencedirect.com/science/article/B6TC3-4W207K1-1/2/55a67c400e2bea07310b9194de47a7ea>)

Abstract:

The present work evaluated the development of different *Curcuma longa* L. explants (leaves basis, root tips and ancillary buds from rhizome) stimulated by exogenous polyamines, combined with naphthalen-acetic acid (NAA) or with 6-benzyl-aminopurine (BAP), to produce callus and its subsequent differentiation. The explants, isolated from field plants, were previously subjected to a basic cleaning method and were inoculated onto Murashige and Skoog culture medium (MS) [Murashige, T.S., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* 15, 473-497] supplemented with NAA (2.0 mg L⁻¹). Buds were subjected to different treatments, with or without 5.0 and 10.0 mmol L⁻¹ exogenous polyamines (mixture of putrescine:spermine:spermidine, 1:1:1) combined with NAA. The calluses obtained were transferred into the same medium, supplemented with the mixture of polyamines combined with BAP, in order to induce plant differentiation. For *C. longa*, buds were the most efficient explants for callus induction ($p < 0.05$). The application of exogenous polyamines (5.0 and 10.0 mmol L⁻¹) produced the most developed callus, with numerous roots. The medium supplemented with 10 mmol L⁻¹ polyamine mixture, combined with BAP, induced good regeneration, producing vigorous plants and excellent shoot formation. Polyamines addition promoted the formation of callus, roots and leaves, representing an important factor in the determination of indirect organogenesis in *C. longa* L., and putrescine content may be considered a valuable marker of the differentiation process in this specie, as well as the enzyme peroxidase.

Keywords: Organogenesis; Putrescine; Spermidine; Spermine; Peroxidase

Stefan Biastoff, Wolfgang Brandt, Birgit Drager, Putrescine N-methyltransferase - The start for alkaloids, *Phytochemistry*, In Press, Corrected Proof, Available online 3 August 2009, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2009.06.012.

(<http://www.sciencedirect.com/science/article/B6TH7-4WXBRJN-2/2/65ada230120830391906062ead4362de>)

Abstract:

Putrescine N-methyltransferase (PMT) catalyses S-adenosylmethionine (SAM) dependent methylation of the diamine putrescine. The product N-methylputrescine is the first specific metabolite on the route to nicotine, tropane, and nortropine alkaloids. PMT cDNA sequences were cloned from tobacco species and other Solanaceae, also from nortropine-forming Convolvulaceae and enzyme proteins were synthesised in *Escherichia coli*. PMT activity was measured by HPLC separation of polyamine derivatives and by an enzyme-coupled colorimetric assay using S-adenosylhomocysteine. PMT cDNA sequences resemble those of plant spermidine synthases (putrescine aminopropyltransferases) and display little similarity to other plant methyltransferases. PMT is likely to have evolved from the ubiquitous enzyme spermidine synthase. PMT and spermidine synthase proteins share the same overall protein structure; they bind the same substrate putrescine and similar co-substrates, SAM and decarboxylated S-adenosylmethionine.

The active sites of both proteins, however, were shaped differentially in the course of evolution. Phylogenetic analysis of both enzyme groups from plants revealed a deep bifurcation and confirmed an early descent of PMT from spermidine synthase in the course of angiosperm development.

Keywords: *Datura stramonium*; Solanales; Putrescine N-methyltransferase; Spermidine synthase; S-Adenosylmethionine; Alkaloid biosynthesis; Enzyme evolution; Protein modelling; Mutagenesis

Preeti A. Mehta, Keerthi C. Rebala, Gayatri Venkataraman, Ajay Parida, A diurnally regulated dehydrin from *Avicennia marina* that shows nucleo-cytoplasmic localization and is phosphorylated by Casein kinase II in vitro, *Plant Physiology and Biochemistry*, Volume 47, Issue 8, August 2009, Pages 701-709, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2009.03.008.

(<http://www.sciencedirect.com/science/article/B6VVD-4VY2C61-2/2/d26ba13ba733840573450ff2b4b9c38>)

Abstract:

Dehydrins have a key role in protecting plants from dehydration stress. We report here the isolation of two cDNAs coding for the same dehydrin, AmDHN1 and AmDHN1a from salt stressed leaves of *Avicennia marina* (Forsk.) Vierh. by EST library screening. AmDHN1 was found to contain a retained intron that was absent in AmDHN1a. AmDHN1 expression in the context of various environmental stresses was investigated. In leaves, AmDHN1 shows a diurnal pattern of regulation and is induced only by mannitol application. In roots, AmDHN1 is rapidly induced by salinity (NaCl) and dehydration stress (PEG and mannitol). A fragment of 795 bp corresponding to the 5' upstream region of AmDHN1 was isolated by TAIL-PCR. In silico analysis of this sequence reveals the presence of putative stress regulatory elements (ABRE, DRE, MYB and MYC binding sequences). Putative phosphorylation sites for Casein kinase II were identified in the AmDHN1a ORF. In vitro phosphorylation of *Escherichia coli* expressed Trx-AmDHN1a by Casein kinase II was observed that was reversed by Shrimp Alkaline Phosphatase treatment. A putative nuclear targeting domain was identified in the translated AmDHN1a ORF and stably transformed AmDHN1a-GFP was found to show nucleo-cytoplasmic localization in tobacco guard cells. As observed for maize Rab17, the phosphorylation of AmDHN1a may contribute to its nuclear localization.

Keywords: *Avicennia marina*; Dehydrin; Diurnal regulation; Green fluorescent protein; Nucleo-cytoplasmic localization; Phosphorylation

Chen Yafei, Zhan Yong, Zhao Xiaoming, Guo Peng, An Hailong, Du Yuguang, Han Yingrong, Liu Hui, Zhang Yuhong, Functions of oligochitosan induced protein kinase in tobacco mosaic virus resistance and pathogenesis related proteins in tobacco, *Plant Physiology and Biochemistry*, Volume 47, Issue 8, August 2009, Pages 724-731, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2009.03.009.

(<http://www.sciencedirect.com/science/article/B6VVD-4VY2C61-1/2/13c20e5840479996a0722fe4a378b481>)

Abstract:

Oligochitosan (OC) can regulate plant defense responses in many aspects, but the basic signal transduction pathway is still unclear. In this study, we used transgenic (TG) tobacco (*Nicotiana Tabacum* var. Samsun NN) as plant material whose oligochitosan induced protein kinase (OIPK) gene was inhibited by antisense transformation, to study the role of OIPK in tobacco defense reactions. The results showed that OIPK could increase tobacco resistance against tobacco mosaic virus (TMV), in that wild-type (WT) tobacco showed longer lesion appearance time, higher lesion inhibition ratio, smaller average final lesion diameter and lower average final lesion area percent to whole leaf area. It led us to analyze some pathogenesis related (PR) enzymes' activities and mRNA level, which played roles in tobacco resistance against TMV. We found that phenylalanine ammonia-lyase (PAL) and peroxidase (POD) activities were positively related to

OIPK, but not polyphenol oxidase (PPO). It was also demonstrated that OIPK mRNA could be induced by OC, wound and TMV infection. In addition, OIPK could up-regulated three PR genes, PAL, chitinase (CHI) and [beta]-1, 3-glucanase (GLU) mRNA level to different extent. Taken together, these results implied that OIPK could function in tobacco resistance against both biotic and abiotic stress, possibly via various PR proteins.

Keywords: Oligochitosan induced protein kinase; Transgenic tobacco; TMV resistance; Pathogenesis related proteins

Francois Cholette, Lay-Keow Ng, A real-time polymerase chain reaction (PCR) method for the identification of *Nicotiana tabacum* in tobacco products, *Industrial Crops and Products*, In Press, Corrected Proof, Available online 29 July 2009, ISSN 0926-6690, DOI: 10.1016/j.indcrop.2009.06.008.

(<http://www.sciencedirect.com/science/article/B6T77-4WW7NND-1/2/e9667b79e29f08b2045d7ee6db78751d>)

Abstract:

A simple and specific real-time PCR assay based on TaqMan(R) technology has been developed for the identification of cultured tobacco (*Nicotiana tabacum*) in various commodities such as cigars, cigarettes and reconstituted tobacco. The TaqMan(R) assay targets a sequence of the putrescine N-methyltransferase gene family encoding an enzyme that plays a crucial role in the biosynthesis of nicotine. To reduce the possibility of false negatives, universal plant chloroplast primers were also used in a separate real-time PCR reaction to give indication if DNA is amplifiable in the matrix. The TaqMan(R) assay successfully identified tobacco in over 40 commercial tobacco products, while negative results were obtained from the assay for DNA extracted from a variety of other botanical products. In our study, two commercial DNA isolation kits were used, namely, the Qiagen DNeasy(R) Plant Mini kit and the Qiagen Genra(R) Puregene(R) kit. They produced good quality DNAs in sufficient quantities for real-time PCR analysis. In a few cases, an additional purification step with the Promega DNA IQ(TM) system had to be implemented to obtain amplifiable DNA.

Keywords: DNA extraction; *Nicotiana tabacum*; Putrescine N-methyltransferase; Real-time PCR; Tobacco products

Manuela Eick, Christine Stohr, Proteolysis at the plasma membrane of tobacco roots: Biochemical evidence and possible roles, *Plant Physiology and Biochemistry*, In Press, Corrected Proof, Available online 24 July 2009, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2009.07.007.

(<http://www.sciencedirect.com/science/article/B6VVD-4WV77T8-1/2/a0c1f086fdef35b413c4ff901dff5ecc>)

Abstract:

Plasma membrane-associated proteases (pm-proteases) exist principally in roots of *Nicotiana tabacum* cv. Samsun, whereas in plasma membrane (pm) vesicles prepared from leaves, protease activity was at the detection limit. Biochemical characterisation revealed a high diversity of particular hydrophobic pm-proteases indicating multiple functions in root tissue. One proportion of chromatographically separated proteases was split up by non-reducing SDS-PAGE in 8-12 single polypeptides, dependent on plant nitrogen nutrition. The active polypeptides could be grouped in those that were (i) inhibited, (ii) stimulated and (iii) independent of bivalent cations. Although, the total specific protease activity of various pm vesicles was almost identical, the composition and activity of individual polypeptides was dependent on nitrogen supply of the plants. Particularly, nitrogen deficiency stimulated the activity of high molecular mass proteases (125 kDa-97 kDa), whereas sufficient nitrate supply enhanced proteolytic activity of 90 kDa, 83 kDa and 65 kDa polypeptides. Endogenous proteolysis within pm vesicles suggested that at least partly protease substrates are localised within the same membrane. A comparison of polypeptides originated from

proteolysis of pm vesicles and those exudated by roots into the external medium points to a role of root pm-proteases in the specific release of polypeptides into the rhizosphere.

Keywords: Plasma membrane; Protease; Roots; Tobacco; Peptide; Exudate

Petra Majer, Laszlo Sass, Gabor V. Horvath, Eva Hideg, Leaf hue measurements offer a fast, high-throughput initial screening of photosynthesis in leaves, *Journal of Plant Physiology*, In Press, Corrected Proof, Available online 23 July 2009, ISSN 0176-1617, DOI: 10.1016/j.jplph.2009.06.015.

(<http://www.sciencedirect.com/science/article/B7GJ7-4WTYXTM-3/2/d6d8e91e91310bec668b9dadae75d062>)

Abstract: Summary

Experiments with tobacco and grapevine leaves having different color due to varying stages of senescence showed that leaf hue is significantly linearly correlated with chlorophyll content up to 80% loss of pigment. Samples from leaves with more pronounced loss of chlorophyll did not fit into this linear relationship, and the hue data set as a whole followed a saturating exponential dependence on chlorophyll content. In leaves with less than 80% chlorophyll loss, the hue parameter was also proportional to the photochemical yield of photosystem (PS) II measured in the light. These results suggest that leaf hue measurements offer a fast, high-throughput initial screening system to precede more specific but more time consuming photosynthesis measurements, with the possibility of applications not only for senescing plants, but also for stress conditions accompanied by chlorophyll loss.

Keywords: Chlorophyll fluorescence; High-throughput screening; Leaf hue; Photochemical yield

Hai Fang, Mir M. Ali, John A. Rizzo, Does smoking affect body weight and obesity in China?, *Economics & Human Biology*, In Press, Corrected Proof, Available online 21 July 2009, ISSN 1570-677X, DOI: 10.1016/j.ehb.2009.07.003.

(<http://www.sciencedirect.com/science/article/B73DX-4WTHS73-1/2/5cfaaf46935ac931be57c6137d8543eb>)

Abstract:

An inverse relationship between smoking and body weight has been documented in the medical literature, but the effect of cigarette smoking on obesity remains inconclusive. In addition, the evidence is mixed on whether rising obesity rates are an unintended consequence of successful anti-smoking policies. This study re-examines these relationships using data from China, the largest consumer and manufacturer of tobacco in the world that is also experiencing a steady rise in obesity rates. We focus on the impact of the total number of cigarettes smoked per day on individuals' body mass index (BMI) and on the likelihood of being overweight and obese. Instrumental variables estimation is used to correct for the endogeneity of cigarette smoking. We find a moderate negative and significant relationship between cigarette smoking and BMI. Smoking is also negatively related to being overweight and obese, but the marginal effects are small and statistically insignificant for being obese. Quantile regression analyses reveal that the association between smoking and BMI is quite weak among subjects whose BMIs are at the high end of the distribution but are considerably stronger among subjects in the healthy weight range. Ordered probit regression analyses also confirm these findings. Our results thus reconcile an inverse average effect of smoking on body weight with the absence of any significant effect on obesity. From a policy perspective these findings suggest that, while smoking cessation may lead to moderate weight gain among subjects of healthy weight, the effects on obese subjects are modest and should not be expected to lead to a large increase in obesity prevalence rates.

Keywords: Cigarette smoking; Body weight; Obesity; China

Xiaoting Qi, Qihong Cui, Ying Luo, Chunfen Guo, Tuanyao Chai, Zn stress-induced inhibition of bean PvSR2-GUS fusion gene splicing is gene-specific in transgenic tobacco, *Journal of Plant*

Physiology, Volume 166, Issue 11, 15 July 2009, Pages 1223-1227, ISSN 0176-1617, DOI: 10.1016/j.jplph.2009.01.010.

(<http://www.sciencedirect.com/science/article/B7GJ7-4VWJ1M7-1/2/dfaff4d88e87d1a4a64202e99d4e5551>)

Abstract: Summary

The stress-related gene no. 2 of *Phaseolus vulgaris* (PvSR2) is metal inducible and contains a single intron. Here, we report that Zn stress inhibited the splicing of the PvSR2-[beta]-glucuronidase (GUS) fusion gene in a concentration- and time-dependent manner in tobacco seedlings. The inhibition appears to be specific for the PvSR2-GUS transgene: splicing of four endogenous tobacco genes was unaffected by Zn stress. Our results provide *in vivo* evidence that Zn stress-dependent intron retention is transgene specific in plants.

Keywords: Intron retention; Intron splicing; PvSR2 intron; Transgene specific

Jadwiga Cholewa, Hans-Joachim Pflüger, Descending unpaired median neurons with bilaterally symmetrical axons in the suboesophageal ganglion of *Manduca sexta* larvae, *Zoology*, Volume 112, Issue 4, 15 July 2009, Pages 251-262, ISSN 0944-2006, DOI: 10.1016/j.zool.2008.10.004.

(<http://www.sciencedirect.com/science/article/B7GJ0-4W7B51F-1/2/88097d8a4548816927f1acc87a77c0f7>)

Abstract:

Three large median cell bodies with a diameter between 40 and 70 [μ]m that exhibit octopamine immunoreactivity were identified in the posterior part of the suboesophageal ganglion of the tobacco hawkmoth larva, *Manduca sexta*. These neurons possess bilaterally symmetrical axons in the posterior neck connectives, and at least one of them extends through the whole ventral nerve cord to the terminal abdominal ganglion. Therefore, these neurons belong to the class of descending ventral unpaired median neurons. From each cell body, a primary neurite ascends anteriorly, which after bending dorsally turns posteriorly and then bifurcates to give rise to two descending axons. From the primary neurite two main dendritic branches ascend anteriorly, and four characteristic branches can be distinguished originating from them: two descending dendritic branches and two ascending dendritic branches. Dense arborizations from all these branches exist in all neuromeres of the suboesophageal ganglion. Intracellular recordings from these neurons show that in contrast to the ventral unpaired median neurons of thoracic and abdominal ganglia, they do not produce overshooting action potentials but exhibit passive soma spikes only. During pharmacologically evoked fictive motor patterns these neurons show coupling to various motor patterns such as crawling, feeding and molting.

Keywords: Insects; Biogenic amines; Octopamine; Neuromodulation; Motor pattern

Pil Joong Chung, Youn Shic Kim, Su-Hyun Park, Baek Hie Nahm, Ju-Kon Kim, Subcellular localization of rice histone deacetylases in organelles, *FEBS Letters*, Volume 583, Issue 13, 7 July 2009, Pages 2249-2254, ISSN 0014-5793, DOI: 10.1016/j.febslet.2009.06.003.

(<http://www.sciencedirect.com/science/article/B6T36-4WGF15D-5/2/f1d8d68378e2423067d539a165fc3ffc>)

Abstract:

Histone deacetylases (HDACs) are known to function in the nucleus. Here, we report on the organellar localization of three rice HDACs, OsSIR2b, OsHDAC6, and OsHDAC10. The 35S:OsSIR2b-GFP and 35S:OsHDAC10-GFP constructs were introduced into tobacco BY2 cells. Co-localization analysis of the green fluorescent protein and MitoTracker fluorescent signals in the transformed BY2 cells indicated that OsSIR2b and OsHDAC10 are localized in the mitochondria. Transgenic *Arabidopsis* lines harboring 35S:OsHDAC6-GFP and 35S:OsHDAC10-GFP constructs were similarly analyzed, revealing that OsHDAC6-GFP is localized exclusively in chloroplasts, whereas OsHDAC10-GFP is localized in both mitochondria and chloroplasts. The presence of OsHDAC6-GFP and OsHDAC10-GFP in chloroplasts was verified by immunodetection.

Keywords: Rice histone deacetylase; Chloroplast; Mitochondria; OsHDAC6; OsHDAC10; OsSIR2b

Boo-Ja Lee, Sung-Kyu Kim, Soo Bok Choi, Jungdon Bae, Ki-Jeong Kim, Young-Jin Kim, Kyung-Hee Paek, Pathogen-inducible CaUGT1 is involved in resistance response against TMV infection by controlling salicylic acid accumulation, *FEBS Letters*, Volume 583, Issue 13, 7 July 2009, Pages 2315-2320, ISSN 0014-5793, DOI: 10.1016/j.febslet.2009.06.028.

(<http://www.sciencedirect.com/science/article/B6T36-4WK43WG-3/2/d03af9f587d46ce313d76f239f555405>)

Abstract:

Capsicum annuum L. Bugang exhibits a hypersensitive response against Tobacco mosaic virus (TMV) P0 infection. The *C. annuum* UDP-glucosyltransferase 1 (CaUGT1) gene was upregulated during resistance response to TMV and by salicylic acid, ethephon, methyl viologen, and sodium nitroprusside treatment. When the gene was downregulated by virus-induced gene silencing, a delayed HR was observed. In addition, free and total SA concentrations in the CaUGT1-downregulated hot pepper were decreased by 52% and 48% compared to that of the control plants, respectively. This suggested that the CaUGT1 gene was involved in resistance response against TMV infection by controlling the accumulation of SA.

Keywords: Salicylic acid; Hot pepper UDP-glucosyltransferase; Tobacco mosaic virus

Ozlem Akpınar, Kader Erdogan, Ufuk Bakir, Levent Yilmaz, Comparison of acid and enzymatic hydrolysis of tobacco stalk xylan for preparation of xylooligosaccharides, *LWT - Food Science and Technology*, In Press, Corrected Proof, Available online 4 July 2009, ISSN 0023-6438, DOI: 10.1016/j.lwt.2009.06.025.

(<http://www.sciencedirect.com/science/article/B6WMV-4WNXV4W-3/2/e7665c958ca20b0e235e1dfcfc383d16>)

Abstract:

Tobacco stalk (TS), a major agricultural waste in the Black Sea region of Turkey, was used for the production of xylooligosaccharides (XOs). It contains about 22 g/100 g xylan whose composition was determined as 93.5 g/100 g xylose, 6.54 g/100 g glucose and 11.2 g/100 g uronic acid after complete acid hydrolysis. XO production was performed by enzymatic and acid hydrolysis of xylan which was obtained by alkali extraction from tobacco stalk. In enzyme hydrolysis, xylan was hydrolyzed using a xylanase preparation and the effects of pH, temperature, hydrolysis period, substrate and enzyme concentrations on the xylooligosaccharide yield and degree of polymerization were investigated. For enzymatic hydrolysis under optimum conditions XO yield with respect to tobacco stalk xylan (TSX) was 8.2 g/100 g after 8 h and 11.4 g/100 g after 24 h reaction period. In the acid hydrolysis, sulfuric acid was used and the hydrolyzate contained different amount of oligosaccharides and monosaccharides. For acid hydrolysis under optimum conditions, XO yield with respect to TSX was 13.0 g/100 g. Enzymatically obtained oligosaccharides were purified via ultrafiltration by using 10 and 3 kDa membranes. After a two-step membrane processing, the permeate containing mostly oligosaccharides was obtained.

Keywords: Xylooligosaccharides; Tobacco stalk; Prebiotics; Lignocellulose

Majid Mahdieh, Akbar Mostajeran, Abscisic acid regulates root hydraulic conductance via aquaporin expression modulation in *Nicotiana tabacum*, *Journal of Plant Physiology*, In Press, Corrected Proof, Available online 2 July 2009, ISSN 0176-1617, DOI: 10.1016/j.jplph.2009.06.001.

(<http://www.sciencedirect.com/science/article/B7GJ7-4WNGD59-1/2/88bb79b3d7690bf69b7c14d355a092be>)

Abstract: Summary

Abscisic acid (ABA) modifies the hydraulic properties of roots by increasing root water flux. The effects of ABA on aquaporin content and root hydraulic conductance are controversial. We addressed these effects via a combination of experiments. Tobacco (*Nicotiana tabacum*) plants

were grown hydroponically, and ABA (1 [μ M]) was exogenously applied to the roots. Then, the water transport properties of tobacco roots and expression of PIP-type aquaporins were examined. ABA increased the sap flow rate (Jv) and also the osmotic root hydraulic conductance (Lpr-o) of excised tobacco roots after 24 h. The expression of three aquaporin PIP-type genes and PIP1s proteins abundance in tobacco roots were analyzed by real-time PCR and protein gel blot analysis, respectively. Interestingly, the accumulation of NtAQP1, NtPIP1;1 and NtPIP2;1 transcripts and NtPIP1;1 and NtAQP1 proteins abundance was significantly increased. Although the antibody used recognize NtPIP1;1 and NtAQP1, most probably it also recognizes other PIP1 proteins present in tobacco. Thus, the increase in the expression of the three PIP-type genes and other PIP1s proteins abundance caused by ABA were correlated with an increase in Lpr-o and Jv. ABA therefore facilitated the cell-to-cell component of water transport across the root cylinder. The subcellular localization of NtPIP1;1- and NtPIP2;1-GFP was investigated by protoplast transformation with chimeric gene, showing NtPIP2;1 localization in plasma membrane and NtPIP1;1 retention in the endoplasmic reticulum (ER). However, ABA did not change subcellular localization of NtPIP1;1 from ER to plasma membrane.

Keywords: Abscisic acid; Aquaporin; *Nicotiana tabacum*; Osmotic root hydraulic conductance; Sap flow rate

Maurice Auroux, Magali Volteau, Beatrice Ducot, Thierry Wack, Alexia Letierce, Laurence Meyer, Marie-Jeanne Mayaux, Progeny's mental aptitudes in man: relationship with parental age at conception and with some environmental factors, *Comptes Rendus Biologies*, Volume 332, Issue 7, July 2009, Pages 603-612, ISSN 1631-0691, DOI: 10.1016/j.crv.2009.02.008.

(<http://www.sciencedirect.com/science/article/B6X1F-4W14HS7-2/2/fbb62353b88dfebe7517c0c1a3ddc556>)

Abstract:

Psychometric tests obtained from 6564 young men were studied as a function of the parents' ages at conception and of some characteristics of the subject's postnatal environment. Individual scores, from 0 to 20, were divided into two groups: n1[greater-or-equal, slanted]11 and n2<11. In univariate analysis, scores <11 were respectively related to low height, high number of siblings and junior in birth order, subject's and parents' tobacco consumption, parents' alcohol consumption, subject's and parents' low academic standard, parents' youth or ageing at conception. In multivariate analysis, these scores remained related to low height, junior in birth order, subject's and parents' tobacco consumption, parents' low academic standard, parents' youth (both <20). Regarding the respective influences of the environment and of the subject's genome on his cerebral development, one can hypothesize a complementarity between these two factors through the possibility of a genetically determined individual synaptic potential, revealing itself, more or less, according to environmental conditions. To cite this article: M. Auroux et al., *C. R. Biologies* 332 (2009).

Keywords: Male progeny; Psychometric tests; Parental age at conception; Environmental factors; Genetic factors; Synaptic potential; Progeniture male; Tests psychometriques; Age parental a la conception; Facteurs environnementaux; Facteurs genetiques; Potentiel synaptique

Ewa Niewiadomska, Lisa Polzien, Christine Desel, Piotr Rozpadek, Zbigniew Miszalski, Karin Krupinska, Spatial patterns of senescence and development-dependent distribution of reactive oxygen species in tobacco (*Nicotiana tabacum*) leaves, *Journal of Plant Physiology*, Volume 166, Issue 10, 1 July 2009, Pages 1057-1068, ISSN 0176-1617, DOI: 10.1016/j.jplph.2008.12.014.

(<http://www.sciencedirect.com/science/article/B7GJ7-4VRWNCS-1/2/475ae65aa8fc4edec3d157a8dc9bbe35>)

Abstract: Summary

Senescence of tobacco leaves is distributed non-uniformly over a leaf blade. While photosynthetic competence and expression of photosynthesis-associated genes decline in interveinal areas of the

leaf lamina with advancing age of the leaf, they are maintained at high levels in the tissue surrounding the veins. In contrast, expression of senescence-associated genes (SAG) was enhanced in both areas of the leaf blade. Accumulation of hydrogen peroxide was shown to precede the phase of senescence initiation in the veinal tissue. In the interveinal tissue, the level of hydrogen peroxide was increased with senescence progression and paralleled by an increase in the level of superoxide anions. It is hypothesized that the spatial differences in superoxide anions are important for the non-uniform down-regulation of photosynthesis-associated genes (PAG), while hydrogen peroxide is responsible for up-regulation of SAG.

Keywords: Hydrogen peroxide; Interveinal tissue; Leaf senescence; Superoxide; Veins

Dae Kwan Ko, Mi Ok Lee, Ji-Sook Hahn, Byung-gee Kim, Choo Bong Hong, Submergence-inducible and circadian rhythmic basic helix-loop-helix protein gene in *Nicotiana tabacum*, *Journal of Plant Physiology*, Volume 166, Issue 10, 1 July 2009, Pages 1090-1100, ISSN 0176-1617, DOI: 10.1016/j.jplph.2008.12.008.

(<http://www.sciencedirect.com/science/article/B7GJ7-4VKDGM7-1/2/25818d519316616a9572d1db4d8f5e0e>)

Abstract: Summary

Submergence stress leads to diverse changes in transcription and translation of genes involved in developmental and physiological metabolisms of plants. The basic helix-loop-helix (bHLH) protein family is one of the largest transcriptional factor families in plants, and has been shown to play pivotal roles in diverse biological responses. However, there has been no report on bHLH protein related to submergence stress response. In this study, a novel bHLH gene, NtbHLH, was isolated from tobacco (*Nicotiana tabacum*) by differential screening of a submergence-stress-induced cDNA library. NtbHLH cDNA is 1027 bp in length, with an open reading frame (ORF) of 702 nucleotides encoding 233 amino acid residues that contain the bHLH domain. RNA-blot analyses showed that transcription of NtbHLH was induced by submergence stress, while cold, heat shock, and drought decreased its expression. The gene expression was down-regulated by gibberellins, but ABA and ethylene seemed not to affect it. It was also apparent that NtbHLH expression follows circadian rhythmicity. The electrophoretic mobility shift and chemical cross-linking assays showed that NtbHLH specifically binds to G-box and forms homo-dimers.

Keywords: Basic Helix-Loop-Helix protein; Circadian clock; *Nicotiana tabacum*; NtbHLH; Submergence stress

Ali Mahjoub, Michel Hernould, Jerome Joubes, Alain Decendit, Mohamed Mars, Francois Barrieu, Said Hamdi, Serge Delrot, Overexpression of a grapevine R2R3-MYB factor in tomato affects vegetative development, flower morphology and flavonoid and terpenoid metabolism, *Plant Physiology and Biochemistry*, Volume 47, Issue 7, July 2009, Pages 551-561, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2009.02.015.

(<http://www.sciencedirect.com/science/article/B6VRD-4VT5TJ6-2/2/68a6b480e928685505d5757ab2f892e2>)

Abstract:

Although the terpenoid pathway constitutes, with the phenylpropanoid metabolism, the major pathway of secondary metabolism in plants, little is known about its regulation. Overexpression of a *Vitis vinifera* R2R3-MYB transcription factor (VvMYB5b) in tomato induced pleiotropic changes including dwarfism, modified leaf structure, alterations of floral morphology, pigmented and glossy fruits at the 'green-mature' stage and impaired seed germination. Two main branches of secondary metabolism, which profoundly influence the organoleptic properties of the fruit, were affected in the opposite way by VvMYB5b overexpression. Phenylpropanoid metabolism was down regulated whereas the amount of beta-carotene was up regulated. This is the first example of the independent regulation of phenylpropanoid and carotenoid metabolism. The strongest modification concerns a decrease in beta-amyrin, the precursor of the oleanolic acid, which is the major

component of grape waxes. Scanning electron microscopy analysis of fruits and leaves confirms the alteration of wax metabolism and a modification of cell size and shape. This may potentially impact resistance/tolerance to biotic and abiotic stresses. The results are compared with a similar approach using heterologous expression of VvMYB5b in tobacco.

Keywords: Flavonoids; Grapes ripening; R2R3-MYB transcription factor; Terpenoids; Wax

Lenka Gemperlova, Milena Cvikrova, Lucie Fischerova, Pavla Binarova, Lukas Fischer, Josef Eder, Polyamine metabolism during the cell cycle of synchronized tobacco BY-2 cell line, *Plant Physiology and Biochemistry*, Volume 47, Issue 7, July 2009, Pages 584-591, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2009.02.014.

(<http://www.sciencedirect.com/science/article/B6VRD-4VT5TJ6-1/2/f83f8fb9cf1c6e26db974b8804202dcf>)

Abstract:

The time courses of the contents of free, soluble and insoluble polyamine (PA) conjugates, PA biosynthetic and catabolic enzyme activities and mRNA levels of PA biosynthetic genes were monitored during the cell cycle of synchronized tobacco BY-2 cell line (*Nicotiana tabacum* L. cv. Bright Yellow 2). Progression through the cell cycle was characterized by specific biphasic changes of PA levels. The first, moderate peak in the amount of free PAs coincided with the S-phase. After a transient decline in G2 phase the contents of free PAs increased rapidly and peaked again during G2/M interface. Then sharply decreased with the minimum at the end of mitosis and during M/G1 transition and started to rise again with the next replication phase. Levels of PA soluble conjugates paralleled those of the free forms. Biosynthetic enzyme activities followed the biphasic manner analogous to the levels of free PAs and seemed to be regulated on both transcriptional and (post)translational level. PA cellular levels were further controlled by both catabolic degradation and conjugation of PAs. PA catabolism played an important role in the PA down-regulation during G2 phase and late mitosis, while the decline in free PAs in G2/M interface and during the whole mitosis resulted mainly from PA conjugation. This study's results demonstrate that during the cell cycle of tobacco BY-2 cells endogenous PA levels are intricately controlled, involving regulation of activities of biosynthetic, catabolic and conjugation enzymes.

Keywords: ADC; Cell cycle; DAO; ODC; PAL; Polyamines; SAMDC; Tobacco

Heike Mikschofsky, Patricia Konig, Gunther M. Keil, Martin Hammer, Horst Schirrmeier, Inge Broer, Cholera toxin B (CTB) is functional as an adjuvant for cytoplasmatic proteins if directed to the endoplasmatic reticulum (ER), but not to the cytoplasm of plants, *Plant Science*, Volume 177, Issue 1, July 2009, Pages 35-42, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2009.03.010.

(<http://www.sciencedirect.com/science/article/B6TBH-4VY2C3F-1/2/2a0cc75257273b0fffb3594f2a6c12c>)

Abstract:

The mucosa-binding subunit B (CTB) of cholera toxin is frequently used to improve immunization. Up to now, the expression of CTB::target antigen fusion proteins in plants has only been performed via the secretory pathway, or in chloroplasts. Thus, it has never been determined whether CTB can also enhance the immunogenicity of naturally cytosolically expressed proteins, such as rabbit haemorrhagic disease virus (RHDV) VP60; furthermore, the optimal compartment for protein partners with different needs has not been characterized. In order to enhance the immunogenicity of plant-derived VP60, we analyzed CTB and CTB::VP60 accumulation via cytosolic versus secretory expression pathways. A synthetic CTB open reading frame (ORF), optimized with tobacco codons, was transferred to *Nicotiana tabacum* and *Solanum tuberosum* both with and without the codons for the CTB signal peptide. The genes were transcriptionally regulated by the CaMV 35S promoter. Biologically active, pentameric CTB complexes accumulated after expression of CTB with the signal peptide and ER-retention; whereas neither monomeric nor pentameric CTB molecules were detectable in transgenic plants expressing the

signal peptide-deleted CTB. A similar result was obtained using tobacco plants expressing fusion proteins of the respective CTB variants and RHDV VP60. ER-directed and glycosylated CTB::VP60 proteins induced anti-VP60 specific antibodies in rabbits using a low immunization dosage of approximately 0.4 ng antigenic VP60.

Keywords: CTB; Adjuvant; Fusion antigen; Plant-derived vaccines; Molecular farming

Mohammad Isbat, Naheed Zeba, Seong Ryong Kim, Choo Bong Hong, A BAX inhibitor-1 gene in *Capsicum annuum* is induced under various abiotic stresses and endows multi-tolerance in transgenic tobacco, *Journal of Plant Physiology*, In Press, Corrected Proof, Available online 13 June 2009, ISSN 0176-1617, DOI: 10.1016/j.jplph.2009.04.017.

(<http://www.sciencedirect.com/science/article/B7GJ7-4WHDHKS-1/2/260433f42e5e8f0967f8baab04c38da8>)

Abstract: Summary

Programmed cell death (PCD) is a highly conserved cellular suicide process important in developmental processes and elimination of damaged cells upon environmental stresses. Among the important regulators of PCD, much interest has been centered on BCL2-associated x protein (BAX) as the pro-PCD factor. On the other hand, BAX inhibitor-1 (BI-1) has been implicated as an anti-PCD factor that balances out the activity of BAX in the developmental processes and responses to environment. A cDNA clone coding a BI-1 gene was isolated from a cDNA library of heat-stressed hot pepper (*Capsicum annuum*) and named as CaBI-1. This gene contains an open reading frame (ORF) of 248 amino acids encoding a BI-1 protein. Genomic DNA-blot analysis for CaBI-1 suggested one or two loci in the *C. annuum* genome. Transcription of CaBI-1 was induced in response to high or low temperatures, drought, high salinity, flooding and heavy metal stresses, and ABA. We introduced the ORF of CaBI-1 under the control of the CaMV 35S promoter (P35S) into tobacco (*Nicotiana tabacum* cv. Wisconsin 38) genome by *Agrobacterium*-mediated transformation. The P35S:CaBI-1 transgenic plants displayed markedly improved tolerance to high temperature, water deficit, and high salinity in comparison to the control plants. The results indicate that CaBI-1 is a BI-1 gene of which expression induced under various abiotic stresses and endows tolerance to several types of environmental stresses.

Keywords: Abiotic stresses; BAX inhibitor-1; *Capsicum annuum*; Transgenic tobacco

Ozlem Akpinar, Kader Erdogan, Seyda Bostanci, Enzymatic production of Xylooligosaccharide from selected agricultural wastes, *Food and Bioproducts Processing*, Volume 87, Issue 2, June 2009, Pages 145-151, ISSN 0960-3085, DOI: 10.1016/j.fbp.2008.09.002.

(<http://www.sciencedirect.com/science/article/B8JGD-4TW53HM-1/2/bfbbe3d2d2b7adabade39a35f36f563f>)

Abstract:

Four different agricultural wastes, namely tobacco stalk (TS), cotton stalk (CS), sunflower stalk (SS) and wheat straw (WS) were tested for the production of Xylooligosaccharide (XO). XO production was performed by enzymatic hydrolysis of xylans which were obtained by alkali extraction from the agricultural wastes. Depending on the source, it was found that these four agricultural wastes contained different amount of xylan, cellulose and lignin and the xylan obtained from these source contained different amount of sugar and uronic acid. The highest amount of arabinose was in xylan from WS while the other xylans mainly had xylose and small amount of glucose. Different xylanase preparations were evaluated for production XO from these xylan sources. *Aspergillus niger* xylanase produced lower amount of XO from wheat straw xylan (WSX) than cotton stalk xylan (CSX), sun flower xylan (SSX) and tobacco stalk xylan (TSX) while *Trichoderma longibrachiatum* xylanase hydrolyzed highly branched WSX better. The HPLC analysis of the hydrolysis products indicated that depending on structure and composition of xylan, *A. niger* xylanase produced less amount of xylose than *T. longibrachiatum* xylanase, and the hydrolysis product of *A. niger* xylanase contained different amount of oligosaccharides ($X_2 > X_3 >$

X4 > X5 > X6, >X6). Regardless of the structural differences of the xylan types presented in this paper, all xylans generated XO with different degree of polymerization (DP), but the DP of XO depended on the enzyme specificity and the structure of substrate.

Keywords: Xylooligosaccharides; Tobacco stalks; Cotton stalks; Sunflower stalks; Wheat straw

Brahma N. Singh, B.R. Singh, R.L. Singh, D. Prakash, D.P. Singh, B.K. Sarma, G. Upadhyay, H.B. Singh, Polyphenolics from various extracts/fractions of red onion (*Allium cepa*) peel with potent antioxidant and antimutagenic activities, *Food and Chemical Toxicology*, Volume 47, Issue 6, June 2009, Pages 1161-1167, ISSN 0278-6915, DOI: 10.1016/j.fct.2009.02.004.

(<http://www.sciencedirect.com/science/article/B6T6P-4VKDN09-8/2/48fded20486486d3c70369f84f42f925>)

Abstract:

In order to determine antioxidant activity, the five extracts/fractions of red onion peel were studied for their total content of phenolics (TPC), flavonoids (TFC), antioxidant activity (AOA), free radical scavenging activity (FRSA), assayed by DPPH radical in the terms of anti-radical power (ARP) and reducing power (RP), expressed as ascorbic acid equivalents (ASE)/ml. High TPC (384.7 +/- 5.0 mg GAE/g), TFC (165.2 +/- 3.2 mg QE/g), AOA (97.4 +/- 7.6%), ARP (75.3 +/- 4.5) and RP (1.6 +/- 0.3 ASE/ml) were found for the ethyl acetate (EA) fraction. EA fraction had markedly higher antioxidant capacity than butylated hydroxytoluene (BHT) in preventive or scavenging capacities against FeCl₃-induced lipid peroxidation, protein fragmentation, hydroxyl (site-specific and non-site-specific), superoxide anion and nitric oxide radicals. EA fraction also showed dose dependent antimutagenic activity by following the inhibition of tobacco-induced mutagenicity in *Salmonella typhimurium* strains (TA102) and hydroxyl radical-induced nicking in plasmid pUC18 DNA. HPLC and MS/MS analysis showed the presence of ferulic, gallic, protocatechuic acids, quercetin and kaempferol. The large amount of polyphenols contained in EA fraction may cause its strong antioxidant and antimutagenic properties. This information shows that EA fraction of red onion peel can be used as natural antioxidant in nutraceutical preparations.

Keywords: *Allium cepa*; HPLC; Antioxidant activity; Protein fragmentation; Ames test; DNA damage

Ana M. Bratic, Dragana B. Majic, Jelena T. Samardzic, Vesna R. Maksimovic, Functional analysis of the buckwheat metallothionein promoter: Tissue specificity pattern and up-regulation under complex stress stimuli, *Journal of Plant Physiology*, Volume 166, Issue 9, 1 June 2009, Pages 996-1000, ISSN 0176-1617, DOI: 10.1016/j.jplph.2008.12.002.

(<http://www.sciencedirect.com/science/article/B7GJ7-4VGMP02-2/2/98a3f80d37c5f0d2fd83435c30a93681>)

Abstract: Summary

To shed light on expression regulation of the metallothionein gene from buckwheat (FeMT3), functional promoter analysis was performed with a complete 5' regulatory region and two deletion variants, employing stably transformed tobacco plants. Histochemical GUS assay of transgenic tobacco lines showed the strongest signals in vascular elements of leaves and in pollen grains, while somewhat weaker staining was observed in the roots of mature plants. This tissue specificity pattern implies a possible function of buckwheat MT3 in those tissues. Quantitative GUS assay showed strong up-regulation of all three promoter constructs (proportional to the length of the regulatory region) in leaves submerged in liquid MS medium containing sucrose, after a prolonged time period. This represented a complex stress situation composed of several synergistically related stress stimuli. These findings suggest complex transcriptional regulation of FeMT3, requiring interactions among a number of different factors.

Keywords: Buckwheat; Metallothionein; Promoter; Stress; Transformation

Adam J. Matich, Marian J. McKenzie, David A. Brummell, Daryl D. Rowan, Organoselenides from *Nicotiana tabacum* genetically modified to accumulate selenium, *Phytochemistry*, Volume 70, Issue 9, June 2009, Pages 1098-1106, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2009.06.001. (<http://www.sciencedirect.com/science/article/B6TH7-4WMXSKR-1/2/e19758d41ecb7052bff3bb1f1eb9cc49>)

Abstract:

Nicotiana tabacum L. (tobacco) plants were transformed to overexpress a selenocysteine methyltransferase gene from the selenium hyperaccumulator *Astragalus bisulcatus* (Hook.) A. Gray (two-grooved milkvetch), and an ATP-sulfurylase gene from *Brassica oleracea* L. var. *italica* (broccoli). Solvent extraction of leaves harvested from plants treated with selenate revealed five selenium-containing compounds, of which four were identified by chemical synthesis as 2-(methylseleno)acetaldehyde, 2,2-bis(methylseleno)acetaldehyde, 4-(methylseleno)-(2E)-nonenal, and 4-(methylseleno)-(2E,6Z)-nonadienal. These four compounds have not previously been reported in nature.

Keywords: *Nicotiana tabacum*; Tobacco; Selenium; Volatiles; Methylselenocysteine; Selenocysteine methyltransferase; ATP-sulfurylase

Shi Xiao, Mee-Len Chye, An Arabidopsis family of six acyl-CoA-binding proteins has three cytosolic members, *Plant Physiology and Biochemistry*, Volume 47, Issue 6, *Plant Lipids*, June 2009, Pages 479-484, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2008.12.002. (<http://www.sciencedirect.com/science/article/B6VRD-4V59VVP-2/2/fa1f7f01286d1792aab284f9dfad8c84>)

Abstract:

In *Arabidopsis thaliana*, a gene family of six members encodes acyl-CoA-binding proteins (ACBPs). These *Arabidopsis* ACBPs (designated ACBP1 to ACBP6) range in size from 10.4 kDa to 73.1 kDa and display varying affinities for acyl-CoA esters, suggesting that they have different roles in plant lipid metabolism. In contrast, only the 10-kDa ACBPs have been well-characterized from other eukaryote species. Our previous studies have revealed that ACBP1 and ACBP2 are membrane-associated proteins, while ACBP3 is extracellularly-targeted. More recently, we have reported that the remaining three members in this protein family (namely ACBP4, ACBP5 and ACBP6) are subcellularly localized to the cytosol in *Arabidopsis*. The subcellular localizations of ACBP4, ACBP5 and ACBP6 in the cytosol were demonstrated using a number of different approaches incorporating biochemical fractionation, confocal microscopy of transgenic *Arabidopsis* expressing autofluorescence-tagged fusions and immunoelectron microscopy using ACBP-specific antibodies. Our results indicate that all three ACBPs in the cytosol are potential candidates for acyl-CoA binding and trafficking in plant cells. In this review, the functional redundancy and differences among the three cytosolic ACBPs are discussed by comparison of their light-regulated expression and substrate affinities to acyl-CoA esters, and from biochemical analyses on their knockout mutants and/or overexpression in transgenic *Arabidopsis*. The transcriptionally light-induced ACBP4 and ACBP5, which encode the two largest forms of *Arabidopsis* ACBPs, bind oleoyl-CoA esters and likely transfer oleoyl-CoAs from the plastids (the site of *de novo* fatty acid biosynthesis) to the endoplasmic reticulum for the biosynthesis of non-plastidial membrane lipids in *Arabidopsis*.

Keywords: Acyl-coenzyme A-binding protein; *Arabidopsis thaliana*; Cytosolic localization; Lipid trafficking; Oleoyl-CoA; Phospholipid binding

Laura Zonia, Teun Munnik, Uncovering hidden treasures in pollen tube growth mechanics, *Trends in Plant Science*, Volume 14, Issue 6, June 2009, Pages 318-327, ISSN 1360-1385, DOI: 10.1016/j.tplants.2009.03.008. (<http://www.sciencedirect.com/science/article/B6TD1-4W91BMD-3/2/1ffd4e3d33fe45173103bd4ae8b73980>)

Abstract:

The long-standing model of tip growth in pollen tubes considers that exocytosis and growth occur at the apex and that the pool of very small vesicles in the apical dome contains secretory (exocytic) vesicles. However, recent work on vesicle trafficking dynamics in tobacco pollen tubes shows that exocytosis occurs in the subapical region. Taking these and other new results into account, we set out to resolve specific problems that are endemic in current models and present a two-part ACE (apical cap extension)-H (hydrodynamics) growth model. The ACE model involves delivery and recycling of materials required for new cell synthesis and the H model involves mechanisms that integrate and regulate key cellular pathways and drive cell elongation during growth.

Tommaso Tosi, Gianluca Cioci, Karina Jouravleva, Cyril Dian, Laurent Terradot, Structures of the tumor necrosis factor α inducing protein Tip α : A novel virulence factor from *Helicobacter pylori*, *FEBS Letters*, Volume 583, Issue 10, 19 May 2009, Pages 1581-1585, ISSN 0014-5793, DOI: 10.1016/j.febslet.2009.04.033.

(<http://www.sciencedirect.com/science/article/B6T36-4W6XYJM-1/2/236a30126ee6b3f26a925a4a95a3d3c9>)

Abstract:

Helicobacter pylori secretes a unique virulence factor, Tip α , that enters gastric cells and both stimulates the production of the TNF- α and activates the NF- κ B pathway. The structures of a truncated version of Tip α (Tip α N34) in two crystal forms are presented here. Tip α adopts a novel β 1 α 1 α 2 β 2 β 3 α 3 α 4 topology that can be described as a combination of three domains. A first region consists in a short flexible extension, a second displays a dodecin-like fold and a third is a helical bundle domain similar to the sterile α motif (SAM). Analysis of the oligomerisation states of Tip α N34 in the crystals and in solution suggests that the disulfide bridges could hold together Tip α monomers during their secretion in the gastric environment. Structured summary

MINT-7033680:

TIP α (uniprotkb:B2UTN0) and TIP α (uniprotkb:B2UTN0) bind (MI:0407) by cosedimentation (MI:0027)

Keywords: Bacterial toxin; X-ray crystallography; Nucleus import; Inflammatory response; Stomach cancer; *Helicobacter pylori*

Defeng Chen, Baishi Hu, Guoliang Qian, Qi Zhang, Chunyan Gu, Jiaqin Fan, Fengquan Liu, A novel probe for harpin receptor in nonhost plants: Monoclonal anti-idiotypic antibodies as internal images of HarpinXoo active sites, *Physiological and Molecular Plant Pathology*, In Press, Corrected Proof, Available online 15 May 2009, ISSN 0885-5765, DOI: 10.1016/j.pmpp.2009.05.001.

(<http://www.sciencedirect.com/science/article/B6WPC-4W99VY0-1/2/bf5b8e182a3683df396d6e09e61cd89b>)

Abstract:

Harpins constitute one group of effector proteins which elicit a hypersensitive response in nonhost plants, but the subcellular localization and tissue distribution of harpin receptors are still controversial. Antigen mimicry by anti-idiotypic antibodies is employed as a reliable strategy to probe receptors that are present in very low concentrations in the organism. In this study, a monoclonal anti-idiotypic antibody (Ab2), named 6B2, was elicited by F(ab')₂ fragments digested from the purified polyclonal antibody specific for HarpinXoo (Ab1). 6B2 competed with HarpinXoo for binding to Ab1 and the total protein extracted from tobacco leaves indicated its anti-idiotypic character and internal image property. The relevance of antigen mimicry was further confirmed by eliciting a third generation antibody (Ab3), which was shown not only to bind to Ab2 competing with Ab1 but also to react with the original antigen, HarpinXoo. Taken together, these results

demonstrate functional and biochemical mimicry of HarpinXoo by Ab2 and suggest that 6B2 can be a useful tool in probing its receptor in nonhost plants and other downstream studies.

Keywords: Anti-idiotypic antibody; Harpin; Mimicry; Receptor; Hypersensitive reaction; Type III secretion system

Mohammad Muzahidul Islam, Md. Anamul Hoque, Eiji Okuma, Mst. Nasrin Akhter Banu, Yasuaki Shimoishi, Yoshimasa Nakamura, Yoshiyuki Murata, Exogenous proline and glycinebetaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells, *Journal of Plant Physiology*, In Press, Corrected Proof, Available online 7 May 2009, ISSN 0176-1617, DOI: 10.1016/j.jplph.2009.04.002.

(<http://www.sciencedirect.com/science/article/B7GJ7-4W7HNTW-2/2/7bf284b60fb50c4cb28103190cc2e47e>)

Abstract: Summary

Environmental stress, including heavy metal stress, can cause oxidative damage to plants. Up-regulation of the antioxidant defense system induced by proline and glycinebetaine (betaine) alleviates the damaging effects of oxidative stress in plants. Here, we investigated the protective effects of exogenously applied proline and betaine on growth, accumulation of proline and betaine, lipid peroxidation and activity of antioxidant enzymes in cultured tobacco Bright Yellow-2 (BY-2) cells exposed to cadmium (Cd) stress. Cadmium stress (at 100 μ M Cd) caused a significant inhibition of the growth of BY-2 cells, and both proline and betaine significantly mitigated this inhibition. In addition, the mitigating effect of proline was more pronounced than that of betaine. Cadmium stress leads to an accumulation of Cd and endogenous proline in cultured cells, increased lipid peroxidation and peroxidase (POX) activity, and decreased activity of superoxide dismutase (SOD) and catalase (CAT). Exogenous application of proline resulted in a decrease in lipid peroxidation and an increase in SOD and CAT activities without reducing Cd contents under Cd stress, while application of betaine resulted in a decrease in lipid peroxidation and an increase in CAT activity with reducing Cd accumulation. Furthermore, exogenous proline and betaine intensified the accumulation of proline and betaine in Cd-stressed BY-2 cells, respectively. The present study suggests that proline and betaine confer tolerance to Cd stress in tobacco BY-2 cells by different mechanisms.

Keywords: Antioxidant enzymes; Cadmium; Glycinebetaine; Proline; Reactive oxygen species

Jennifer E. Murray, Rachel D. Penrod, Rick A. Bevins, Nicotine-evoked conditioned responding is dependent on concentration of sucrose unconditioned stimulus, *Behavioural Processes*, Volume 81, Issue 1, May 2009, Pages 136-139, ISSN 0376-6357, DOI: 10.1016/j.beproc.2009.01.002.

(<http://www.sciencedirect.com/science/article/B6T2J-4VD542D-3/2/61d83a25791f7e36b15067cdce73322c>)

Abstract:

Previous studies have shown that the interoceptive nicotine conditional stimulus (CS) functions similarly to exteroceptive CSs such as lights or environments. For instance, the appetitive conditioned response (CR) evoked when nicotine is repeatedly paired with sucrose presentations (the unconditioned stimulus; US) is sensitive to changes in training dose (CS salience) and the contiguity between the CS effects and sucrose. The current study was conducted to extend this research by examining the possible role of US intensity in CR acquisition and maintenance. Rats were trained using one of four sucrose concentrations: 0, 4, 16, or 32% (w/v). On nicotine sessions (0.4 mg base/kg), rats received 36 deliveries (4 s each) of their assigned concentration intermittently throughout the session; sucrose was withheld on saline sessions. In all groups, an appetitive goal-tracking CR was acquired at a similar rate. However, the asymptotic CR level varied with sucrose concentration. The magnitude of the CR was increased in rats trained with higher sucrose US concentrations. These findings are consistent with previous Pavlovian

conditioning research, and extend the conditions under which the nicotine state functions as an interoceptive conditional stimulus.

Keywords: Appetitive reinforcement; Drug discrimination; Pavlovian conditioning; Response magnitude; Tobacco; Stimulus salience

Keiichirou Nemoto, Masamitsu Hara, Shingo Goto, Kouji Kasai, Hikaru Seki, Masashi Suzuki, Atsuhiko Oka, Toshiya Muranaka, Yoshihiro Mano, The aux1 gene of the Ri plasmid is sufficient to confer auxin autotrophy in tobacco BY-2 cells, *Journal of Plant Physiology*, Volume 166, Issue 7, 1 May 2009, Pages 729-738, ISSN 0176-1617, DOI: 10.1016/j.jplph.2008.09.006.

(<http://www.sciencedirect.com/science/article/B7GJ7-4TVFVMG-1/2/5406b76946036e969a44cca64169ba5e>)

Abstract: Summary

Tobacco (*Nicotiana tabacum*) Bright Yellow-2 (BY-2) cells are rapidly proliferating meristematic cells that require auxin for culture in vitro. We have established several transgenic BY-2 cell lines that carry the T-DNA of *Agrobacterium rhizogenes* 15834, which harbors an agropine-type root-inducing (Ri) plasmid. Two of these lines, BYHR-3 and BYHR-7, were used to test the role of auxin in the proliferation of plant cells. The lines grew rapidly in Linsmaier-Skoog (LS) medium lacking auxin and other phytohormones. The TR-DNA, containing the aux1 (tryptophan monooxygenase) and aux2 (indoleacetamide hydrolase) genes, was present in the genomes of both transgenic lines, whereas the TL-DNA, containing the rolA, B, C and D genes, was present in the genome of BYHR-7 but not BYHR-3. Since the introduction of the rolABCD genes alone did not affect the auxin requirement of BY-2 cells, the aux1 and aux2 genes, but not the rolABCD genes, appear to be relevant to the auxin autotrophy of these transgenic lines. Furthermore, the overexpression of aux1 allowed BY-2 cells to grow rapidly in the absence of auxin, suggesting the existence in plant cells of an unidentified gene whose product is functionally equivalent or similar to that of aux2 of the Ri plasmid.

Keywords: *Agrobacterium rhizogenes*; Auxin-autonomous cell lines; Ri plasmid; T-DNA; Tobacco BY-2 cells

Jan B. Wooten, Newton E. Kalengamaliro, David E. Axelson, Characterization of bright tobaccos by multivariate analysis of ¹³C CPMAS NMR spectra, *Phytochemistry*, Volume 70, Issue 7, May 2009, Pages 940-951, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2009.04.015.

(<http://www.sciencedirect.com/science/article/B6TH7-4WBM06D-1/2/615f5ce843f7813b44298cbee0021b0c>)

Abstract:

Univariate and multivariate statistics were applied to characterize cured bright tobacco samples on the basis of their ¹³C CPMAS NMR spectra and leaf constituent analysis. NMR spectra were obtained for 55 samples selected from a set of 134 samples of graded bright tobacco leaves from crop year 1999. Historical leaf constituent analyses were available for total alkaloids, reducing sugars, total nitrogen, and insoluble ash. In addition, we applied HPLC to quantify the two abundant plant polyphenols, chlorogenic acid, and rutin. Principal component analysis (PCA) and partial least squares (PLS) of the NMR spectra revealed systematic relationships between groups of samples related to these substances and afforded predictive quantitative models for the analyzed constituents. Analysis of the PLS significant variables showed that leaf polysaccharides, alkaloids, and minerals are major determinants influencing the grading of cured bright tobacco leaves.

Keywords: ¹³C CPMAS NMR; *Nicotiana tabacum*; Tobacco; Solanaceae; Multivariate analysis

Yan Liu, Qiuqiang Gao, Bin Wu, Taobo Ai, Xingqi Guo, NgRDR1, an RNA-dependent RNA polymerase isolated from *Nicotiana glutinosa*, was involved in biotic and abiotic stresses, *Plant*

Physiology and Biochemistry, Volume 47, Issue 5, May 2009, Pages 359-368, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2008.12.017.

(<http://www.sciencedirect.com/science/article/B6VVRD-4V9S36R-1/2/e6999dad26cf11ace30e311a4a18136a>)

Abstract:

The RNA-dependent RNA polymerases (RDRs) play a key role in RNA silencing, heterochromatin formation and natural gene regulation. Here, a novel RDR gene was isolated from *Nicotiana glutinosa*, designated as NgRDR1. The full-length cDNA of NgRDR1 encodes a 1117-amino acid protein which harbors the five conserved regions in plant RDRs, including the most remarkable motif DbDGD (b is a bulky residue). Amino acid sequence alignment revealed that NgRDR1 exhibited a high degree of identity with other higher plant RDR genes. Five exons were detected in the genomic DNA sequence, and the fourth exon is 151 bp, the location and the length of which are conserved among different plant species. From the phylogenetic tree constructed with different kinds of plant RDRs, it is determined that NgRDR1 falls into group I, and is closely associated with the dicotyledons RDRs. The analysis of the 5'-flanking region of NgRDR1 revealed a group of putative cis-acting elements. The results of expression analysis showed that the transcripts of NgRDR1 can be induced by biotic stresses, such as exogenous signaling molecules including salicylic acid (SA), SA analogues, hydrogen peroxide (H₂O₂), and methyl jasmonate (MeJA). Furthermore, NgRDR1 expression can be up-regulated by potato virus Y (PVY), tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV), but not by potato virus X (PVX). Besides, different kinds of fungi can also induce NgRDR1 expression. These results indicate that NgRDR1 may play an important role in response to biotic and abiotic stresses.

Keywords: Abiotic and biotic stress response; cDNA cloning; cis-acting elements; *Nicotiana glutinosa*; RNA-dependent RNA polymerase (RDR); Semi-quantitative RT-PCR

Qun-dan Lv, Ren-jie Tang, Hua Liu, Xiao-shu Gao, Yi-zhou Li, Hui-qiong Zheng, Hong-xia Zhang, Cloning and molecular analyses of the *Arabidopsis thaliana* chloride channel gene family, *Plant Science*, Volume 176, Issue 5, May 2009, Pages 650-661, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2009.02.006.

(<http://www.sciencedirect.com/science/article/B6TBH-4VNH3MM-4/2/65ad277e3e64fef4ddc0b64c889a80a9>)

Abstract:

The *Arabidopsis* chloride channels (CLC) family includes seven members, AtCLCa-g, that can be divided into two distinct subclasses. Here we show the developmental and tissue-specific expression of *Arabidopsis* CLC (AtCLC) gene family, and the subcellular localization of individual members. Reverse transcription PCR (RT-PCR) analyses indicated that the family members were ubiquitously but differentially expressed in *Arabidopsis*. Promoter-driven [β]-glucuronidase (GUS) expression in transgenic *Arabidopsis* plants further demonstrated the overlapping but distinct expression pattern of this family, and all the family members were predominantly expressed in vascular tissues, as well as a preferential expression of AtCLCc in guard cells. When expressed as yellow fluorescent protein (YFP)-tagged fusion proteins in tobacco mesophyll protoplasts, AtCLCe was specifically localized to chloroplast; AtCLCa-c and g were localized to the vacuole membranes, whereas AtCLCd and AtCLCf were targeted to Golgi apparatus. Among the seven AtCLC members, only AtCLCc and AtCLCd could suppress, with discrepant efficacy, the cation-sensitive phenotype of [Δ]gef1, a yeast mutant lacking the single chloride channel GEF1.

Keywords: *Arabidopsis thaliana*; Chloride channels; Chloroplast; Vacuole membranes; Yellow fluorescent protein

Mark Seger, Jose Luis Ortega, Suman Bagga, Champa Sengupta-Gopalan, Corrigendum to 'Repercussion of mesophyll-specific overexpression of a soybean cytosolic glutamine synthetase

gene in alfalfa (*Medicago sativa* L.) and tobacco (*Nicotiana tabaccum* L.)' [Plant Sci. 176 (2009) 119-129], Plant Science, Volume 176, Issue 5, May 2009, Page 707, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2008.12.008.

(<http://www.sciencedirect.com/science/article/B6TBH-4V761XC-1/2/628dd7e234934cb1828013f9dcc64190>)

Bart Cottyn, Kim Heylen, Jeroen Heyrman, Katrien Vanhouteghem, Ellen Pauwelyn, Peter Bleyaert, Johan Van Vaerenbergh, Monica Hofte, Paul De Vos, Martine Maes, *Pseudomonas cichorii* as the causal agent of midrib rot, an emerging disease of greenhouse-grown butterhead lettuce in Flanders, Systematic and Applied Microbiology, Volume 32, Issue 3, May 2009, Pages 211-225, ISSN 0723-2020, DOI: 10.1016/j.syapm.2008.11.006.

(<http://www.sciencedirect.com/science/article/B7GVX-4VDSCY1-2/2/44935c33d5c1bb0c0f40f3630501e32a>)

Abstract:

Bacterial midrib rot of greenhouse-grown butterhead lettuce (*Lactuca sativa* L. var. capitata) is an emerging disease in Flanders (Belgium) and fluorescent pseudomonads are suspected to play an important role in the disease. Isolations from infected lettuces, collected from 14 commercial greenhouses in Flanders, yielded 149 isolates that were characterized polyphasically, which included morphological characteristics, pigmentation, pathogenicity tests by both injection and spraying of lettuce, LOPAT characteristics, FAME analysis, BOX-PCR fingerprinting, 16S rRNA and *rpoB* gene sequencing, as well as DNA-DNA hybridization. Ninety-eight isolates (66%) exhibited a fluorescent pigmentation and were associated with the genus *Pseudomonas*. Fifty-five of them induced an HR+ (hypersensitive reaction in tobacco leaves) response. The other 43 fluorescent isolates were most probably saprophytic bacteria and about half of them were able to cause rot on potato tuber slices. BOX-PCR genomic fingerprinting was used to assess the genetic diversity of the *Pseudomonas* midrib rot isolates. The delineated BOX-PCR patterns matched quite well with *Pseudomonas* morphotypes defined on the basis of colony appearance and variation in fluorescent pigmentation. 16S rRNA and *rpoB* gene sequence analyses allowed most of the fluorescent isolates to be allocated to *Pseudomonas*, and they belonged to either the *Pseudomonas fluorescens* group, *Pseudomonas putida* group, or the *Pseudomonas cichorii/syringae* group. In particular, the isolates allocated to this latter group constituted the vast majority of HR+ isolates and were identified as *P. cichorii* by DNA-DNA hybridization. They were demonstrated by spray-inoculation tests on greenhouse-grown lettuce to induce the midrib rot disease and could be re-isolated from lesions of inoculated plants. Four HR+ non-fluorescent isolates associated with one sample that showed an atypical midrib rot were identified as *Dickeya* sp.

Keywords: Bacterial rot; Butterhead lettuce; Greenhouse; *Pseudomonas cichorii*; Pectolytic fluorescent pseudomonads; *Dickeya* sp.

Camilla Christiansen, Maher Abou Hachem, Mikkel A. Glaring, Anders Vikso-Nielsen, Bent W. Sigurskjold, Birte Svensson, Andreas Blennow, A CBM20 low-affinity starch-binding domain from glucan, water dikinase, FEBS Letters, Volume 583, Issue 7, 2 April 2009, Pages 1159-1163, ISSN 0014-5793, DOI: 10.1016/j.febslet.2009.02.045.

(<http://www.sciencedirect.com/science/article/B6T36-4VT14K5-2/2/68c93e39a285cd6e534513830d04e9b5>)

Abstract:

The family 20 carbohydrate-binding module (CBM20) of the *Arabidopsis* starch phosphorylator glucan, water dikinase 3 (GWD3) was heterologously produced and its properties were compared to the CBM20 from a fungal glucoamylase (GA). The GWD3 CBM20 has 50-fold lower affinity for cyclodextrins than that from GA. Homology modelling identified possible structural elements responsible for this weak binding of the intracellular CBM20. Differential binding of fluorescein-

labelled GWD3 and GA modules to starch granules in vitro was demonstrated by confocal laser scanning microscopy and yellow fluorescent protein-tagged GWD3 CBM20 expressed in tobacco confirmed binding to starch granules in planta.

Keywords: Bioimaging; Carbohydrate-binding module 20; Glucan, water dikinase; Starch-binding domain; Surface plasmon resonance

Veerle M.J. Grispén, Barbara Irtelli, Henk W.J. Hakvoort, Riet Vooijs, Tijs Bliëk, Wilma M. ten Bookum, Jos A.C. Verkleij, Henk Schat, Expression of the Arabidopsis metallothionein 2b enhances arsenite sensitivity and root to shoot translocation in tobacco, *Environmental and Experimental Botany*, Volume 66, Issue 1, April 2009, Pages 69-73, ISSN 0098-8472, DOI: 10.1016/j.envexpbot.2008.12.021.

(<http://www.sciencedirect.com/science/article/B6T66-4VGPV3V-1/2/c877483e7ec99f47b85177ebd8084da4>)

Abstract:

We expressed the AtMT2b gene under the 35 S cauliflower mosaic virus promoter in *Nicotiana tabacum* (Sr1), using leaf disc transformation. Arsenite tolerance and uptake, as well as arsenite-induced phytochelatin (PC) accumulation in roots were measured in transgenic lines, and compared to untransformed ('wild type') tobacco. Measured after 5 days of exposure, arsenite tolerance was slightly but significantly decreased in the transgenic lines compared to wild type. The highest AtMT2b expressing line exhibited a significantly decreased arsenic accumulation in roots, but an increased accumulation in shoots, while the total amount of arsenic taken up remained unchanged, suggesting that AtMT2b expression enhanced the arsenic root to shoot transport. The same transformant line also exhibited a decreased rate of phytochelatin accumulation in the roots, but the phytochelatin-SH to As molar ratio was higher than in wild type, suggesting that the lower arsenite tolerance in the transformant lines was not due to a potential shortage of cysteine for PC synthesis, imposed by expression of the transgene.

Keywords: Arabidopsis thaliana metallothionein 2b; *Nicotiana tabacum* SR1; Arsenite toxicity; Phytochelatins

Erjun Ling, Xiang-Jun Rao, Jing-Qun Ao, Xiao-Qiang Yu, Purification and characterization of a small cationic protein from the tobacco hornworm *Manduca sexta*, *Insect Biochemistry and Molecular Biology*, Volume 39, Issue 4, April 2009, Pages 263-271, ISSN 0965-1748, DOI: 10.1016/j.ibmb.2008.12.006.

(<http://www.sciencedirect.com/science/article/B6T79-4V94WXB-2/2/00dfa694181870792c45bb4817107a7a>)

Abstract:

The prophenoloxidase (proPO) activation system is an important defense mechanism in arthropods, and activation of proPO to active phenoloxidase (PO) involves a serine proteinase cascade. Here, we report the purification and characterization of a small cationic protein CP8 from the tobacco hornworm, *Manduca sexta*, which can stimulate proPO activation. BLAST search showed that *Manduca* CP8 is similar to a fungal proteinase inhibitor-1 (AmFPI-1), an inducible serine proteinase inhibitor-1 (ISPI-1), and other small cationic proteins with unknown functions. However, we showed that *Manduca* CP8 did not inhibit proteinase activity, but stimulated proPO activation in plasma. When small amount (0.1 [μ g]) of purified native CP8 or BSA was added to cell-free plasma samples and incubated for 20 min, low PO activity was observed in both groups. But significantly higher PO activity was observed in the CP8-group than in the BSA-group when more proteins (0.5 [μ g]) were added and incubated for 20 min. However, when the plasma samples were incubated with proteins for 30 min, high PO activity was observed in both the CP8 and BSA groups regardless of the amount of proteins added. Moreover, when PO in the plasma was pre-activated with *Micrococcus luteus*, addition of CP8 did not have an effect on PO activity, and CP8/bacteria mixture did not stimulate PO activity to a higher level than did BSA/bacteria.

These results suggest that CP8 helps activate proPO more rapidly at the initial stage. CP8 mRNA was specifically expressed in fat body and its mRNA level decreased when larvae were injected with saline or bacteria. However, CP8 protein concentration in hemolymph did not change significantly in larvae injected with saline or microorganisms.

Keywords: Prophenoloxidase; Phenoloxidase; Serine proteinase; Cationic protein; Innate immunity; *Manduca sexta*

Anita Myer, Heather A. Mason, Wendy Smith, Christine Brown, Lawrence M. Schwartz, Differential control of cell death and gene expression during two distinct phases of hormonally-regulated muscle death in the tobacco hawkmoth *Manduca sexta*, *Journal of Insect Physiology*, Volume 55, Issue 4, April 2009, Pages 314-320, ISSN 0022-1910, DOI: 10.1016/j.jinsphys.2008.12.006.

([http://www.sciencedirect.com/science/article/B6T3F-4VGMPCG-](http://www.sciencedirect.com/science/article/B6T3F-4VGMPCG-2/2/1707cbfca7be6c4a2849c0c9e056ce42)

[2/2/1707cbfca7be6c4a2849c0c9e056ce42](http://www.sciencedirect.com/science/article/B6T3F-4VGMPCG-2/2/1707cbfca7be6c4a2849c0c9e056ce42))

Abstract:

In larvae of the tobacco hawkmoth *Manduca sexta*, the intersegmental muscles (ISMs) span eight abdominal segments and represent the major muscle group. Following pupation, the ISMs in the first two and last two segments undergo programmed cell death (PCD), while the remaining four segments persist until the time of adult eclosion, when they too undergo PCD. ISM death at adult eclosion is initiated by a decline in the circulating ecdysteroid titer and requires de novo gene expression. In this study we have investigated the hormonal regulation and the patterns of gene expression that accompany both early and late ISM death. We find that distinct endocrine cues regulate these two periods of muscle death. Even though the middle segments of ISMs are exposed to the same endocrine environment as the adjacent cells that die following pupation, they do not express death-associated transcripts until they are specifically signaled to die following adult eclosion. These data indicate that subsets of homologous muscles appear to make segment-specific decisions to couple their endogenous cell death programs to distinctly different developmental cues. Nevertheless, once cell death is initiated, they utilize many of the same molecular components.

Keywords: Acheron; Autophagy; Apoptosis; Ubiquitin; Proteasome; Ecdysone

Yoshiko Mitsuya, Yoshihiro Takahashi, Thomas Berberich, Atsushi Miyazaki, Hideo Matsumura, Hideki Takahashi, Ryohei Terauchi, Tomonobu Kusano, Spermine signaling plays a significant role in the defense response of *Arabidopsis thaliana* to cucumber mosaic virus, *Journal of Plant Physiology*, Volume 166, Issue 6, 1 April 2009, Pages 626-643, ISSN 0176-1617, DOI: 10.1016/j.jplph.2008.08.006.

([http://www.sciencedirect.com/science/article/B7GJ7-4TNTN08-](http://www.sciencedirect.com/science/article/B7GJ7-4TNTN08-1/2/1f4777110fa4933ef73308614cb74e27)

[1/2/1f4777110fa4933ef73308614cb74e27](http://www.sciencedirect.com/science/article/B7GJ7-4TNTN08-1/2/1f4777110fa4933ef73308614cb74e27))

Abstract: Summary

We have proposed that the polyamine spermine (Spm) functions as a signaling molecule to evoke defense reactions/cell death in avirulent pathogen-attacked tobacco plants. To understand its molecular basis in depth, Spm-responsive genes in *Arabidopsis thaliana* were identified by SuperSAGE analysis. Close to 90% of the Spm-responsive genes also responded during cucumber mosaic virus (CMV)-elicited hypersensitive response. Spm modulated the expression of genes of redox components, and genes involved in protein folding and secretion, protein degradation and defense. Two other prominent changes, the coordinately enhanced expression of members of the photorespiration pathway and a diversion in electron flow from the primary electron transfer chain of respiration to an alternative oxidase pathway, occurred in response to Spm. Spm activated the expression of 6 transcription factor genes including ZAT7, ZAT12, AtWRKY40 and AtbZIP60, of which the former three genes' products are currently assigned as components of H₂O₂ signaling pathway, suggesting the involvement of H₂O₂ in Spm-triggered responses. Since AtbZIP60 plays a proven master role in the unfolded protein response in

Arabidopsis thaliana, it may function to control the expression of genes participating in protein folding and secretion, which were mentioned above. Spm induction and CMV-triggered up-regulation of the genes described mainly coincided and their induction was suppressed by inhibitors of Spm oxidation. Furthermore, treatment with those inhibitors prior to CMV inoculation allowed higher viral multiplication in *Arabidopsis thaliana* plants. These results support the existence of a Spm-signaling pathway in *Arabidopsis thaliana* and its significant role in defense against CMV.

Keywords: *Arabidopsis thaliana*; Cucumber mosaic virus; Hypersensitive response; Polyamine; Spermine

Uri Hanania, Margarita Velcheva, Nachman Sahar, Moshe Flaishman, Etti Or, Oded Dgani, Avihai Perl, Suppression and overexpression of ubiquitin extension protein S27a affects cell proliferation and in vitro regeneration in *Nicotiana benthamiana*, *Plant Science*, Volume 176, Issue 4, April 2009, Pages 566-574, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2009.01.019.

(<http://www.sciencedirect.com/science/article/B6TBH-4VJBTJ8-2/2/9da0e5fb9aa63391a4223ac014aacbd>)

Abstract:

Ubiquitin is a highly conserved 76-amino-acid protein found in all eukaryotic cells. Ubiquitin's expression is encoded and expressed as multimeric head-to-tail repeats (polyubiquitins) that are post-translationally cleaved into monomers, or fused with ribosomal proteins S27a and L40. S27a is highly expressed in meristematic tissues, pollen and ovules and its ubiquitin moiety is thought to act as a chaperone in ribosome biogenesis prior to cleavage. This study suggests that the ribosomal protein S27a plays a critical role in the allocation of meristematic cells that differentiate into lateral structures such as leaves and flowers. S27a was also found to regulate floral meristem development, possibly through the control of cell proliferation as well as cell identity. Overexpression of S27a was correlated with increased proliferation of undifferentiated cells and arrest of morphologically 'normal' shoot and leaf development. The ubiquitin moiety did not affect the localization of S27a, but it did affect its protein level: expression of S27a without the ubiquitin moiety caused a severe reduction in S27a protein level.

Keywords: Ubiquitin extension S27a; Tobacco rattle virus; *N. benthamiana*; Shoot apical meristem

J.P.G. Minella, G.H. Merten, D.E. Walling, J.M. Reichert, Changing sediment yield as an indicator of improved soil management practices in southern Brazil, *CATENA*, In Press, Corrected Proof, Available online 28 March 2009, ISSN 0341-8162, DOI: 10.1016/j.catena.2009.02.020.

(<http://www.sciencedirect.com/science/article/B6VCG-4VY16GT-2/2/8372d2ef2a2828eac8018fa772488c12>)

Abstract:

Catchment-level soil and water conservation programmes have been widely employed in Brazil. An important component of these programmes is the implementation of water and sediment monitoring projects to evaluate the impact of changes in soil management on water resources. In general, results from monitoring projects have been inconclusive, due to a series of difficulties associated with data collection and limited timeframes. This study presents results from a hydrosedimentological monitoring project undertaken in a small (1.19 km²) rural catchment in Southern Brazil before and after the introduction of conservation tillage practices implemented by the RS-RURAL -- Program Against Rural Poverty. These practices, including use of winter cover crops and minimum tillage tobacco cultivation, were gradually adopted by local farmers. Data on precipitation, runoff volume, maximum flow and sediment yield were assembled for representative storm events occurring between May 2002 and March 2006, and analyzed to identify changes in the storm runoff and storm-period sediment response of the study catchment. The results provide evidence of statistically significant reductions in storm runoff, maximum flow rate and sediment yield after implementation of the conservation practices. Sediment sources were also investigated

using the fingerprinting technique and this work demonstrated a statistically significant reduction over the study period in the proportion of the sediment contributed by fields (62% to 54%) and unmetalled roads (36% to 24%). This reduction was offset by an increased contribution of sediment from channel sources (2% to 22%). The increased proportion of sediment mobilized from the channel is in part a function of the reduced contribution from the two other sources, but it also reflects the reduction in sediment inputs to the channel from the fields and unmetalled roads, which results in an increase in the energy available for channel scour. Results emphasize the complexity of the relationship between sediment yield, the effects of climactic variability, and changes in land use and management.

Keywords: Monitoring and modelling; Catchments; Soil conservation; Suspended sediment; Fingerprinting approach; Brazil

D. Kami, L. Shi, T. Sato, T. Suzuki, K. Oosawa, Cryopreservation of shoot apices of hawthorn in vitro cultures originating from East Asia, *Scientia Horticulturae*, Volume 120, Issue 1, 3 March 2009, Pages 84-88, ISSN 0304-4238, DOI: 10.1016/j.scienta.2008.09.019.

(<http://www.sciencedirect.com/science/article/B6TC3-4TY8WFM-2/2/d11620fb5234611a60b204e489ee4876>)

Abstract:

The objective of this study was to establish a cryopreservation protocol for hawthorn shoot apices (*Crataegus pinnatifida* Bge.). Cryopreservation was carried out via encapsulation-dehydration, vitrification, and encapsulation-vitrification on shoot apices excised from in vitro cultures. We began by showing that cold-acclimation enhanced the regrowth of cryopreserved apices from 10.0 to 65.5% in encapsulation-dehydration. We then decided that the encapsulation-dehydration method was an optimal cryopreservation method for hawthorn shoot apices in terms of its high recovery after cryopreservation as well as its ease of use compared with vitrification and encapsulation-vitrification. In encapsulation-dehydration, the protocol leading to optimal regrowth was as follows: after cold-acclimation at 5 [degree sign]C in the dark for 2 weeks, excised shoot tips were pretreated for 24 h at 25 [degree sign]C on hormone-free Murashige and Skoog [Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15, 473-497] (MS) basal medium with 0.4 mol/L sucrose, then encapsulated and precultured in liquid MS medium with 0.8 mol/L sucrose for 16 h at 25 [degree sign]C. Precultured beads were dehydrated for 6 h at 25 [degree sign]C in the dessicator containing 50 g silica gel to a moisture content of 15.3% (fresh-weight basis) before cryostorage for 1 h. In addition, we examined the effect of adding glycerol to both the alginate beads and loading solution to enhance regrowth after cryopreservation in encapsulation-dehydration. In the present study, it was shown that adding 0.5 mol/L glycerol resulted in high regrowth percentages (82.5-90.0%) in four *Crataegus* species.

Keywords: Cryopreservation; Encapsulation-dehydration; Encapsulation-vitrification; Glycerol; Hawthorn; Vitrification

Wang Xin-Zhong, Liu Guo-Shun, Hu Hong-Chao, Wang Zhen-Hai, Liu Qing-Hua, Liu Xu-Feng, Hao Wei-Hong, Li Yan-Tao, Determination of management zones for a tobacco field based on soil fertility, *Computers and Electronics in Agriculture*, Volume 65, Issue 2, March 2009, Pages 168-175, ISSN 0168-1699, DOI: 10.1016/j.compag.2008.08.008.

(<http://www.sciencedirect.com/science/article/B6T5M-4TMRJX8-1/2/bee48e1369f33deca4307950d13b6623>)

Abstract:

Present nutrient management recommendations for flue-cured tobacco (*Nicotiana tabacum*) in Central China are typically uniform for large regions. This results in over-application in areas with high nutrient levels and under-application in areas with low nutrient levels. An 87-ha tobacco field was selected to define management zones (MZs) for more precise soil nutrient management. Our

objectives were to: (1) quantify the spatial variability of soil fertility variables, and (2) delineate the MZs by the combined usage of principal component analysis (PCA) and fuzzy cluster algorithm. To achieve these objectives, soil samples (0-20 cm) were taken from 81 points on an approximately 100-m grid in March 2007. Soil samples were analyzed for pH, organic matter (OM), total nitrogen (TN), alkalytic nitrogen (AN), available phosphorous (AP), available potassium (AK), and cation exchange capacity (CEC). Spatial variability was assessed and spatial distribution maps constructed using geostatistical techniques. PCA and fuzzy cluster algorithm were then performed to delineate MZs; fuzzy performance index (FPI) and normalized classification entropy (NCE) were used to determine the optimum cluster number. Results showed that the optimum number of MZs for this study area was three and analysis of variance indicated the heterogeneity of soil fertility among different MZs. The defined MZs provide a basis of information for site-specific fertilizer management in the tobacco-planted field.

Keywords: Management zones; Soil fertility; Spatial variability; Tobacco field

Marco Zancani, Elisa Petrusa, Jana Krajnakova, Valentino Casolo, Riccardo Spaccini, Alessandro Piccolo, Francesco Macri, Angelo Vianello, Effect of humic acids on phosphate level and energetic metabolism of tobacco BY-2 suspension cell cultures, *Environmental and Experimental Botany*, Volume 65, Issues 2-3, March 2009, Pages 287-295, ISSN 0098-8472, DOI: 10.1016/j.envexpbot.2008.09.012.

(<http://www.sciencedirect.com/science/article/B6T66-4TPHRRW-2/2/5bd9a9447eaf6da9ec655a8baf726dcb>)

Abstract:

To investigate the role of natural humic substances on plant cell phosphate level and metabolism, tobacco BY-2 suspension cell cultures were grown in the presence of humic samples of different chemical composition: soil humic acid and its three size-fractions (I-III) separated by High Pressure Size Exclusion Chromatography. The humic samples were characterized by CPMAS-NMR spectroscopy and on-line pyrolysis-gas chromatography-mass spectrometry. Suspension cell cultures, after 7 days of incubation, were facing Pi starvation. The fraction III, the most hydrophilic and smallest in molecular size among humic samples, induced a partial relief from Pi starvation, increasing total cell phosphate amount, ATP and glucose-6-phosphate levels, as well as the activity of secreted acid phosphatases. Furthermore, fraction III induced a decrease of KCN-insensitive respiration, evaluated in both suspension cells and isolated mitochondria. The low amount of acidic groups in fraction III excluded that its observed effect in relieving cells from Pi deficiency may be attributed to a partial replacement of the chelating ability of secreted acids (mainly citric and malic acids) in releasing Pi from metal-phosphate complexes. The molecular characteristics of fraction III are conducive to a flexible conformational structure due to hydrophilic domains, which are still contoured by hydrophobic moieties such as alkyl and aromatic compounds. Such flexible molecular associations may induce an efficient release of Pi from organic sources (e.g. nucleic acids), released in the media by damaged or dead cells, exerting a sort of positive effect on either the production or activity of extracellular Pi hydrolytic enzymes. This work shows that only by combining advanced molecular characterization of natural humic molecules with their effect on plant cells, it is possible to formulate sound hypotheses for structure-activity relationships.

Keywords: ATP; Glucose-6-phosphate; High Pressure Size Exclusion Chromatography; Humic acid; Mitochondria; Phosphate; Tobacco BY-2 suspension cell culture

Petr Babula, Vojtech Adam, Rene Kizek, Zdenek Sladky, Ladislav Havel, Naphthoquinones as allelochemical triggers of programmed cell death, *Environmental and Experimental Botany*, Volume 65, Issues 2-3, March 2009, Pages 330-337, ISSN 0098-8472, DOI: 10.1016/j.envexpbot.2008.11.007.

(<http://www.sciencedirect.com/science/article/B6T66-4V0TD46-1/2/b5f73fa9f021ab563baa33b765a8a6fa>)

Abstract:

Juglone and plumbagin are plant bioactive derivatives of 1,4-naphthoquinone occurring in plants, whereas lots of these plants belong to invasive species. Clarifying of action of juglone and plumbagin applied on plant cell model represented by tobacco BY-2 cells was the basic aim of this work. It was shown that naphthoquinones are able to induce various structural, functional and enzymatic changes leading to processes of apoptic-like cell death. Using dihydroethidium as fluorescent probe the mechanism of naphthoquinones action was explained. They are able to generate reactive oxygen species, which play important role in processes of programmed cell death. Disruption of mitochondrial respiratory chain was detected too. This study shown that mechanism of naphthoquinones action to plant cells is very complex and predestine them to be very effective compounds in plant competition fight.

Keywords: Programmed cell death; Juglone; Plumbagin; Tobacco BY-2

Dong-Ru Feng, Bing Liu, Wen-Yan Li, Yan-Ming He, Kang-Biao Qi, Hong-Bin Wang, Jin-Fa Wang, Over-expression of a cold-induced plasma membrane protein gene (MpRCI) from plantain enhances low temperature-resistance in transgenic tobacco, *Environmental and Experimental Botany*, Volume 65, Issues 2-3, March 2009, Pages 395-402, ISSN 0098-8472, DOI: 10.1016/j.envexptbot.2008.12.009.

(<http://www.sciencedirect.com/science/article/B6T66-4V4VY88-1/2/4b79c0b93c8cfd6af4f0c69f64c4798a>)

Abstract:

The plasma membrane is the most direct target of low temperature injury in plants. We have cloned a cold-induced gene (MpRCI) from plantain (*Musa paradisiaca* L.; ABB Group). Expression of an MpRCI::GFP fusion protein in onion epidermal cells showed localization of the protein product in the plasma membrane. The expression profile of MpRCI was analyzed by RT-PCR and the results indicated that MpRCI is induced by low temperatures in leaves and leafstalk, but not in the shoot meristematic or roots. We also cloned a 1.2 kb fragment upstream of MpRCI, predicted to contain several elements related to abiotic stresses, and demonstrated that the sequence has characteristics of low temperature- and ABA-induced promoter activity. Furthermore, the results of the phenotypic espial and ion leakage assays, using transgenic tobacco containing the gene, indicated that over-expression of the cold-induced plasma membrane protein gene MpRCI enhanced low temperature-resistance in the these plants. These results suggest that MpRCI is involved in maintaining the stability of the plasma membrane at low temperatures.

Keywords: Plantain (*Musa paradisiaca* L.; ABB Group); Cold-induced gene; MpRCI; Plasma membrane protein; Low temperature-resistance

S.S. Agrawal, K. Rajagopal, Nicotine contents in various toothpowders (dant manjans): Measurement and safety evaluation, *Food and Chemical Toxicology*, Volume 47, Issue 3, March 2009, Pages 511-524, ISSN 0278-6915, DOI: 10.1016/j.fct.2008.11.042.

(<http://www.sciencedirect.com/science/article/B6T6P-4V74XSH-1/2/2fa91e9b8045f9077ec3666bf65a7043>)

Abstract:

The use of tobacco products as dentrifice is prevalent in various parts of India. Among them toothpowder (dant manjan) is very common. These nicotine containing toothpastes/toothpowders are health hazards and is also habit forming. Health experts of India rightly banned use of nicotine containing toothpowder as early as 1992 by making proper legislation. We just made an attempt to verify whether the manufacturers complying the legislation or not. Eight leading brands of toothpowders were analyzed qualitatively by gas chromatography-mass spectrum detector and also quantitatively by gas chromatography-nitrogen phosphorus detector. Our results indicated

four brands were found to contain nicotine in the range of 2.53 [mu]g/g to 11.50 mg/g of toothpowder. This finding further confirms that addition of nicotine in dentifrice violates the regulatory norms. Regulatory authorities should give more attention to ensure that all toothpowders are free from nicotine which is also a statutory requirement.

Keywords: Toothpowders; Nicotine and gas chromatography

D. Bilalis, A. Karkanis, A. Efthimiadou, Ar. Konstantas, V. Triantafyllidis, Effects of irrigation system and green manure on yield and nicotine content of Virginia (flue-cured) Organic tobacco (*Nicotiana tabacum*), under Mediterranean conditions, *Industrial Crops and Products*, Volume 29, Issues 2-3, March 2009, Pages 388-394, ISSN 0926-6690, DOI: 10.1016/j.indcrop.2008.07.007.

(<http://www.sciencedirect.com/science/article/B6T77-4TDYNWF-1/2/6422b00afa6611cc825c8de41303f451>)

Abstract:

Organic tobacco consists of a new industrial crop product. Field experiments were conducted to determine the effects of irrigation system and fertilization on the growth (biomass and roots) of organic tobacco plants (*Nicotiana tabacum* cv NC 71). The experiment was designed as a split plot design with four replicates, two main plots (drip and sprinkler irrigation) and three sub-plots (vetch as green manure, red clover as green manure and control; without fertilization). Drip irrigation was characterised by lower amount of water applied to the soil. Furthermore the tobacco yield was not affected by the reduced water application and the crops under drip irrigation were higher than those with sprinkler irrigation. Positive effect of green manure in the nicotine content of tobacco leaves was also observed, and that reported for first time under organic system. Equally the higher amount of nitrogen from green fertilization with vetch led to increase of nicotine concentration (0.91% max concentration). Agronomic water use efficiency (WUE) for drip irrigation was always higher than those for sprinkler irrigation. There were no significant differences between the drip and sprinkler irrigation concerning the root biomass. Moreover, green manures increased roots dry weight. Yield of tobacco crop was significantly increased by the green manures, with the lowest yield (1850 kg ha⁻¹) to be found in the control plots. Finally, green manures increased the SPAD values and number of leaves, with most significant impact the time when vetch applied to soil.

Keywords: Drip irrigation; Sprinkler irrigation; Nicotine; Tobacco; Green manure

Xu LingFei, Ma FengWang, Liang Dong, Plant regeneration from in vitro cultured leaves of Lanzhou lily (*Lilium davidii* var. unicolor), *Scientia Horticulturae*, Volume 119, Issue 4, 17 February 2009, Pages 458-461, ISSN 0304-4238, DOI: 10.1016/j.scienta.2008.08.026.

(<http://www.sciencedirect.com/science/article/B6TC3-4TKXDDG-1/2/b5e568ed9f856da1269d3eb25984218c>)

Abstract:

Lanzhou lily (*Lilium davidii* var. unicolor) is one of the best lilies which are edible in China but the efficient shoot regeneration system has not been developed. The purpose of the present study is to establish an efficient and reproducible protocol for induction of shoots in vitro from *L. davidii* var. unicolor leaves. Shoot regeneration from in vitro cultured leaves of *L. davidii* var. unicolor was tested on the 26 media based on NN [Nitsch, J.P., Nitsch, C., 1969. Haploid plants from pollen grains. *Science* 163, 85-87] basal medium, containing different concentrations of thidiazuron (TDZ) in combination with different concentrations of [alpha]-naphthaleneacetic acid (NAA). Shoot organogenesis occurred directly from the leaves without forming callus. Shoot regeneration mainly occurred from the cuts across the midvein and the base of the leaf explants. The highest frequency of regeneration (93.3%) and the largest number of shoots per leaf (3.83) were obtained on NN basal medium supplemented with 0.5 mg l⁻¹ TDZ and 1.0 mg l⁻¹ NAA. All the regenerated shoots formed complete plantlets on half-strength MS [Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* 15, 473-497]

basal medium containing 0.1-0.5 mg l⁻¹ indole-3-butyric acid (IBA) within 30 days, and 92% of the regenerated plantlets survived in the soil. This study will be useful for Agrobacterium-mediated transformation and exploitation of somaclonal variation of Lanzhou lily.

Keywords: *Lilium davidii* var. *unicolor*; Leaf explants; Plant regeneration; Tissue culture

Pourvi Jain, Sumita Kachhwaha, S.L. Kothari, Improved micropropagation protocol and enhancement in biomass and chlorophyll content in *Stevia rebaudiana* (Bert.) Bertonii by using high copper levels in the culture medium, *Scientia Horticulturae*, Volume 119, Issue 3, 3 February 2009, Pages 315-319, ISSN 0304-4238, DOI: 10.1016/j.scienta.2008.08.015.

(<http://www.sciencedirect.com/science/article/B6TC3-4TJ5YW5-2/2/a44acdf7988b96fdcd7652f4e6f0724a>)

Abstract:

Incorporation of a range of higher concentrations of CuSO₄·5H₂O in MS medium [Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassay with 240 tobacco tissue cultures. *Physiol. Plant* 15, 473-497] significantly enhanced direct shoot bud induction and proliferation from cultured leaf and nodal explants taken from mature plants of *Stevia rebaudiana*. Shoot bud induction medium was supplemented with BAP (2.2 [μM]) and IAA (2.8 [μM]). When the concentration of CuSO₄·5H₂O in the induction medium was raised to 0.5 [μM] (five times the MS level, i.e. 0.1 [μM]) there was significant increase in percentage response along with increase in shoot bud number per explant. The shoots were healthy, well developed with dark green broader leaves. There was remarkable increase in total biomass and chlorophyll content at increased (0.5 [μM]) copper level in the medium. During proliferation stage also presence of high copper levels in the medium favoured increase in shoot bud number per explant.

Keywords: Honey leaf; Plant regeneration; Organogenesis; Copper; Micronutrient; Stevioside; Trace elements

Xiao Ming Xia, Kai Yun Wang, Hong Yan Wang, Resistance of *Helicoverpa assulta* (Guenee) (Lepidoptera: Noctuidae) to fenvalerate, phoxim and methomyl in China, *Crop Protection*, Volume 28, Issue 2, February 2009, Pages 162-167, ISSN 0261-2194, DOI: 10.1016/j.cropro.2008.10.003.

(<http://www.sciencedirect.com/science/article/B6T5T-4V6YST9-1/2/f46c1cb2853f694e7ff00ba11bd06103>)

Abstract:

Field populations of *Helicoverpa assulta* (Guenee) (Lepidoptera: Noctuidae) from the main tobacco-growing regions of China were investigated for their resistance to the conventional insecticidal chemicals fenvalerate (pyrethroid), phoxim (organophosphate), and methomyl (carbamate) during 2004-2005 using a micro-topical method under laboratory conditions. The studied strains exhibited an increased resistance to fenvalerate by 5.83-17,622-fold compared to the control strain. The highest levels of resistance to fenvalerate were 1042-17,622-fold and observed for 12 out of the 25 studied strains. Four strains showed a resistance level increased by 189-823-fold and 5 strains showed very low resistance to fenvalerate (<10). Substantial resistance to phoxim was observed only in one strain (50.8-fold), which was collected from Anhui in East China (AH1). Significant resistance to methomyl was detected in two strains with increases of 37.1- and 31.6-fold, respectively. These were collected from Anhui (AH1) and Yunnan (YN1) in East China and Southwest China. The impacts of *H. assulta* resistance to these insecticides on its management in China are discussed.

Keywords: *Helicoverpa assulta*; Resistance; Fenvalerate; Phoxim; Methomyl; China

Mourad A.M. Aboul-Soud, Hany A. El-Shemy, Identification and subcellular localisation of SlINT7: A novel tomato (*Solanum lycopersicum* Mill.) fruit ripening-related and stress-inducible gene, *Plant*

Science, Volume 176, Issue 2, February 2009, Pages 241-247, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2008.10.010.

(<http://www.sciencedirect.com/science/article/B6TBH-4TT9GBH-2/2/d11062ea671a777cd662300e39eda755>)

Abstract:

The key step in ethylene (C₂H₄) signalling during tomato fruit ripening is initialized via the direct interaction between C₂H₄ and specialized membrane-bound receptors, including Never-Ripe (NR), which is strongly induced during ripening. In order to identify novel ripening-related C₂H₄-dependent components, a yeast two-hybrid interaction screen has previously been employed, in which NR cDNA has been used as bait. This screen has identified a clone corresponding to interacting protein 7 (SI;INT7), through its specific and strong interaction with the NR receptor (L. Alexander, Z. Lin, R. Hackett, I. Wilson and D. Grierson, unpublished work). In this work, our objective was to identify the corresponding NR-interacting gene and subsequently characterize its expression response to various stress treatments, as well as unravelling its subcellular location in the cell. By sequencing and plant database interrogation, SI;INT7 was found to be a small gene with an open reading frame (ORF) of ~243 bb encoding a protein composed of 77 aa that shares no sequence homology with any known gene. Notably, northern analyses demonstrated that SI;INT7 gene expression is up-regulated in response to various stress signalling molecules such as salicylic acid (SA), abscissic acid, jasmonic acid, nitric oxide (NO) and salt, implicating SI;INT7 in biotic and abiotic stress signalling transduction responses. To gain more insight into the possible function of SI;INT7, a construct in which SI;INT7 is C-terminally fused to the green fluorescent protein (GFP) was generated. Subsequently, 35S::SI;INT7::GFP-containing constructs were transiently expressed in both tobacco leaves and onion peels via microprojectiles bombardment. Subsequently, confocal laser microscopic examination of bombarded tobacco and onion tissues revealed that the expression of GFP-SI;INT7 was observed predominantly in the plasma membrane, compared to the location throughout the cell observed with the control GFP construct alone. These results are discussed in the light of our present knowledge of C₂H₄-mediated control over fruit ripening and degree of cross-talk with other stress signalling pathways.

Keywords: Ethylene; Fruit ripening; Subcellular localisation; Tomato

Seong Hee Lee, Gap Chae Chung, Janusz J. Zwiazek, Effects of irradiance on cell water relations in leaf bundle sheath cells of wild-type and transgenic tobacco (*Nicotiana tabacum*) plants overexpressing aquaporins, *Plant Science*, Volume 176, Issue 2, February 2009, Pages 248-255, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2008.10.013.

(<http://www.sciencedirect.com/science/article/B6TBH-4TVJ3H1-1/2/ca8919710d19b8f4ae17b9e44f1a2908>)

Abstract:

Effects of low and high irradiance on cell water transport properties were studied in the leaves of wild-type and transgenic tobacco plants constitutively overexpressing PIP2;5 and PIP1;4 aquaporins (AQPs). Exposure of plants to high irradiance (~350 [μ]mol m⁻² s⁻¹ photosynthetic photon flux density, PPF) reduced hydraulic conductivity of the bundle sheath cells (L_{pc}) in the midrib, secondary, tertiary and quaternary veins of tobacco (*Nicotiana tabacum*) leaves. However, this decrease was not reflected in the decrease of the leaf hydraulic conductance and both wild-type and transgenic plants responded to high irradiance with an increase in transpiration rates. When the plants were exposed to ~10 [μ]mol m⁻² s⁻¹ PPF, L_{pc} in the wild-type plants was reduced by 3-4-fold with 50 and 100 [μ]M HgCl₂ treatments. A higher, 200 [μ]M HgCl₂ concentration was required to reduce L_{pc} in the transgenic plants. When the plants were exposed to ~350 [μ]mol m⁻² s⁻¹ PPF, HgCl₂ treatments did not affect L_{pc} implying that the mercury-sensitive water transport processes involving aquaporins had been inhibited by high irradiance. Exposure of leaves to ~350 [μ]mol m⁻² s⁻¹ PPF did not affect the modulus of elasticity in bundle sheath cells, but decreased turgor pressure (P) and this decrease was reversible by

exposing the leaves to $\sim 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPDF. The reduction in P was not affected by the HgCl₂ treatments. However, 10 mM tetraethylammonium, a K⁺ channel and AQP inhibitor, prevented the high irradiance-induced decrease in P and decreased Lpc. Exposure of leaves to high irradiance also increased the ratio of cell wall to protoplast K⁺ in the bundle sheath cells. Our results suggest that the effects of irradiance on cell water relations in tobacco leaves involve an inhibition of AQP-mediated water transport and changes in K fluxes leading to the decline in cell P. Keywords: Aquaporins; Irradiance; Leaf hydraulic conductivity; Mercuric chloride; Potassium; Tetraethylammonium

Junbin Wang, Monogalactosyldiacylglycerol deficiency affects jasmonic acid biosynthesis and defense responses to insect herbivores in *Nicotiana tabacum*, *Plant Science*, Volume 176, Issue 2, February 2009, Pages 279-285, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2008.11.003.

(<http://www.sciencedirect.com/science/article/B6TBH-4TY9MJB-1/2/e1f0ed4415f3a11c301f0dd4e2574d3d>)

Abstract:

Jasmonic acid (JA) plays critical roles in plant development and defense. Linolenic acid (18:3) and hexadecatrienoic acid (16:3) released from chloroplast lipids are now known to be precursors for JA biosynthesis, but the relationship between chloroplast lipids, especially galactolipids and JA biosynthesis still remains unclear. Here, this question was addressed by characterizing the transgenic tobacco plants, which had reduction in monogalactosyldiacylglycerol (MGDG) and trienoic fatty acids owing to the fact that MGDG synthase activity was down-regulated by using RNA interference technology. In response to wounding, the transgenic plants produced lower levels of JA than wild-type plants. Moreover, the expression of genes encoding lipoxygenase (LOX1), allene oxide cyclase (AOC), hydroperoxide lyase (HPL) and proteinase inhibitor (PI-I and PI-II) was strongly activated by mechanical wounding in wild-type plants but was diminished in transgenic plants. In addition, the transgenic plants were shown to be more susceptible to attack by *Helicoverpa armigera* larvae. Treatment of transgenic plants with methyl jasmonate restored resistance to *H. armigera* and expression of HPL, PI-I and PI-II. Our results suggest that MGDG plays important roles as source of 18:3 and 16:3 in JA biosynthesis and JA-mediated defense responses to insect herbivores in tobacco.

Keywords: Monogalactosyldiacylglycerol; Jasmonic acid; Insect; Defense gene; *Nicotiana tabacum*

Mst. Nasrin Akhter Banu, Md. Anamul Hoque, Megumi Watanabe-Sugimoto, Ken Matsuoka, Yoshimasa Nakamura, Yasuaki Shimoishi, Yoshiyuki Murata, Proline and glycinebetaine induce antioxidant defense gene expression and suppress cell death in cultured tobacco cells under salt stress, *Journal of Plant Physiology*, Volume 166, Issue 2, 30 January 2009, Pages 146-156, ISSN 0176-1617, DOI: 10.1016/j.jplph.2008.03.002.

(<http://www.sciencedirect.com/science/article/B7GJ7-4SG017J-1/2/4401509e64fdd7801c8ae41e6fc195fa>)

Abstract: Summary

Salt stress causes oxidative damage and cell death in plants. Plants accumulate proline and glycinebetaine (betaine) to mitigate detrimental effects of salt stress. The aim of this study was to investigate the protective effects of proline and betaine on cell death in NaCl-unadapted tobacco (*Nicotiana tabacum*) Bright Yellow-2 suspension-cultured cells subjected to salt stress. Salt stress increased reactive oxygen species (ROS) accumulation, lipid peroxidation, nuclear deformation and degradation, chromatin condensation, apoptosis-like cell death and ATP contents. Neither proline nor betaine affected apoptosis-like cell death and G1 phase population, and increased ATP contents in the 200 mM NaCl-stressed cells. However, both of them effectively decreased ROS accumulation and lipid peroxidation, and suppressed nuclear deformation and chromatin condensation induced by severe salt stress. Evans Blue staining experiment showed that both proline and betaine significantly suppressed increment of membrane permeability induced by 200

mM NaCl. Furthermore, among the ROS scavenging antioxidant defense genes studied here, mRNA levels of salicylic acid-binding (SAbind) catalase (CAT) and lignin-forming peroxidase (POX) were found to be increased by proline and betaine under salt stress. It is concluded that both proline and betaine provide a protection against NaCl-induced cell death via decreasing level of ROS accumulation and lipid peroxidation as well as improvement of membrane integrity.

Keywords: Antioxidant defense gene; Cell death; Glycinebetaine; Proline; Salt stress

Keiichirou Nemoto, Masamitsu Hara, Masashi Suzuki, Hikaru Seki, Toshiya Muranaka, Yoshihiro Mano, The NtAMI1 gene functions in cell division of tobacco BY-2 cells in the presence of indole-3-acetamide, FEBS Letters, Volume 583, Issue 2, 22 January 2009, Pages 487-492, ISSN 0014-5793, DOI: 10.1016/j.febslet.2008.12.049.

(<http://www.sciencedirect.com/science/article/B6T36-4V8FNYS-6/2/677fb3141467f89b30e7c0a673ee8745>)

Abstract:

Tobacco (*Nicotiana tabacum*) Bright Yellow-2 (BY-2) cells can be grown in medium containing indole-3-acetamide (IAM). Based on this finding, the NtAMI1 gene, whose product is functionally equivalent to the AtAMI1 gene of *Arabidopsis thaliana* and the aux2 gene of *Agrobacterium rhizogenes*, was isolated from BY-2 cells. Overexpression of the NtAMI1 gene allowed BY-2 cells to proliferate at lower concentrations of IAM, whereas suppression of the NtAMI1 gene by RNA interference (RNAi) caused severe growth inhibition in the medium containing IAM. These results suggest that IAM is incorporated into plant cells and converted to the auxin, indole-3-acetic acid, by NtAMI1.

Keywords: NtAMI1 gene; Tobacco BY-2 cell; Indole-3-acetamide; Indoleacetamide hydrolase; Auxin

S. Amutha, M. Muruganantham, G. Ananthakrishnan, S. Yablonsky, S. Singer, V. Gaba, Improved shoot regeneration due to prolonged seed storage, Scientia Horticulturae, Volume 119, Issue 2, 6 January 2009, Pages 117-119, ISSN 0304-4238, DOI: 10.1016/j.scienta.2008.07.027.

(<http://www.sciencedirect.com/science/article/B6TC3-4TC8J5Y-3/2/90afc2b4fd7b3c1f96ab08456e534fde>)

Abstract:

Regeneration in vitro from cotyledon explants of commercial squash (*Cucurbita pepo* L.) cultivars is generally efficient on Murashige and Skoog [Murashige, M., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant.* 15, 473-497] medium augmented with 4.4 [μ]M benzyladenine. However, cotyledon explants from certain seed batches of cultivars Bareqet and Ma'yan could regenerate only buds, leaf primordia or very small shoots with storage for up to 2 years at 4 [$^{\circ}$]C. Seed storage of cultivars Bareqet and Ma'yan at 4 [$^{\circ}$]C for 6-8 years resulted in significant increases in shoot regeneration, shoot growth and explant growth, returning to the normal range of values for *C. pepo*. For example, for cv. Bareqet shoots regenerated per explant increased from 0.4 after storage for 2 years to 1.21 after storage for 8 years; shoot length increased from a mean of less than 2 mm after storage for 2 years to 22 mm after storage for 8 years. Additionally, the final explant fresh weight of cv. B increased from 181 mg after storage for 2 years, to 1389 mg after storage for 8 years. Similar responses were observed for seedling-derived explants of cv. Ma'yan following storage' for 2-7 years. However, total regeneration (number of explants regenerating buds, leaf primordia or shoots) was not affected by prolonged storage for either cultivar. This is the first report of stimulation of in vitro shoot regeneration of a seedling-derived organ caused by prolonged seed storage. Moreover, the great improvement in regeneration due to long-term seed storage provides a new mechanism for the understanding of non-repeatability of plant tissue culture results.

Keywords: Non-repeatability; *Cucurbita pepo*; Seed storage; Shoot regeneration; Explant growth; Squash

Elisabeth P.J. Burgess, Bruce A. Philip, Emma I. Barraclough, Richelle K. Marshall, John T. Christeller, Impacts on the predatory carabid beetle *Ctenognathus novaezelandiae* of pure and mixed diets of natural field-collected prey and *Spodoptera litura* fed control or transgenic avidin tobacco, *Biological Control*, Volume 48, Issue 1, January 2009, Pages 55-62, ISSN 1049-9644, DOI: 10.1016/j.biocontrol.2008.09.007.

(<http://www.sciencedirect.com/science/article/B6WBP-4TJTX78-4/2/0b29b2b1049611672067ad077f9ed050>)

Abstract:

The potential compatibility with biological control of transgenic insecticidal plants expressing the biotin-binding protein avidin was investigated in tri-trophic experiments with the predatory carabid beetle, *Ctenognathus novaezelandiae*. Beetles were provided with pure and mixed diets of 33%, 67% or 100% of *Spodoptera litura* larvae, fed either avidin-expressing or isogenic control tobacco, and invertebrates field-collected from the forest floor. Beetles given only tobacco-fed *S. litura*, whether avidin was present or not, had lower fecundity, egg fertility, body mass and male survival than beetles that received some field food. Fewer of the avidin tobacco-fed prey were consumed than the control tobacco-fed, whatever mixture or proportion offered, probably as a result of the reduced quality of biotin-deprived prey. Beetles consuming 100% avidin tobacco-fed prey had lower fecundity than those given 100% control tobacco-fed prey, although predation on eggs as well as reduced prey quality could have contributed to this result. Despite the nutritionally limiting nature of an exclusive diet of tobacco-fed prey, there was no effect of avidin on fecundity in beetles consuming 67% or 33% avidin prey, or any effect on female or male mass, survival or egg fertility, even in the 100% avidin prey treatment. Fecundity in beetles fed 33% field food with 67% tobacco-fed prey was lower than in those fed 67% or 100% field food. However, there was no added impact of avidin on fecundity, mass or survival, or egg fertility of the 33% field food diet, suggesting that under field conditions, where a mixture of prey is available, negative impacts of avidin-fed prey are unlikely.

Keywords: Avidin; Environmental risk-assessment; Natural enemies; Predatory beetle; Transgenic plants; Tri-trophic impacts; *Ctenognathus novaezelandiae*; *Spodoptera litura*; *Nicotiana tabacum*, 'Samsun'

Houda Maaroufi Dguimi, Mohamed Debouba, Mohamed Habib Ghorbel, Houda Gouia, Tissue-specific cadmium accumulation and its effects on nitrogen metabolism in tobacco (*Nicotiana tabacum*, Bureley v. Fb9), *Comptes Rendus Biologies*, Volume 332, Issue 1, January 2009, Pages 58-68, ISSN 1631-0691, DOI: 10.1016/j.crv.2008.08.021.

(<http://www.sciencedirect.com/science/article/B6X1F-4V1MFHK-6/2/2c6a09416246e1a1525551c1b51afc89>)

Abstract:

Tobacco (*Nicotiana Tabacum*, Bureley v. Fb9) seedlings were grown for 30 days on control medium, and then treated for seven days with different concentrations (0, 10, 20, 50 and 100 [μ]M) of CdCl₂. Cadmium (Cd) was mostly accumulated in the leaves. However, nitrate reductase and nitrite reductase activities (NR, EC 1.6.1.6 and NiR, EC 1.7.7.1) were more inhibited by Cd stress in the roots than in leaves. Glutamine synthetase activity (GS, EC 6.3.1.2) was inhibited by Cd treatment in roots and leaves. In both organs, aminating activity of glutamate dehydrogenase (GDH, EC 1.4.1.2) and protease activity were significantly stimulated in the leaves and roots of stressed plants. The lesser extents of Cd stress effects on leaves, despite their high Cd accumulation, suggest that: (i) tobacco leaves may evolve adaptive process to partially inactivate Cd ions; and (ii) tobacco is useful for phytoremediation. To cite this article: H. Maaroufi Dguimi et al., *C. R. Biologies* 332 (2009).

Keywords: Cadmium; Nitrate reductase; Glutamate dehydrogenase; Glutamine synthetase; Nicotiana tabacum; Nitrogen metabolism; Cadmium; Nitrate reductase; Glutamate deshydrogenase; Glutamine synthetase; Nicotiana Tabacum; Metabolisme azote

J.A. LaMondia, Efficacy of fungicides and a systemic acquired resistance activator (acibenzolar-S-methyl) against tobacco blue mould, *Crop Protection*, Volume 28, Issue 1, January 2009, Pages 72-76, ISSN 0261-2194, DOI: 10.1016/j.cropro.2008.08.017.

(<http://www.sciencedirect.com/science/article/B6T5T-4TMYJV2-1/2/416cfb587edb1dc97c89e70c8567f7ca>)

Abstract:

Tobacco blue mould, caused by *Peronospora tabacina* Adam (*Peronospora hyoscyami* f. sp. *tabacina* Skalicky 1964) can be an economically devastating leaf spot disease in shade and broadleaf cigar wrapper tobacco (*Nicotiana tabacum* L.) types grown in Connecticut and Massachusetts. We investigated the effects of dimethomorph plus mancozeb and azoxystrobin fungicides as well as acibenzolar-S-methyl, a systemic acquired resistance inducer, on disease severity over 2 years in both shade-grown and broadleaf tobaccos. All fungicide and fungicide plus acibenzolar-S-methyl treatments applied were effective in reducing the number of blue mould lesions per plant. Treatments containing acibenzolar-S-methyl were the most effective, resulting in almost complete control. Substituting two or three applications of acibenzolar-S-methyl at label rates for dimethomorph plus mancozeb treatments in a spray program increased blue mould control over the same number of dimethomorph plus mancozeb applications by 28-94 percent. The effects of acibenzolar-S-methyl application on cured leaf quality were determined in commercial shade tobacco fields in 2000 and 2001. Leaves were cured, processed and commercially evaluated for quality in a blind test. Standard fungicide applications of dimethomorph plus mancozeb applied on a 14-d interval were compared to three acibenzolar-S-methyl treatments. Economic value was not different between treatments in 2000, but acibenzolar-S-methyl applied at 10-d intervals was associated with reduced value in 2001 when plants were more subject to drought and heat stress.

Keywords: Acrobat; Actigard; Azoxystrobin; Blue mould; Dimethomorph; Disease control; Dithane; Forum; Fungicides; Mancozeb; *Nicotiana tabacum*; *Peronospora tabacina*; Quadris; SAR; Tobacco

J.B. Havla, C.E. Hill, S.Z. Abdel-Rahman, E. Richter, Evaluation of the mutagenic effects of myosmine in human lymphocytes using the HPRT gene mutation assay, *Food and Chemical Toxicology*, Volume 47, Issue 1, January 2009, Pages 237-241, ISSN 0278-6915, DOI: 10.1016/j.fct.2008.11.008.

(<http://www.sciencedirect.com/science/article/B6T6P-4TX798M-2/2/ee89e4b62174dc8d1ef07c5df12a742b>)

Abstract:

The minor tobacco alkaloid myosmine is implicated in DNA damage through pyridyloxobutylation similar to the tobacco-specific nitrosamines (TSNA). In contrast to TSNA, occurrence of myosmine is not restricted to tobacco. Myosmine is genotoxic to human cells in the comet assay. In this study, the mutagenic effect of myosmine was evaluated using the cloning hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene mutation assay. Four hour exposure of isolated peripheral blood lymphocytes from 14 subjects homozygous for the Leu84 wild-type of the O6-methylguanine-DNA-methyltransferase (MGMT) gene to 1 mM of myosmine increased mutant frequency from $0.73 \pm 0.58 \times 10^{-6}$ in control to $1.14 \pm 0.89 \times 10^{-6}$ lymphocytes ($P < 0.05$). These new data further confirm the mutagenic effects of myosmine.

Keywords: Myosmine; HPRT mutation assay; Human lymphocytes

Zhudong Liu, Peiyu Gong, David G. Heckel, Wei Wei, Jianghua Sun, Dianmo Li, Effects of larval host plants on over-wintering physiological dynamics and survival of the cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), *Journal of Insect Physiology*, Volume 55, Issue 1, January 2009, Pages 1-9, ISSN 0022-1910, DOI: 10.1016/j.jinsphys.2008.07.017.

(<http://www.sciencedirect.com/science/article/B6T3F-4T5JJ3J-1/2/62388c6f110f8318103f8f24cdbb30a1>)

Abstract:

Laboratory colonies of cotton bollworm larvae, *Helicoverpa armigera*, were kept at 20 [degree sign]C under a photoperiod of L:D = 10:14 and fed on three host plants (cotton, tobacco, kidney bean) and an artificial diet (control) to determine the dynamic effects of larval host quality on over-wintering physiology and mortality. Energy reserves (glycogen and lipid), super-cooling points (SCPs), low-molecular-weight sugars, temperature, and mortality were monitored from November 2002 to April 2003. Lipid content did not change much for each group during over-wintering, but differed according to larval host plants. Larval host plants obviously influence the amount of glycogen, as does time of year: glycogen was lowest in February and increased in early spring. During winter, the mean pupal SCPs increased the most in February, then decreased, and were also affected by larval host plant, i.e. over-wintering pupae reared on kidney bean had the highest SCPs. Levels of glycerol and inositol differed significantly among host plants and months, which peaked in February. Pupal mortality also varied according to larval host plants and time: pupae reached their highest mortality in March and showed host plant differences in January. Records show that February was the coldest month during the period we observed, which corresponded closely to changes in over-wintering characteristics.

Keywords: *Helicoverpa armigera*; Host plants; Reserve storage; Low-molecular-weight sugars and sugar-alcohols; Over-wintering dynamics; Pupal mortality

Lisa Lazzarato, Grazia Trebbi, Cristina Pagnucco, Cinzia Franchin, Patrizia Torrigiani, Lucietta Betti, Exogenous spermidine, arsenic and [beta]-aminobutyric acid modulate tobacco resistance to tobacco mosaic virus, and affect local and systemic glucosylsalicylic acid levels and arginine decarboxylase gene expression in tobacco leaves, *Journal of Plant Physiology*, Volume 166, Issue 1, 1 January 2009, Pages 90-100, ISSN 0176-1617, DOI: 10.1016/j.jplph.2008.01.011.

(<http://www.sciencedirect.com/science/article/B7GJ7-4SFHHF4-2/2/5ad2f202f8c02e7167cb572c1aa7accb>)

Abstract: Summary

The polyamine spermidine and the metalloid arsenic increased resistance responses in the well-known pathosystem NN tobacco/tobacco mosaic virus (TMV). Both the hypersensitive response to TMV in a leaf disk model system (inoculated disks floating in the 0.1 mM treatments) and systemic acquired resistance (SAR) in whole plants were significantly affected. In the latter case, 1 mM foliar sprays of spermidine and arsenic were as effective as TMV and dl-[beta]-aminobutyric acid (BABA), both taken as positive controls, in improving the plant's response to subsequent challenge inoculation with TMV. Moreover, this phenotypic response was correlated with changes in the endogenous concentration of the SAR-related molecule salicylic acid and in transcript levels of some pathogenesis/stress-related genes (pathogenesis-related proteins PR-1a and PR-2 and arginine decarboxylase (ADC)). Concentrations of free salicylic acid and of 2-O-[beta]-d-glucosylsalicylic acid and mRNA amount of PR-1a, PR-2 and ADC were analyzed in plants treated with either spermidine or arsenic, and compared with those from untreated plants and from positive (TMV-inoculated or BABA-treated) controls. Conjugated salicylic acid content and ADC transcripts were found to significantly increase, at both the local and systemic levels, relative to untreated controls.

Keywords: Arginine decarboxylase; Arsenic; Salicylic acid; Spermidine; Systemic acquired resistance

Gustavo Zaparoli, Odalys Garcia Cabrera, Francisco Javier Medrano, Ricardo Tiburcio, Gustavo Lacerda, Goncalo Guimaraes Pereira, Identification of a second family of genes in *Moniliophthora perniciosa*, the causal agent of witches' broom disease in cacao, encoding necrosis-inducing proteins similar to cerato-platanins, *Mycological Research*, Volume 113, Issue 1, January 2009, Pages 61-72, ISSN 0953-7562, DOI: 10.1016/j.mycres.2008.08.004.

(<http://www.sciencedirect.com/science/article/B7XMR-4TB77J8-1/2/8dd7695f5ead564e4f3bad3f545e6756>)

Abstract:

The hemibiotrophic basidiomycete *Moniliophthora perniciosa* is the causal agent of witches' broom disease in cacao. This is a dimorphic species, with monokaryotic hyphae during the biotrophic phase, which is converted to dikaryotic mycelia during the saprophytic phase. The infection in pod is characterized by the formation of hypertrophic and hyperplastic tissues in the biotrophic phase, which is followed by necrosis and complete degradation of the organ. We found at least five sequences in the fungal genome encoding putative proteins similar to cerato-platanin (CP)-like proteins, a novel class of proteins initially found in the phytopathogen *Ceratocystis fimbriata*. One *M. perniciosa* CP gene (MpCP1) was expressed *in vitro* and proved to have necrosis-inducing ability in tobacco and cacao leaves. The protein is present in solution as dimers and is able to recover necrosis activity after heat treatment. Transcription analysis *ex planta* showed that MpCP1 is more expressed in biotrophic-like mycelia than saprotrophic mycelia. The necrosis profile presented is different from that caused by *M. perniciosa* necrosis and ethylene-inducing proteins (MpNEPs), another family of elicitors expressed by *M. perniciosa*. Remarkably, a mixture of MpCP1 with MpNEP2 led to a synergistic necrosis effect very similar to that found in naturally infected plants. This is the first report of a basidiomycete presenting both NEP1-like proteins (NLPs) and CPs in its genome.

Keywords: Cerato-platanin; *Moniliophthora perniciosa*; NEP1 like proteins; Witches' broom disease

Nan Zhao, Ju Guan, Farhad Forouhar, Timothy J. Tschaplinski, Zong-Ming Cheng, Liang Tong, Feng Chen, Two poplar methyl salicylate esterases display comparable biochemical properties but divergent expression patterns, *Phytochemistry*, Volume 70, Issue 1, January 2009, Pages 32-39, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2008.11.014.

(<http://www.sciencedirect.com/science/article/B6TH7-4VBK4J8-2/2/3675b1b611f0f8083830a2b01572e31f>)

Abstract:

Two genes encoding proteins of 98% sequence identity that are highly homologous to tobacco methyl salicylate (MeSA) esterase (SABP2) were identified and cloned from poplar. Proteins encoded by these two genes displayed specific esterase activities towards MeSA to produce salicylic acid, and are named PtSABP2-1 and PtSABP2-2, respectively. Recombinant PtSABP2-1 and PtSABP2-2 exhibited apparent K_m values of 68.2 ± 3.8 μM and 24.6 ± 1 μM with MeSA, respectively. Structural modeling using the three-dimensional structure of tobacco SABP2 as a template indicated that the active sites of PtSABP2-1 and PtSABP2-2 were highly similar to that of tobacco SABP2. Under normal growing conditions, PtSABP2-1 showed the highest level of expression in leaves and PtSABP2-2 was most highly expressed in roots. In leaf tissues of poplar plants under stress conditions, the expression of PtSABP2-1 was significantly down-regulated by two stress factors, whereas the expression of PtSABP2-2 was significantly up-regulated by four stress factors. The plausible mechanisms leading to these two highly homologous MeSA esterase genes involved in divergent biological processes in poplar are discussed.

Keywords: Black cottonwood; *Populus trichocarpa*; Methyl esterase; SABP2; Methyl salicylate; Salicylic acid; Gene family; Molecular modeling

Luisa Ederli, Lara Reale, Laura Madeo, Francesco Ferranti, Chris Gehring, Marco Fornaciari, Bruno Romano, Stefania Pasqualini, NO release by nitric oxide donors in vitro and in planta, *Plant Physiology and Biochemistry*, Volume 47, Issue 1, January 2009, Pages 42-48, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2008.09.008.

(<http://www.sciencedirect.com/science/article/B6VRD-4TMBVP3-2/2/1475c1f049a0cff83b6bd93df8b0fe49>)

Abstract:

Artificial nitric oxide (NO) donors are widely used as tools to study the role of NO in plants. However, reliable and reproducible characterisations of metabolic responses induced by different NO donors is complicated by the variability of their NO release characteristics. The latter are affected by different physical and biological factors including temperature and light. Here we critically evaluate NO release characteristics of the donors sodium nitroprusside (SNP), S-nitrosoglutathione (GSNO) and nitric oxide synthase (NOS), both in vitro and in planta (*Nicotiana tabacum* L. cv. BelW3) and assess their effects on NO dependent processes such as the transcriptional regulation of the mitochondrial alternative oxidase gene (AOX1a), accumulation of H₂O₂ and induction of cell death. We demonstrate that, contrary to NOS and SNP, GSNO is not an efficient NO generator in leaf tissue. Furthermore, spectrophotometric measurement of NO with a haemoglobin assay, rather than diaminofluorescein (DAF-FM) based detection, is best suited for the quantification of tissue NO. In spite of the different NO release signatures by SNP and NOS in tissue, the NO dependent responses examined were similar, suggesting that there is a critical threshold for the NO response.

Keywords: Alternative oxidase; Cell death; Nitric oxide; SNP; NOS; Tobacco

Zhao-Shi Xu, Teng-Fei Xiong, Zhi-Yong Ni, Xue-Ping Chen, Ming Chen, Lian-Cheng Li, Dong-Yao Gao, Xiu-Dao Yu, Pei Liu, You-Zhi Ma, Isolation and identification of two genes encoding leucine-rich repeat (LRR) proteins differentially responsive to pathogen attack and salt stress in tobacco, *Plant Science*, Volume 176, Issue 1, January 2009, Pages 38-45, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2008.09.004.

(<http://www.sciencedirect.com/science/article/B6TBH-4TG9HMN-1/2/52c94e2205ba9271511786fe34db50c3>)

Abstract:

Leucine-rich repeat proteins (LRRs) play important roles in signal perception and activation of defense responses. We isolated two new LRR genes, NtLRR1 and NtLRR2, in tobacco (*Nicotiana tabacum*). NtLRR1 and NtLRR2 are characterized by 9 and 3 LRR domains, respectively. The phylogenetic relationships show that NtLRR1 and NtLRR2 belong to different subfamilies of the polygalacturonase inhibitor proteins (PGIPs) and LRP-related proteins (LRPs), respectively. NtLRR1 and NtLRR2 are responsive to pathogen attack and salt stress, but display differential expression patterns in tobacco. NtLRR1 is activated rapidly by infection with the tobacco wildfire pathogen (*Pseudomonas syringae* pv. *tabaci*), but slowly by tobacco mosaic virus (TMV). In contrast, NtLRR2 transcripts rapidly accumulate after infection with TMV, and only sluggishly with infection by the wildfire pathogen. In addition, NtLRR1 transcripts abundantly accumulate in stems, whereas NtLRR2 appears mainly in the roots. Isolation of the NtLRR2 promoter revealed some cis-acting elements responding to stresses and defense signal molecules. Subcellular localization indicated that NtLRR1 and NtLRR2 proteins localize in the cell walls and plasma membranes, respectively. It was concluded that NtLRR1 and NtLRR2 are important proteins having different functions in response to different stresses and mediating in binding interactions in a wide variety of biological processes.

Keywords: Induction kinetics; Leucine-rich repeat; Pathogen infection; Salt stress; Subcellular localization; Tobacco

Xia Liu, Zhi Wang, Lili Wang, Renhua Wu, Jonathan Phillips, Xin Deng, LEA 4 group genes from the resurrection plant *Boea hygrometrica* confer dehydration tolerance in transgenic tobacco, *Plant Science*, Volume 176, Issue 1, January 2009, Pages 90-98, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2008.09.012.

(<http://www.sciencedirect.com/science/article/B6TBH-4TMJ3SX-1/2/61031b9b29e543b75273b8cc362945fa>)

Abstract:

The resurrection plant *Boea hygrometrica* can survive extreme dehydration and is used as a model system to study desiccation tolerance. Screening of a cDNA library prepared from desiccated leaves via a macroarray technique has resulted in the identification of two dehydration responsive genes that encode group 4 late embryogenesis abundant (LEA) proteins, designated as BhLEA1 and BhLEA2, respectively. BhLEA1 and BhLEA2 were induced by dehydration and signaling molecules, including abscisic acid (ABA). Transgenic tobacco that ectopically express BhLEA1 and BhLEA2 were generated and used to study the role of LEA proteins in dehydration tolerance. After a period of drought, the relative water content of leaves and photosystem II activity in transgenic tobacco were higher than wild-type plants. Furthermore the membrane permeability was lower in selected transgenic lines that expressed BhLEA1 and BhLEA2 than in wild-type plants. Superoxide dismutase and peroxidase activities were increased in transgenic plants relative to that observed in the wild-type control and proteins including ribulose-bisphosphate carboxylase (large subunit), light-harvesting complex II and photosystem II extrinsic protein were stabilized in transgenic plants compared to wild-type plants. Surprisingly, the steady state levels of BhLEA1 and BhLEA2 protein substantially increased in response drying, despite being under the transcriptional control of the CaMV 35S promoter. Data presented here suggests that BhLEA genes are likely to play a role in the general protection of the plant cell during dehydration and affect membrane and protein stability.

Keywords: *Boea hygrometrica*; Dehydration; LEA proteins; Resurrection plant; Transgenic tobacco

Mark Seger, Jose Luis Ortega, Suman Bagga, Champa-Sengupta Gopalan, Repercussion of mesophyll-specific overexpression of a soybean cytosolic glutamine synthetase gene in alfalfa (*Medicago sativa* L.) and tobacco (*Nicotiana tabacum* L.), *Plant Science*, Volume 176, Issue 1, January 2009, Pages 119-129, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2008.10.006.

(<http://www.sciencedirect.com/science/article/B6TBH-4TTF81H-1/2/db43950b80790f5950d09abb0d71c68f>)

Abstract:

Glutamine synthetase (GS) plays a central role in plant nitrogen metabolism. Plant GS occurs as a number of isoenzymes present in either the cytosol (GS1) or chloroplast/plastid (GS2). There are several reports of improved performance in transgenic plants overexpressing GS1 transgenes driven by the constitutive CaMV35S promoter. Improvement has been attributed to the GS1 transgene product functioning to enhance re-assimilation of NH₄⁺ released by photorespiration or protein degradation. In this paper, alfalfa and tobacco transformants expressing a soybean gene driven by a photosynthetic cell-specific promoter have been compared to transformants with the same transgene driven by the stronger CaMV35S promoter. The two classes of alfalfa and tobacco transformants showed differences in the level of GS1 transcript and GS1 protein accumulation, but the difference in the total GS activity was small. The discrepancy in the transgene expression level and GS activity has been attributed to posttranslational regulation at the level of holoprotein stability. Both classes of transformants exhibited similar level of improvement in soluble protein and in the rates of photosynthesis and photorespiration. The data supports the hypothesis that GS1 made in the mesophyll cells is involved in the re-assimilation of NH₄⁺ released via photorespiration and/or protein degradation.

Keywords: Glutamine synthetase; Mesophyll-specific overexpression; Nitrogen assimilation; Photorespiratory ammonium; Transgenic alfalfa

Jian-Jun MAO, De-Wen QIU, Xiu-Feng YANG, Hong-Mei ZENG, Jing-Jing YUAN, Expression of Protein Elicitor-Encoding Gene pemG1 in Tobacco (*Nicotiana tabacum* cv. Samsun NN) Plants and Enhancement of Resistance to TMV, *Acta Agronomica Sinica*, Volume 34, Issue 12, December 2008, Pages 2070-2076, ISSN 1875-2780, DOI: 10.1016/S1875-2780(09)60018-3.

(<http://www.sciencedirect.com/science/article/B94TW-4WBT18W-1/2/c2bf289c11f8878ceb1bda78408a9f9c>)

Abstract:

The pemG1 gene from *Magnaporthe grisea* was transferred into tobacco (*Nicotiana tabacum* cv. Samsun NN) to test its functions. Plant expression vector pCAMBIA2300-Ubi-pemG1-Oc harboring elicitor-encoding gene pemG1 was constructed. The maize ubiquitin promoter/octopine synthase terminator system and kanamycin-resistant gene npt II were used for constitutive expression systems. The vector was then introduced into *Agrobacterium tumefaciens* strain AGL-1 with freeze-thaw method. Tobacco primary transformants were produced by leaf disc transformation. The kanamycin-resistant regenerated plants were confirmed to be electropositive by PCR. Integration and expression of the pemG1 gene were further confirmed by Southern blotting and Western blotting analyses. Transgenic tobacco plants of T2 generation were successively inoculated with Tobacco Mosaic Virus (TMV) at virus concentrations of 0.2 and 0.5 mg per leaf. In comparison with TMV-infected wild-type plants, PemG1-expressed plants displayed reduced hypersensitive-response lesions in both inoculation treatments. Furthermore, accumulation level of pemG1 steady-state transcripts was examined at 24 h after inoculation. The results indicated that the reduction of lesions corresponded to the accumulation of pemG1 steady-state transcripts as monitored by Northern blotting analysis. All these indicated that the expression of pemG1 in tobacco plants improved the resistance to TMV.

Keywords: protein elicitor; pemG1; tobacco; expression; TMV

Alain-Jacques Valleron, La mortalite et la morbidite mondiale, maintenant et demain : que connait-on ?, *Comptes Rendus Biologies*, Volume 331, Issue 12, La sante dans les politiques de developpement / Health care in development policy, December 2008, Pages 991-1006, ISSN 1631-0691, DOI: 10.1016/j.crv.2008.09.002.

(<http://www.sciencedirect.com/science/article/B6X1F-4TK2PHX-3/2/965e1f02bac82b4921faa2fa503f1424>)

Abstract:

The knowledge of the global distribution of death, diseases and risk factors is important to make clear to the general public and to governments that health inequalities are incredibly high, at the dawn of this 21st century, and to help fight these. More than 20% of the 56 millions of deaths in 2001 were of children less than 5 years old. There are at least 1 million deaths per year from malaria. Diarrhoea kill more than 1.5 million, and measles more than half a million. The large majority of deaths by infectious diseases occur in underdeveloped countries. Moreover, chronic diseases kill an increasing number in underdeveloped countries, because populations are aging, because expansive health care which is needed to prevent and control these diseases is unavailable, and because the inhabitants are increasingly exposed to risk factors. In particular, smoking is increasing dramatically in underdeveloped countries as a result of the aggressive marketing of tobacco companies, the delay in implementing antismoking regulations, and because the public perception of the risk of smoking is still low. More than 4 million deaths per year are presently attributed to smoking, and reports forecast a death toll of 10 million in 2030.

The WHO, Harvard University and the World Bank are at the origin of comprehensive data analyses on the 'global burden of diseases' which help to identify health priorities. Unfortunately, global data are still scarce and of low quality, particularly in those underdeveloped countries where they would be most useful.

Precise knowledge of the variations of mortality, morbidity and exposure to risk factors would be essential to monitor the improvements, or failures of health care progress. The optimal interpretation of the available data requires expertise in demography, epidemiology, statistics, and computer sciences, which are rarely found in this area. Thus, improvements in the collection of data and in the research effort in this field are necessary. To cite this article: A.-J. Valleron, C. R. Biologies 331 (2008).

Keywords: Epidemiologie; Mortalite; Morbidite; Poids sanitaire mondial de la maladie; Epidemiology; Mortality; Morbidity; Global Burden of Diseases; GDB

Chuan Liu, Letter to the Editor, Food and Chemical Toxicology, 2007 'DNA solutionR in cigarette filters reduces polycyclic aromatic hydrocarbon (PAH) levels in mainstream tobacco smoke' M. Lodovici, V. Akpan, S. Caldini, B. Akanju, and P. Dolara, Food and Chemical Toxicology, Volume 46, Issue 12, December 2008, Pages 3851-3852, ISSN 0278-6915, DOI: 10.1016/j.fct.2008.09.011.

(<http://www.sciencedirect.com/science/article/B6T6P-4TG9HYT-1/2/b7f4ddb606a17d1cf562c222c6bd2088>)

Patricia Agudelo-Romero, Francisca de la Iglesia, Santiago F. Elena, The pleiotropic cost of host-specialization in Tobacco etch potyvirus, Infection, Genetics and Evolution, Volume 8, Issue 6, December 2008, Pages 806-814, ISSN 1567-1348, DOI: 10.1016/j.meegid.2008.07.010.

(<http://www.sciencedirect.com/science/article/B6W8B-4T708B8-1/2/c47bd46f7824f671dd86787a8c3964c9>)

Abstract:

Host-range expansion is thought to allow viruses to broaden their ecological niches by allowing access to new resources. However, traits governing the infection of multiple hosts may decrease fitness in the original one due to the pleiotropic effect of adaptive mutations that may give rise to fitness tradeoffs across hosts. Here, we have experimentally examined the consequences of host-specialization in the plant pathogen Tobacco etch potyvirus (TEV). Replicate populations of TEV were allowed to evolve for 15 serial undiluted passages on the original tobacco host or on pepper, a novel host. Virulence and biologically active viral load were evaluated during the course of the experiment for each lineage on both potential hosts. In agreement with the tradeoff hypothesis, lineages evolved in the novel host experienced substantial increases in virulence and virus accumulation in its own host, but suffered reduced virulence and accumulation on the original host. By contrast, lineages evolved on the ancestral host did not increase virulence or viral load on either host. Genomic consensus sequences were obtained for each lineage at the end time point. The potential relevance for the evolution of virulence and virus fitness of the characterized mutations is discussed.

Keywords: Adaptation; Experimental evolution; Evolution of virulence; Plant virus; Specialization; Tradeoffs; Virus evolution

Shahnaz Shahidi-Noghabi, Els J.M. Van Damme, Guy Smagghe, Carbohydrate-binding activity of the type-2 ribosome-inactivating protein SNA-I from elderberry (*Sambucus nigra*) is a determining factor for its insecticidal activity, Phytochemistry, Volume 69, Issue 17, December 2008, Pages 2972-2978, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2008.09.012.

(<http://www.sciencedirect.com/science/article/B6TH7-4TRXDNF-3/2/57679021205599b1012114bfb8bcd0f2>)

Abstract:

In recent years, different classes of proteins have been reported to promote toxic effects when ingested. Type-2 ribosome-inactivating proteins (RIPs) are a group of chimeric proteins built up of an A-chain with RNA N-glycosidase activity and a B-chain with lectin activity. These proteins are thought to play a role in plant protection. *Sambucus nigra* agglutinin I (SNA-I) is a type-2 RIP,

isolated from the bark of elderberry (*S. nigra* L.). This study demonstrated the insecticidal potency of SNA-I on two Hemipteran insect species using two different methods. An artificial diet supplemented with different concentrations of the purified RIP reduced survival and fecundity of pea aphids *Acyrtosiphon pisum*. In addition, feeding of tobacco aphids, *Myzus nicotianae*, on leaves from transfected plants constitutively expressing SNA-I, resulted in a delayed development and reduced adult survival and also the fertility parameters of the surviving aphids were reduced, suggesting that a population of aphids would build up significantly slower on plants expressing SNA-I. Finally, a series of experiments with transgenic lines in which a mutant RIP was expressed, revealed that the carbohydrate-binding activity of SNA-I is necessary for its insecticidal activity. In a first set of mutants, the B-chain was mutated at one position (Asp231[Delta]Glu), and in the second set both carbohydrate-binding sites were mutated (Asn48[Delta]Ser and Asp231[Delta]Glu). Mutation of one carbohydrate-binding site strongly reduced the insecticidal activity of SNA-I, whereas mutation of both lectin sites (almost) completely abolished the SNA-I effect on tobacco aphids.

Keywords: Ribosome-inactivating protein; Mutant; *Sambucus nigra* agglutinin; *Acyrtosiphon pisum*; *Myzus nicotianae*; Lectin

Sravan Kumar Jami, Greg B. Clark, Swathi Anuradha Turlapati, Craig Handley, Stanley J. Roux, Pulugurtha Bharadwaja Kirti, Ectopic expression of an annexin from *Brassica juncea* confers tolerance to abiotic and biotic stress treatments in transgenic tobacco, *Plant Physiology and Biochemistry*, Volume 46, Issue 12, December 2008, Pages 1019-1030, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2008.07.006.

(<http://www.sciencedirect.com/science/article/B6VRD-4T2DKT6-1/2/e60e145a44e27b8a35517946b777e205>)

Abstract:

Plant annexins belong to a multigene family and are suggested to play a role in stress responses. A full-length cDNA for a gene encoding an annexin protein was isolated and characterized from *Brassica juncea* (AnnBj1). AnnBj1 message levels were regulated by abscisic acid, ethephon, salicylic acid, and methyl jasmonate as well as chemicals that induce osmotic stress (NaCl, Mannitol or PEG), heavy metal stress (CdCl₂) and oxidative stress (methyl viologen or H₂O₂). In order to determine if AnnBj1 functions in protection against stress, we generated transgenic tobacco plants ectopically expressing AnnBj1 under the control of constitutive CaMV 35S promoter. The transgenic tobacco plants showed significant tolerance to dehydration (mannitol), salt (NaCl), heavy metal (CdCl₂) and oxidative stress (H₂O₂) at the seedling stage and retained higher chlorophyll levels in response to the above stresses as determined in detached leaf senescence assays. The transgenic plants also showed decreased accumulation of thiobarbituric acid-reactive substances (TBARS) compared to wild-type plants in response to mannitol treatments in leaf disc assays. AnnBj1 recombinant protein exhibited low levels of peroxidase activity *in vitro* and transgenic plants showed increased total peroxidase activity. Additionally, the transgenic plants showed enhanced resistance to the oomycete pathogen, *Phytophthora parasitica* var. *nicotianae*, and increased message levels for several pathogenesis-related proteins. Our results demonstrate that ectopic expression of AnnBj1 in tobacco provides tolerance to a variety of abiotic and biotic stresses.

Keywords: Abiotic stress; Annexin; *Brassica juncea*; Oomycetes; Peroxidase activity; Transgenic tobacco

Marcello Iriti, Franco Faoro, Abscisic acid is involved in chitosan-induced resistance to tobacco necrosis virus (TNV), *Plant Physiology and Biochemistry*, Volume 46, Issue 12, December 2008, Pages 1106-1111, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2008.08.002.

(<http://www.sciencedirect.com/science/article/B6VRD-4TB183M-1/2/bf79cff5b73b01c9032a725d5d5f1304>)

Abstract:

Chitosan (CHT) antiviral activity has been further investigated in the pathosystem *Phaseolus vulgaris* - tobacco necrosis virus (TNV). CHT application elicited both callose apposition and ABA accumulation in leaf tissues, at 12 and 24 h after treatment, respectively, and induced a high level of resistance against TNV. Besides, treatment with the ABA inhibitor nordihydroguaiaretic acid (NDGA), before CHT application, reduced both callose deposition and plant resistance to the virus, thus indicating the involvement of ABA in these processes. Exogenous application of ABA also induced a significant resistance to TNV, though this resistance was abolished by NDGA pre-treatment. These results, overall, indicate that the rise of ABA synthesis induced by chitosan plays an important role in enhancing callose deposition but the latter has only a partial effect on virus spreading, which must be constrained by other resistance mechanisms.

Keywords: Abscisic acid; Antiviral activity; Callose; Induced resistance; Chitosan; Nordihydroguaiaretic acid; Plant immunity

Maria Chiara Zonno, Maurizio Vurro, Sergio Lucretti, Anna Andolfi, Carmen Perrone, Antonio Evidente, Phyllostictine A, a potential natural herbicide produced by *Phyllosticta cirsii*: In vitro production and toxicity, *Plant Science*, Volume 175, Issue 6, December 2008, Pages 818-825, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2008.08.003.

(<http://www.sciencedirect.com/science/article/B6TBH-4T7XGK6-2/2/4a716564f0ab9a342f34f8d948c6ef7b>)

Abstract:

Phyllostictine A is a powerful toxin produced by *Phyllosticta cirsii*, a potential mycoherbicide of *Cirsium arvense*. To support its potential use as a natural herbicide, toxin production has been studied using different media and cultural conditions. The toxin content in the crude extracts has been determined by using a HPLC method set up for this purpose. Furthermore, its phytotoxicity has been evaluated on tobacco protoplasts by flow cytometric analysis, and on *C. arvense* protoplasts, by fluorescence microscopy. The best cultural conditions found allowed to produce more than 28 mg ml⁻¹ of toxin in culture filtrate. The pure metabolite proved to have rapid dose-dependant toxic effects on host and non-host plant protoplasts.

Keywords: Phyllostictine A; Flow cytometry; *Cirsium arvense*; *Nicotiana tabacum*; HPLC analytical method; Protoplasts

Antonio Riglietti, Pacifico Ruggiero, Carmine Crecchio, Investigating the influence of transgenic tobacco plants codifying a protease inhibitor on soil microbial community, *Soil Biology and Biochemistry*, Volume 40, Issue 12, December 2008, Pages 2928-2936, ISSN 0038-0717, DOI: 10.1016/j.soilbio.2008.07.027.

(<http://www.sciencedirect.com/science/article/B6TC7-4T9JSS5-2/2/13ef52fea3a964570f82753373de2243>)

Abstract:

Serine protease inhibitors (PIs) are involved in several physiological processes, such as regulation of endogenous proteinases and defence against phytophagous insects. Transgenic modifications have enhanced protease inhibitor expression to develop insect resistant cultivars in several important crops. The fate of protease inhibitors released from genetically engineered plants is an important issue because of possible inhibition of soil proteases and effects of the insecticidal protein and its codifying sequence on soil microorganisms. The persistence of transgenic sequence mustard trypsin inhibitor-2 in soil and its hypothetical acquisition by soil microorganisms by horizontal gene transfer and the effect of transgenic plant material on soil microbial community structure and soil protease activity were investigated. With the aim to simulate the effects of plant litter on soil microorganisms, a microcosm experimental model was used. Despite the persistence of transgenic DNA sequences, no recombination event was detected between plant DNA and soil bacteria; molecular analysis of bacterial community also showed no significant influence on the

dominant members of the bacterial community and soil protease activity was not inhibited by the release of constitutively over-expressed protease inhibitor.

Keywords: Transgenic plants; Horizontal gene transfer; Microbial community structure; Litterbags

M. Al Rashidi, A. Shihadeh, N.A. Saliba, Volatile aldehydes in the mainstream smoke of the narghile waterpipe, *Food and Chemical Toxicology*, Volume 46, Issue 11, November 2008, Pages 3546-3549, ISSN 0278-6915, DOI: 10.1016/j.fct.2008.09.007.

(<http://www.sciencedirect.com/science/article/B6T6P-4TDVMJ7-6/2/7bb145a8d52ee79ec032ccf06628fdcc>)

Abstract:

Very little is known about the quality and quantity of toxicants yielded by the narghile, a subject of increasing importance as this method of tobacco smoking has become popular all over the world. This study is concerned with the identification and quantification of volatile aldehydes in the gas and particle phases of mainstream narghile smoke generated using a popular type of flavored ma'ssel tobacco mixture. These compounds were analyzed based on a modified version of the Environmental Protection Agency compendium method TO-11A. Using a standardized smoking machine protocol consisting of 171 puffs, 2.6 s puff duration and 17 s inter puff interval, the average yields of formaldehyde, acetaldehyde, acrolein, propionaldehyde and methacrolein were 630, 2520, 892, 403, and 106 [μ]g/smoking session, respectively. The results showed that none of the aldehydes identified in this study are found in the particulate phase of the smoke, except for formaldehyde for which the partitioning coefficient was estimated as $K_p = 3.3 \times 10^{-8}$ [μ]g/m³. Given previously reported lung absorption fractions of circa 90% for volatile aldehydes, the yields measured in this study are sufficient to induce various diseases depending on the extent of exposure, and on the breathing patterns of the smokers.

Keywords: Aldehyde; Waterpipe; Mainstream smoke; Partitioning; Health hazards

Wei Li, Libo Zhang, Jinhui Peng, Ning Li, Shimin Zhang, Shenghui Guo, Tobacco stems as a low cost adsorbent for the removal of Pb(II) from wastewater: Equilibrium and kinetic studies, *Industrial Crops and Products*, Volume 28, Issue 3, November 2008, Pages 294-302, ISSN 0926-6690, DOI: 10.1016/j.indcrop.2008.03.007.

(<http://www.sciencedirect.com/science/article/B6T77-4SDNG2K-2/2/e0ab12f9e15f739b37bdd1e1ef81226a>)

Abstract:

Adsorption of Pb(II) ions from aqueous solution onto tobacco stems has been investigated to evaluate the effects of initial lead ion concentration, adsorbent dosage, contact time, pH and temperature on the removal of Pb(II) systematically. The optimal pH value for Pb(II) adsorption onto the tobacco stems was found to be 5.0. The removal of lead ions for concentrations 10, 30 and 50 mg L⁻¹ using 0.8 g adsorbent at contact time of 120 min and at temperature of 299 K were 94.37%, 92.10% and 90.43%, respectively. Thermodynamic parameters such as standard Gibbs free energy ($[\Delta]G[\text{degree sign}]$), standard enthalpy ($[\Delta]H[\text{degree sign}]$), and standard entropy ($[\Delta]S[\text{degree sign}]$) were evaluated by applying the Van't Hoff equation, which describes the dependence of equilibrium constant on temperature. The thermodynamics of Pb(II) adsorption onto the tobacco stems indicated that the adsorption was spontaneous and endothermic. Langmuir and Freundlich isotherms were used to analyze the equilibrium data at different temperatures and the equilibrium data were found to fit Freundlich isotherm equation better than Langmuir isotherm. The adsorption was analyzed using pseudo-second-order kinetic equation.

Keywords: Tobacco stems; Adsorption; Lead ions(II); Isotherm; Kinetics

Qifang Guo, Jin Zhang, Qiang Gao, Shichao Xing, Feng Li, Wei Wang, Drought tolerance through overexpression of monoubiquitin in transgenic tobacco, *Journal of Plant Physiology*, Volume 165,

Issue 16, 1 November 2008, Pages 1745-1755, ISSN 0176-1617, DOI: 10.1016/j.jplph.2007.10.002.

(<http://www.sciencedirect.com/science/article/B7GJ7-4RV1JH7-1/2/af11c778921b93bf625f32749027a4cd>)

Abstract: Summary

Ubiquitin (Ub) is present in all eukaryotic species examined. It is a multifunctional protein and one of its main known functions is to tag proteins for selective degradation by the 26S proteasome. In this study, Ta-Ub2, a cDNA sequence containing a single Ub repeat and a 3' non-coding region of a polyubiquitin gene, was isolated from wheat (*Triticum aestivum*) by reverse transcription-polymerase chain reaction (RT-PCR). A PBI sense vector with Ta-Ub2 was constructed and transformed into tobacco plants. Ub expression in wheat leaves, monitored by semi-quantitative RT-PCR, responded to drought stress. In transgenic tobacco, determined by protein gel blot analysis, we found higher amounts of Ub-protein conjugates than in control (tobacco carrying a PBI GUS vector without Ta-Ub2) and wild-type (WT) lines. However, free Ub levels did not significantly differ in the 3 genotypes. Seeds from transgenic, Ub-overexpressing tobacco germinated faster and seedlings grew more vigorously than control and WT samples, both under drought and non-drought conditions. Furthermore, CO₂ assimilation of transgenic plants was significantly higher under drought stress. Our results indicate that Ub may be involved in the response of plants to drought stress and that overexpression of monoubiquitin might be an effective strategy for enhancing drought tolerance.

Keywords: Drought tolerance; Gene expression; Transgenic tobacco; Ubiquitin; Wheat

S.-L. Yan, A.T. Lehrer, M.R. Hajirezaei, A. Springer, E. Komor, Modulation of carbohydrate metabolism and chloroplast structure in sugarcane leaves which were infected by Sugarcane Yellow Leaf Virus (SCYLV), *Physiological and Molecular Plant Pathology*, Volume 73, Issues 4-5, November 2008, Pages 78-87, ISSN 0885-5765, DOI: 10.1016/j.pmpp.2009.02.004.

(<http://www.sciencedirect.com/science/article/B6WPC-4VTVPXM-1/2/8e78ac4a10882267d30fd65c718b3e8d>)

Abstract:

Non-symptomatic sugarcane plants infected with Sugarcane Yellow Leaf Virus showed starch in mesophyll and bundle sheath cells. In situ-hybridization of mRNAs of sucrose-phosphate phosphatase and ADP-glucose pyrophosphorylase revealed that infected leaves contained SPPase and AGPase in mesophyll cells, Kranz cells and bundle sheath cells. In contrast virus-free leaves contained SPPase only in Kranz cells and AGPase only in bundle sheath cells. Infected leaves exhibited ultrastructural changes in Kranz cell chloroplasts and a shift of the chlorophyll a/b ratio. No obstruction of plasmodesmata was observed. The results indicate that SCYLV-infected plants, even when visually non-symptomatic, underwent strong metabolic and ultrastructural changes.

Keywords: ADP-glucose pyrophosphorylase; Chlorophyll breakdown; Chloroplast ultrastructure; In situ hybridization; Plasmodesmata; *Saccharum spec. hybrid*; Starch; Sucrose-phosphate phosphatase

Ashraful Haque, Nobumitsu Sasaki, Hiromi Kanegae, Seisuke Mimori, Jun-Shan Gao, Hiroshi Nyunoya, Identification of a Tobacco mosaic virus elicitor-responsive sequence in the resistance gene N, *Physiological and Molecular Plant Pathology*, Volume 73, Issues 4-5, November 2008, Pages 101-108, ISSN 0885-5765, DOI: 10.1016/j.pmpp.2009.03.001.

(<http://www.sciencedirect.com/science/article/B6WPC-4VVGKM0-1/2/77e31f116a1055b7646d8dbbec048a30>)

Abstract:

The N gene of *Nicotiana tabacum* cv. Samsun NN is a resistance gene for Tobacco mosaic virus. The transcription of the gene increases immediately after virus infection or transient expression of

the elicitor p50, a helicase domain of the virus 126/180 K replicases. In this study, we cloned the upstream sequences of the N gene from Samsun NN and fused them to the GFP reporter gene for promoter assays. *Agrobacterium*-mediated transient gene expression in *N. tabacum* cv. Samsun nn lacking the N gene allowed us to evaluate promoter activity with or without the expression of p50 and/or N. Individual expression of p50 or N specifically stimulated the N promoter, although the stimulation by N was less prominent than that of p50. The coexpression of p50 and N resulted in higher stimulation of the N promoter than the individual expression. Through deletion and gain-of-function analyses of the upstream region, we identified a 20-bp elicitor-responsive sequence that was essential and sufficient for promoter stimulation by p50 and N. Based on these data, we discuss the possibility that the virus elicitor and N may mediate cooperatively the stimulation of the N promoter upon virus infection.

Keywords: Hypersensitive response; HR; Dof binding motif; Elicitor

Kliti Grice, Hong Lu, Youping Zhou, Hilary Stuart-Williams, Graham D. Farquhar, *Biosynthetic and environmental effects on the stable carbon isotopic compositions of anteiso- (3-methyl) and iso- (2-methyl) alkanes in tobacco leaves*, *Phytochemistry*, Volume 69, Issue 16, November 2008, Pages 2807-2814, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2008.08.024.

(<http://www.sciencedirect.com/science/article/B6TH7-4TS80VP-1/2/c85ad06e0a02b65c08df12d31d2927f0>)

Abstract:

Nicotiana tabacum is the only plant known to synthesise large quantities of anteiso- (3-methyl) alkanes and iso- (2-methyl) alkanes. We investigated the carbon isotope ratios of individual long-chain n-alkanes, anteiso- and iso-alkanes (in the C29-C33 carbon number range) extracted from tobacco grown in chambers under controlled conditions to confirm the pathway used by the tobacco plant to synthesise these particular lipids and to examine whether environmental data are recorded in these compounds. Tobacco was grown under differing temperatures, water availabilities and light intensities in order to control its stable carbon isotope ratios and evaluate isotopic fractionations associated with the synthesis of these particular lipids. The anteiso-alkanes were found to have a predominant even-carbon number distribution (maximising at C32), whereas the iso-alkanes exhibit an odd-carbon number distribution (maximising at C31). Iso-alkanes were relatively more abundant than the anteiso-alkanes and only two anteiso-alkanes (C30 and C32) were observed.

The anteiso-alkanes and iso-alkanes were found to be enriched in ^{13}C by 2.8-4.3[per mille sign] and 0-1.8[per mille sign] compared to the n-alkanes, respectively, consistent with different biosynthetic precursors. The assumed precursor for the odd-carbon-numbered iso-alkanes is iso-butyryl-CoA (a C4 unit derived from valine) followed by subsequent elongation of C2 units and then decarboxylation. The assumed precursor for even-carbon-numbered anteiso-alkanes is [alpha]-methylbutyryl-CoA (a C5 unit derived from isoleucine) and subsequent elongation by C2 units followed by decarboxylation. The ratio of carbon atoms derived from [alpha]-methylbutyryl-CoA and subsequent C2 units (from malonyl-CoA) is 1:5 for the biosynthesis of a C30 anteiso-alkane. The ratio of carbon atoms derived from iso-butyryl-CoA and subsequent C2 units (from malonyl-CoA) is 4:25 for the synthesis of a C29 iso-alkane. An order of ^{13}C depletion n-alkanes > iso-alkanes > anteiso-alkanes is evident from compound specific isotope data. This trend can probably be attributed to the ratio of the two different sources of carbon atoms in the final wax components.

Higher water availability generally results in more depleted stable carbon isotope ratios due to maximised discrimination during carboxylation, associated with less diffusional limitation. This was confirmed in the present study by compound specific isotope analyses of iso-alkanes, anteiso-alkanes and n-alkane lipids extracted from the tobacco leaves. Likewise, light intensity has been shown to influence plant bulk $[\delta^{13}\text{C}]$ in previous studies. The carbon isotope ratios of n-alkanes in tobacco grown under low-light conditions were about 2[per mille sign] more depleted in

^{13}C than those of lipids extracted from tobacco grown under elevated light conditions. A similar order of difference is observed for the iso-alkanes and anteiso-alkanes (1.8[per mille sign] and 1.9[per mille sign], respectively). A negligible depletion in carbon isotope ratios was observed for the iso-alkanes and anteiso-alkanes extracted from tobacco grown under elevated temperatures. These results are consistent with the work of Farquhar [Farquhar, G.D., 1980. Carbon isotope discrimination by plants: effects of carbon dioxide concentration and temperature via the ratio of intercellular and atmospheric CO_2 concentrations. In: Pearman, G.I. (Ed.), Carbon Dioxide and Climate: Australian Research. Springer, Berlin, pp. 105-110] where temperature appears to have only a minor effect on plant bulk $[\delta]^{13}\text{C}$.

Keywords: *Nicotiana tabacum*; Biosynthesis; Anteiso-alkanes; Iso-alkanes; Stable carbon isotopes

Attila Hegedus, Tibor Janda, Gabor V. Horvath, Denes Dudits, Accumulation of overproduced ferritin in the chloroplast provides protection against photoinhibition induced by low temperature in tobacco plants, *Journal of Plant Physiology*, Volume 165, Issue 15, 9 October 2008, Pages 1647-1651, ISSN 0176-1617, DOI: 10.1016/j.jplph.2008.05.005.

(<http://www.sciencedirect.com/science/article/B7GJ7-4SXRTH6-2/2/02c6426d4ae371c795a449a455f462e9>)

Abstract: Summary

Wild-type tobacco plants (*Nicotiana tabacum* L. cv. Petit Havanna line SR1) and plants transformed with full-length alfalfa ferritin cDNA with the chloroplast transit peptide under the control of a Rubisco small subunit gene promoter (C3 and C8) were cold-treated at 0 [degree sign]C with continuous light (250 [μ]mol $\text{m}^{-2} \text{s}^{-1}$). These transgenic plants had higher chlorophyll content and higher Fv/Fm chlorophyll-a fluorescence induction parameters than wild-type plants after 2 or 3 d of cold treatment in C3 and C8 transgenic plants, respectively. Thermoluminescence studies on the high-temperature bands suggest that these plants suffered less oxidative damage in comparison to the wild-type genotype. The present experiments provide evidence that transgenic tobacco lines overexpressing alfalfa ferritin, which is accumulated in the chloroplasts, may show higher tolerance to various stress factors, generating ROS including low temperature-induced photoinhibition.

Keywords: Ferritin; Low temperature; Photoinhibition; Stress tolerance; Transgenic plants

J.J. Bull, The optimal burst of mutation to create a phenotype, *Journal of Theoretical Biology*, Volume 254, Issue 3, 7 October 2008, Pages 667-673, ISSN 0022-5193, DOI: 10.1016/j.jtbi.2008.06.006.

(<http://www.sciencedirect.com/science/article/B6WMD-4SSNDCR-1/2/ff9876b6152fd14234ec5265b8e6d02d>)

Abstract:

Mutagenesis is commonly applied to genes and genomes to create novel variants with desired properties. This paper calculates the level of mutagenesis that maximizes the appearance of favorable mutants, assuming that the mutagenesis is applied in a single episode. The downside of mutagenesis is that a substantial fraction of mutations will destroy gene/genome function. The upside of mutagenesis is the production of beneficial mutations, but the desired phenotype may require that 1, 2 or more beneficial mutations be present simultaneously (the phenotype dimensionality). The optimum level of mutagenesis is sensitive to both properties. In the simplest model, the mutation optimum occurs when number of lethal equivalents per genome equals the phenotype dimensionality, a result first derived by Mundry and Gierer [1958. Production of mutations in tobacco mosaic virus by chemical treatment of its nucleic acid in vitro. *Z. Vererbungsl.* 89 (4), 614-630]. This level of mutation is shown to be an upper bound for the optimum in various extensions of the model, and the recovery of mutants is also reasonably tolerant to deviations from the optimum.

Keywords: Mutation; Mutagenesis; Evolution; Optimal; Survival

Rumi Kaida, Takahisa Hayashi, Takako S. Kaneko, Purple acid phosphatase in the walls of tobacco cells, *Phytochemistry*, Volume 69, Issue 14, October 2008, Pages 2546-2551, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2008.07.008.

(<http://www.sciencedirect.com/science/article/B6TH7-4TBHJYH-1/2/4011f61bba3da574b8faefc989a9fa68>)

Abstract:

Purple acid phosphatase isolated from the walls of tobacco cells appears to be a 220 kDa homotetramer composed of 60 kDa subunits, which is purple in color and which contains iron as its only metal ion. Although the phosphatase did not require dithiothreitol for activity and was not inhibited by phenylarsine oxide, the enzyme showed a higher catalytic efficiency (kcat/Km) for phosphotyrosine-containing peptides than for other substrates including p-nitrophenyl-phosphate and ATP. The phosphatase formed as a 120 kDa dimer in the cytoplasm and as a 220 kDa tetramer in the walls, where Brefeldin A blocked its secretion during wall regeneration. According to our double-immunofluorescence labeling results, the enzyme might be translocated through the Golgi apparatus to the walls at the interphase and to the cell plate during cytokinesis.

Keywords: *Nicotiana tabacum*; Solanaceae; Tobacco; Purple acid phosphatase; Metallophosphatase; Wall; Golgi apparatus; JIM 84; Brefeldin A; Translocation

S. Isaac Kirubakaran, S. Mubarak Begum, K. Ulaganathan, N. Sakthivel, Characterization of a new antifungal lipid transfer protein from wheat, *Plant Physiology and Biochemistry*, Volume 46, Issue 10, October 2008, Pages 918-927, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2008.05.007.

(<http://www.sciencedirect.com/science/article/B6VRD-4SKK205-1/2/424760489d1710262c5ba16e51517e1b>)

Abstract:

Lipid transfer proteins (LTPs) are members of the family of pathogenesis-related proteins (PR-14) that are believed to be involved in plant defense responses. In this study, a novel gene Ltp 3F1 encoding an antifungal protein from wheat (Sumai 3) was subcloned, overexpressed in *Escherichia coli* BL-21 (DE3) and enriched using ammonium sulfate fractionation followed by gel permeation chromatography. Molecular phylogeny analyses of wheat Ltp 3F1 gene showed a strong identity to other plant LTPs. Predicted three-dimensional structural model showed the presence of 6 [alpha]-helices and 9 loop turns. The active site catalytic residues Gly30, Pro50, Ala52 and Cys55 may be suggested for catalyzing the reaction involved in lipid binding. SDS-PAGE analysis confirmed the production of recombinant fusion protein. The LTP fusion protein exhibited a broad-spectrum antifungal activity against *Alternaria* sp., *Rhizoctonia solani*, *Curvularia lunata*, *Bipolaris oryzae*, *Cylindrocladium scoparium*, *Botrytis cinerea* and *Sarocladium oryzae*. Gene cassette with cyanamide hydratase (cah) marker and Ltp 3F1 gene was constructed for genetic transformation in tobacco. Efficient regeneration was achieved in selective media amended with cyanamide. Transgenic plants with normal phenotype were obtained. Results of PCR and Southern, Northern and Western hybridization analyses confirmed the integration and expression of genes in transgenic plants. Experiments with detached leaves from transgenic tobacco expressing Ltp 3F1 gene showed fungal resistance. Due to the innate potential of broad-spectrum antifungal activity, wheat Ltp 3F1 gene can be used to enhance resistance against fungi in crop plants.

Keywords: Heterologous expression; Lipid transfer protein; Antifungal activity; Transgenic tobacco

Marion Le Foll, Sophie Blanchet, Laurine Millan, Chantal Mathieu, Catherine Bergounioux, Nathalie Glab, The plant CDK inhibitor NtKIS1a interferes with dedifferentiation, is specifically down regulated during development and interacts with a JAB1 homolog, *Plant Science*, Volume 175, Issue 4, October 2008, Pages 513-523, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2008.05.022.

(<http://www.sciencedirect.com/science/article/B6TBH-4SR7119-2/2/11f2f4174c73d45d54716d4df70fd728>)

Abstract:

CKIs from plant and animal share functional similarities although their roles have evolved in each kingdom to become adapted to the specific requirement of developmental programs. In plants, two types of CKI have been described so far and only one is related to a limited extend to the animal p27KIP1 a fact essentially based on sequence similarities and interacting partners. To decipher the role of plant CKIs, a gain of function approach was mainly applied in *Arabidopsis thaliana* and showed that overexpressed CKIs inhibit cell division in planta. Here, *Arabidopsis* overexpressing NtKIS1a, a tobacco CKI, was used to address the question of how accumulation of the CDK/cyclin complex inhibitor NtKIS1a still allowed the development of a mature plant. We analysed the re-induction of division from differentiated cells and showed that NtKIS1a overexpression interfered with dedifferentiation. In order to test whether some discrete cells could get depleted of the inhibitory NtKIS1a protein, we analysed the presence of NtKIS1a protein in the various plant meristems. Surprisingly, its absence was revealed in the vegetative apical meristem. Characterisation of an interaction between NtKIS1a and AtAJH1, a plant JAB1 homolog, suggested that like p27KIP1, AtAJH1 might target NtKIS1a degradation. Furthermore, NtKIS1a sub-cellular localisation was modified in the presence of AtAJH1.

Keywords: *Arabidopsis thaliana*; Cell cycle; CDK inhibitor; Dedifferentiation; Meristems; JAB1 homolog

Hong-yuan SONG, Xue-song REN, Jun SI, Cheng-qiong LI, Ming SONG, Construction of Marker-Free GFP Transgenic Tobacco by Cre/lox Site-Specific Recombination System, *Agricultural Sciences in China*, Volume 7, Issue 9, September 2008, Pages 1061-1070, ISSN 1671-2927, DOI: 10.1016/S1671-2927(08)60147-9.

(<http://www.sciencedirect.com/science/article/B82XG-4THKV6G-5/2/e010f1e384a8aa91f0f1791bad7710ef>)

Abstract:

Marker-free GFP transgenic tobacco plants were constructed based on Cre/lox site-specific recombination system. A GFP gene was introduced into the tobacco genome using the Bar gene as a linked selectable marker flanked by recombination sites in a directed orientation. The Bar gene expression box was subsequently excised from the plant genome by a strategy of Cre gene retransformation. After removal of the Cre-NPT II locus by genetic segregation through self-cross, plants that incorporated only the GFP transgene were obtained. Transgenic tobacco plants mediated by *Agrobacterium tumefaciens* were obtained, which resisted herbicide Basta and GFP expressed well, then the Cre gene was subsequently introduced into 5 plants of them, respectively, by retransformation. The leaf disks from Cre transgenic plants were used to test the resistance to Basta on the medium with 8 mg L⁻¹ of PPT. The results showed that few discs were able to regenerate normally, and the excision at 76-100% efficiency depended on individual retransformation events. Evidence for a precise recombination event was confirmed by cloning the nucleotides sequence surrounding the lox sites of the Basta sensitive plants. The result indicated that the excision event in the recombination sites was precise and conservative, without loss or alteration of any submarginal nucleotides of the recombination sites. Bar gene excised plants were self-pollinated to allow segregation of the GFP gene from the Cre-NPT II locus. The progenies from self-pollinated plants were scored for Kan sensitivity, then the segregation of GFP gene from Cre-NPT II locus in the Kan sensitive plants were confirmed by PCR analysis subsequently. Hence, constructing marker-free transgenic tobacco plants by Cre/lox site-specific recombination system was reliable, and the strategy presented here should be applicable to other plants for the construction of marker-free transgenic plants as well.

Keywords: Cre/lox site-specific recombination system; marker-free transgenic tobacco; GFP

Wei-qun LIU, Hong-xiang GUO, Hao LI, Proteomics Identification of Differentially Expressed Proteins Relevant for Nicotine Synthesis in Flue-Cured Tobacco Roots Before and After Decapitation, *Agricultural Sciences in China*, Volume 7, Issue 9, September 2008, Pages 1084-1090, ISSN 1671-2927, DOI: 10.1016/S1671-2927(08)60150-9.

(<http://www.sciencedirect.com/science/article/B82XG-4THKV6G-8/2/8fd72bcd37f610d6a76480e82591504d>)

Abstract:

Nicotine is a secondary substance synthesized in tobacco roots. In flue-cured tobacco planting, tobacco decapitation is an effective practice to promote nicotine biosynthesis by regulation of the redistribution of total nitrogen amounts. However, proteins relevant to nicotine synthesis in tobacco roots has not been identified and characterized yet. It is important to explore the regulation of nicotine biosynthesis in tobacco roots. To identify the proteins relevant to nicotine synthesis, the protein patterns in roots of flue-cured tobacco (cv. K326) before and after decapitation were analyzed. In the present study, the protein patterns in roots of flue-cured tobacco were analyzed by two-dimensional electrophoresis (2-DE), and the differentially-expressed spots were identified by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). Paired comparison of 2-DE maps revealed 26 spots of differentially-expressed proteins in roots before and after decapitation. Furthermore, nine differentially-expressed spots were identified. There were four proteins which were enzymes possibly involved in nicotine biosynthesis. In addition, the roles of the four enzymes in nicotine biosynthesis were discussed in a putative network. Our results would contribute to the understanding of the regulation pathway of nicotine biosynthesis and further to the molecular manipulation on the nicotine contents in flue-cured tobacco.

Keywords: flue-cured tobacco; decapitation; roots; nicotine; differential proteomics

B.C. Qi, C. Aldrich, Biosorption of heavy metals from aqueous solutions with tobacco dust, *Bioresource Technology*, Volume 99, Issue 13, September 2008, Pages 5595-5601, ISSN 0960-8524, DOI: 10.1016/j.biortech.2007.10.042.

(<http://www.sciencedirect.com/science/article/B6V24-4RDB8TX-1/2/17299c7f341760066c6d4fe1d2cb1497>)

Abstract:

A typical lignocellulosic agricultural residue, namely tobacco dust, was investigated for its heavy metal binding efficiency. The tobacco dust exhibited a strong capacity for heavy metals, such as Pb(II), Cu(II), Cd(II), Zn(II) and Ni(II), with respective equilibrium loadings of 39.6, 36.0, 29.6, 25.1 and 24.5 mg of metal per g of sorbent. Moreover, the heavy metals loaded onto the biosorbent could be released easily with a dilute HCl solution. Zeta potential and surface acidity measurements showed that the tobacco dust was negatively charged over a wide pH range (pH > 2), with a strong surface acidity and a high OH⁻ adsorption capacity. Changes in the surface morphology of the tobacco dust as visualized by atomic force microscopy suggested that the sorption of heavy metal ions on the tobacco could be associated with changes in the surface properties of the dust particles. These surface changes appeared to have resulted from a loss of some of the structures on the surface of the particles, owing to leaching in the acid metal ion solution. However, Fourier transform infrared spectroscopy (FTIR) showed no substantial change in the chemical structure of the tobacco dust subjected to biosorption. The heavy metal uptake by the tobacco dust may be interpreted as metal-H ion exchange or metal ion surface complexation adsorption or both.

Keywords: Biosorption; Heavy metals; Lignocellulose; Tobacco

Radek Kana, Imre Vass, Thermoimaging as a tool for studying light-induced heating of leaves: Correlation of heat dissipation with the efficiency of photosystem II photochemistry and non-

photochemical quenching, *Environmental and Experimental Botany*, Volume 64, Issue 1, September 2008, Pages 90-96, ISSN 0098-8472, DOI: 10.1016/j.envexpbot.2008.02.006.

(<http://www.sciencedirect.com/science/article/B6T66-4S0YXP1-1/2/b8cfed30c41ef758d4b3a07be40516c6>)

Abstract:

Thermoimaging - a highly sensitive and non-invasive method of temperature measurement - was applied to explore the role of changing photosynthetic efficiency in light-induced heating of tobacco (*Nicotiana tabacum* cv. Samsun) leaves. In the absence of evaporative cooling through the stomata, which was achieved by covering leaves with Vaseline, illumination with 50-1400 [μ]M photons $m^{-2} s^{-1}$ intensity of photosynthetically active radiation resulted in [approximate]1-5 [$^{\circ}$]C leaf temperature increase in about 2 min. The heating effect showed a non-linear correlation with the extent of non-photochemical quenching (NPQ) resulting in higher leaf temperatures at higher NPQ values. When leaves were adapted to excessive irradiance (1300 [μ]M photons $m^{-2} s^{-1}$ for 6 h), which resulted in reduction of photosynthetic efficiency and amplification of NPQ the light-induced heating effect was enhanced. The experimental results have been explained on the basis of a simple theoretical model characterizing the balance of energy fluxes in leaves in relation to the efficiency of photosystem II photochemistry and non-photochemical quenching. The role of alternative energy dissipation pathways outside of PSII in the phenomenon of light-induced leaf heating is also discussed.

Keywords: Thermoimaging; NPQ; Photosystem II efficiency; Leaf temperature; Energy balance equation of leaf

Bassel Monzer, Elizabeth Sepetdjian, Najat Saliba, Alan Shihadeh, Charcoal emissions as a source of CO and carcinogenic PAH in mainstream narghile waterpipe smoke, *Food and Chemical Toxicology*, Volume 46, Issue 9, September 2008, Pages 2991-2995, ISSN 0278-6915, DOI: 10.1016/j.fct.2008.05.031.

(<http://www.sciencedirect.com/science/article/B6T6P-4SNNT9K-3/2/de42fbedd322e5f2eaf624ca231878b7>)

Abstract:

Burning charcoal is normally placed atop the tobacco to smoke the narghile waterpipe. We investigated the importance of charcoal as a toxicant source in the mainstream smoke, with particular attention to two well-known charcoal emissions: carbon monoxide (CO) and polyaromatic hydrocarbons (PAH). CO and PAH yields were compared when a waterpipe was machine smoked using charcoal and using an electrical heating element. The electrical heating element was designed to produce spatial and temporal temperature distributions similar to those measured using charcoal. With a popular type of ma'assel tobacco mixture, and using a smoking regimen consisting of 105 puffs of 530 ml volume spaced 17 s apart, it was found that approximately 90% of the CO and 75-92% of the 4- and 5-membered ring PAH compounds originated in the charcoal. Greater than 95% of the benzo(a)pyrene in the smoke was attributable to the charcoal. It was also found that the relative proportions of individual PAH species, the 'PAH fingerprint', of the mainstream smoke were highly correlated to those extracted from the unburned charcoal ($R^2 > 0.94$). In contrast, there was no correlation between the PAH fingerprint of the electrically heated and charcoal-heated conditions ($R^2 < 0.02$). In addition to inhaling toxicants transferred from the tobacco, such as nicotine, 'tar', and nitrosamines, waterpipe smokers thus also inhale large quantities of combustion-generated toxicants. This explains why, despite the generally low temperatures attained in the narghile tobacco, large quantities of CO and PAH have been found in the smoke.

Keywords: Hooka; Shisha; Tobacco smoke; Carbon monoxide; PAH; Charcoal

Wei Li, Libo Zhang, Jinhui Peng, Ning Li, Shimin Zhang, Shenghui Guo, Effects of microwave irradiation on the basic properties of woodceramics made from carbonized tobacco stems

impregnated with phenolic resin, *Industrial Crops and Products*, Volume 28, Issue 2, September 2008, Pages 143-154, ISSN 0926-6690, DOI: 10.1016/j.indcrop.2008.02.002.

(<http://www.sciencedirect.com/science/article/B6T77-4S9R1WS-1/2/cbd6ec6f455f70e08849d2d88a94e975>)

Abstract:

A series of woodceramics derived from carbonized tobacco stems and phenolic resin composite heated by microwave irradiation was prepared and effects of microwave irradiation time, power and mass fraction of phenolic resin in woodceramics on the mass loss ratio, volume shrinkage ratio, apparent density, open porosity and volume electrical resistivity were investigated systematically. The pyrolysis behaviours of tobacco stems, phenolic resin and carbonized tobacco stems/phenolic resin composite were also evaluated using thermogravimetry. The experimental results showed that the mass loss ratio, volume shrinkage ratio and apparent density increased, while the volume electrical resistivity and open porosity decreased with increasing microwave irradiation time. The apparent density increased, while the mass loss ratio, volume shrinkage ratio, volume electrical resistivity and open porosity decreased with an increase in the mass fraction of phenolic resin. The mass loss ratio, volume shrinkage ratio and open porosity increased, while apparent density and volume electrical resistivity decreased with an increase in the microwave power. Microstructures of woodceramics obtained at various microwave irradiation time were characterized by scanning electron microscopy (SEM) technique, which confirmed the results of pyrolysis analyses of samples and effects of microwave irradiation on basic properties of woodceramics prepared.

Keywords: Woodceramics; Microwave irradiation; Property; Microstructure; Tobacco stems

Junmo Cho, Sujin Park, Chunkeun Lim, Yong Chul Park, Jang Hyun Hur, Soonsung Hong, Thomas M. Brown, Saeyoull Cho, Kdr allelic variation in a sodium channel gene from a population of South Carolina *Heliothis virescens* (Fabricius), *Journal of Asia-Pacific Entomology*, Volume 11, Issue 3, September 2008, Pages 117-121, ISSN 1226-8615, DOI: 10.1016/j.aspen.2008.06.004.

(<http://www.sciencedirect.com/science/article/B8JJN-4SVV8SR-3/2/15527124e345b439aca302c41930f25c>)

Abstract:

Mutations at V421M and L1029H in the hscp sodium channel gene are known to contribute to knockdown resistance (kdr) in the Woodrow, Dalzell, and PTJ strains of *H. virescens* (tobacco budworm) from the cotton fields of South Carolina, USA. In the IS6 region of the sodium channel gene, the frequencies of the mutant allele methionine in the Woodrow and Dalzell strains were 0.07 and 0.1, respectively. For the IIS6 region, the frequencies of the mutant allele histidine in Woodrow and Dalzell strains were 0.175 and 0.263, respectively. In the PTJ strain, the frequencies of methionine and histidine alleles were 0 and 0.1, respectively. The Hpy3 allele, which is strongly linked to the histidine mutant allele, was also found in Woodrow and Dalzell strains. In addition, we found a new allele, which is one nucleotide different from Hpy3, called Hpy3-1, and found that it is also linked to histidine.

Keywords: *Heliothis virescens*; kdr; V461M; L1029H; Hpy; Pyrethroid

Ganapathi Sridevi, Chidambaram Parameswari, Natarajan Sabapathi, Vengoji Raghupathy, Karuppannan Veluthambi, Combined expression of chitinase and [beta]-1,3-glucanase genes in indica rice (*Oryza sativa* L.) enhances resistance against *Rhizoctonia solani*, *Plant Science*, Volume 175, Issue 3, September 2008, Pages 283-290, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2008.04.011.

(<http://www.sciencedirect.com/science/article/B6TBH-4SH6B77-1/2/d6d779ac8dbb5a8222a5a4c14cc0dcfa>)

Abstract:

Agrobacterium-mediated transformation of rice was done using the binary vector pNSP3, harbouring the rice chitinase (*chi11*) gene under maize ubiquitin promoter and the tobacco [β]-1,3-glucanase gene under CaMV 35S promoter in the same T-DNA. Four of the six T0 plants had single copies of complete T-DNAs, while the other two had complex integration patterns. Three of the four single-copy lines showed a 3:1 segregation ratio in the T1 generation. Northern and western blot analyses of T1 plants revealed constitutive expression of chitinase and [β]-1,3-glucanase genes. Homozygous T2 plants of the single-copy lines CG20, CG27 and CG53 showed 62-, 9.6- and 11-fold higher chitinase activity over the control plants. [β]-1,3-Glucanase activity was 1.1- to 2.5-fold higher in the transgenic plants. Bioassay of homozygous T2 plants of the three single-copy transgenic lines against *Rhizoctonia solani* revealed a 60% reduction in sheath blight Disease Index in the first week. The Disease Index increased from 61.8 in the first week to 90.6 in the third week in control plants, while it remained low (26.8-34.2) in the transgenic T3 plants in the corresponding period, reflecting the persistence of sheath blight resistance for a longer period.

Keywords: Agrobacterium; Chitinase; Fungal resistance; [β]-1,3-Glucanase; Sheath blight disease; Transgenic rice

Andres A. Estrada-Luna, Jose de Jesus Martinez-Hernandez, Maria Esthela Torres-Torres, Francisco Chable-Moreno, In vitro micropropagation of the ornamental prickly pear cactus *Opuntia lanigera* Salm-Dyck and effects of sprayed GA3 after transplantation to ex vitro conditions, *Scientia Horticulturae*, Volume 117, Issue 4, 18 August 2008, Pages 378-385, ISSN 0304-4238, DOI: 10.1016/j.scienta.2008.05.042.

(<http://www.sciencedirect.com/science/article/B6TC3-4T0X2ST-1/2/f29b39c04360712f792b98398037e2a3>)

Abstract:

We established the conditions to micropropagate the ornamental prickly pear cactus *Opuntia lanigera* Salm-Dyck through axillary shoot development from isolated areoles. For the shoot proliferation stage different explant orientation (vertical and horizontal), type of cytokinin (BA, DAP and K), and concentrations (0, 1.25, 2.5, 5.0 and 7.5 mg/L) were evaluated. Media [Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco cultures. *Phys. Plant.* 15, 473-497: 50 and 100%], and carbohydrate concentration (0.25, 0.5, 0.75 and 1.0%) were studied to optimize individual shoot growth and elongation. Following micropropagation and plantlet acclimatization, the effects of GA3 on plant growth were determined by spraying a series of increasing concentrations (0, 150, 300 and 450 ppm). A reliable and efficient protocol of micropropagation was established for this particular plant species. The greatest propagation ratio (shoot proliferation) was obtained when explants were cultured in vertical orientation (4.975 shoots per explant) as compared to horizontal position (3.692 shoots per explant). The addition of BA to the media resulted in increased shoot number per explant (8) in comparison to K and DA, which produced only 2 shoots in average. However, after 42 days of culture, significantly higher shoot length was obtained with DAP (14 mm) compared to K and BA (4 mm). After the shoot proliferation stage, an elongation subculture was performed prior rooting in which shoot growth was enhanced when crowns of shoots were cultured in 50% of basal salt formulation of Murashige and Skoog (1962) and low sucrose concentration (2.5 and 5%). Exogenous application of GA3 after plantlet acclimatization on glasshouse conditions increased spine-hair (developed from areoles in young plants) length as part of short-term effects. However, significantly higher values were obtained in plantlets treated with 300 ppm of GA3 when compared with the rest of the treatments. At the end of the study, the most important long-term effect produced by GA3 was the suppression of total shoot growth. The micropropagation protocol described here and the conditions to grow the plants through fertigation plus the application of GA3 that induced changes in the phenotype may be used in commercial exploitations to regenerate 12,500 plantlets in average after 12 months of culture and produce healthy plants with better ornamental characteristics and higher commercial value.

Keywords: In vitro propagation; Prickly pear cactus; Nopal; Plant growth regulators

Sanjib Kumar Panda, Yoko Yamamoto, Hideki Kondo, Hideaki Matsumoto, Mitochondrial alterations related to programmed cell death in tobacco cells under aluminium stress, *Comptes Rendus Biologies*, Volume 331, Issue 8, August 2008, Pages 597-610, ISSN 1631-0691, DOI: 10.1016/j.crv.2008.04.008.

(<http://www.sciencedirect.com/science/article/B6X1F-4SN92JM-1/2/05369fbbf840caa353e0c35dda652edd>)

Abstract:

The present investigation was undertaken to verify whether mitochondria play a significant role in aluminium (Al) toxicity, using the mitochondria isolated from tobacco cells (*Nicotiana tabacum*, non-chlorophyll cell line SL) under Al stress. An inhibition of respiration was observed in terms of state-III, state-IV, succinate-dependent, alternative oxidase (AOX)-pathway capacity and cytochrome (CYT)-pathway capacity, respectively, in the mitochondria isolated from tobacco cells subjected to Al stress for 18 h. In accordance with the respiratory inhibition, the mitochondrial ATP content showed a significant decrease under Al treatment. An enhancement of reactive oxygen species (ROS) production under state-III respiration was observed in the mitochondria isolated from Al-treated cells, which would create an oxidative stress situation. The opening of mitochondrial permeability transition pore (MPTP) was seen more extensively in mitochondria isolated from Al-treated cells than in those isolated from control cells. This was Ca²⁺ dependent and well modulated by dithioerythritol (DTE) and Pi, but insensitive to cyclosporine A (CsA). The collapse of inner mitochondrial membrane potential ($[\Delta][\Psi]_m$) was also observed with a release of cytochrome c from mitochondria. A great decrease in the ATP content was also seen under Al stress. Transmission electron microscopy analysis of Al-treated cells also corroborated our biochemical data with distortion in membrane architecture in mitochondria. TUNEL-positive nuclei in Al-treated cells strongly indicated the occurrence of nuclear fragmentation. From the above study, it was concluded that Al toxicity affects severely the mitochondrial respiratory functions and alters the redox status studied in vitro and also the internal structure, which seems to cause finally cell death in tobacco cells. To cite this article: S.K. Panda et al., *C. R. Biologies* 331 (2008).

Keywords: Aluminium; Mitochondria; *Nicotiana tabacum*; Programmed cell death

Gunnar Broehan, Michael Kemper, Daniel Driemeier, Inga Vogelpohl, Hans Merzendorfer, Cloning and expression analysis of midgut chymotrypsin-like proteinases in the tobacco hornworm, *Journal of Insect Physiology*, Volume 54, Issue 8, August 2008, Pages 1243-1252, ISSN 0022-1910, DOI: 10.1016/j.jinsphys.2008.06.007.

(<http://www.sciencedirect.com/science/article/B6T3F-4SVK SXN-1/2/fb8f94430882110fb6ab9c6518a20dce>)

Abstract:

Digestion of proteins in the midgut of lepidopteran larvae relies on different trypsin and chymotrypsin isoforms. In this study we describe three chymotrypsin-like proteinases (CTLP2-4) from the larval midgut of *Manduca sexta*, which are closely related to CTLP1 and less closely related to another chymotrypsin (CT), two previously described proteinases present in the larval midgut of *M. sexta*. CTLP1-4 fit perfectly into a novel subgroup of insect CTLPs by sequence similarity and by the replacement of GP by SA in the highly conserved GDSGGP motif. When we examined CTLP expression in different tissues, most of the proteinases were predominantly expressed in the anterior and median midgut, while some were found in the Malpighian tubules. When we examined CTLP expression at different physiological states, we observed that the CTLP mRNA amounts did not differ considerably in feeding and starving larvae except for CTLP2, whose mRNA dropped significantly upon starvation. During moulting, however, the mRNA amounts of all CTLPs dropped significantly. When we immunologically examined CTLP amounts, mature

proteinases were only detectable in the gut lumen of feeding and re-fed larvae, but not in that of starving or moulting larvae, suggesting that CTLP secretion is suspended during starvation or moult.

Keywords: Chymotrypsin-like proteinase; Serine proteinase; Midgut; *Manduca sexta*

Kumaresan Kavitha, Gayatri Venkataraman, Ajay Parida, An oxidative and salinity stress induced peroxisomal ascorbate peroxidase from *Avicennia marina*: Molecular and functional characterization, *Plant Physiology and Biochemistry*, Volume 46, Issues 8-9, August-September 2008, Pages 794-804, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2008.05.008.

(<http://www.sciencedirect.com/science/article/B6VVRD-4SM62CN-2/2/92479afa490f04ad4e2d2977bff34192>)

Abstract:

APX (EC, 1.11.1.11) has a key role in scavenging ROS and in protecting cells against their toxic effects in algae and higher plants. A cDNA encoding a peroxisomal ascorbate peroxidase, Am-pAPX1, was isolated from salt stressed leaves of *Avicennia marina* (Forsk.) Vierh. by EST library screening and its expression in the context of various environmental stresses was investigated. Am-pAPX1 contains an ORF of 286 amino acids coding for a 31.4 kDa protein. The C-terminal region of the Am-pAPX1 ORF has a putative transmembrane domain and a peroxisomal targeting signal (RKKMK), suggesting peroxisomal localization. The peroxisomal localization of Am-pAPX1 was confirmed by stable transformation of the GFP-(Ala)₁₀-Am-pAPX1 fusion in tobacco. RNA blot analysis revealed that Am-pAPX1 is expressed in response to salinity (NaCl) and oxidative stress (high intensity light, hydrogen peroxide application and excess iron). The isolated genomic clone of Am-pAPX1 was found to contain nine exons. A fragment of 1616 bp corresponding to the 5' upstream region of Am-pAPX1 was isolated by TAIL-PCR. In silico analysis of this sequence reveals the presence of putative light and abiotic stress regulatory elements.

Keywords: Ascorbate peroxidase; *Avicennia marina*; Green fluorescent protein; Hydrogen peroxide; Peroxisome; Subcellular localization

Yu-Wei ZHAO, Jian-Guo HAO, Huai-Yu BU, Ying-Juan WANG, Jing-Fen JIA, Cloning of HvBADH1 Gene from Hulless Barley and Its Transformation to Tobacco, *Acta Agronomica Sinica*, Volume 34, Issue 7, July 2008, Pages 1153-1159, ISSN 1875-2780, DOI: 10.1016/S1875-2780(08)60041-3.

(<http://www.sciencedirect.com/science/article/B94TW-4TS6MWT-3/2/31e8d58c3f95609a17069214d34b7201>)

Abstract:

To reveal the relationship between the expression of betaine aldehyde dehydrogenase (BADH) and the tolerance to salt stress resistance in hulless barley (*Hordeum vulgare* L. var. *nudum* Hook. f), a 1,512 bp cDNA encoding BADH was cloned from hulless barley using the methods of reverse transcription PCR (RT-PCR) and rapid application of cDNA ends (RACE). The cDNA, designated HvBADH1 under the accession number EF492983 in GenBank, encodes a 54.2 kD protein with 232 amino acid residues. Gene HvBADH1 exhibited a homology (98.4%) in amino acid sequence with BBD2 gene encoding an isoenzyme of BADH from barley. It also shared a high homology of 97.0%, 84.7%, and 85.1% with BADH in wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and rice (*Oryza sativa* L.), respectively. Gene HvBADH1 was inserted into pMAL c2x, and the recombinant plasmid was then transferred into *Escherichia coli* TB1 cells. The recombinant TB1 harboring pMAL c2x-HvBADH1 and the control TB1 harboring empty pMAL c2x cells were induced with IPTG. The results revealed that the recombinant *E. coli* cells expressed a fusion protein with molecular weight of 96.3 kD. This fusion protein was fused by maltose binding protein (MBP, about 42.1 kD) and the peptide (about 54.2 kD) encoded by HvBADH1. The open reading frame (ORF) of HvBADH1 was inserted between CaMV35S promoter and NOS polyA in T-DNA region of the binary expression vector pCAMBIA1301. The recombinant plasmid, designated pCAM-ba, was

transferred into *Agrobacterium tumefaciens* strain LBA4404. The HvBADH1 gene was transformed to tobacco (*Nicotiana tabacum* L.) mediated by *Agrobacterium*. Two hygromycin B (Hyg)-resistant plants were obtained. PCR detection and Southern blot analysis indicated that all the Hyg-resistant tobacco plants contained the alien BADH gene. RT-PCR analysis showed that HvBADH1 gene was expressed on the mRNA level in the transgenic tobacco plants. This suggested that HvBADH1 gene was related to the salt tolerance in hullless barley, which can be expressed in transgenic plants.

Keywords: *Hordeum vulgare* L. var. nudum Hook. f; gene clone; genetic transformation; salt-tolerant resistance; RT-PCR; RACE

Wei Li, Jinhui Peng, Libo Zhang, Hongying Xia, Ning Li, Kunbin Yang, Xueyun Zhu, Investigations on carbonization processes of plain tobacco stems and H₃PO₄-impregnated tobacco stems used for the preparation of activated carbons with H₃PO₄ activation, *Industrial Crops and Products*, Volume 28, Issue 1, July 2008, Pages 73-80, ISSN 0926-6690, DOI: 10.1016/j.indcrop.2008.01.006.

(<http://www.sciencedirect.com/science/article/B6T77-4S32DFW-1/2/a71c5db5986dd70fb39fbc2882833297>)

Abstract:

In order to elucidate the mechanism of carbonization process of tobacco stems-based activated carbons with H₃PO₄-activation, the pyrolysis characteristics of plain tobacco stems and H₃PO₄-impregnated tobacco stems were investigated through thermogravimetry (TG) at different heating rates (5, 15, 30 K/min) under nitrogen atmosphere. Based on TG curves, the kinetic parameters of the processes, including pre-exponential factors and activation energies, were calculated using the method of Coats-Redfern. The pyrolytic kinetic models of plain tobacco stems and H₃PO₄-impregnated tobacco stems were set up. The main pyrolysis stage could be described by the first-order and 2.5-order global models for plain tobacco stems and H₃PO₄-impregnated tobacco stems, respectively. The results indicated that the activation energy of carbonization process of H₃PO₄-impregnated tobacco stems was lower about 32.67-38.71 kJ mol⁻¹ than that of plain tobacco stems; furthermore, the kinetic parameters exhibited kinetic compensation effects.

Keywords: Tobacco stems; H₃PO₄; Carbonization; Pyrolysis; Kinetics; Kinetic compensation effect

J.O. Silva-Werneck, D.J. Ellar, Characterization of a novel Cry9Bb [δ]-endotoxin from *Bacillus thuringiensis*, *Journal of Invertebrate Pathology*, Volume 98, Issue 3, Special Issue for SIP 2008, SIP 2008, July 2008, Pages 320-328, ISSN 0022-2011, DOI: 10.1016/j.jip.2008.03.012.

(<http://www.sciencedirect.com/science/article/B6WJV-4S5FJKP-1/2/291e9284a5e3105cab494dfc20454900>)

Abstract:

The Brazilian *Bacillus thuringiensis* serovar japonensis strain S725 was selected for its toxicity to the velvetbean caterpillar, *Anticarsia gemmatalis*. This strain produces spherical crystals harbouring a major protein of about 130 kDa which yields fragments of between 50 and 70 kDa upon trypsin activation. The protein showed a high level of identity and immunoaffinity to the Cry9 class of [δ]-endotoxins. The cloned cry9-like gene sequence contains a 3492 bp ORF, which encodes a polypeptide of 1163 amino acids, with a predicted molecular mass of 131.4 kDa. The deduced amino acid sequence is unique and shows 73% identity to Cry9Ba, 64% identity to Cry9Ea, 63% identity to Cry9Da, and 59% identity to Cry9Ca proteins. The novel [δ]-endotoxin was assigned to a new subclass, Cry9Bb, by the Bt Toxin Nomenclature Committee. The Cry9Bb protein was expressed in an acrySTALLIFEROUS Bt strain, and exhibited activity against the tobacco hornworm, *Manduca sexta*, and the velvetbean caterpillar, *A. gemmatalis*. The biological effect of an amino acid residue change, A84P, was investigated. The LC₅₀ for the Cry9Bb crystals against *M. sexta* neonate larvae was 6.84 [μ g]/cm², while the LC₅₀ for the mutant's Cry9Bb crystals was

0.78 [mu]g/cm². PCR screening revealed that in addition to cry9Bb, Bt strain S725 also contains cry1I and vip3 genes. Transcription analysis, using RT-PCR, showed that the cry1I gene was transcribed at T2 and T5 stages of sporulation.

Keywords: *Bacillus thuringiensis*; Characterization; Cry toxins; cry9 gene; *Manduca sexta*; *Anticarsia gemmatalis*; Biological control

Md. Mijan Hossain, Chiharu Tani, Tomoko Suzuki, Fumiko Taguchi, Tatsuhiko Ezawa, Yuki Ichinose, Polyphosphate kinase is essential for swarming motility, tolerance to environmental stresses, and virulence in *Pseudomonas syringae* pv. *tabaci* 6605, *Physiological and Molecular Plant Pathology*, Volume 72, Issues 4-6, July-September 2008, Pages 122-127, ISSN 0885-5765, DOI: 10.1016/j.pmpp.2008.04.007.

(<http://www.sciencedirect.com/science/article/B6WPC-4SWWT3T-1/2/22f57f8a574e6d85faf614362b157991>)

Abstract:

Polyphosphate kinase (PPK), encoded by the *ppk* gene, is a principal enzyme responsible for synthesis of inorganic polyphosphate (poly P) from ATP in many Gram-negative bacteria. In order to elucidate the functions of poly P in *Pseudomonas syringae* pv. *tabaci* 6605, an in-frame deletion mutant of the *ppk* gene ([up triangle, open]ppk) was constructed. The [up triangle, open]ppk mutant did not accumulate poly P, whereas the wild-type strain accumulated a large quantity. The mutant had reduced swarming motility, even though it retains swimming motility like the parental strain. The mutant exhibited increased sensitivity to prolonged incubation and environmental stresses, such as heat shock and oxidative stress and reduced exopolysaccharide (EPS) production compared to the wild-type. Northern blot analysis revealed that expression of the *rpoS* gene, encoding the stationary phase sigma factor RpoS, was reduced in [up triangle, open]ppk in the logarithmic phase, indicating that *rpoS* is regulated by the *ppk* gene. The poly P deficient mutant had significantly reduced ability to cause disease in its host tobacco plant and in planta growth of the mutant was also significantly reduced in host tobacco leaves as compared to the wild-type strain. Thus, our results suggest that poly P plays an important role in the virulence of *P. syringae* pv. *tabaci* 6605.

Keywords: Environmental stress; Polyphosphate kinase; *ppk*; *Pseudomonas syringae* pv. *tabaci* 6605; Tolerance

Suvi T. Hakkinen, Sofie Tilleman, Agnieszka Swiatek, Valerie De Sutter, Heiko Rischer, Isabelle Vanhoutte, Harry Van Onckelen, Pierre Hilson, Dirk Inze, Kirsi-Marja Oksman-Caldentey, Alain Goossens, Erratum to 'Functional characterisation of genes involved in pyridine alkaloid biosynthesis in tobacco' [*Phytochemistry* 68 (2007) 2773-2785], *Phytochemistry*, Volume 69, Issue 10, July 2008, Page 2095, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2008.05.001.

(<http://www.sciencedirect.com/science/article/B6TH7-4SMGCC2-3/2/f591b970f3fdd604ca03842fc6a18f08>)

Natalia Loukanina, Claudio Stasolla, Mark F. Belmonte, Edward C. Yeung, Trevor A. Thorpe, Changes in the de novo, salvage, and degradation pathways of pyrimidine nucleotides during tobacco shoot organogenesis, *Plant Physiology and Biochemistry*, Volume 46, Issue 7, July 2008, Pages 665-672, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2007.10.017.

(<http://www.sciencedirect.com/science/article/B6VRD-4R0CK2W-1/2/984b085a112dad36f5d1ee07021d288f>)

Abstract:

Pyrimidine nucleotide metabolism was studied in tobacco callus cultured for 21 days under shoot-forming (SF) and non-shoot-forming (NSF) conditions by following the metabolic fate of orotic acid, a precursor of the de novo pathway, and uridine and uracil, intermediates of the salvage and degradation pathways respectively. Nucleic acid synthesis was also investigated by measuring the

incorporation of labeled thymidine into different cellular components. Our results indicate that with respect to nucleotide metabolism, the organogenic process in tobacco can be divided in two 'metabolic phases': a de novo phase followed by a salvage phase. The initial stages of meristemoid formation during tobacco organogenesis (up to day 8) are characterized by a heavy utilization of orotic acid into nucleotides and nucleic acids. Utilization of this intermediate for the de novo synthesis of nucleotides, which is limited in NSF tissue, is mainly due to the activity of orotate phosphoribosyltransferase (OPRT), which increases in tissue cultured under SF conditions. After day 8, nucleotide synthesis during shoot growth seems to be mainly due to the salvage activity of both uridine and uracil. Both intermediates are preferentially utilized in SF tissue for the formation of nucleotides and nucleic acids through the activities of their respective salvage enzymes: uridine kinase (URK), and uracil phosphoribosyltransferase (UPRT). Metabolic studies on thymidine indicate that in SF tissue maximal nucleic acid synthesis occurs at day 4, in support of the initiation of meristemoid formation. Overall these results suggest that the organogenic process in tobacco is underlined by precise fluctuations in pyrimidine metabolism which delineate structural events culminating in shoot formation.

Keywords: *Nicotiana tabacum*; De novo biosynthesis; Nucleic acid synthesis; Pyrimidine nucleotide metabolism; Salvage biosynthesis; Shoot formation; Tobacco callus

Petra Suchomelova-Maskova, Ondrej Novak, Helena Lipavska, Tobacco cells transformed with the fission yeast *Spcdc25* mitotic inducer display growth and morphological characteristics as well as starch and sugar status evocable by cytokinin application, *Plant Physiology and Biochemistry*, Volume 46, Issue 7, July 2008, Pages 673-684, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2008.04.017.

(<http://www.sciencedirect.com/science/article/B6VRD-4SD29M7-1/2/8d36e4895602821d671bd9ec79c79c73d>)

Abstract:

In plants, the G2/M control of cell cycle remains an elusive issue as doubts persist about activatory dephosphorylation--in other eukaryotes provided by CDC25 phosphatase and serving as a final all-or-nothing mitosis regulator. We report on the effects of tobacco (*Nicotiana tabacum* L., cv. Samsun) transformation with fission yeast (*Schizosaccharomyces pombe*) *cdc25* (*Spcdc25*) on cell characteristics. Transformed cell suspension cultures showed higher dry mass accumulation during the exponential phase and clustered more circular cell phenotypes compared to chains of elongated WT cells. Similar cell parameters, as in the transformants, can be induced in WT by cytokinins. *Spcdc25* cells, after cytokinin treatment, showed giant cell clusters and growth inhibition. In addition, *Spcdc25* expression led to altered carbohydrate status: increased starch and soluble sugars with higher sucrose:hexoses ratio, inducible in WT by cytokinin treatment. Taken together, the *Spcdc25* transformation had a cytokinin-like effect on studied characteristics. However, endogenous cytokinin determination revealed markedly lower cytokinin levels in *Spcdc25* transformants. This indicates that the cells sense *Spcdc25* expression as an increased cytokinin availability, manifested by changed cell morphology, and in consequence decrease endogenous cytokinin levels. Clearly, the results on cell growth and morphology are consistent with the model of G2/M control including cytokinin-regulated activatory dephosphorylation. Nevertheless, no clear link is obvious between *Spcdc25* transformation and carbohydrate status and thus the observed cytokinin-like effect on carbohydrate levels poses a problem. Hence, we propose that *Spcdc25*-induced higher CDK(s) activity at G2/M generates a signal-modifying carbohydrate metabolism to meet high energy and C demands of forthcoming cell division.

Keywords: Carbohydrate status; *cdc25*; Cell cycle; Cell morphology; Cytokinin; *Nicotiana tabacum*; *Schizosaccharomyces pombe*

Cigdem Alev Ozel, Khalid Mahmood Khawar, Orhan Arslan, A comparison of the gelling of isubgol, agar and gelrite on in vitro shoot regeneration and rooting of variety Samsun of tobacco (*Nicotiana*

tabacum L.), *Scientia Horticulturae*, Volume 117, Issue 2, 26 June 2008, Pages 174-181, ISSN 0304-4238, DOI: 10.1016/j.scienta.2008.03.022.

(<http://www.sciencedirect.com/science/article/B6TC3-4SH1J2R-1/2/52d0711a3e206b9d7a844a36bc16fbed>)

Abstract:

Agar is being used to solidify media for plant tissue culture since long. Both purity and type of agar or gelling agent influence the behaviour and growth of tissues in culture. The study using leaf disc explants of variety Samsun of tobacco compares adventitious shoot regeneration on 0.5 cm x 0.5 cm leaf discs cultured on MSD4X2 medium and rooting on MSO medium gelled with different blends of agar-isubgol, gelrite-isubgol, phytigel-isubgol or isubgol singly. It was found that irrespective of some problems associated with isubgol gel, the maximum number of shoot per explant were recorded on MSD4X2 medium gelled with 7 g/l isubgol. The longest shoots were recorded on MSD4X2 medium gelled with 9 g/l isubgol. Likewise, the highest number of roots were also recorded on MSO medium gelled with 7 g/l isubgol. For a given quantity of a medium, agar, gelrite/isubgol blends are very cheap compared to agar used singly. Moreover, blends of agar/isubgol, gelrite/isubgol gelled at low temperatures indicated safe use of these gels for heat labile substances in genetic transformation or tissue culture experiments. The results emphasized the potential of the isubgol used singly or in combination with agar and gelrite for economic commercial application, replacing the costliest, though not indispensable, gelling agent agar.

Keywords: Gelling agents; Isubgol; Mass propagation; Plantlet regeneration; Rooting

W. Schiettecatte, L. D'hondt, W.M. Cornelis, M.L. Acosta, Z. Leal, N. Lauwers, Y. Almoza, G.R. Alonso, J. Diaz, M. Ruiz, D. Gabriels, Influence of landuse on soil erosion risk in the Cuyaguaje watershed (Cuba), *CATENA*, Volume 74, Issue 1, 15 June 2008, Pages 1-12, ISSN 0341-8162, DOI: 10.1016/j.catena.2007.12.003.

(<http://www.sciencedirect.com/science/article/B6VCG-4RWK0B9-1/2/faef1d91cd4532b26a83e81527da8c21>)

Abstract:

Landuse changes may dramatically enhance erosion risk. Besides deforestation, also arable landuse may have an important influence on soil loss. We investigated the erosion risk in a 151 km² subwatershed of the Cuyaguaje watershed (Cuba) using the RUSLE model. It was found that the valleys used for agriculture have the highest erosion risk, with actual erosion surpassing soil loss tolerance. Over the period 1985-2000, about 14 km² of forest has been converted into arable land. As a result, the area with a very high erosion risk increased with 12%. On arable land it was found that the crop management factor C of a 'tobacco/maize' rotation was 0.478, compared to 0.245 for a rotation of various crops (sweet potato, beans, maize, cassava and fallow). When maize in the 'tobacco/maize' rotation was intercropped with a leguminous crop (hyacinth bean) the C factor decreased to a value of 0.369. Also contouring may halve soil loss on moderate slopes (< 10%) when high ridges are applied, which is in Cuba generally the case for maize, cassava and sweet potato.

Keywords: RUSLE; C factor; Soil loss; Erosivity

Yan LU, Pei LIU, Zhao-Shi XU, Rui-Yue ZHANG, Li LIU, Lian-Cheng LI, Ming CHEN, Xing-Guo YE, Yao-Feng CHEN, You-Zhi MA, Overexpression of W6 Gene Increases Salt Tolerance in Transgenic Tobacco Plants, *Acta Agronomica Sinica*, Volume 34, Issue 6, June 2008, Pages 984-990, ISSN 1875-2780, DOI: 10.1016/S1875-2780(08)60037-1.

(<http://www.sciencedirect.com/science/article/B94TW-4TJT5JS-6/2/1a4a9582040e976a28c0df345d19a724>)

Abstract:

An ethylene responsive factor (ERF) gene W6, which belongs to ERF IV subfamily, was isolated from the cDNA library of wheat (*Triticum aestivum* L.) landrace Xiaobaimai. The full-length cDNA

of W6 encoded an ERF protein containing a conserved ERF DNA-binding motif, 2 putative nuclear localization sequences, and a C-terminal acidic transcription activation domain. The expression of W6 in wheat was induced by various treatments, such as cold, drought, salt, abscisic acid (ABA), and fungal pathogens. To identify the function of the W6 gene, a transgenic vector (35S::pBI121::W6) containing CaMV 35S promoter was constructed and the W6-overexpressed tobacco (*Nicotiana tabacum*) plants by *Agrobacterium*-mediated transformation was obtained. Under the treatment with 200 mmol L⁻¹ NaCl for 50 d, the transgenic plants grew well but the control plants almost died. The root length of the transgenic tobacco plants ranged from 1.40 to 3.93 cm but that of control was only 0.20 cm. The root weight of the transgenic tobacco plants ranged from 2.41 to 7.79 g compared with the control of 0.06 g. The superoxide dismutase activity and chlorophyll content in the transgenic plants were obviously higher than those of the control. The results showed that the overexpression of W6 improved the salt tolerance of tobacco, and W6 probably acted as a connector among different signal transduction pathways. The overexpression of W6 activated the expression of GCC box-containing genes PR2, PR3, PR5, and drought-responsive element/C-repeat (DRE/CRT) genes (NtERD10A and NtERD10C) under normal growth conditions. This improvement of the transgenic tobacco plants to salt stress suggested that W6 regulates osmotic tolerance by activation of downstream gene expression through interaction with the GCC box or DRE elements.

Keywords: ethylene responsive factor (ERF); transgenic plant; salt tolerance; tobacco

Yun-xiang ZHAO, Pei LIU, Zhao-shi XU, Ming CHEN, Lian-cheng LI, Yao-feng CHEN, Xiang-jin XIONG, You-zhi MA, Analysis of Specific Binding and Subcellular Localization of Wheat ERF Transcription Factor W17, *Agricultural Sciences in China*, Volume 7, Issue 6, June 2008, Pages 647-655, ISSN 1671-2927, DOI: 10.1016/S1671-2927(08)60098-X.

(<http://www.sciencedirect.com/science/article/B82XG-4SWFK1G-2/2/8812f7cd58b83e53098e132bb0e3d66d>)

Abstract:

The study aims to detect the subcellular localization of ERF (ethylene-responsive element binding factor) transcription factor W17 protein, the interaction between W17 and cis-acting regulatory elements GCC-box and DRE in vitro, the binding and transactivating ability in vivo, and the role of W17 in higher plant stress-signal pathway. Recombinant plasmid W17/163hGFP was introduced into onion epidermal cells by the particle bombardment method with a PDS1000/He. Transformed cells were incubated for 24 h at 22[degree sign]C in the dark and green fluorescence was monitored under a confocal microscope. The gene W17 was fused N-terminus of GST (glutathione-S-transferase) in prokaryotic expression vector pGEX-4T-1 and then transformed into *E. coli* strain BL21 (DE3). IPTG (0.5 mmol L⁻¹) was added to induce the expression of recombinant GST/W17 for 3 h. The fused proteins were purified by GST purification columns, and then subjected to gel retardation assay with a 32P-labeled GCC or DRE sequence. The different reporter and effector plasmids were introduced into tobacco leaves through agroinfiltration, then transformed leaves stained by X-Gluc, faded with 75% alcohol and monitored under a Stereozooming microscope. The GFP fused with W17 protein was localized in the nuclei; SDS-PAGE assay demonstrated that the fused protein GST/W17 could be induced and purified with molecular weight at around 42.2 kD under the induction of IPTG. Purified fused protein was able to specifically bind to both the wild-type GCC-box and DRE element, but had no interaction with either the mutant DRE or GCC-box; W17 protein can bind to GCC-box and transactive downstream GUS gene in vivo. W17 can localize into the nuclei, and it may be involved not only in biotic stresses controlled by GCC-box, but also in abiotic stresses (e.g., salt-) induced signaling pathway.

Keywords: ERF/AP2 domain; ERF; DRE element; GCC-box; subcellular localization

Jeffrey I. Seeman, Richard A. Carchman, The possible role of ammonia toxicity on the exposure, deposition, retention, and the bioavailability of nicotine during smoking, *Food and Chemical Toxicology*, Volume 46, Issue 6, June 2008, Pages 1863-1881, ISSN 0278-6915, DOI: 10.1016/j.fct.2008.02.021.

(<http://www.sciencedirect.com/science/article/B6T6P-4S0209T-3/2/1722fe5b51c9eff22a689c1f5f98e06f>)

Abstract:

A complete and rigorous review is presented of the possible effect(s) of ammonia on the exposure, deposition and retention of nicotine during smoking and the bioavailability of nicotine to the smoker. There are no toxicological data in humans regarding ammonia exposure within the context of tobacco smoke. Extrapolation from occupational exposure of ammonia to smoking in humans suggests minimal, non-toxicological effects, if any. No direct study has examined the effect of the ammonia on the total rate or amount of nicotine reaching the arterial bloodstream or brains of smokers. Machine-smoking methods have been reported which accurately quantify >99% of the nicotine in mainstream (MS) smoke for a wide variety of commercial and test cigarettes, including a series of experimental cigarettes having a range in MS smoke ammonia yields using the US Federal Trade Commission (FTC) protocol. However, the actual exposure of nicotine to smokers depends on their own smoking behavior. The nicotine ring system is relatively thermally stable. Protonated nicotine forms nicotine which evaporates before the nicotine ring system decomposes. The experimental data indicate that neither nicotine transfer from tobacco to MS smoke nor nicotine bioavailability to the smoker increases with an increase in any of the following properties: tobacco soluble ammonia, MS smoke ammonia, 'tobacco pH' or 'smoke pH' at levels found in commercial cigarettes. Gas phase nicotine deposits primarily in the mouth and upper respiratory tract. To the extent that ammonia increases the deposition of nicotine in the buccal cavity and upper respiratory tract during smoking, the total rate and amount of nicotine into the arterial bloodstream and to the central nervous system will decrease. Charged nicotine analogues are actively transported in a number of tissues. This active transport system appears to be insensitive to pH and the form of nicotine in the biological milieu, suggesting that protonated nicotine may be a substrate for active transport. Neither 'smoke pH' of commercial cigarettes nor 'smoke pHeff' nor the fraction of non-protonated nicotine in tobacco smoke particulate matter are useful, practical smoke parameters for providing understanding or predictability of nicotine bioavailability to smokers. Greater than 95% of both ammonia and nicotine are in the gas phase of environmental tobacco, and both are likely to deposit in the buccal cavity and upper respiratory tract following exposure.

Keywords: Tobacco smoke; Aerosol; Nicotine; Ammonia; Smoking; pH; Active transport; Passive transport

Zhen Zou, Fares Najjar, Yang Wang, Bruce Roe, Haobo Jiang, Pyrosequence analysis of expressed sequence tags for *Manduca sexta* hemolymph proteins involved in immune responses, *Insect Biochemistry and Molecular Biology*, Volume 38, Issue 6, June 2008, Pages 677-682, ISSN 0965-1748, DOI: 10.1016/j.ibmb.2008.03.009.

(<http://www.sciencedirect.com/science/article/B6T79-4S5FJB6-2/2/4f604b889fcec757a57e9ad7b68bf1b0>)

Abstract:

The tobacco hornworm *Manduca sexta* is widely used as a model organism to investigate the biochemical basis of insect physiological processes but little transcriptome information is available. To get a broad view of the larval hemolymph proteins, particularly those related to immunity, we synthesized and sequenced cDNA fragments from a mixture of eight total RNA samples: fat body and hemocytes from larvae injected with killed bacteria, fat body, hemocytes, integument and trachea from naive larvae, and fat body and hemocytes from wandering larvae. Using massively parallel pyrosequencing, we obtained 95,458 *M. sexta* expressed sequence tags (ESTs) at an

average size of 185 bp per read. A majority of the sequences (69,429 reads) could be assembled into 7231 contigs with an average size of 300 bp, 1178 of which had significant similarity with *Drosophila* genes from various functional groups. Only ~8% (606) of the contigs matched known *M. sexta* cDNA sequences, representing 186 of the 375 unique NCBI entries. The remaining 6625 contigs represented newly discovered cDNA segments from this well studied biochemical model insect. A search of the 7231 contigs using *Tribolium castaneum*, *Drosophila melanogaster*, and *Bombyx mori* immunity-related sequences revealed 424 cDNA contigs with significant similarity (E-value <1x10⁻⁵). These included 218 previously unknown *M. sexta* sequences coding for putative defense molecules such as pattern recognition receptors, serine proteinases, serpins, Spatzle, Toll-like receptors, intracellular signaling molecules, and antimicrobial peptides.

Keywords: Insect immunity; Hemolymph proteins; Gene discovery; Transcript profiling; 454 sequencing

Irene Stenzel, Bettina Hause, Reinhard Proels, Otto Miersch, Mariko Oka, Thomas Roitsch, Claus Wasternack, The AOC promoter of tomato is regulated by developmental and environmental stimuli, *Phytochemistry*, Volume 69, Issue 9, June 2008, Pages 1859-1869, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2008.03.007.

(<http://www.sciencedirect.com/science/article/B6TH7-4SCN7T1-1/2/0b90b176caca27880e00de988d6c0a03>)

Abstract:

The allene oxide cyclase (AOC) catalyzes the formation of cis-(+)-12-oxophytodienoic acid, an intermediate in jasmonate biosynthesis and is encoded by a single copy gene in tomato. The full length AOC promoter isolated by genome walk contains 3600 bp. Transgenic tomato lines carrying a 1000 bp promoter fragment and the full length promoter, respectively, in front of the [β]-glucuronidase (GUS)-encoding uidA gene and several tobacco lines carrying the full length tomato AOC promoter before GUS were used to record organ- and tissue-specific promoter activities during development and in response to various stimuli. High promoter activities corresponding to immunocytochemically detected occurrence of the AOC protein were found in seeds and young seedlings and were confined to the root tip, hypocotyl and cotyledons of 3-d-old seedlings. In 10-d-old seedlings promoter activity appeared preferentially in the elongation zone. Fully developed tomato leaves were free of AOC promoter activity, but showed high activity upon wounding locally and systemically or upon treatment with JA, systemin or glucose. Tomato flowers showed high AOC promoter activities in ovules, sepals, anthers and pollen. Most of the promoter activity patterns found in tomato with the 1000 bp promoter fragment were also detected with the full length tomato AOC promoter in tobacco during development or in response to various stimuli. The data support a spatial and temporal regulation of JA biosynthesis during development and in response to environmental stimuli.

Keywords: Tomato (*Solanum lycopersicum*); Tobacco (*Nicotiana tabacum*); Allene oxide cyclase promoter; GUS activity; Expression analysis; Developmental and environmental regulation

Md. Anamul Hoque, Mst. Nasrin Akhter Banu, Yoshimasa Nakamura, Yasuaki Shimoishi, Yoshiyuki Murata, Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells, *Journal of Plant Physiology*, Volume 165, Issue 8, 26 May 2008, Pages 813-824, ISSN 0176-1617, DOI: 10.1016/j.jplph.2007.07.013.

(<http://www.sciencedirect.com/science/article/B7GJ7-4R008BR-1/2/87f934c9ec3ad8fd20aa4999461eadc7>)

Abstract: Summary

Salt stress impairs reactive oxygen species (ROS) and methylglyoxal (MG) detoxification systems, and causes oxidative damage to plants. Up-regulation of the antioxidant and glyoxalase systems provides protection against NaCl-induced oxidative damage in plants. Thiol-disulfide contents,

glutathione content and its associated enzyme activities involved in the antioxidant defense and glyoxalase systems, and protein carbonylation in tobacco Bright Yellow-2 cells grown in suspension culture were investigated to assess the protection offered by proline and glycinebetaine against salt stress. Salt stress increased protein carbonylation, contents of thiol, disulfide, reduced (GSH) and oxidized (GSSG) forms of glutathione, and the activity of glutathione-S-transferase and glyoxalase II enzymes, but decreased redox state of both thiol-disulfide and glutathione, and the activity of glutathione peroxidase and glyoxalase I enzymes involved in the ROS and MG detoxification systems. Exogenous application of proline or glycinebetaine resulted in a reduction of protein carbonylation, and in an increase in glutathione redox state and activity of glutathione peroxidase, glutathione-S-transferase and glyoxalase I under salt stress. Neither proline nor glycinebetaine, however, had any direct protective effect on NaCl-induced GSH-associated enzyme activities. The present study, therefore, suggests that both proline and glycinebetaine provide a protective action against NaCl-induced oxidative damage by reducing protein carbonylation, and enhancing antioxidant defense and MG detoxification systems.

Keywords: Antioxidant defense; Glycinebetaine; Glyoxalase; Proline; Salt stress

Anne Straczek, Geraldine Sarret, Alain Manceau, Philippe Hinsinger, Nicolas Geoffroy, Benoit Jaillard, Zinc distribution and speciation in roots of various genotypes of tobacco exposed to Zn, *Environmental and Experimental Botany*, Volume 63, Issues 1-3, May 2008, Pages 80-90, ISSN 0098-8472, DOI: 10.1016/j.envexpbot.2007.10.034.

(<http://www.sciencedirect.com/science/article/B6T66-4R3C06Y-1/2/daf0b432b9ad57f7a6f904022c65014c>)

Abstract:

Cell walls of roots have a great reactivity towards metals, and may act as a barrier limiting the entry of metals, especially in non-hyperaccumulating species. The aim of this study was to determine the localization and speciation of Zn in roots of tobacco (*Nicotiana tabacum*) grown in Zn-contaminated substrates. Chemical extractions and EXAFS spectroscopy were applied on whole roots and on isolated cell walls of roots. Our results show that cell walls of roots exhibited a distribution of Zn affinity sites, from water-soluble to non-exchangeable Zn. In whole roots, Zn was bound with oxalate and other COOH/OH groups: the first species was probably intracellular while the second was attributed to Zn bound to the cell walls and, to a lesser extent, to intracellular organic acids. Moreover, Zn-phosphate was also identified, and this species was CuSO₄-extractable. It probably resulted from chemical precipitation in the apoplast, and explained the steady increase in exchangeable root Zn observed in root of tobacco during the culture. This study shows the strength of combining EXAFS and chemical extractions for studying localization and speciation of metals in plants.

Keywords: Cation exchange capacity of roots (CECRs); Cell walls; Chemical extractions; EXAFS; Pectin; Cellulose

Marton Laszlo, Manganese requirement of sunflower (*Helianthus annuus* L.), tobacco (*Nicotiana tabacum* L.) and triticale (x *Triticosecale* W.) at early stage of growth, *European Journal of Agronomy*, Volume 28, Issue 4, May 2008, Pages 586-596, ISSN 1161-0301, DOI: 10.1016/j.eja.2008.01.006.

(<http://www.sciencedirect.com/science/article/B6T67-4S0HC69-1/2/de97836f025b1f524a0798523b8a9827>)

Abstract:

Manganese deficiency symptoms are more often observed in crops at early stages of growth since Mn²⁺ can be easily mobilized from the surface soil. The objectives of this study were to evaluate some of the popular rotation crops grown in Hungary for tolerance to low external Mn²⁺ levels and to determine the critical tissue concentration for Mn²⁺ deficiency during early stages of growth. Indicator plants of sunflower (*Helianthus annuus* L.) were grown with NPKCaMg-fertilization

induced of 0.0425-0.0700 g kg⁻¹; of tobacco (*Nicotiana tabacum* L.) 0.0237-0.0337 g kg⁻¹; of triticale (x *Triticosecale* W.) 0.0103-0.0327 g NH₄-acetate + EDTA extractable soil Mn²⁺ kg⁻¹; and were grown for 73, 50, and 191 days. The minimum Mn²⁺ concentration required in soil nutrient contents was 0.0425 g kg⁻¹ for sunflower, 0.0243 g kg⁻¹ for tobacco, and 0.0103 g kg⁻¹ for triticale. Sunflower, tobacco and triticale achieved optimum growth from 0.048 to 0.065 g Mn²⁺ kg⁻¹, from 0.0249 to 0.0321 g Mn²⁺ kg⁻¹, and from 0.0287 to 0.0296 g Mn²⁺ kg⁻¹, respectively. Critical ABP's dry weight Mn²⁺ concentration at early stages of growth was 0.0536 g kg⁻¹ in sunflower, 0.458 g kg⁻¹ in tobacco, and 0.1938 g kg⁻¹ in triticale. Our results demonstrate that the tolerance to low external Mn²⁺ (triticale <0.0302 g kg⁻¹; sunflower <0.0562 g kg⁻¹; tobacco <0.0693 g kg⁻¹) and the critical tissue Mn²⁺ levels for deficiency varied significantly among crop species tested.

Keywords: Crop; Manganese; Deficiency; Requirement; Tolerance

Rawad Saleh, Alan Shihadeh, Elevated toxicant yields with narghile waterpipes smoked using a plastic hose, *Food and Chemical Toxicology*, Volume 46, Issue 5, May 2008, Pages 1461-1466, ISSN 0278-6915, DOI: 10.1016/j.fct.2007.12.007.

(<http://www.sciencedirect.com/science/article/B6T6P-4RC2S43-1/2/1c29626507d99df9716aca8fe51ec391>)

Abstract:

The effect of hose permeability on toxicant yields for the narghile waterpipe is investigated, with special reference to the recent adoption of plastic as a hose construction material. Measurements of air infiltration rates for 23 leather and plastic hoses representing 11 types commonly available in Beirut, Lebanon were made, revealing that while leather hoses allowed significant outside air infiltration during a puff constituting up to 31% of the puff volume, plastic hoses were found to be air-tight, indicating that the smoke reaching the waterpipe user can be considerably more concentrated when delivered via a plastic hose. Total particulate matter (TPM), nicotine and carbon monoxide (CO) yields were compared when a waterpipe was machine smoked using a highly permeable leather and an air-tight plastic hose. It was found that the plastic hose resulted in similar yields of nicotine, but more than double the CO yielded with the highly permeable leather hose. Thus, even if narghile smokers titrate for nicotine intake, the use of a plastic hose will likely greatly increase the exposure to CO, a major causative agent in cardiovascular disease.

Keywords: Argileh; Arguileh; Narguile; Nargileh; Shisha; Hooka; Hubble-bubble; Water-pipe; Tobacco smoke; Carbon monoxide; Nicotine

Elizabeth Sepetdjian, Alan Shihadeh, Najat A. Saliba, Measurement of 16 polycyclic aromatic hydrocarbons in narghile waterpipe tobacco smoke, *Food and Chemical Toxicology*, Volume 46, Issue 5, May 2008, Pages 1582-1590, ISSN 0278-6915, DOI: 10.1016/j.fct.2007.12.028.

(<http://www.sciencedirect.com/science/article/B6T6P-4RJYV9N-1/2/6176edcf3ad5204c6116fb985caecc0>)

Abstract:

An analytical method for the determination of 16 polycyclic aromatic hydrocarbons (PAHs) in the mainstream of narghile smoke is presented. The smoke was generated using a digital waterpipe smoking machine connected to the mouthpiece of a narghile that was loaded with 10 g of a popular flavored tobacco and kept alight with quick-light charcoal briquettes that are commonly used for this purpose. A standard smoking regimen consisting of 171 puffs of 530 ml volume and 2.6 s duration spaced 17 s apart was used, and the smoke condensates were collected on glass fiber filters. PAHs were extracted with toluene assisted by sonication. For purification, the extract was passed through a silica cartridge and eluted with hexane. The eluent was preconcentrated, reconstituted in acetonitrile, and analyzed using a GC-MS-SICP method. The method showed good selectivity, repeatability, accuracy and sensitivity. The limit of detection ranged from 15 to 96 ng for benzo[a]pyrene and indeno[1,2,3-cd]pyrene, respectively. It was found that a single narghile

smoking session delivers approximately 50 times the quantities of carcinogenic 4- and 5-membered ring PAHs as a single 1R4F cigarette smoked using the FTC protocol. The pattern of PAH concentrations suggested that formation pathways differ from those of the cigarette, possibly reflecting the differing combustion conditions of the two smoking devices.

Keywords: PAH; Narghile smoke; Ma'assel tobacco; Carcinogens; Analytical separation tobacco smoke

Wei Li, Li-bo Zhang, Jin-hui Peng, Ning Li, Xue-yun Zhu, Preparation of high surface area activated carbons from tobacco stems with K₂CO₃ activation using microwave radiation, *Industrial Crops and Products*, Volume 27, Issue 3, May 2008, Pages 341-347, ISSN 0926-6690, DOI: 10.1016/j.indcrop.2007.11.011.

([http://www.sciencedirect.com/science/article/B6T77-4RJSJ16-](http://www.sciencedirect.com/science/article/B6T77-4RJSJ16-1/2/38bcc0e96c0a99d993140cf2a6c5efe)

[1/2/38bcc0e96c0a99d993140cf2a6c5efe](http://www.sciencedirect.com/science/article/B6T77-4RJSJ16-1/2/38bcc0e96c0a99d993140cf2a6c5efe))

Abstract:

In this paper, activated carbons with high surface area from carbonized tobacco stems with K₂CO₃ activation by microwave radiation were investigated. Effects of microwave radiation time and K₂CO₃/C ratio on the yield and adsorption capacities of activated carbons were evaluated. Experimental results indicated that the optimum conditions were as follow: microwave power 700 W; microwave radiation time 30 min; K₂CO₃/C ratio 1.5:1. Iodine number, amount of methylene blue adsorption and the yield of activated carbon prepared under optimum conditions were 1834 mg/g, 517.5 mg/g and 16.65%, respectively. Surface area, micropore volume and pore size distribution (PSD) of the carbons were determined by the BET, H-K and DFT methods. Results showed that activated carbons had a micropore content about 59.98% and a small number of mesopores and macropores; BET specific surface area and total pore volume were 2557 m²/g and 1.647 cm³/g, respectively.

Keywords: Carbonized tobacco stems; Microwave radiation; K₂CO₃; High surface area; Pore size distribution

Huarong Li, Hailin Tang, S. Sivakumar, Judith Philip, Robert L. Harrison, John A. Gatehouse, Bryony C. Bonning, Insecticidal activity of a basement membrane-degrading protease against *Heliothis virescens* (Fabricius) and *Acyrtosiphon pisum* (Harris), *Journal of Insect Physiology*, Volume 54, Issue 5, May 2008, Pages 777-789, ISSN 0022-1910, DOI: 10.1016/j.jinsphys.2008.02.008.

([http://www.sciencedirect.com/science/article/B6T3F-4RX079F-](http://www.sciencedirect.com/science/article/B6T3F-4RX079F-1/2/b68df2ec6a1447e57bc7670568acfb4f)

[1/2/b68df2ec6a1447e57bc7670568acfb4f](http://www.sciencedirect.com/science/article/B6T3F-4RX079F-1/2/b68df2ec6a1447e57bc7670568acfb4f))

Abstract:

ScathL is a cathepsin L-like cysteine protease derived from the flesh fly *Sarcophaga peregrina* that functions in basement membrane (BM) remodeling during insect development. A recombinant baculovirus expressing ScathL (AcMLF9.ScathL) kills larvae of the tobacco budworm, *Heliothis virescens*, significantly faster than the wild-type virus. Here, we show that the occurrence of larval melanization prior to death was closely associated with the onset of high cysteine protease activity of ScathL in the hemolymph of fifth instars infected with AcMLF9.ScathL, but not with AcMLF9.ScathL.C146A, a recombinant baculovirus expressing a catalytic site mutant of ScathL. Fragmented fat body, ruptured gut and malpighian tubules, and melanized tracheae were observed in AcMLF9.ScathL-infected larvae. Phenoloxidase activity in hemolymph was unchanged, but the pool of prophenoloxidase was significantly reduced in virus-infected larvae and further reduced in AcMLF9.ScathL-infected larvae. The median lethal dose (LD₅₀) for purified ScathL injected into fifth-instar *H. virescens* was 11.0 [μg]/larva. ScathL was also lethal to adult pea aphids, *Acyrtosiphon pisum* with a similar loss of integrity of the gut and fat body. Injection with purified ScathL.C146A or bovine trypsin at 20 [μg]/larva did not produce any effect in either insect. These results illustrate the potent insecticidal effects of ScathL cysteine protease activity

and the potential for use of ScathL in development of insect resistant transgenic plants when combined with an appropriate delivery system.

Keywords: Cathepsin L; Cysteine protease; Basement membrane; Insecticidal protein; Tobacco budworm; Pea aphid; Baculovirus; Melanization

Jianfeng Xu, Li Tan, Derek T.A. Lamport, Allan M. Showalter, Marcia J. Kieliszewski, The O-Hyp glycosylation code in tobacco and Arabidopsis and a proposed role of Hyp-glycans in secretion, *Phytochemistry*, Volume 69, Issue 8, May 2008, Pages 1631-1640, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2008.02.006.

(<http://www.sciencedirect.com/science/article/B6TH7-4S4J6NK-1/2/f985d2316b9e340381e15f7666782317>)

Abstract:

Most aspects of plant growth involve cell surface hydroxyproline (Hyp)-rich glycoproteins (HRGPs) whose properties depend on arabinogalactan polysaccharides and arabinosides that define the molecular surface. Potential glycosylation sites are defined by an O-Hyp glycosylation code: contiguous Hyp directs arabinosylation. Clustered non-contiguous Hyp directs arabinogalactosylation. Elucidation of this code involved a single species, tobacco (*Nicotiana tabacum*) BY-2 cells. However, recent work suggests species variation, perhaps tissue specific Hyp glycosylation. Thus, the extent to which the Hyp glycosylation code is 'global' needs testing. We compared the ability of distantly related Arabidopsis cell cultures to process putative HRGP glycosylation motifs encoded by synthetic genes. The genes included: repetitive Ser-Pro, Ser-Pro2, Ser-Pro4 and an analog of the tomato arabinogalactan-protein, LeAGP-1[Delta]GPI. All were expressed as enhanced green fluorescent protein (EGFP) fusion glycoproteins, designated: AtSO-EGFP (O = Hyp), AtSO2-EGFP, AtSO4-EGFP and AtEGFP-LeAGP-1[Delta]GPI, respectively. The Arabidopsis glycosylation patterns were essentially similar to those observed in *Nicotiana*: non-contiguous Hyp residues in AtSO-EGFP were glycosylated exclusively with arabinogalactan polysaccharides while contiguous Hyp in AtSO2-EGFP and AtSO4-EGFP was exclusively arabinosylated. Mixed contiguous and non-contiguous Hyp residues in AtEGFP-LeAGP-1[Delta]GPI were also arabinosylated and arabinogalactosylated consistent with the code. However, slightly more arabinogalactosylated Hyp and less non-glycosylated Hyp in AtEGFP-LeAGP-1[Delta]GPI than tobacco NtEGFP-LeAGP-1[Delta]GPI suggested Arabidopsis prolyl hydroxylases have a slightly broader specificity. Arabidopsis Hyp-arabinogalactans differed from tobacco in decreased glucuronic acid content and lack of rhamnose. Yields of the EGFP fusion glycoproteins were dramatically higher than targeted EGFP lacking Hyp-glycomodules. This validates earlier suggestions that the glycosylation of proteins facilitates their secretion.

Keywords: Arabidopsis thaliana; Cruciferae; O-Glycosylation; Arabinogalactan-protein; Hydroxyproline-rich glycoprotein; ([beta]-d-Galactosyl)3-Yariv reagent

C.I. Gonzalez-Verdejo, J.V. Die, S. Nadal, A. Di Pietro, X. Barandiaran, J.I. Cubero, B. Roman, Isolation and expression analysis of a cobalamin-independent methionine synthase gene from the parasitic plant *Orobanche ramosa*, *Scientia Horticulturae*, Volume 116, Issue 3, 1 May 2008, Pages 337-341, ISSN 0304-4238, DOI: 10.1016/j.scienta.2008.01.002.

(<http://www.sciencedirect.com/science/article/B6TC3-4S02K2V-2/2/fa2bff5450cfb93ef47e53c5b8391e13>)

Abstract:

Broomrapes (*Orobanche* spp) are holoparasitic weeds that cause devastating losses in many economically important crops. The branched broomrape (*Orobanche ramosa*) represents a real threat for many vegetable crops including tobacco, tomato and potato.

During its parasitic phase (tubercles and flowers), *Orobanche* behaves like an additional sink of the host and competes with other actively growing parts of the host plant for photoassimilates. In

order to elucidate molecular mechanism that are implicated in the tubercles and flowers formation, a gene involved in sink development was studied.

In this work, the cobalamin-independent methionine synthase clone Or-MET1 was isolated from a cDNA library representing different developmental stages of the parasitic plant. The pattern of expression of the gene was studied by quantitative RT-PCR analysis during both pre-infection and parasitic phases. Since this gene is sucrose-regulated in plants, the effect of this sugar in Or-MET1 expression was also analysed. The results showed that Or-MET1 was expressed during all the stages tested but was more prominently induced in tubercles and flowers. No effect of sucrose was observed. These findings are consistent with a typical gene expression pattern of cobalamin-independent methionine synthase in host sink and with a putative role of Or-MET1 during post-infection stages.

Keywords: Parasitic plant; *Orobanche ramosa*; Sink; Methionine synthase; Sucrose-regulated gene; Quantitative RT-PCR

Haiyan Wang, Mouming Zhao, Bao Yang, Yueming Jiang, Guohua Rao, Identification of polyphenols in tobacco leaf and their antioxidant and antimicrobial activities, *Food Chemistry*, Volume 107, Issue 4, 15 April 2008, Pages 1399-1406, ISSN 0308-8146, DOI: 10.1016/j.foodchem.2007.09.068.

(<http://www.sciencedirect.com/science/article/B6T6R-4PT0Y83-7/2/61268314d219a135c36ee725e5bf0d58>)

Abstract:

Crude polyphenols were extracted from tobacco leaf by 80% ethanol solution with ultrasonic treatment and then purified by a macroporous resin. The polyphenols from tobacco leaf (PTL) were subjected to analyses by reverse-phase high-performance liquid chromatography (RP-HPLC) and electrospray ionization mass spectrometry (ESI-MS). The dominant polyphenols in tobacco leaf were identified as chlorogenic acid and rutin. Furthermore, the antioxidant activities of PTL were investigated, including scavenging activities of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (5.02 [μ g/ml IC₅₀ value), hydroxyl radicals (49.6 [μ g/ml IC₅₀ value) and superoxide anion radicals (44.0 [μ g/ml IC₅₀ value), inhibition activity of lipid peroxidation (132 [μ g/ml IC₅₀ value) and reducing power. The proliferation inhibition activities on *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were also measured for evaluating the antimicrobial activity of PTL. The diameters of inhibition zones were 20.23 \pm 0.42, 17.66 \pm 0.86 and 12.89 \pm 0.29 mm, respectively. The results showed that PTL had great potential as antioxidant and antimicrobial agent.

Keywords: Tobacco; Polyphenol; Antioxidant activity; Antimicrobial activity; HPLC-MS

Xiao-Tang JU, Feng-Chun CHAO, Chun-Jian LI, Rong-Feng JIANG, P. CHRISTIE, Fu-Suo ZHANG, Yield and Nicotine Content of Flue-Cured Tobacco as Affected by Soil Nitrogen Mineralization, *Pedosphere*, Volume 18, Issue 2, April 2008, Pages 227-235, ISSN 1002-0160, DOI: 10.1016/S1002-0160(08)60011-9.

(<http://www.sciencedirect.com/science/article/B82XV-4RXTDD6-C/2/aafc6e67efe061072110b6883c9db0c4>)

Abstract:

Nitrogen (N) supply is the most important factor affecting yield and quality of flue-cured tobacco (FCT). A field experiment and an in situ incubation method were used to study the effects of soil N mineralization in the later stages of growth on yield and nicotine content of FCT in Fenggang and Jinsha, Guizhou Province. The yield and market value of FCT at Fenggang were much lower than those at Jinsha. However, the nicotine content of middle and upper leaves was much higher at Fenggang than at Jinsha when the same rate of fertilizer N was applied, which might be due to a higher N supply capacity at the Fenggang site. At later stages of growth (7-16 weeks after transplanting), the soil net N mineralization at Fenggang (56 kg N ha⁻¹) was almost double that at

Jinsha (30 kg N ha⁻¹). While soil NH₄-N and NO₃-N were almost exhausted by the plants or leached 5 weeks after transplanting, the N taken up at the later growth stages at Fenggang were mainly derived from soil N mineralization, which contributed to a high nicotine content in the upper leaves. The order of soil N contribution to N buildup in different leaves was: upper leaves > middle leaves > lower leaves. Thus, soil N mineralization at late growth stages was an important factor affecting N accumulation and therefore the nicotine content in the upper leaves.

Keywords: flue cured tobacco; nicotine content; soil N mineralization; tobacco quality; tobacco yield

Giandomenico Corrado, Mariarosaria Scarpetta, Daniela Alioto, Antimo Di Maro, Letizia Polito, Augusto Parente, Rosa Rao, Inducible antiviral activity and rapid production of the Ribosome-Inactivating Protein I from *Phytolacca heterotepala* in tobacco, *Plant Science*, Volume 174, Issue 4, April 2008, Pages 467-474, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2008.01.009.

(<http://www.sciencedirect.com/science/article/B6TBH-4RPM7PG-2/2/0bb40c83357f0fe1a3705ac60dd9e1cb>)

Abstract:

We studied the *in vitro* and *in planta* antiviral activity of the PhRIP I, a type 1 Ribosome-Inactivating Protein originally purified from leaves of the *Phytolacca heterotepala*. This protein inhibited protein translation in a cell-free assay and limited the local lesion formation from PVX infection on tobacco leaves. We used a transient expression system based on leaf infiltration with recombinant *Agrobacteria* to show that tobacco can produce a correctly processed PhRIP I enzyme that retains its antiviral activity. Hence, it is possible to rapidly yield in plants a type 1 RIP by means of this transient expression system. To analyse the possible increase of virus resistance in plants, *Nicotiana tabacum* lines that were transformed with the PhRIP I coding sequence under the control of the wound-inducible PGIP promoter were challenged by PVX. A significantly lower number of viral lesions compared to untransformed plants was observed only after the induction of the transgene, indicating that the controlled gene expression of an antiviral protein can increase virus resistance.

Keywords: RIP; Virus; Resistance; Inducible; Transient expression; Transgenic

K. Kalai, A. Meszaros, F. Denes, E. Balazs, Comparative study of constitutive and inducible promoters in tobacco, *South African Journal of Botany*, Volume 74, Issue 2, April 2008, Pages 313-319, ISSN 0254-6299, DOI: 10.1016/j.sajb.2008.01.003.

(<http://www.sciencedirect.com/science/article/B7XN9-4RV7H38-2/2/c62eab007ed22ca794f400e912ddcb33>)

Abstract:

The 42 kDa endochitinase gene of *Trichoderma hamatum* was expressed in tobacco, led by the constitutive 35S promoter of Cauliflower Mosaic Virus and the inducible Actin 7 promoter of *Arabidopsis thaliana*. Transgenic tobacco events containing the 35S promoter had increased chitinase levels in the leaves and enhanced tolerance to *Botrytis cinerea*, but their stems were still susceptible to infection. When the Actin 7 promoter was used, the resulting transgenic tobacco events exhibited tolerance to the pathogen even in the stem. The presence of the transgene was monitored by PCR analysis and the sequence of the PCR product confirmed the integration of the chitinase gene. Analysis of the transgenic plants showed that the lines were heterogeneous. Southern hybridization confirmed a single copy of the transgene in lines transformed with the 35S promoter, while the copy number was 1-4 in lines transformed with the Actin 7 promoter. Compared to the untransformed control, chitinase activity was higher in the leaves of the transgenic plants. This increase was not observed in the stems. In this comparative promoter analysis the stress-induced promoter proved to be more effective in obtaining mould-resistant plants.

Keywords: Actin 7 promoter; CaMV 35S promoter; Chitinase; Mould resistance; Transformation

Zhong-Tian Xue, Anna Holefors, Margareta Welander, Intron splicing in 5' untranslated region of the *rolA* transcript in transgenic apple, *Journal of Plant Physiology*, Volume 165, Issue 5, 31 March 2008, Pages 544-552, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.11.010.

(<http://www.sciencedirect.com/science/article/B7GJ7-4NNYFVP-1/2/3ccbcdf49b29a495b7eb3421577a916d>)

Abstract: Summary

The *rolA* gene encoded on the Ri plasmid of *Agrobacterium rhizogenes* causes developmental alterations, including dwarfing characteristics in the transgenic plants. In an attempt to introduce dwarfing characteristics into apple rootstocks for breeding purposes, the *rolA* gene was incorporated into the apple rootstock M26 and obtained four transgenic clones. All the clones exhibited reduced growth compared to untransformed control plants but different degree of dwarfing and wrinkled leaves. In the present study, expression of the *rolA* gene was further investigated by analysing the structure of the *rolA* transcript and the levels of the *rolA* mRNAs from these clones. The nucleotide (nt) sequence of the *rolA* transcript showed two forms of the transcript: one, the unspliced form, was co-linear with the *rolA* sequence in the genomic DNA; the other was spliced mRNA in which an 85-base pair (bp) intron sequence in the 5' untranslated region (5'UTR) was spliced out. The position of splicing is different from that in *Arabidopsis thaliana* but similar to the splicing site found in tobacco. The transcription start region of the *rolA* gene in apple was 206 bp upstream of that in *Arabidopsis* and 277 bp upstream to *Nicotiana tabacum* transcription start. A hairpin-like secondary structure and an upstream open reading frame (uORF) were revealed in the *rolA* 5'UTR. The levels of the *rolA* mRNA in the apple transgenic clones were analysed by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). The results showed slight variation in the shoot tissues of the transgenic clones.

Keywords: Dwarfing phenotype; *rolA* transcript; Semi-quantitative RT-PCR; Transgenic apple; 5' untranslated region (5'UTR)

Young Geun Mok, Byoung Doo Lee, Young Jin Kim, Chang Eun Lee, Dong Gwan Kim, Joohyun Lee, Jaekyung Shim, Yuling Meng, Barry P. Rosen, Jong Soon Choi, Hyoung Sun Shin, Seong-Ki Kim, June Seung Lee, Seongbin Hwang, The tobacco gene *Ntcyc07* confers arsenite tolerance in *Saccharomyces cerevisiae* by reducing the steady state levels of intracellular arsenic, *FEBS Letters*, Volume 582, Issue 6, 19 March 2008, Pages 916-924, ISSN 0014-5793, DOI: 10.1016/j.febslet.2008.02.030.

(<http://www.sciencedirect.com/science/article/B6T36-4RWHFCC-6/2/bf2a48ae4a9ef21ed1772d82323fbd41>)

Abstract:

We cloned a plant gene, *Ntcyc07*, conferring arsenite tolerance by expressing a tobacco expression library in WT yeast (Y800). Expression of *Ntcyc07* increased the tolerance to As(III) and decreased its accumulation, suggesting that the enhanced As(III) tolerance resulted from a reduction of the intracellular arsenic level. Interestingly, expression of *Ntcyc07* increased the expression of the As(III) export carrier ACR3, but repressed that of As(III) uptake channel FPS1. *Ntcyc07p* interacted with *Acr1p*, which is the transcriptional activator of ACR3, but not with the ACR3 promoter. Taken together, the data indicated that *Ntcyc07p* promoted As(III) tolerance by decreasing the intracellular level of As(III) via increasing the expression of ACR3 and reducing that of FPS1.

Keywords: Arsenite; *cyc07*; ACR1; ACR3; FPS1; *N. tabacum*; *S. cerevisiae*

Ridvan Kizilkaya, Dehydrogenase activity in *Lumbricus terrestris* casts and surrounding soil affected by addition of different organic wastes and Zn, *Bioresource Technology*, Volume 99, Issue 5, March 2008, Pages 946-953, ISSN 0960-8524, DOI: 10.1016/j.biortech.2007.03.004.

(<http://www.sciencedirect.com/science/article/B6V24-4NJP3KP-7/2/ce53274863ff4ef8cfc1f71a724a504d>)

Abstract:

A laboratory experiment was conducted to determine the effects of different organic wastes such as wheat straw (WS), tea production waste (TEW), tobacco production waste (TOW), cow manure (CM) and hazelnut husk (HH) on dehydrogenase activity (DHA) in casts of earthworm *Lumbricus terrestris* and surrounding soil using 5% (dry weight) application rates associated with increasing doses of Zn (0, 50, 100, 250, 500 and 1000 [μ g g⁻¹). Twenty one days after treatment of Zn and organic wastes, the DHA analyses were carried out on collected casts and soil samples.

In general, all organic waste treatments influenced the DHA, the contents of organic C, N and available Zn in earthworm *L. terrestris* casts and the surrounding soil in comparison with the control. DHA in casts exceeded that in the surrounding soil without Zn additions. After Zn application of 50 [μ g Zn g⁻¹ in all organic waste treatments and the control, the DHA level in casts and surrounding soil increased significantly. It decreased by application rates of 100, 250, 500 and 1000 [μ g Zn g⁻¹ consecutively in all organic waste applications. The addition of wastes with low C/N ratio and high Zn content (TEW, TOW, CM) inhibited the DHA in both cast and surrounding soil.

Keywords: Earthworm; Wormcasts; C/N ratio; Zinc; Dehydrogenase activity

D.S. Ogunniyi, T.E. Odetoje, Preparation and evaluation of tobacco seed oil-modified alkyd resins, *Bioresource Technology*, Volume 99, Issue 5, March 2008, Pages 1300-1304, ISSN 0960-8524, DOI: 10.1016/j.biortech.2007.02.044.

(<http://www.sciencedirect.com/science/article/B6V24-4NP3P1V-2/2/771d84edc57ea489441846fe269fae90>)

Abstract:

Four sets of alkyd resins modified by varying the percentage of tobacco seed oil (TSO) contents were prepared according to the alcoholysis-polyesterification process. The effect of oil contents on properties such as the drying performance, thickness of film, solubility, viscosity and color of the alkyd resins was evaluated. The alkyd with the least oil content was the most rapid drying, most viscous and darkest in color. Also, the prepared alkyds and the commercial alkyds were used separately in the formulation of white gloss paints and the properties of the alkyds were found to be comparable with commercial samples.

Keywords: Tobacco seed oil; Alkyd; Resin

Hong-Bo Shao, Dong-Mei He, Kai-Xian Qian, Gui-Fang Shen, Zhong-Liang Su, The expression of classical swine fever virus structural protein E2 gene in tobacco chloroplasts for applying chloroplasts as bioreactors, *Comptes Rendus Biologies*, Volume 331, Issue 3, March 2008, Pages 179-184, ISSN 1631-0691, DOI: 10.1016/j.crv.2007.12.007.

(<http://www.sciencedirect.com/science/article/B6X1F-4RS3TKH-1/2/4ab4d33044a49ba4d227b4b7fa70042c>)

Abstract:

It has been reported that genes encoding antigens of bacterial and viral pathogens can be expressed in plants and are shown to induce protection antibodies. The structural protein E2 of classical swine fever virus (CSFV), which has been shown to carry critical epitopes, has been expressed in different systems. Here, we report the expression of CSFV E2 gene in tobacco chloroplasts. Mice immunized with leaf extracts elicited specific antibodies. This indicated that the expressed E2 proteins had a certain degree of immunogenicity. To our knowledge, this is the first report showing induction of protective antibody in response to classical swine fever virus (CSFV) by immunization with antigen protein E2 expressed in tobacco chloroplasts, which will open a new way to protection from CSFV by plant chloroplasts as bioreactors. To cite this article: H.-B. Shao et al., *C. R. Biologies* 331 (2008).

Keywords: Classical swine fever virus (CSFV); E2 gene; Chloroplast; Immunogenicity; Bioreactor; Anti-disease plant-gene engineering

Cem Erdogan, Graham D. Moores, M. Oktay Gurkan, Kevin J. Gorman, Ian Denholm, Insecticide resistance and biotype status of populations of the tobacco whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Turkey, *Crop Protection*, Volume 27, Issues 3-5, March-May 2008, Pages 600-605, ISSN 0261-2194, DOI: 10.1016/j.cropro.2007.09.002.

(<http://www.sciencedirect.com/science/article/B6T5T-4PXG79B-1/2/df087e7c8929a8aa43cc09a1d6ee6933>)

Abstract:

Analysis using polyacrylamide gel electrophoresis (PAGE) of four strains of the tobacco whitefly, *Bemisia tabaci*, collected from cotton in Turkey showed all insects to be assignable to the geographically widespread B biotype of this species. Bioassays with appropriate life-stages were used to investigate the status of resistance to two pyrethroid insecticides (bifenthrin and fenpropathrin), two organophosphates (OPs) (formothion and triazophos) and an insect growth regulator (buprofezin). All four strains showed significant resistance to pyrethroids (57- to 360-fold) and OPs (20- to 310-fold). Resistance to buprofezin was found only in a strain from Izmir. Total non-specific esterase activities were 7.4-11-fold greater than in an insecticide-susceptible strain, and were likely to account, in part at least, for resistance to pyrethroids. Inhibition assays with acetylcholinesterase also implicated target-site modification as a mechanism of resistance to OPs. The data update previous results on the resistance status of *B. tabaci* in Turkey and the implications for managing resistance on cotton and other whitefly hosts are discussed.

Keywords: *Bemisia tabaci*; Tobacco whitefly; Insecticide resistance; Biotype; Pyrethroid; Organophosphate; Buprofezin; Esterase; Acetylcholinesterase

S. Visoni, N. Meireles, L. Monteiro, A. Rossini, L.F.R. Pinto, Different modes of inhibition of mouse Cyp2a5 and rat CYP2A3 by the food-derived 8-methoxypsoralen, *Food and Chemical Toxicology*, Volume 46, Issue 3, March 2008, Pages 1190-1195, ISSN 0278-6915, DOI: 10.1016/j.fct.2007.12.001.

(<http://www.sciencedirect.com/science/article/B6T6P-4R9GGY1-3/2/0d7bbd0c128310b2ec0435a3aecc83f5>)

Abstract:

CYP2A enzymes are responsible for nicotine metabolism and for activating tobacco-related carcinogens. Inhibition of CYP2A is a promising approach in chemoprevention, which could lead to a decrease in cigarette consumption and to a reduction in tobacco-related cancer risk. 8-Methoxypsoralen (8-MOP) is a mechanism-based inhibitor of human CYP2A6 and CYP2A13. 8-MOP is also an inhibitor of Cyp2a5, but the mode of this inhibition is unknown. There is no published data on the inhibition of CYP2A3 by 8-MOP. The objective of this work was to investigate the characteristics of 8-MOP inhibition on mouse hepatic Cyp2a5 and rat nasal CYP2A3, in order to determine the best experimental model for chemoprevention studies using 8-MOP. The results show that 8-MOP inhibits CYP2a5 through three different mechanisms: competitive, non-competitive ($K_{iu} = 1.7 \text{ } [\mu\text{M}]$), and mechanism-based ($K_{inactivation} \text{ of } 0.17 \text{ min}^{-1}$). By contrast, 8-MOP was able to inhibit CYP2A3-mediated coumarin 7-hydroxylase only in a non-competitive way ($K_{iu} = 0.22 \text{ } [\mu\text{M}]$). In conclusion, we showed that 8-MOP inhibits Cyp2a5 and CYP2A3 through different mechanisms.

Keywords: CYP2A3; Cyp2a5; 8-Methoxypsoralen; Chemoprevention; Coumarin 7-hydroxylase

, Position of the American Dietetic Association: Nutrition and Lifestyle for a Healthy Pregnancy Outcome, *Journal of the American Dietetic Association*, Volume 108, Issue 3, March 2008, Pages 553-561, ISSN 0002-8223, DOI: 10.1016/j.jada.2008.01.030.

(<http://www.sciencedirect.com/science/article/B758G-4RY6RKN-13/2/d54d6ecaccea3635f3fac1234b681dfb>)

Abstract:

It is the position of the American Dietetic Association that women of child-bearing ages should maintain good nutritional status through a lifestyle that optimizes maternal health and reduces the risk of birth defects, suboptimal fetal growth and development, and chronic health problems in their children. The key components of a health-promoting lifestyle during pregnancy include appropriate weight gain; appropriate physical activity; consumption of a variety of foods in accordance with the Dietary Guidelines for Americans 2005; appropriate and timely vitamin and mineral supplementation; avoidance of alcohol, tobacco, and other harmful substances; and safe food handling. Pregnant women with inappropriate weight gain, hyperemesis, poor dietary patterns, phenylketonuria, certain chronic health problems, or a history of substance abuse should be referred to a registered dietitian for medical nutrition therapy. Prenatal weight gain within the Institute of Medicine recommended ranges has been associated with better pregnancy outcomes. Most pregnant women need 2,200 to 2,900 kcal a day, but prepregnancy body mass index, rate of weight gain, maternal age, and appetite must be considered when tailoring this recommendation to the individual. The consumption of more food to meet energy needs, and the increased absorption and efficiency of nutrient utilization that occurs in pregnancy, are generally adequate to meet the needs for most nutrients. However, vitamin and mineral supplementation is appropriate for some nutrients and situations. This position paper also includes recommendations pertaining to use of alcohol, tobacco, caffeine, and illicit drugs.

Jeremy Catinot, Antony Buchala, Eliane Abou-Mansour, Jean-Pierre Metraux, Salicylic acid production in response to biotic and abiotic stress depends on isochorismate in *Nicotiana benthamiana*, *FEBS Letters*, Volume 582, Issue 4, 20 February 2008, Pages 473-478, ISSN 0014-5793, DOI: 10.1016/j.febslet.2007.12.039.

(<http://www.sciencedirect.com/science/article/B6T36-4RKMB58-9/2/12063674b115914e3e3c109b0ea50912>)

Abstract:

Salicylic acid (SA) is an important signal involved in the activation of defence responses against abiotic and biotic stress. In tobacco, benzoic acid or glucosyl benzoate were proposed to be precursors of SA. This is in sharp contrast with studies in *Arabidopsis thaliana*, where SA derives from isochorismate. We have determined the importance of isochorismate for SA biosynthesis in *Nicotiana benthamiana* using virus-induced gene silencing of the isochorismate synthase (ICS) gene. Plants with silenced ICS expression do not accumulate SA after exposure to UV or to pathogen stress. Plants with silenced ICS expression also exhibit strongly decreased levels of phyloquinone, a product of isochorismate. Our data provide evidence for an isochorismate-derived synthesis of SA in *N. benthamiana*

Keywords: Isochorismate synthase; Pathogen stress; Phyloquinone; Salicylic acid; UV stress; *Nicotiana*

Maurice Tubiana, Generalites sur la cancerogenese, *Comptes Rendus Biologies*, Volume 331, Issue 2, Dossier : Nouveautes en cancerogenese / New developments in carcinogenesis, February 2008, Pages 114-125, ISSN 1631-0691, DOI: 10.1016/j.crv.2007.03.003.

(<http://www.sciencedirect.com/science/article/B6X1F-4NH6CVN-1/2/177dcdc358827b4b291702f07006b80a>)

Abstract:

Currently, carcinogenesis appears to be a process much more complex than what was believed a decade ago. The study of the genome of human tumour cells has revealed a number of genetic and epigenetic modifications much greater than suspected. Moreover, the delay between the first initiating event (when its timing is precisely known) and the clinical emergence of a cancer can be

very long, up to 60 years. This long delay shows that risk factors during infancy and childhood deserve critical analysis. The epidemiological data emphasize the role of promotion. For example, alcohol, asbestos are not mutagenic, but cause irritation and cell proliferation. Even for tobacco, the role of promotion appears to be more important than that of mutations. In human carcinogenesis, initial mutations do not appear to be a limiting or crucial step.

Finally, the biological study of carcinogenesis has shown that the initiating cell is not passively affected by the accumulation of damages by the carcinogenic physical or chemical agents. It reacts through at least three mechanisms: (a) by fighting against reactive oxygen species (ROS) generated by any oxidative stress, such as UV or ionizing radiations, (b) by eliminating injured cells (mutated or unstable), through two ways - (i) apoptosis, which can be initiated by doses as low as a few millisieverts, thus eliminating cells with genomes that have been damaged or ill-repaired, (ii) death of cells during mitosis when lesions have not been repaired -, (c) by stimulating or activating DNA repair systems. Furthermore, intercellular communication systems inform a cell about the presence of an insult in neighbouring cells. A system of intercellular induction of apoptosis exists whereby non-transformed cells can selectively remove transformed cells.

Modern transcriptional analysis of cellular genes using microarray technology reveals that many genes are activated by doses of carcinogenic agents much lower than those for which mutagenesis is observed. These methods have been a source of considerable progress. Moreover, it was thought that carcinogenesis was initiated by lesions of the genome affecting at random a few specific targets (proto-oncogenes, suppressor genes, etc.). This relatively simple model has been replaced by a more complex one, in which the relationship between the initiated cells and their microenvironment plays an essential role.

Thus, the carcinogenic process is counteracted by effective defence mechanisms in the cell, tissue and the organism. With regard to tissue, the mechanisms that govern embryogenesis and direct tissue repair after injury appear to play also an important role in the control of cell proliferation. This is particularly important when a transformed cell is surrounded by normal cells that appear to be able to inhibit its proliferation. Tissue disorganization by inflammation or by the death of a large proportion of cells is often associated with the escape of the initiated cells and the emergence of a clone of pre-neoplastic-neoplastic cells. The effectiveness of immunosurveillance is also shown by the large increase in the incidence of several types of cancers among immunodepressed people. To cite this article: M. Tubiana, C. R. *BioEssays* 33:1 (2008).

Keywords: Carcinogenesis; DNA repair; Apoptosis; Oxidative stress

Maciej A. Pszczolkowski, Emily Olson, Crystal Rhine, Sonny B. Ramaswamy, Role for calcium in the development of ovarian patency in *Heliothis virescens*, *Journal of Insect Physiology*, Volume 54, Issue 2, February 2008, Pages 358-366, ISSN 0022-1910, DOI: 10.1016/j.jinsphys.2007.10.005.

(<http://www.sciencedirect.com/science/article/B6T3F-4PYGW67-1/2/1089cec3e79c7f5078b5491b1121022a>)

Abstract:

Insect oocytes sequester nutritive proteins from the hemolymph under the regulation by juvenile hormone (JH), in a process called patency. Here, a pharmacological approach was used to decipher the role for calcium in ovarian patency in the moth, *Heliothis virescens*.

Follicular epithelial cells were exposed in calcium-free or calcium-containing media to JH I, JH II or JH III alone, or in combination with various inhibitors of signal transduction. Protein kinase inhibitors, Na⁺/K⁺-ATPase inhibitor, ouabain, an inhibitor of voltage-dependent calcium channels in plasma membrane, [omega]-Conotoxin MVII, endoplasmic reticulum (ER) Ca²⁺-ATPase inhibitor, thapsigargin, ER inositol 1,4,5-triphosphate receptor (IP3R) inhibitor, 2-ABP and ER ryanodine receptor (RyR) inhibitor, ryanodine, were used.

The results of our study suggest that JH II evokes patency via protein kinase C-dependent signaling pathway, and activation of Na⁺/K⁺-ATPase, similar to JH III. Response to JH II and JH III predominantly relies upon external and internal calcium stores, using voltage-dependent calcium channels, IP3Rs and RyRs. In contrast, regulation of patency by JH I appears to be largely calcium independent, and the calcium-dependent component of the signaling pathway likely does not use IP3Rs, but RyRs only. The JH II, JH III and calcium-dependent component of JH I signaling pathway probably utilize calcium/calmodulin-dependent kinase II for activation of Na⁺/K⁺-ATPase.

Keywords: Juvenile hormone; Signal transduction; Oocyte maturation; Tobacco budworm; Na⁺/K⁺-ATPase

E.P.J. Burgess, B.A. Philip, J.T. Christeller, N.E.M. Page, R.K. Marshall, M.W. Wohlers, Tri-trophic effects of transgenic insect-resistant tobacco expressing a protease inhibitor or a biotin-binding protein on adults of the predatory carabid beetle *Ctenognathus novaezelandiae*, *Journal of Insect Physiology*, Volume 54, Issue 2, February 2008, Pages 518-528, ISSN 0022-1910, DOI: 10.1016/j.jinsphys.2007.12.002.

(<http://www.sciencedirect.com/science/article/B6T3F-4R98KDN-3/2/c8cb41116bed4f865fe9e9ada60d417c>)

Abstract:

Tri-trophic impacts on adult predatory carabid beetles, *Ctenognathus novaezelandiae*, of insect-resistant transgenic tobacco plants expressing a serine protease inhibitor, bovine spleen trypsin inhibitor (BSTI), or a biotin-binding protein, avidin, were investigated. Both proteins could potentially affect this beetle, since avidin is known to be insecticidal to many beetle species and *C. novaezelandiae* midguts were shown to contain high levels of trypsin, a protease powerfully inhibited by bovine pancreatic trypsin inhibitor (a BSTI homologue) *in vitro*. Newly emerged field-collected adult *C. novaezelandiae* were fed exclusively for 280 days on *Spodoptera litura* larvae raised either on non-transgenic control, transgenic avidin (55 ppm) or transgenic BSTI (68 ppm) tobacco. Despite this long-term exclusive diet, there was no treatment effect on survival or fecundity and only minor and transient effects on beetles were observed. Data pooled across time and genders showed control-prey-fed beetles weighed 3% more than BSTI-prey-fed beetles and avidin-prey-fed beetles consumed 3-4% fewer prey than control- or BSTI-prey-fed individuals. Females in all treatments gained more mass and survived longer than males. Low exposure to the proteins because of dilution and deactivation within the prey is the most likely explanation for the lack of tri-trophic effects observed. Additionally, the presence of a digestive chymotrypsin only partially inhibited by BSTI may provide an alternative path for proteolysis.

Keywords: Tri-trophic non-target impacts; Carabid beetle; Transgenic plant; Avidin; Bovine spleen trypsin inhibitor (BSTI); Aprotinin (BPTI)

Bo Huang, Longguo Jin, Jin-Yuan Liu, Identification and characterization of the novel gene GhDBP2 encoding a DRE-binding protein from cotton (*Gossypium hirsutum*), *Journal of Plant Physiology*, Volume 165, Issue 2, 1 February 2008, Pages 214-223, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.11.003.

(<http://www.sciencedirect.com/science/article/B7GJ7-4MV1GNW-1/2/0cd38dff335ca2374fb9a1c8dc2443b6>)

Abstract: Summary

A cDNA encoding one novel DRE-binding protein, GhDBP2, was isolated from cotton seedlings. It is classified into the A-6 group of DREB subfamily based on multiple sequence alignment and phylogenetic characterization. Using semi-quantitative RT-PCR, we found that the GhDBP2 transcripts were greatly induced by drought, NaCl, low temperature and ABA treatments in cotton cotyledons. The DNA-binding properties of GhDBP2 were analyzed by electrophoretic mobility shift assay (EMSA), showing that GhDBP2 successfully binds to the previously characterized DRE

cis-element as well as the promoter region of the LEA D113 gene. Consistent with its role as a DNA-binding protein, GhDBP2 is preferentially localized to the nucleus of onion epidermal cells. In addition, when GhDBP2 is transiently expressed in tobacco cells, it activates reporter gene expression driven by the LEA D113 promoter. Taken together, our results indicate that GhDBP2 is a DRE-binding transcriptional activator involved in activation of down-stream genes such as LEA D113 expression through interaction with the DRE element, in response to environmental stresses as well as ABA treatment.

Keywords: ABA; DRE-binding protein; *Gossypium hirsutum*; LEA D113; Transcriptional activator

Wenxia Wang, Shuguang Li, Xiaoming Zhao, Yuguang Du, Bincheng Lin, Oligochitosan induces cell death and hydrogen peroxide accumulation in tobacco suspension cells, *Pesticide Biochemistry and Physiology*, Volume 90, Issue 2, February 2008, Pages 106-113, ISSN 0048-3575, DOI: 10.1016/j.pestbp.2007.10.003.

(<http://www.sciencedirect.com/science/article/B6WP8-4R68N96-1/2/38607fcc8442f3ed35b8d56438b3a45c>)

Abstract:

Oligochitosan has been shown to induce several plant defense responses. In the present work, the effect of oligochitosan on tobacco cell survival was investigated. The results showed that oligochitosan caused tobacco cell death in a dose-dependent manner. About 40.6 % tobacco cells died when cultured for 72 h after 500 [μ]g ml⁻¹ oligochitosan treatment. Certain aspects of this cell death process appeared to be similar to apoptosis in animal cells. These included shrinkage of cytoplasm and condensation of chromatin. Oligochitosan also induced H₂O₂ accumulation in tobacco cell suspension culture. The role of H₂O₂ in the signal transduction that leads to cell death was investigated. Co-treatment of tobacco cells with oligochitosan and catalase inhibited H₂O₂ accumulation but did not inhibit the induction of cell death. The results suggested that apoptosis-like cell death of tobacco cells induced by oligochitosan is independent of H₂O₂ signal pathway.

Keywords: Oligochitosan; Apoptosis; Hydrogen peroxide; Plant defense responses

Hiroshi Ashihara, Hiroshi Sano, Alan Crozier, Caffeine and related purine alkaloids: Biosynthesis, catabolism, function and genetic engineering, *Phytochemistry*, Volume 69, Issue 4, February 2008, Pages 841-856, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2007.10.029.

(<http://www.sciencedirect.com/science/article/B6TH7-4RCW7WP-1/2/48370b6c2717b11c86549153c7678352>)

Abstract:

Details of the recently elucidated biosynthetic pathways of caffeine and related purine alkaloids are reviewed. The main caffeine biosynthetic pathway is a sequence consisting of xanthosine --> 7-methylxanthosine --> 7-methylxanthine --> theobromine --> caffeine. Genes encoding N-methyltransferases involved in three of these four reactions have been isolated and the molecular structure of N-methyltransferases investigated. Pathways for the catabolism of caffeine have also been studied, although there are currently no reports of enzymatic and genetic studies having been successfully carried out. Metabolism of purine alkaloids in species including *Camellia*, *Coffea*, *Theobroma* and *Ilex* plants is summarised, and evidence for the involvement of caffeine in chemical defense and allelopathy is discussed. Finally, information is presented on metabolic engineering that has produced coffee seedlings with reduced caffeine content, and transgenic caffeine-producing tobacco plants with enhanced disease resistance.

Keywords: *Camellia sinensis*; Theaceae; *Coffea* sp.; Rubiaceae; *Theobroma cacao*; Sterculiaceae; Review; Metabolism; Caffeine

Yun-Soo Kim, Hiroshi Sano, Pathogen resistance of transgenic tobacco plants producing caffeine, *Phytochemistry*, Volume 69, Issue 4, February 2008, Pages 882-888, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2007.10.021.

(<http://www.sciencedirect.com/science/article/B6TH7-4R718M3-1/2/a0c93ea17a0ee87954951e1f6cbb6024>)

Abstract:

Caffeine (1,3,7-trimethylxanthine) is a typical purine alkaloid, and produced by a variety of plants such as coffee and tea. Its physiological function, however, is not completely understood, but chemical defense against pathogens and herbivores, and allelopathic effects against competing plant species have been proposed. Previously, we constructed transgenic tobacco plants, which produced caffeine up to 5 [μ]g per gram fresh weight of leaves, and showed them to repel caterpillars of tobacco cutworms (*Spodoptera litura*). In the present study, we found that these transgenic plants constitutively expressed defense-related genes encoding pathogenesis-related (PR)-1a and proteinase inhibitor II under non-stressed conditions. We also found that they were highly resistant against pathogens, tobacco mosaic virus and *Pseudomonas syringae*. Expression of PR-1a and PR-2 was higher in transgenic plants than in wild-type plants during infection. Exogenously applied caffeine to wild-type tobacco leaves exhibited the similar resistant activity. These results suggested that caffeine stimulated endogenous defense system of host plants through directly or indirectly activating gene expression. This assumption is essentially consistent with the idea of chemical defense, in which caffeine may act as one of signaling molecules to activate defense response. It is thus conceivable that the effect of caffeine is bifunctional; direct interference with pest metabolic pathways, and activation of host defense systems.

Keywords: Caffeine; *Nicotiana tabacum*; Solanaceae; Pathogenesis-related genes; *Pseudomonas syringae* pv. *glycinea*; Tobacco mosaic virus

Gao-Hua Zhang, Qiao Su, Li-Jia An, Song Wu, Characterization and expression of a vacuolar Na⁺/H⁺ antiporter gene from the monocot halophyte *Aeluropus littoralis*, *Plant Physiology and Biochemistry*, Volume 46, Issue 2, February 2008, Pages 117-126, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2007.10.022.

(<http://www.sciencedirect.com/science/article/B6VRD-4R0CK2W-3/2/19a1a8045635275e6021375041f84212>)

Abstract:

Plant vacuolar Na⁺/H⁺ antiporter plays an important role in salt tolerance. In order to understand the molecular basis of vacuolar Na⁺/H⁺ antiporter responded to salinity and reveal a possible role of salt tolerance in monocots, a vacuolar Na⁺/H⁺ antiporter gene (AINHX) was isolated by reverse transcription-PCR and RNA ligase mediated rapid amplification of cDNA ends (RLM-RACE) based on the homology from *Aeluropus littoralis* (Gouan) Parl, a graminaceous halophyte. The AINHX sequence contained 2706 bp with an open read frame of 1623 bp and the deduced transcripts encoding 540 amino acids shared a high homology with those putative vacuolar Na⁺/H⁺ antiporters of higher plants. AINHX was predicted containing ten putative hydrophobic regions, which was different with AtNHX1 and OsNHX1. DNA gel blot analysis indicated that there were two or three copies of AINHX in the *A. littoralis* genome. The increased transcript levels of AINHX were much higher in roots than that in shoots under salt stress. In addition, overexpression of AINHX in tobacco conferred high salt tolerance to the transgenic plants. The analysis of ion contents indicated that under high salt stress for one month, the transgenic plants compartmentalized more Na⁺ in the roots and kept a relative high K⁺/Na⁺ ratio in the leaves compared with wild-type plants.

Keywords: *Aeluropus littoralis*; Halophyte; Salt-tolerance; Transgenic tobacco; Vacuolar Na⁺/H⁺ antiporter

Shigeo Takumi, Chisa Shimamura, Fuminori Kobayashi, Increased freezing tolerance through up-regulation of downstream genes via the wheat CBF gene in transgenic tobacco, *Plant Physiology and Biochemistry*, Volume 46, Issue 2, February 2008, Pages 205-211, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2007.10.019.

(<http://www.sciencedirect.com/science/article/B6VVD-4R0CK2W-2/2/06cddb258a7b0ad58fc1ba031d64d74>)

Abstract:

The wheat (*Triticum aestivum* L.) CBF gene family is assumed to play important roles in development of low-temperature and freezing tolerance through activation of the downstream Cor/Lea genes. However, no direct evidence shows association of the wheat CBF genes with stress tolerance or any interaction between wheat CBF transcription factors and Cor/Lea gene activation. Here, we introduced Wcbf2, one of the wheat CBF genes, into the tobacco (*Nicotiana tabacum* L.) genome. Expression of Wcbf2 significantly increased the level of freezing tolerance in the transgenic tobacco plants without phenotypic retardation, and altered the expression patterns of tobacco genes, including cold-responsive genes. A transgenic tobacco plant expressing Wcbf2 was crossed to other transgenic plants expressing a GUS reporter gene under control of the wheat Cor/Lea gene promoter. Analysis of the F1 plants showed that the WCBF2 protein positively regulated at least the expression of Wdhn13 and Wrab17. These results strongly indicate that WCBF2 functions as a transcription factor in the development of freezing tolerance in common wheat.

Keywords: CBF transcription factor; Cor/Lea genes; Differential display; Freezing tolerance; Transgenic plant; *Triticum aestivum* L

Lin Cong, Hong-Chun Zheng, Yu-Xiu Zhang, Tuan-Yao Chai, Arabidopsis DREB1A confers high salinity tolerance and regulates the expression of GA dioxygenases in Tobacco, *Plant Science*, Volume 174, Issue 2, February 2008, Pages 156-164, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2007.11.002.

(<http://www.sciencedirect.com/science/article/B6TBH-4R40SHH-1/2/e1490e9e013998852b36996e97e213f1>)

Abstract:

AtDREB1A driven by the CaMV 35S or the stress-inducible rd29A promoter were introduced into tobacco plants to investigate the role of AtDREB1A in salt stress. The AtDREB1A-expressing plants showed higher root length and chlorophyll content, and had elevated levels of soluble sugars under high salinity conditions compared to control plants. The expression of LEA (NtERD10B and NtLEA5) and Suc-phosphate-synthase A (NtSPSA, which encodes a key enzyme involved in sucrose biosynthesis) increased under salt stress in control plants. Moreover, their expression was up-regulated by AtDREB1A in transgenic plants under normal conditions and further enhanced by high salt, which at least partially contributed to the high salt tolerance in AtDREB1A-expressing plants. On the other hand, overexpression of AtDREB1A caused a dwarf phenotype. The expression of NtGA3ox and NtGA2ox increased while NtGA20ox seemed slightly decreased in AtDREB1A-overexpressing plants or under salt stress in wild-type tobacco plants. Exogenous GA3 treatment did not restore the dwarf phenotype except for the enlarged leaf area and the lengthened petiole. These results suggest that overexpression of AtDREB1A confers salt tolerance and regulates the expression of GA dioxygenases in the similar way as salt stress in tobacco plants. The possible mechanism leading to the dwarf phenotype in AtDREB1A-overexpressing plants is discussed.

Keywords: AtDREB1A; High salinity tolerance; Tobacco; LEA genes; Sucrose biosynthesis; GA dioxygenases

Ji Huang, Mei-Mei Wang, Yan Jiang, Qi-Hong Wang, Xi Huang, Hong-Sheng Zhang, Stress repressive expression of rice SRZ1 and characterization of plant SRZ gene family, *Plant Science*,

Volume 174, Issue 2, February 2008, Pages 227-235, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2007.11.010.

(<http://www.sciencedirect.com/science/article/B6TBH-4R8HHNH-1/2/25211c1e2f5b01a50cab03c805c04ebc>)

Abstract:

A zinc finger protein gene was cloned from rice and designated as SRZ1 (stress repressive zinc finger protein 1). SRZ1 encodes a protein with three C2C2-type zinc finger motifs that are structurally similar to human ZNF265 zinc finger motifs. SRZ1 was expressed with high level in leaves and markedly repressed by salt, cold, drought and abscisic acid stresses but not by salicylic acid and blast inoculation in rice seedlings. Ectopic expression of SRZ1 in tobacco plants repressed expression of abiotic stress-related genes including osmotin, NtERB10B, NtERB10C, and increased plant sensitivity to cold and salt stresses. Database searches showed SRZ genes were widely distributed among plant species and their expressions in rice and Arabidopsis were significantly down-regulated by at least one type of abiotic stresses. This study indicates that SRZ1 and its relatives in plants may play negative roles in abiotic stress signaling and the down-regulation of them might be crucial for plant tolerance against stresses.

Keywords: Abiotic stress; Repression; Rice; Transgenic plant; Zinc finger protein

C.L. Gaworski, R. Lemus-Olalde, E.L. Carmines, Toxicological evaluation of potassium sorbate added to cigarette tobacco, Food and Chemical Toxicology, Volume 46, Issue 1, January 2008, Pages 339-351, ISSN 0278-6915, DOI: 10.1016/j.fct.2007.08.012.

(<http://www.sciencedirect.com/science/article/B6T6P-4PFW6GD-2/2/2279fb87957f1c2712688624cdc86fa2>)

Abstract:

Potassium sorbate (PS) may be incorporated in blended cigarette tobacco either as a mold growth inhibitor in processed tobacco sheet material, or as a preservative in flavor systems or paper adhesives. To evaluate the effect of PS addition, neat material pyrolysis studies, smoke chemistry and biological activity studies (bacterial mutagenicity, cytotoxicity, in vivo micronucleus, and 90-day nose-only rat inhalation) with mainstream smoke, or mainstream smoke preparations from cigarettes containing various measured levels of PS (0%, 0.15%, 1.6%, and 3.7%) were performed. At simulated tobacco burning temperatures up to 1000 [degree sign]C, neat PS completely pyrolyzed to form aromatic ring materials including benzene, toluene, substituted benzenes, naphthalene, and substituted naphthalenes. Under machine smoking conditions (FTC/ISO), high levels of PS may alter the burning characteristics of the cigarette leading to decreased puff count, total particulate matter, carbon monoxide, hydrogen cyanide, 2-nitropropane, and tobacco specific nitrosamines yields in the smoke, while increasing the yield of nicotine, 1,3-butadiene, isoprene, and some PAHs. Biological studies indicated no relevant differences in the genotoxic or cytotoxic potential of either mainstream smoke from cigarettes with or without added PS. Rats exposed to mainstream cigarette smoke developed respiratory tract changes consistent with those seen in previous smoke inhalation studies, with no relevant histopathological differences between the control and the PS test cigarette groups. These studies demonstrated that high levels of PS could alter the burning rate of the tobacco leading to alteration in the smoke chemistry profile. Yet, based on the panel of biological endpoints monitored here, it appeared that added PS produced little relevant change in the overall toxicity profile of smoke.

Keywords: Cigarette tobacco; Potassium sorbate; Inhalation

Deepali Merchant, Ronald L. Ertl, Stephen I. Rennard, David W. Stanley, Jon S. Miller, Eicosanoids mediate insect hemocyte migration, Journal of Insect Physiology, Volume 54, Issue 1, January 2008, Pages 215-221, ISSN 0022-1910, DOI: 10.1016/j.jinsphys.2007.09.004.

(<http://www.sciencedirect.com/science/article/B6T3F-4PPF6GX-1/2/0b7f963fb9d27a18118984234d09b4a2>)

Abstract:

Hemocyte migration toward infection and wound sites is an essential component of insect defense reactions, although the biochemical signal mechanisms responsible for mediating migration in insect cells are not well understood. Here we report on the outcomes of experiments designed to test the hypotheses that (1) insect hemocytes are able to detect and migrate toward a source of N-formyl-Met-Leu-Phe (fMLP), the major chemotactic peptide from *Escherichia coli* and (2) that pharmaceutical modulation of eicosanoid biosynthesis inhibits hemocyte migration. We used primary hemocyte cultures prepared from fifth-instar tobacco hornworms, *Manduca sexta* in Boyden chambers to assess hemocyte migration toward buffer (negative control) and toward buffer amended with fMLP (positive control). Approximately 42% of negative control hemocytes migrated toward buffer and about 64% of positive control hemocytes migrated toward fMLP. Hemocyte migration was inhibited (by >40%) by treating hornworms with pharmaceutical modulators of cyclooxygenase (COX), lipoxygenase and phospholipase A2 (PLA2) before preparing primary hemocyte cultures. The influence of the COX inhibitor, indomethacin, and the glucocorticoid, dexamethasone, which leads to inhibition of PLA2, was expressed in a dose-dependent way. The influence of dexamethasone was reversed by injecting arachidonic acid (precursor to eicosanoid biosynthesis) into hornworms before preparing primary hemocyte cultures. The saturated fatty acid, palmitic acid, did not reverse the inhibitor effect. These findings support both our hypotheses, first that insect hemocytes can detect and respond to fMLP, and second, that insect hemocyte migration is mediated by eicosanoids.

Keywords: *Manduca sexta*; Insect immunity; Migration; Boyden chamber

Rizana M. Mahroof, Thomas W. Phillips, Life history parameters of *Lasioderma serricorne* (F.) as influenced by food sources, *Journal of Stored Products Research*, Volume 44, Issue 3, 2008, Pages 219-226, ISSN 0022-474X, DOI: 10.1016/j.jspr.2007.12.001.

(<http://www.sciencedirect.com/science/article/B6T8Y-4S0359W-1/2/a113953d1bc97aff2fecaa333de6c689>)

Abstract:

Fecundity, egg to adult survival rate, developmental time, and adult body weight of the cigarette beetle, *Lasioderma serricorne* (Coleoptera: Anobiidae) were evaluated on seven food sources at 28 [degree sign]C. Ground chili, paprika, cayenne pepper, chewing leaf tobacco, cigar tobacco, a commercial insect bait referred to as NOW bait, and wheat flour were used to evaluate mean lifetime fecundity. The highest fecundity (52.4+/-4.8 eggs/female) was observed in wheat flour, whereas the lowest fecundity (5.8+/-0.8 eggs/female) was observed in cigar tobacco. Among the seven food sources, beetles reared in wheat flour showed the highest survival rate of 91.0+/-2.7%. Only 15% of the eggs laid in NOW bait developed to the adult stage. In the three food sources containing *Capsicum* spp. the survival rate ranged from 30% to 40%. The egg, larval, and pupal development times varied from 3 to 5, 38 to 92 and 4 to 18 d, respectively, among food sources. Body weight and adult longevity studies showed that the heavier adults also had the longest life span. Ovipositing female *L. serricorne* appear to discriminate among different food sources. Although *L. serricorne* laid eggs in all food sources evaluated, larval and pupal survival were lowest in NOW bait. Information on the biology and host use pattern of *L. serricorne* may help to explain how various stored commodities are affected by this species and may lead to develop appropriate pest management strategies for this insect pest.

Keywords: *Lasioderma serricorne*; Cigarette beetle; Development; Survival; Fecundity

Yunxia Ma, Tao Zhou, Yiguo Hong, Zaifeng Fan, Huaifang Li, Decreased level of ferredoxin I in Tobacco mosaic virus-infected tobacco is associated with development of the mosaic symptom, *Physiological and Molecular Plant Pathology*, Volume 72, Issues 1-3, January-March 2008, Pages 39-45, ISSN 0885-5765, DOI: 10.1016/j.pmp.2008.05.004.

(<http://www.sciencedirect.com/science/article/B6WPC-4SVV8GM-1/2/e2a965861679376017f85c3bcbe1a693>)

Abstract:

A decrease of ferredoxin I (Fd I) level was detected in the Tobacco mosaic virus (TMV)-infected tobacco (*Nicotiana tabacum*) leaves and the extent of decrease was uneven in the leaves showing mosaic symptoms. The Fd I level in the dark-green regions which contain less TMV was higher than that in the chlorotic regions of the same leaf, but was lower than that in the healthy control plants. The *N. benthamiana* plants with the Fed-1 gene being silenced by a chimeric viral vector showed chlorotic and yellowish phenotype in which H₂O₂ accumulated to a high level. In antisense Fed-1 transgenic *N. benthamiana* plants, the multiplication of TMV in infected plants and the accumulation of the viral coat protein in the chloroplasts were enhanced by the decrease of Fd I. Taken together, these results suggest that the unevenly decreased level of Fd I in TMV-infected tobacco leaves may contribute to the expression of chlorosis and mosaic symptoms.

Keywords: Ferredoxin I; Virus infection; Chlorosis induction; *Nicotiana tabacum*; *Nicotiana benthamiana*

Shiu-Cheung Lung, Andy Leung, Rainbow Kuang, Yu Wang, Priscilla Leung, Boon-Leong Lim, Phytase activity in tobacco (*Nicotiana tabacum*) root exudates is exhibited by a purple acid phosphatase, *Phytochemistry*, Volume 69, Issue 2, January 2008, Pages 365-373, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2007.06.036.

(<http://www.sciencedirect.com/science/article/B6TH7-4PRRH3-1/2/8f58824963fc1d16c2127d2661316cc2>)

Abstract:

Phytases are enzymes that catalyze liberation of inorganic phosphates from phytate, the major organic phosphorus in soil. Tobacco (*Nicotiana tabacum*) responds to phosphorus starvation with an increase in extracellular phytase activity. By a three-step purification scheme, a phosphatase with phytase activity was purified 486-fold from tobacco root exudates to a specific activity of 6,028 nkat mg⁻¹ and an overall yield of 3%. SDS-PAGE revealed a single polypeptide of 64 kDa, thus indicating apparent homogeneity of the final enzyme preparation. Gel filtration chromatography suggested that the enzyme was a ca. 56 kDa monomeric protein. De novo sequencing by tandem mass spectrometry resulted in a tryptic peptide sequence that shares high homology with several plant purple acid phosphatases. The identity of the enzyme was further confirmed by molybdate-inhibition assay and cDNA cloning. The purified enzyme exhibited pH and temperature optima at 5.0-5.5 and 45 [degree sign]C, respectively, and were found to have high affinities for both p-nitrophenyl phosphate (pNPP; K_m = 13.9 [μ]M) and phytate (K_m = 14.7 [μ]M), but a higher k_{cat} for pNPP (2,056 s⁻¹) than phytate (908 s⁻¹). Although a broad specificity of the enzyme was observed for a range of physiological substrates in soil, maximum activity was achieved using mononucleotides as substrates. We conclude that the phytase activity in tobacco root exudates is exhibited by a purple acid phosphatase and its catalytic properties are pertinent to its role in mobilizing organic P in soil.

Keywords: *Nicotiana tabacum*; Solanaceae; Phytase; Purple acid phosphatase; Phytate; Phosphorus; Root exudates; Tobacco

Lennart Wirthmueller, Yan Zhang, Jonathan D.G. Jones, Jane E. Parker, Nuclear Accumulation of the Arabidopsis Immune Receptor RPS4 Is Necessary for Triggering EDS1-Dependent Defense, *Current Biology*, Volume 17, Issue 23, 4 December 2007, Pages 2023-2029, ISSN 0960-9822, DOI: 10.1016/j.cub.2007.10.042.

(<http://www.sciencedirect.com/science/article/B6VRT-4R352VN-3/2/d13a9f9b40c5e323f8f315c2c61fa728>)

Abstract: Summary

Recognition of specific pathogen molecules inside the cell by nucleotide-binding domain and leucine-rich repeat (NB-LRR) receptors constitutes an important layer of innate immunity in plants [1]. Receptor activation triggers host cellular reprogramming involving transcriptional potentiation of basal defenses and localized programmed cell death [1], [2] and [3]. The sites and modes of action of NB-LRR receptors are, however, poorly understood. Arabidopsis Toll/Interleukin-1 (TIR) type NB-LRR receptor RPS4 recognizes the bacterial type III effector AvrRps4 [4]. We show that epitope-tagged RPS4 expressed under its native regulatory sequences distributes between endomembranes and nuclei in healthy and AvrRps4-triggered tissues. RPS4 accumulation in the nucleus, mediated by a bipartite nuclear localization sequence (NLS) at its C terminus, is necessary for triggering immunity through authentic activation by AvrRps4 in Arabidopsis or as an effector-independent 'deregulated' receptor in tobacco. A strikingly conserved feature of TIR-NB-LRR receptors is their recruitment of the nucleocytoplasmic basal-defense regulator EDS1 in resistance to diverse pathogens [5] and [6]. We find that EDS1 is an indispensable component of RPS4 signaling and that it functions downstream of RPS4 activation but upstream of RPS4-mediated transcriptional reprogramming in the nucleus.

Keywords: SIGNALING

Julia Koehl, Alma Djulic, Veronika Kirner, Tach Thao Nguyen, Ingrid Heiser, Ethylene is required for elicitor-induced oxidative burst but not for cell death induction in tobacco cell suspension cultures, *Journal of Plant Physiology*, Volume 164, Issue 12, 3 December 2007, Pages 1555-1563, ISSN 0176-1617, DOI: 10.1016/j.jplph.2007.05.012.

(<http://www.sciencedirect.com/science/article/B7GJ7-4PTF972-2/2/a662ac386b9f10dc5e16924157c64515>)

Abstract: Summary

The signal compound ethylene and its relationships with oxidative burst and cell death were analyzed in cultured tobacco cells treated with the proteinaceous elicitor quercinin. Quercinin belongs to the protein family of elicitors and was isolated from the soil-born oak pathogen *Phytophthora quercina*. It was shown to induce a dose-dependent oxidative burst in tobacco cell culture in concentrations from 0.05 to 0.5 nM, and subsequently, cell death. The characteristics of quercinin-induced cell death included both membrane damage and DNA fragmentation in tobacco cell culture.

At higher quercinin concentrations (2 nM), H₂O₂ formation and ethylene biosynthesis were inhibited. Ethylene at low concentrations proved to be necessary for induction and maintenance of H₂O₂ production in tobacco cells treated with quercinin. It was demonstrated that external addition of inhibitors of ethylene biosynthesis such as [alpha]-amino-oxy-acetic acid (AOA) and CoCl₂ also decreased or even inhibited the quercinin-induced oxidative burst, but did not influence cell death induction. These results demonstrate evidence for a requirement of the plant hormone ethylene for the onset of the quercinin-induced oxidative burst.

Keywords: Elicitor; Ethylene; Oxidative burst; Plant cell death

Tie-zhao YANG, Li-ming LU, Wei XIA, Jin-hua FAN, Characteristics of Potassium-Enriched, Flue-Cured Tobacco Genotype in Potassium Absorption, Accumulation, and In-Ward Potassium Currents of Root Cortex, *Agricultural Sciences in China*, Volume 6, Issue 12, December 2007, Pages 1479-1486, ISSN 1671-2927, DOI: 10.1016/S1671-2927(08)60011-5.

(<http://www.sciencedirect.com/science/article/B82XG-4RJBPV7-C/2/0d3f3be92de5f93b918251c7b4f330f2>)

Abstract:

This study was to investigate the main traits of potassium-enriched, flue-cured tobacco genotypes related to potassium absorption, accumulation, and in-ward potassium currents of the root cortex. Hydroponic methods, K⁺-depletion methods, and patch-clamp, whole-cell recordings were conducted to study the accumulation of dry matter and potassium in different organs, and to

measure potassium absorption and dynamic and in-ward potassium currents in potassium-enriched, flue-cured tobacco genotypes. The average dry weights of leaves and whole plant of potassium-enriched, flue-cured tobacco genotype ND202 were 10.20, and 14.85 g, respectively, higher than JYH (8.50 and 13.11 g, respectively) and NC2326 (8.39 and 12.72 g, respectively), when potassium concentration in the solution ranged from 0.1 to 50 mmol L⁻¹. Potassium accumulation in the leaves of ND202 was 18.6% higher than JYH and 34% higher than NC2326 when potassium concentration in the solution was superior to 0.5 mmol L⁻¹. The V_{max} (the maximum velocity) of ND202 was 118.11 [μ]mol FW g⁻¹ h⁻¹, obviously higher than that of JYH (58.87 [μ]mol FW g⁻¹ h⁻¹) and NC2326 (64.40 [μ]mol FW g⁻¹ h⁻¹). In the in-ward potassium currents, the absolute value of current density (pA/pF) of ND202 was 60, higher than that of JYH (50) and NC2326 (40). Potassium concentration in leaves, V_{max}, and in-ward potassium currents, could be used to screen potassium-enriched, flue-cured tobacco genotypes.

Keywords: flue-cured tobacco; potassium-enriched genotype; potassium absorption; potassium content; in-ward potassium currents

Sijun Dong, Akio Inoue, Yun Zhu, Masao Tanji, Ryoiti Kiyama, Activation of rapid signaling pathways and the subsequent transcriptional regulation for the proliferation of breast cancer MCF-7 cells by the treatment with an extract of *Glycyrrhiza glabra* root, Food and Chemical Toxicology, Volume 45, Issue 12, December 2007, Pages 2470-2478, ISSN 0278-6915, DOI: 10.1016/j.fct.2007.05.031.

(<http://www.sciencedirect.com/science/article/B6T6P-4P1X7J2-1/2/41a44e01e4a30c21d6952b689aed40bf>)

Abstract:

Glycyrrhiza glabra root is one of the common traditional Chinese medicines and used as flavoring and sweetening agents for tobaccos, chewing gums, candies, toothpaste and beverages. While glycyrrhizin is one of the main components in the extract of *G. glabra* root and has been characterized, the other components have not been well characterized. The mechanism of growth activation of breast cancer MCF-7 cells, including the activation of Erk1/2 and Akt, and the transcriptional regulation of estrogen-responsive genes, was examined by means of sulforhodamine B, luciferase reporter gene, real-time RT-PCR and Western blotting assays after the induction of the cells with the extract of *G. glabra* root. The extract has similar activity to that induced by 17[β]-estradiol (E2), although glycyrrhizin did not show such an activity. Moreover, the estrogen receptor [α]-dependent neurite outgrowth induced by the extract was similar to that by E2, whereas glycyrrhizin had no effect. Furthermore, the expression profile examined by cDNA microarray assay using a set of 120 estrogen-responsive genes, which were related to proliferation, transcription, transport, enzymes and signaling, showed a statistically significant correlation ($R = 0.47$, $P < 0.0001$) between the profiles for E2 and the extract. However, the expression profile for glycyrrhizin was different from that of the extract and E2. The results indicate that rapid signaling pathways, including Erk1/2 and Akt, and the subsequent transcriptional regulation are involved in the proliferation of MCF-7 cells induced by the extract of *G. glabra* root. Furthermore, the extract had estrogenic activity and a distinguishable profile of gene expression, suggesting the presence of potentially useful components other than glycyrrhizin in *G. glabra* root for hormone and anti-cancer therapies.

Keywords: *Glycyrrhiza glabra* root; Glycyrrhizin; Estrogenic activity; Signaling; Erk1/2; Akt

Gang Ren, Rosanne A. Healy, Harry T. Horner, Martha G. James, Robert W. Thornburg, Expression of starch metabolic genes in the developing nectaries of ornamental tobacco plants, Plant Science, Volume 173, Issue 6, December 2007, Pages 621-637, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2007.08.012.

(<http://www.sciencedirect.com/science/article/B6TBH-4PKXBKF-1/2/46f25c7f1964e8ac165f997ae5d42811>)

Abstract:

To understand the mechanisms that control starch metabolism in the nectary, this study combined TEM analysis of floral nectaries in ornamental tobacco with gene expression analysis of a number of starch metabolism genes over the time course of nectary development. Using TEM, we observed an increase in amyloplasts accumulating from early developmental stages until stage 9 (S9), and their subsequent disappearance prior to anthesis (S12). Nectary starch gene expression analysis was initiated by isolating 18 nectary-expressed cDNAs encoding different starch metabolic enzymes. Strong sucrose synthase gene expression was observed early in nectary development at the mRNA and protein levels. ADP-glucose pyrophosphorylase (AGPase) mRNA levels, protein levels, and enzymatic activities also were high at early stages of nectary development then declined as the nectary matured. Starch synthase 3 (SS3), the most strongly expressed SS, followed a similar expression pattern. Quantitative RT-PCR revealed three different regulatory patterns of gene expression, an anabolic pattern, a catabolic pattern and a constitutive pattern. The anabolic genes, including AGPS and SS3, were expressed early in development (S2 and S6), and down regulated after S9. In contrast, catabolic gene expression, including ISA1, AMY and BMY, was detected at S9 and later but not at early stages. The third class of genes was expressed throughout nectary development. The switch from starch anabolism to catabolism seems to be an important key to normal nectary development and function. These combined results provide a better understanding of the importance of starch metabolism to overall nectary activity.

Keywords: Nicotiana; Ornamental tobacco; Nectar; Nectary; Starch metabolism gene expression

Hailin Tang, Huarong Li, Soi Meng Lei, Robert L. Harrison, Bryony C. Bonning, Tissue specificity of a baculovirus-expressed, basement membrane-degrading protease in larvae of *Heliothis virescens*, *Tissue and Cell*, Volume 39, Issue 6, December 2007, Pages 431-443, ISSN 0040-8166, DOI: 10.1016/j.tice.2007.08.003.

(<http://www.sciencedirect.com/science/article/B6WXF-4R05BJ8-1/2/26879401371c48b4905d5a55bcba04fe>)

Abstract:

ScathL is a cathepsin L-like cysteine protease from the flesh fly, *Sarcophaga peregrina*, which digests components of the basement membrane during insect metamorphosis. A recombinant baculovirus (AcMLF9.ScathL) expressing ScathL kills larvae of the tobacco budworm *Heliothis virescens* significantly faster than the wild type virus and triggers melanization and tissue fragmentation shortly before death. The tissue fragmentation was assumed to be a direct consequence of basement membrane degradation by ScathL. The goal of this study was to investigate the tissue specificity of ScathL when expressed by AcMLF9.ScathL using light, transmission and scanning electron microscopy. Baculovirus expression of ScathL resulted in damage to the basement membrane overlying the midgut, fat body and muscle fibers in larvae infected with AcMLF9.ScathL, but not in larvae infected with the control virus AcMLF9.ScathL.C146A or wild type virus AcMNPV C6. Injection of recombinant ScathL and high levels of baculovirus-mediated expression of ScathL resulted in complete loss of the gut. Extensive damage to the basement membrane mediated by ScathL likely resulted in loss of viability of the underlying tissue and subsequent death of the insect. These results confirm the conclusion of an earlier study (Philip, J.M.D., Fitches, E., Harrison, R.L., Bonning, B.C., Gatehouse, J.A., 2007. Characterisation of functional and insecticidal properties of a recombinant cathepsin L-like proteinase from flesh fly (*Sarcophaga peregrina*), which plays a role in differentiation of imaginal discs. *Insect Biochem. Mol. Biol.* 37, 589-600) of the remarkable specificity of this protease.

Keywords: *Heliothis virescens*; Basement membrane; *Autographa californica* multiple nucleopolyhedrovirus; Cathepsin L; Ultrastructure

Remy Merret, Jean-Roger Cirioni, Thomas J. Bach, Andrea Hemmerlin, A serine involved in actin-dependent subcellular localization of a stress-induced tobacco BY-2 hydroxymethylglutaryl-CoA reductase isoform, *FEBS Letters*, Volume 581, Issue 27, 13 November 2007, Pages 5295-5299, ISSN 0014-5793, DOI: 10.1016/j.febslet.2007.10.023.

(<http://www.sciencedirect.com/science/article/B6T36-4PYPRMR-7/2/862b186bc474d7dce4c2297c6908b0f6>)

Abstract:

3-Hydroxy-3-methylglutaryl-CoA reductase (HMGR) is unique in the first part of the cytoplasmic isoprenoid pathway, as it contains a membrane domain that includes ER-specific retention motifs. When fused to GFP, this domain targets two tobacco BY-2 HMGR isoforms differentially. While the first isoform is ER-localized, a second stress-induced one forms globular structures connected by tubular structures. A serine positioned upstream of the ER retention motif seems to be implicated in this specific subcellular localization. Surprisingly, these structures are closely connected to F-actin, and their intactness is dependent upon the integrity of the filaments or the action of a calmodulin antagonist.

Keywords: Actin; Endoplasmic reticulum; Membrane domain; Plant HMG-CoA reductase; Serine

Md. Anamul Hoque, Mst. Nasrin Akhter Banu, Eiji Okuma, Katsumi Amako, Yoshimasa Nakamura, Yasuaki Shimoishi, Yoshiyuki Murata, Exogenous proline and glycinebetaine increase NaCl-induced ascorbate-glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells, *Journal of Plant Physiology*, Volume 164, Issue 11, 9 November 2007, Pages 1457-1468, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.10.004.

(<http://www.sciencedirect.com/science/article/B7GJ7-4MV19M3-7/2/80fcc709337bcc7a22cb322da46a5c1e>)

Abstract: Summary

Up-regulation of the antioxidant system provides protection against NaCl-induced oxidative damage in plants. Antioxidants and activity of enzymes involved in the ascorbate-glutathione (ASC-GSH) cycle in tobacco Bright Yellow-2 (BY-2) were investigated to assess the antioxidant protection offered by exogenous proline and glycinebetaine (betaine from now on) against salt stress using cells grown in suspension culture. Reduced ascorbate (ASC) was detected in BY-2 cells but dehydroascorbate (DHA) was not. Large quantities of a reduced form of glutathione (GSH) and smaller quantities of an oxidized form of glutathione (GSSG) were detected in BY-2 cells. Salt stress significantly reduced the contents of ASC and GSH as well as activities of ASC-GSH cycle enzymes such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). Exogenous proline or betaine increased the activities of all enzymes except MDHAR involved in NaCl-induced ASC-GSH cycle. Levels of ASC and GSH in BY-2 cells under salt stress were lower in the presence of proline or betaine than in the absence of proline or betaine whereas there was no difference in redox status. Proline proved more effective than betaine in maintaining the activity of enzymes involved in NaCl-induced ASC-GSH cycle. Neither proline nor betaine had any direct protective effect on NaCl-induced enzyme activity involved in the antioxidant system; however, both improved salt tolerance by increasing enzyme activity. The present study, together with our earlier findings [Hoque MA, Okuma E, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y. Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. *J Plant Physiol* 2006;164:553-61.], suggests that proline offered greater protection against salt stress than betaine did because proline was more effective in increasing the activity of enzymes involved in the antioxidant system.

Keywords: Ascorbate-glutathione cycle; Glycinebetaine; Proline; Reactive oxygen species; Salt stress

Angel Medina, Rufino Mateo, Francisco M. Valle-Algarra, Eva M. Mateo, Misericordia Jimenez, Effect of carbendazim and physicochemical factors on the growth and ochratoxin A production of *Aspergillus carbonarius* isolated from grapes, *International Journal of Food Microbiology*, Volume 119, Issue 3, 1 November 2007, Pages 230-235, ISSN 0168-1605, DOI: 10.1016/j.ijfoodmicro.2007.07.053.

(<http://www.sciencedirect.com/science/article/B6T7K-4PC8RBR-3/2/ad753c76f147a01b56fca2ed9d620584>)

Abstract:

Carbendazim is a systemic fungicide that is commonly used on several crops (tobacco, fruit, vegetables, cereals, etc.). This fungicide is used to control fungal infections in vineyards. It is indicated against *Botrytis cinerea*, *Uncinula necator*, *Plasmopara viticola* and other fungi and can be used either alone or coupled with other fungicides. However, there is a lack of in-depth studies to evaluate its effectiveness against growth of *Aspergillus carbonarius* isolated from grapes and OTA production. A medium based on red grape juice was used in this study. Preliminary studies were performed at 0.98 aw and 25 [degree sign]C using carbendazim concentrations over a wide range (1-2000 ng/ml medium) to control both growth of a strain of *A. carbonarius* isolated from grape and its ability to produce OTA. As the lag phase increased considerably at levels > 1000 ng/ml of medium, detailed studies were carried out in the range 50-450 ng/ml of medium at 0.98-0.94 aw and 20-28 [degree sign]C. Statistical analysis (multifactor ANOVA) of the data revealed that the three factors assayed and the interactions aw-carbendazim concentration and aw-temperature had significant effects on lag phase duration. The highest lag-times were observed at 0.94 aw, 20 [degree sign]C, and with 450 ng carbendazim/ml. The three factors also had significant effects of the growth rate and there was an interaction between aw and temperature. The growth rate of *A. carbonarius* in these cultures is favoured by high water availability and relatively high temperatures. However, addition of carbendazim at the assayed levels did not significantly influenced fungal growth rate. Accumulation of OTA was studied as a function of four factors (the three previously considered, and time). All factors had significant effects on the accumulation of OTA. There were also two significant interactions (aw-temperature and temperature-time). On the basis of the results obtained, carbendazim does not increase the lag phase of *A. carbonarius* except at relatively low aw and temperatures. It does not substantially decrease fungal growth rate once growth is apparent but it appears to cause an increase in OTA accumulation in the medium at the doses assayed. Carbendazim, which is widely used against fungal infections in grape, can positively influence OTA production by *A. carbonarius* in field, which can increase OTA content in grape juices and wines.

Keywords: *Aspergillus carbonarius*; Carbendazim; Ochratoxin A; Mycotoxins; Fungal growth

Laurence V. Bindschedler, Jutta Tuerck, Martin Maunders, Katia Ruel, Michel Petit-Conil, Saida Danoun, Alain-Michel Boudet, Jean-Paul Joseleau, G. Paul Bolwell, Modification of hemicellulose content by antisense down-regulation of UDP-glucuronate decarboxylase in tobacco and its consequences for cellulose extractability, *Phytochemistry*, Volume 68, Issue 21, November 2007, Pages 2635-2648, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2007.08.029.

(<http://www.sciencedirect.com/science/article/B6TH7-4R00FN2-4/2/3dd09a146c798327eb81549696a35a82>)

Abstract:

Extractability and recovery of cellulose from cell walls influences many industrial processes and also the utilisation of biomass for energy purposes. The utility of genetic manipulation of lignin has proven potential for optimising such processes and is also advantageous for the environment. Hemicelluloses, particularly secondary wall xylans, also influence the extractability of cellulose. UDP-glucuronate decarboxylase produces UDP-xylose, the precursor for xylans and the effect of its down-regulation on cell wall structure and cellulose extractability in transgenic tobacco has been investigated. Since there are a number of potential UDP-glucuronate decarboxylase genes, a

490 bp sequence of high similarity between members of the family, was chosen for general alteration of the expression of the gene family. Sense and antisense transgenic lines were analysed for enzyme activity using a modified and optimised electrophoretic assay, for enzyme levels by western blotting and for secondary cell wall composition. Some of the down-regulated antisense plants showed high glucose to xylose ratios in xylem walls due to less xylose-containing polymers, while arabinose and uronic acid contents, which could also have been affected by any change in UDP-xylose provision, were unchanged. The overall morphology and stem lignin content of the modified lines remained little changed compared with wild-type. However, there were some changes in vascular organisation and reduction of xylans in the secondary walls was confirmed by immunocytochemistry. Pulping analysis showed a decreased pulp yield and a higher Kappa number in some lines compared with controls, indicating that they were less delignified, although the level of residual alkali was reduced. Such traits probably indicate that lignin was less available for removal in a reduced background of xylans. However, the viscosity was higher in most antisense lines, meaning that the cellulose was less broken-down during the pulping process. This is one of the first studies of a directed manipulation of hemicellulose content on cellulose extractability and shows both positive and negative outcomes.

Keywords: *Nicotiana tabacum*; Solanaceae; Tobacco; Cell wall; Hemicellulose; UDP-glucuronate decarboxylase; Antisense

Suvi T. Hakkinen, Sofie Tilleman, Agnieszka Swiatek, Valerie De Sutter, Heiko Rischer, Isabelle Vanhoutte, Harry Van Onckelen, Pierre Hilson, Dirk Inze, Kirsi-Marja Oksman-Caldentey, Alain Goossens, Functional characterisation of genes involved in pyridine alkaloid biosynthesis in tobacco, *Phytochemistry*, Volume 68, Issues 22-24, Highlights in the Evolution of Phytochemistry: 50 Years of the Phytochemical Society of Europe, November-December 2007, Pages 2773-2785, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2007.09.010.

(<http://www.sciencedirect.com/science/article/B6TH7-4R4DG43-1/2/94eea8cfd7ded0ec42dacc737e0cd3c1>)

Abstract:

Although secondary metabolism in *Nicotiana tabacum* (L.) (tobacco) is rather well studied, many molecular aspects of the biosynthetic pathways and their regulation remain to be disclosed, even for prominent compounds such as nicotine and other pyridine alkaloids. To identify players in tobacco pyridine alkaloid biosynthesis a functional screen was performed, starting from a tobacco gene collection established previously by means of combined transcript profiling and metabolite analysis. First, full-length cDNA clones were isolated for 34 genes, corresponding to tobacco transcript tag sequences putatively associated with pyridine alkaloid metabolism. Full-length open reading frames were transferred to pCaMV35S-steered overexpression vectors. The effects of plant transformation with these expression cassettes on the accumulation of nicotine and other pyridine alkaloids were assessed in transgenic tobacco Bright-Yellow 2 (BY-2) cell suspensions and hairy root cultures. This screen identified potential catalysers of tobacco pyridine metabolism, amongst which a lysine decarboxylase-like gene and a GH3-like enzyme. Overexpression of the GH3-like enzyme, presumably involved in auxin homeostasis and designated NtNEG1 (*Nicotiana tabacum* Nicotine-Enhancing GH3 enzyme 1), increased nicotine levels in BY-2 hairy roots significantly. This study shows how functional genomics-based identification of genes potentially involved in biosynthetic pathways followed by systematic functional assays in plant cells can be used at large-scale to decipher plant metabolic networks at the molecular level.

Keywords: *Nicotiana tabacum*; Solanaceae; Tobacco; Functional genomics; Pyridine alkaloids; Nicotine; Lysine decarboxylase; GH3-like enzyme; Jasmonate; Auxin conjugates; cDNA-AFLP

J. Gubis, R. Vankova, V. Cervena, M. Dragunova, M. Hudcovicova, H. Lichtnerova, T. Dokupil, Z. Jurekova, Transformed tobacco plants with increased tolerance to drought, *South African Journal*

of Botany, Volume 73, Issue 4, November 2007, Pages 505-511, ISSN 0254-6299, DOI: 10.1016/j.sajb.2007.03.011.

(<http://www.sciencedirect.com/science/article/B7XN9-4NP9KP3-2/2/ccccf13a04abcc9feed25445f1ad3405d>)

Abstract:

P5CSF129A cDNA and the nptII marker gene were used for tobacco (*Nicotiana tabacum* L. cv. Bel B and cv. M51) transformation via *Agrobacterium tumefaciens* strain LBA4404. Twenty transformed tobacco plants were obtained after transformation of leaf discs. Presence of the transgene was confirmed by polymerase chain reaction (PCR) analysis. Physiological responses to water stress were compared in transgenic and wild-type tobacco plants. Transgenic plants of both cultivars accumulated high levels of free proline. They did not exhibit dry mass relocation or chlorophyll content reduction. Neither precocious senescence, nor leaf necrosis or morphological changes were observed in control and stress conditions (RWC decrease by 7-8%). Transgenic plants with elevated accumulation of osmoprotectants seem to be better adapted to water stress, providing a perspective for future research of stress effects that have a principle role in the functional activity of plants. This study confirmed P5CSF129A to be a candidate gene in crop engineering for enhanced water stress tolerance.

Keywords: *Nicotiana tabacum* L.; Pigment; Proline; Relative water content; Stress

Kris De Jonghe, Dirk Hermans, Monica Hofte, Efficacy of alcohol alkoxylate surfactants differing in the molecular structure of the hydrophilic portion to control *Phytophthora nicotianae* in tomato substrate culture, *Crop Protection*, Volume 26, Issue 10, October 2007, Pages 1524-1531, ISSN 0261-2194, DOI: 10.1016/j.cropro.2007.01.001.

(<http://www.sciencedirect.com/science/article/B6T5T-4N55TB4-1/2/d12b3e233078450fc8308e5fdd8ca3c0>)

Abstract:

Phytophthora nicotianae is a common and destructive pathogen of numerous ornamental, agronomic and horticultural crops such as tomato, tobacco and citrus. Three monobranch C13 alcohol alkoxylate non-ionic surfactants were evaluated for in vitro inhibitory activity against the different asexual structures of *P. nicotianae*. The same surfactants, labelled MBA1301, MBA1303 and MBA1306, were tested for their in vivo control capacity against *P. nicotianae* root rot of tomato (*Lycopersicon esculentum*) under glasshouse conditions. MBA1301, MBA1303 and MBA1306 differ in the molecular structure of the hydrophilic portion. The molecular weight of MBA1301 is comparable to that of MBA1303 and is eight times lower than that of MBA1306. The main in vitro activity for MBA1301 and MBA1303 was a direct lytic effect on the zoospores. Zoospore lysis was already observed in the presence of 1 [μ]g ml⁻¹ of these two surfactants and almost no zoospores survived an addition of 5 [μ]g ml⁻¹ surfactant. In addition, MBA1301 and MBA1303 reduced sporangia formation at a concentration of 5 [μ]g ml⁻¹. Both surfactants only affect mycelium growth at concentrations as high as 100 [μ]g ml⁻¹. MBA1306 did not show any effect on sporangia formation, zoospore release and mycelium growth of *P. nicotianae* at a concentration 10 times that of the other two surfactants. A good in vivo control of *P. nicotianae* on tomato in substrate culture was obtained for MBA1301 and MBA1303 whereas the control capacity of MBA1306 was significantly lower. The results of this research indicate that non-ionic alcohol alkoxylate surfactants can be used to control tomato root rot caused by *P. nicotianae* in substrate culture. In addition, the size rather than the arrangement of the polymers in the hydrophilic portion of the surfactant molecule determines the efficacy to control tomato root rot caused by *P. nicotianae* in substrate culture.

Keywords: *Phytophthora nicotianae*; Zoosporic pathogens; Non-ionic surfactants; Tomato root rot; Substrate culture

Gregory M. Polzin, Stephen B. Stanfill, Candace R. Brown, David L. Ashley, Clifford H. Watson, Determination of eugenol, anethole, and coumarin in the mainstream cigarette smoke of Indonesian clove cigarettes, *Food and Chemical Toxicology*, Volume 45, Issue 10, October 2007, Pages 1948-1953, ISSN 0278-6915, DOI: 10.1016/j.fct.2007.04.012.

(<http://www.sciencedirect.com/science/article/B6T6P-4NMWRGG-1/2/afb232dc7b4a22964c6e67a27174390a>)

Abstract:

Indonesian clove cigarettes (kreteks), typically have the appearance of a conventional domestic cigarette. The unique aspects of kreteks are that in addition to tobacco they contain dried clove buds (15-40%, by wt.), and are flavored with a proprietary 'sauce'. Whereas the clove buds contribute to generating high levels of eugenol in the smoke, the 'sauce' may also contribute other potentially harmful constituents in addition to those associated with tobacco use. We measured levels of eugenol, trans-anethole (anethole), and coumarin in smoke from 33 brands of clove-flavored cigarettes (filtered and unfiltered) from five kretek manufacturers. In order to provide information for evaluating the delivery of these compounds under standard smoking conditions, a quantification method was developed for their measurement in mainstream cigarette smoke. The method allowed collection of mainstream cigarette smoke particulate matter on a Cambridge filter pad, extraction with methanol, sampling by automated headspace solid-phase microextraction, and subsequent analysis using gas chromatography/mass spectrometry. The presence of these compounds was confirmed in the smoke of kreteks using mass spectral library matching, high-resolution mass spectrometry (± 0.0002 amu), and agreement with a relative retention time index, and native standards. We found that when kreteks were smoked according to standardized machine smoke parameters as specified by the International Standards Organization, all 33 clove brands contained levels of eugenol ranging from 2490 to 37,900 [μ g/cigarette] ([μ g/cig). Anethole was detected in smoke from 13 brands at levels of 22.8-1030 [μ g/cig], and coumarin was detected in 19 brands at levels ranging from 9.2 to 215 [μ g/cig]. These detected levels are significantly higher than the levels found in commercial cigarette brands available in the United States.

Keywords: Kretek; Clove; Cigarette; Eugenol; Anethole; Coumarin

Ayşe Eren Putun, Eylem Onal, Basak Burcu Uzun, Nurgül Ozbay, Comparison between the 'slow' and 'fast' pyrolysis of tobacco residue, *Industrial Crops and Products*, Volume 26, Issue 3, October 2007, Pages 307-314, ISSN 0926-6690, DOI: 10.1016/j.indcrop.2007.03.011.

(<http://www.sciencedirect.com/science/article/B6T77-4NP9KCM-3/2/114709d52a64a1f997d7c2730677e41b>)

Abstract:

In this study, the trends in yields and product compositions of slow pyrolysis and fast pyrolysis were determined and compared with each other. Slow pyrolysis of tobacco residues with particles of between 0.425 and 0.850 mm were conducted in a fixed-bed tubular reactor with a heating rate of 7 [$^{\circ}$ C min⁻¹] for various pyrolysis temperatures (400, 500, 550 and 700 [$^{\circ}$ C]) and various nitrogen flow rates (50, 100, 200 and 400 cm³ min⁻¹). The maximum oil yield was 27% at the pyrolysis temperature of 550 [$^{\circ}$ C] with the sweeping gas flow rate of 100 cm³ min⁻¹. Increasing heating rate up to 300 [$^{\circ}$ C min⁻¹] caused 10% increase of liquid yields. Chemical compositions of the oils were investigated by using chromatographic and spectroscopic techniques such as column chromatography, Fourier transform infrared spectroscopy (FT-IR), gas chromatography (GC), ¹H NMR and elemental analysis. Bio-oil obtained from fast pyrolysis has lower C distribution and higher H/C ratio than bio-oil obtained from slow pyrolysis. Consequently, the chemical characterization has shown that the obtained bio-oils could be used as conventional fuels.

Keywords: Pyrolysis; Tobacco residue; Bio-oil

Yang Wang, Haobo Jiang, Reconstitution of a branch of the *Manduca sexta* prophenoloxidase activation cascade in vitro: Snake-like hemolymph proteinase 21 (HP21) cleaved by HP14 activates prophenoloxidase-activating proteinase-2 precursor, *Insect Biochemistry and Molecular Biology*, Volume 37, Issue 10, October 2007, Pages 1015-1025, ISSN 0965-1748, DOI: 10.1016/j.ibmb.2007.05.013.

(<http://www.sciencedirect.com/science/article/B6T79-4NVH7RR-5/2/8bbd0711c76dad3117b46d18a55f16fc>)

Abstract:

Upon wounding or infection, a serine proteinase cascade in insect hemolymph leads to prophenoloxidase (proPO) activation and melanization, a defense response against invading microbes. In the tobacco hornworm *Manduca sexta*, this response is initiated via hemolymph proteinase 14 (HP14), a mosaic protein that interacts with bacterial peptidoglycan or fungal [β]-1,3-glucan to autoactivate. In this paper, we report the expression, purification, and functional analysis of *M. sexta* HP21 precursor, an HP14 substrate similar to *Drosophila* snake. The recombinant proHP21 is a 51.1 kDa glycoprotein with an amino-terminal clip domain, a linker region, and a carboxyl-terminal serine proteinase domain. HP14, generated by incubating proHP14 with [β]-1,3-glucan and [β]-1,3-glucan recognition protein-2, activated proHP21 by limited proteolysis between Leu152 and Ile153. Active HP21 formed an SDS-stable complex with *M. sexta* serpin-4, a physiological regulator of the proPO activation system. We determined the P1 site of serpin-4 to be Arg355 and, thus, confirmed our prediction that HP21 has trypsin-like specificity. After active HP21 was added to the plasma, there was a major increase in PO activity. HP21 cleaved proPO activating proteinase-2 precursor (proPAP-2) after Lys153 and generated an amidase activity, which activated proPO in the presence of serine proteinase homolog-1 and 2. In summary, we have discovered and reconstituted a branch of the proPO activation cascade in vitro: [β]-1,3-glucan recognition--proHP14 autoactivation--proHP21 cleavage--PAP-2 generation--proPO activation--melanin formation.

Keywords: Clip domain; Insect immunity; Melanization; Phenoloxidase; Proteinase cascade

Zhudong Liu, Peiyu Gong, Kujun Wu, Wei Wei, Jianghua Sun, Dianmo Li, Effects of larval host plants on over-wintering preparedness and survival of the cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), *Journal of Insect Physiology*, Volume 53, Issue 10, October 2007, Pages 1016-1026, ISSN 0022-1910, DOI: 10.1016/j.jinsphys.2007.05.005.

(<http://www.sciencedirect.com/science/article/B6T3F-4NS2J7D-1/2/1c93a0a129583f4eade838a00a3c4a35>)

Abstract:

Laboratory colonies of cotton bollworm larvae, *Helicoverpa armigera*, kept at 20 [degree sign]C under a photoperiod of L:D=10:14 were fed on five host plants (cotton, corn, kidney bean, tobacco and tomato) and an artificial diet (control) to determine the effects of larval host quality on survival and pupal over-wintering preparedness. A separate experiment showed that diapausing pupae weighed more and contained greater nutrient stores than did non-diapausing pupae. Diapausing pupae reared on different host plants showed significant differences in terms of over-wintering reserve storage, and degree of cold-hardiness (extent of low-molecular-weight substances and SCPs), and survivorship. The more nutrients the host plant had, the more the pupae weighed and the higher the levels of total lipids and glycogen. Body water content was also significantly affected by larval food quality. The mean pupal super-cooling capacities varied significantly from -16.7 to -18.9 [degree sign]C according to host plants the larvae feed on, and these significantly related to water content, pupal weight, lipid and glycogen content, and the levels of glycerol. Levels of trehalose, glycerol, and inositol, which were mainly low-molecular-weight substances, showed no significant differences among different host plants, except for trehalose. Pupal mortality varied from 39.7% on corn to 3.3% on the artificial diet, which was significantly related to pupal weight,

total lipid content, trehalose levels, and super-cooling points. These results suggest that larval food quality can affect survival and influence the over-wintering preparedness of the cotton bollworm.

Keywords: *Helicoverpa armigera*; Host plants; Reserve storage; Low molecular sugars and sugar-alcohols; Over-wintering preparedness; Survival

Yupyn Chintapakorn, John D. Hamill, Antisense-mediated reduction in ADC activity causes minor alterations in the alkaloid profile of cultured hairy roots and regenerated transgenic plants of *Nicotiana tabacum*, *Phytochemistry*, Volume 68, Issue 19, Reports on Structure Elucidation, October 2007, Pages 2465-2479, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2007.05.025.

(<http://www.sciencedirect.com/science/article/B6TH7-4P47V5W-1/2/8c11c44c9e35db682698f10eeb36e081>)

Abstract:

In species of the genus *Nicotiana*, as in most plants, the important polyamine precursor putrescine can be derived from the amino acids ornithine and/or arginine via the activity of ornithine decarboxylase (ODC) and/or arginine decarboxylase (ADC), respectively. *Nicotiana* species also utilize putrescine to provide the pyrrolidine ring of the defensive alkaloid nicotine and its derivatives. Previous biochemical studies, involving callus tissues cultured *in vitro*, suggested that the ADC-mediated route to putrescine is used preferentially to provide the putrescine that is utilized for nicotine synthesis in *N. tabacum*. To ascertain if this is the case in *N. tabacum* plants, where nicotine synthesis takes place exclusively in roots, we used an antisense approach to diminish ADC activity in transformed roots which were cultured *in vitro*. Several independent lines were recovered possessing markedly reduced levels of ADC transcript and ADC activity compared to controls. Transcript levels of other genes in this general area of metabolism, including ODC, were not altered as a result of the antisense-mediated downregulation of ADC. Concentrations of nicotine were comparable in antisense-ADC and control hairy root lines throughout most of their respective culture cycles, except at the latter stages of growth when the nicotine content of antisense-ADC hairy root lines was observed to be ~20% lower than in controls. Levels of anatabine, the second most abundant alkaloid typically found in *N. tabacum*, which is not derived from putrescine, were slightly elevated in two antisense-ADC hairy root lines at the latter stages of their culture cycles compared to controls. Comparison of alkaloid levels in leaves of transgenic plants that were regenerated from separate antisense-ADC and control transformed root lines indicated that the former possessed slightly elevated levels of anatabine but did not contain average levels of leaf nicotine that were different from that of controls. Our overall conclusion is that the ADC mediated route to putrescine plays a role, but is not of prime importance, in providing the pyrrolidine ring which is used for nicotine synthesis in cultured hairy roots of *N. tabacum* and in roots of healthy greenhouse-grown plants.

Keywords: Alkaloid; Arginine decarboxylase; Anatabine; Antisense; Hairy roots; Metabolism; *Nicotiana*; Nicotine; Solanaceae; Tobacco

Hirofumi Harashima, Ko Kato, Atsuhiko Shinmyo, Masami Sekine, Auxin is required for the assembly of A-type cyclin-dependent kinase complexes in tobacco cell suspension culture, *Journal of Plant Physiology*, Volume 164, Issue 9, 5 September 2007, Pages 1103-1112, ISSN 0176-1617, DOI: 10.1016/j.jplph.2007.01.005.

(<http://www.sciencedirect.com/science/article/B7GJ7-4N7YFFG-3/2/db21c3778c80fff5d31376eb7275956f>)

Abstract: Summary

Although activation of A-type cyclin-dependent kinase (CDKA) is required for plant cell division, little is known about how CDKA is activated before commitment to cell division. Here, we show that auxin is required for the formation of active CDKA-associated complexes, promoting assembly of the complex in tobacco suspension culture Bright Yellow-2 (BY-2) cells. Protein gel blot analysis revealed that CDKA levels increased greatly after stationary-phase BY-2 cells were subcultured

into fresh medium to re-enter the cell cycle. However, these increasing levels subsided when cells were subcultured into auxin-deprived medium, and a subtle increase was observed after subculturing into sucrose-deprived medium. Additionally, p13suc1-associated kinase activity did not increase significantly after subculturing into either auxin- or sucrose-deprived medium, but increased strongly after subculturing into medium containing both auxin and sucrose. Using gel filtration, we found that p13suc1-associated kinase activity against tobacco retinoblastoma-related protein was maximal in fractions corresponding to the molecular mass of the cyclin/CDKA complex. Interestingly, this peak distribution of high molecular-mass fractions of CDKA disappeared after cells were subcultured into auxin-deprived medium. These findings suggest an important role for auxin in the assembly of active CDKA-associated complexes.

Keywords: Auxin; Cell division; CDKA; Gel filtration; Tobacco BY-2 cells

Marc R. Roussel, Alexander A. Ivlev, Abir U. Igamberdiev, Oscillations of the internal CO₂ concentration in tobacco leaves transferred to low CO₂, *Journal of Plant Physiology*, Volume 164, Issue 9, 5 September 2007, Pages 1188-1196, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.08.004.

(<http://www.sciencedirect.com/science/article/B7GJ7-4M0BH7D-2/2/bb72fa7755fdab2045a2698b2480423>)

Abstract: Summary

Measurement of the internal CO₂ concentration (C_i) in tobacco leaves using a fast-response CO₂ exchange system showed that in the light, switching from 350 [μ]L L⁻¹ to a low CO₂ concentration of 36.5 [μ]L L⁻¹ (promoting high photorespiration) resulted in the C_i oscillating near the value of CO₂ compensation point ([Gamma]*). The oscillations are highly irregular, the range of C_i varying by 2-4 [μ]L L⁻¹ in substomatal cavities with a period of a few seconds. The statistical properties of the time series became stationary after a transient of ~100 s following transfer to low CO₂. Attractor reconstruction shows that the observed oscillations are not chaotic but exhibit stochastic behavior. The period of oscillations is consistent with the duration of photorespiratory post-illumination burst (PIB). We suggest that the observed oscillations may be due to a similar mechanism to that which leads to PIB, and may play a role in switching mitochondrial operation between oxidation of the photorespiratory glycine and of the tricarboxylic acid cycle substrates.

Keywords: Attractor reconstruction; CO₂ compensation point; Fast Fourier transformation; Oscillations; Photorespiration

Beatrix E. Czikkell, Denis P. Maxwell, NtGRAS1, a novel stress-induced member of the GRAS family in tobacco, localizes to the nucleus, *Journal of Plant Physiology*, Volume 164, Issue 9, 5 September 2007, Pages 1220-1230, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.07.010.

(<http://www.sciencedirect.com/science/article/B7GJ7-4M0BH7D-1/2/de54a9063d277b471a377f443733c62e>)

Abstract: Summary

We report the isolation and initial characterization of a new member of the GRAS gene family from tobacco, NtGRAS1. Analysis of the predicted amino acid sequence shows that NtGRAS1 shares the highly conserved carboxy-terminal motifs common to all members of the GRAS family. NtGRAS1 expression was strongly induced in tobacco (BY-2) suspension cells by antimycin A, H₂O₂, salicylic acid, and L-cysteine which were all found to raise intracellular reactive oxygen levels. An increase in NtGRAS1 expression was also triggered by treating cells with the nitric oxide donor sodium nitroprusside. By employing inhibitors of protein kinase and phosphatase action, we show that reversible phosphorylation is required for the stress-induced induction of NtGRAS1 and that reactive oxygen as well as NO-dependent signaling pathways probably share key intracellular components. Interestingly, in soil-grown plants, high constitutive expression of NtGRAS1 was found only in roots while expression was strongly induced in leaf tissue upon antimycin A

treatment or following *Pseudomonas syringae* infection. Many members of the GRAS family are implicated in regulating transcription and this function for NtGRAS1 is supported by our finding that an NtGRAS1-GFP fusion protein localizes to the nucleus of onion epidermal cells. Our data suggest that NtGRAS1 may represent an important transcriptional regulator involved in the plant stress response.

Keywords: GRAS; Mitochondria; Oxidative stress; Stress signaling; Tobacco

Johannes A. van der Merwe, Ian A. Dubery, Expression of mitochondrial *tatC* in *Nicotiana tabacum* is responsive to benzothiadiazole and salicylic acid, *Journal of Plant Physiology*, Volume 164, Issue 9, 5 September 2007, Pages 1231-1234, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.11.009.

(<http://www.sciencedirect.com/science/article/B7GJ7-4N74HT4-1/2/e137ef0a488aec9ee2967d6dea431e0e>)

Abstract: Summary

A cDNA, up-regulated upon treatment of tobacco cells with salicylic acid and benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester, was identified by differential RNA display and the full sequence obtained. This mitochondrial gene, twin arginine translocation (*tatC*), resembles orthologues across different species, including the gene that codes for a sec-independent membrane translocating protein in bacteria. Hypothetical *tatC* proteins have also been identified in the mitochondria of *Arabidopsis thaliana*, *Oenothera berteriana*, *Beta vulgaris*, *Oryza sativa* and *Marchantia polymorpha*. Comparative protein analysis indicates a similar function for the *tatC* gene. The up-regulation of the *tatC* gene in a 3 kbp transcript was confirmed by RNA gel blot analysis.

Keywords: Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester mitochondria; Salicylic acid; Systemic acquired resistance; *tatC*

M. Lodovici, V. Akpan, S. Caldini, B. Akanju, P. Dolaro, DNA solutionR in cigarette filters reduces polycyclic aromatic hydrocarbon (PAH) levels in mainstream tobacco smoke, *Food and Chemical Toxicology*, Volume 45, Issue 9, September 2007, Pages 1752-1756, ISSN 0278-6915, DOI: 10.1016/j.fct.2007.03.007.

(<http://www.sciencedirect.com/science/article/B6T6P-4N9886K-2/2/50364800e73492b98b071f77592bb2e2>)

Abstract:

Tobacco consumption represents a major health hazard to humans and, despite anti-smoking campaigns, the number of smokers remains high; thus the reduction of toxic compounds from tobacco smoke may reduce the health hazards of smoking. In the last 25 years cigarette manufacturers have introduced a variety of filter designs to reduce toxic and carcinogenic substances in tobacco smoke (normal filters, NF). However, large quantities of harmful constituents are inefficiently retained by commonly used cigarette filters. Following a patented method we modified commercial cigarette filters (modified filter, MF) by injecting a DNA solution into the filter tips; we then evaluated the reduced polycyclic aromatic hydrocarbon (PAH) levels in mainstream tobacco smoke of MF relative to NF. The PAH measured were: fluoranthene (FLUO), pyrene (PY), benzo(a)anthracene (B(a)A), chrysene (CRY), benzo(a)pyrene (B(a)P), benzo(b)fluoranthene (B(b)F), benzo(k)fluoranthene (B(k)F), benzo(g,h,i)perylene (BGP), dibenzo(a,h)anthracene (DBA). The levels of PAH in cigarette smoke after MF were significantly reduced ($P < 0.001$) compared to NF, using a variety of cigarette brands in a smoking machine (44.5% +/- 8.4 % and 41.8% +/- 5% for total and carcinogenic PAH, respectively, means +/- SE). Using B(a)P/TEF values the reduction in PAH concentrations were similar for all cigarette brands with the exception of Camel, where the reduction was lower considering B(a)P/TEF values. Amongst carcinogenic PAH, B(a)A, B(b)F and B(k)F were reduced by 50-58%, CRY, B(a)P and DBA by about 40%. In conclusion MF filters treated with DNA have the potential of decreasing the

exposure to PAH in cigarette smoke. Since, unlike some previously proposed biological filters MF do not retain additional nicotine, the main addictive compound of tobacco smoke, these filters may not induce increased smoking to compensate for the reduction in the nicotine delivery to smokers. Keywords: PAH; B(a)P; TEF, toxic equivalent factors; Treated cigarette filters; DNA solutionR; Carcinogenic PAH; Mainstream tobacco smoke

Takayuki Miyaji, Yoshiaki Kouzuma, Jun Yaguchi, Rika Matsumoto, Michael R. Kanost, Karl J. Kramer, Masami Yonekura, Purification of a cysteine protease inhibitor from larval hemolymph of the tobacco hornworm (*Manduca sexta*) and functional expression of the recombinant protein, *Insect Biochemistry and Molecular Biology*, Volume 37, Issue 9, September 2007, Pages 960-968, ISSN 0965-1748, DOI: 10.1016/j.ibmb.2007.05.003.

(<http://www.sciencedirect.com/science/article/B6T79-4NS2GF1-1/2/7113cc727797d65b8a5b09ff24a97fc7>)

Abstract:

A cysteine protease inhibitor (CPI) with an apparent molecular mass of 11.5 kDa was purified from larval hemolymph of the tobacco hornworm (*Manduca sexta*) by gel filtration on Sephadex G-50 followed by hydrophobic and ion-exchange column chromatographies. The purified cysteine proteinase inhibitor, denoted as MsCPI, strongly inhibited the plant cysteine protease, papain, with a K_i value of 5.5×10^{-9} M. Nucleotide sequence analysis of a partial cDNA encoding MsCPI indicated that MsCPI consists of 105 amino acid residues in a sequence that is similar to sarcocystatin A from *Sarcophaga peregrina*. However, northern blotting and PCR analyses using the specific primers of MsCPI suggested that the mRNA encoding MsCPI had a size of more than 12 kilobases, which included at least six tandemly repeated MsCPI segments. MsCPI was expressed in *Escherichia coli* and the recombinant protein effectively inhibited cysteine proteases from plants as well as from animals such as cathepsins B (K_i , 6.8 nM), H (3.0 nM), and L (0.87 nM). There was no inhibition exhibited toward trypsin, chymotrypsin, subtilisin, pepsin or themolysin.

Keywords: Cysteine protease; Cysteine protease inhibitor; Cystatin; Hemolymph; *Manduca sexta*; Overexpression

Robert A. Raguso, Tamaire Ojeda-Avila, Sheetal Desai, Melissa A. Jurkiewicz, H. Arthur Woods, The influence of larval diet on adult feeding behaviour in the tobacco hornworm moth, *Manduca sexta*, *Journal of Insect Physiology*, Volume 53, Issue 9, September 2007, Pages 923-932, ISSN 0022-1910, DOI: 10.1016/j.jinsphys.2007.03.007.

(<http://www.sciencedirect.com/science/article/B6T3F-4N919VB-3/2/d8ba8bb2ce010894728925e9fe4bdab6>)

Abstract:

Lab-reared sphingid and noctuid moths appear to feed less than wild moths, and often are starved to enhance responsiveness in feeding assays. To measure the impact of larval nutrition on adult feeding, we raised a model sphingid species, *Manduca sexta*, on control or modified diets (reduced sugar, protein or water, supplemented [β]-carotene) or cut tobacco leaves, then conducted feeding assays with artificial flowers. Behaviour was scored and analysed in a double-blind manner. Larval diet affected adult eclosion time, size and fat content, the latter of which was inversely proportional to moth approaches to the floral array in a flight cage. In contrast, behaviours refractory to feeding (sitting, escaping) were associated with sex and barometric pressure, but not with diet or fat content. Frequency of floral approaches and probing was not associated with any variable. However, moths reared on [β]-carotene-supplemented diet were 2-3 times more likely to feed, and significantly less likely to sit or show 'escape' behaviour than were moths from most other treatments. Our results suggest that decreased visual sensitivity, rather than increased fat content, accounts for reduced adult feeding by lab-reared *M. sexta*.

Keywords: Artificial diet; Fat composition; *Manduca sexta*; Nectar foraging; Starvation; Visual pigment

Gang Ren, Rosanne A. Healy, Anna M. Klyne, Harry T. Horner, Martha G. James, Robert W. Thornburg, Transient starch metabolism in ornamental tobacco floral nectaries regulates nectar composition and release, *Plant Science*, Volume 173, Issue 3, September 2007, Pages 277-290, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2007.05.008.

(<http://www.sciencedirect.com/science/article/B6TBH-4NWNCN3-2/2/2c36035e49b6b2ea9c7979363727a444>)

Abstract:

Enlargement of the floral nectary gland of ornamental tobacco during its development is accompanied by a major accumulation of starch granules in nectary amyloplasts. Quantification of starch in the nectary at various developmental stages showed little starch accumulation at early stages but increasing amounts of starch over the course of nectary development that reached a peak approximately 24 h prior to anthesis. After this point, the amount of starch declined dramatically, suggesting its conversion to sugars (sucrose, glucose, and fructose) for the nectar production that occurs at anthesis. Compositional and structural analyses of nectary starch showed that amylose content and degree of amylopectin branching also varied during nectary development. Increasingly complex starch structures were observed up to intermediate stages of nectary development, followed by decreased starch complexity and amount in the mature nectary. Although the total amount of carbohydrate stored in the nectary at mid-development is roughly equivalent to the carbohydrate in nectar sugars at anthesis, four- to five-times more sugar is secreted into nectar following anthesis. Radiolabeling of sugars prior to their transport into the flower bud, nectary, and nectar showed the flow of sugars into the nectary increased markedly after anthesis. The finding that the nectary is the strongest sink tissue of all floral organs suggests that two processes, starch degradation and rapid sugar influx, are determinants of sugar composition in floral nectar. A model is presented in which these two processes are coordinated for high-level nectar production and release.

Keywords: Nectar; Nectary; *Nicotiana*; Ovary; Starch; Flowers

Kaoru Suzuki, Akira Yano, Takumi Nishiuchi, Toshitsugu Nakano, Hiroaki Kodama, Kazuo Yamaguchi, Hideaki Shinshi, Comprehensive analysis of early response genes to two different microbial elicitors in tobacco cells, *Plant Science*, Volume 173, Issue 3, September 2007, Pages 291-301, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2007.06.002.

(<http://www.sciencedirect.com/science/article/B6TBH-4P0X56S-1/2/89468eea99ca66c3404144f4c1dcd678>)

Abstract:

The key to understanding the molecular mechanism of the defense response triggered by recognition of pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) is the identification and comparison of a set of early response genes to different microbial elicitors. We performed comprehensive and detailed monitoring of gene expression over time after application of two different microbial elicitors, PiE (an elicitor from the cell walls of an oomycete, *Phytophthora infestans*) and TvX (a xylanase from a fungus, *Trichoderma viride*), in tobacco cultured cells using the suppression subtractive hybridization and cDNA macroarray techniques. We identified various kinds of genes that are up- or down-regulated at the early stages of response to the elicitors. The majority of up-regulated genes are predicted to have a role in the defense response as signaling components and transcription factors as well as the metabolism involved in the production of secondary signaling molecules and anti-microbial compounds. The overall results revealed that there is no substantial difference in the expression profiles between cells treated with two different microbial elicitors, at least during the early phase of the defense response.

Keywords: cDNA macroarray; Elicitors; MAMPs; PAMPs; Suppression subtractive hybridization; Tobacco

Mathieu Frison, Jean Luc Parrou, Damien Guillaumot, Daniele Masquelier, Jean Francois, Francois Chaumont, Henri Batoko, The Arabidopsis thaliana trehalase is a plasma membrane-bound enzyme with extracellular activity, FEBS Letters, Volume 581, Issue 21, 21 August 2007, Pages 4010-4016, ISSN 0014-5793, DOI: 10.1016/j.febslet.2007.07.036.

(<http://www.sciencedirect.com/science/article/B6T36-4P8H76K-6/2/19c370ecc974fdda836caa15ae4c4008>)

Abstract:

The lack of trehalose accumulation in most plant species has been partly attributed to the presence of an active trehalase. Although trehalose synthesis enzymes are thought to be cytosolic, and previous studies have indicated that trehalase activity is extracellular, the exact location of the enzyme has not yet been established in plant cell. We present evidence that the yet uncharacterised full-length Arabidopsis trehalase is a plasma membrane-bound protein, probably anchored to the membrane through a predicted N-terminal membrane spanning domain. The full-length AtTRE1, when expressed in yeast can functionally substitute for the extracellularly active trehalase Ath1p, by sustaining the growth of an ath1 null mutant strain on trehalose and at pH 4.8. We further demonstrate that AtTRE1 expressed in yeast is plasma membrane-bound as in plant cell. In light of these findings, the regulation of plant cell endogenous trehalose by trehalase is discussed.

Keywords: Arabidopsis; Plasma membrane; Trehalase; Trehalose; Yeast

Phyllis A.W. Martin, Michael B. Blackburn, Using combinatorics to screen Bacillus thuringiensis isolates for toxicity against Manduca sexta and Plutella xylostella, Biological Control, Volume 42, Issue 2, August 2007, Pages 226-232, ISSN 1049-9644, DOI: 10.1016/j.biocontrol.2007.05.004.

(<http://www.sciencedirect.com/science/article/B6WBP-4NRT3BX-2/2/1940bbb02c7e034cf50881ddf427f3d5>)

Abstract:

Screening Bacillus thuringiensis (Bt) isolates or strains for toxicity has traditionally been performed with one bacterial isolate at a time versus a specific insect. By testing Bt strains in groups, we more rapidly identified 28 of 147 Bt isolates as toxic to either diamondback moth, Plutella xylostella (L.), or tobacco hornworm, Manduca sexta (L.). The use of freeze-dried diet and directed pooling of isolates for toxicity testing decreased the number of bioassays required to identify toxic strains by as much as 60% for a given group of isolates. Three of the B. thuringiensis isolates were more toxic to diamondback moth than a standard commercial strain. This method parallels the concept of combinatorics used for screening compounds in the pharmaceutical industry by the use of bacterial strains rather than chemicals.

Keywords: Tobacco hornworm; Diamondback moth; Entomopathogens; Insect bioassays; Freeze-dried diet; Insect diet

Paola Tarantino, Rosa Caiazzo, Angela Carella, Ernesto Lahoz, Control of Rhizoctonia solani in a tobacco-float system using low rates of iprodione- and iprodione-resistant strains of Gliocladium roseum, Crop Protection, Volume 26, Issue 8, August 2007, Pages 1298-1302, ISSN 0261-2194, DOI: 10.1016/j.cropro.2006.11.004.

(<http://www.sciencedirect.com/science/article/B6T5T-4MNJ2R3-2/2/591efe5a4c26d4f0b3cf5cef3d7d2c38>)

Abstract:

Results obtained using low rates of the fungicide iprodione- and iprodione-resistant strains of Gliocladium roseum to control Rhizoctonia solani Kuhn (Teleomorph: Thanatephorus cucumeris (Frank) Donk) in a tobacco float system are reported. The objectives were three-fold: to obtain G.

roseum (GNL) strains resistant to the fungicide iprodione; to verify that the mutants maintained the same characteristics of the wild type; and to control *R. solani* in a tobacco float system by the application of low doses of fungicide combined with a resistant biological control agent (BCA) strain. Two resistant strains were obtained and the IC₅₀ values were 44.7 and 5000 [μg ml⁻¹] for GNL wild type and mutants, respectively. The results showed the same level of disease control in plots with a high rate of iprodione and in plots with a low rate of fungicide, applied together with GNLr1 (96%) and GNLr2 (98%) iprodione-resistant strains of *G. roseum*. Less-disease control was obtained by a reduced dose of iprodione, when applied alone.

Keywords: *Rhizoctonia solani*; Biological control; *Gliocladium roseum*; Fungicide resistance

Werner Romisch-Margl, Nicholas Schramek, Tanja Radykewicz, Christian Ettenhuber, Eva Eylert, Claudia Huber, Lilla Romisch-Margl, Christine Schwarz, Maria Dobner, Norbert Demmel, Bernhard Winzenhorlein, Adelbert Bacher, Wolfgang Eisenreich, ¹³CO₂ as a universal metabolic tracer in isotopologue perturbation experiments, *Phytochemistry*, Volume 68, Issues 16-18, Dynamic Metabolic Networks, August-September 2007, Pages 2273-2289, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2007.03.034.

(<http://www.sciencedirect.com/science/article/B6TH7-4NRCRX7-1/2/56c9d4b72cf35711836e93b2e1181c05>)

Abstract:

A tobacco plant was illuminated for 5 h in an atmosphere containing ¹³CO₂ and then maintained for 10 days under standard greenhouse conditions. Nicotine, glucose, and amino acids from proteins were isolated chromatographically. Isotopologue abundances of isolated metabolites were determined quantitatively by NMR spectroscopy and mass spectrometry. The observed non-stochastic isotopologue patterns indicate (i) formation of multiply labeled photosynthetic carbohydrates during the ¹³CO₂ pulse phase followed by (ii) partial catabolism of the primary photosynthetic products, and (iii) recombination of the ¹³C-labeled fragments with unlabeled intermediary metabolites during the chase period. The detected and simulated isotopologue profiles of glucose and amino acids reflect carbon partitioning that is dominated by the Calvin cycle and glycolysis/gluconeogenesis. Retrobiosynthetic analysis of the nicotine pattern is in line with its known formation from nicotinic acid and putrescine via aspartate, glyceraldehyde phosphate and [α]-ketoglutarate as basic building blocks. The study demonstrates that pulse/chase labeling with ¹³CO₂ as precursor is a powerful tool for the analysis of quantitative aspects of plant metabolism in completely unperturbed whole plants.

Chandan Sahi, Manu Agarwal, Amanjot Singh, Anil Grover, Molecular characterization of a novel isoform of rice (*Oryza sativa* L.) glycine rich-RNA binding protein and evidence for its involvement in high temperature stress response, *Plant Science*, Volume 173, Issue 2, August 2007, Pages 144-155, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2007.04.010.

(<http://www.sciencedirect.com/science/article/B6TBH-4NN0W3K-2/2/ceb82873027a3acd578cd07fcf361156>)

Abstract:

A novel full-length cDNA encoding for glycine rich (GR)-RNA binding protein (RBP) (*Osgr-rbp4*) is isolated from rice heat shock cDNA library. Amino acid sequence of the deduced protein reveals existence of RNA recognition motif (RRM) comprising of highly conserved RNA binding RNPI and RNPII domains at the N-terminus. C-terminus of this protein is rich in arginine and glycine residues. Blast search analysis on rice genome sequence database shows that GR-RBP protein family is constituted of multiple members with high level of amino acid conservation in RNA recognition motif and glycine domain regions. Similar analysis across wider biological systems from NCBI database indicated that rice GR-RBP4 has homologs in different living genera. *Osgr-rbp4* transcript in rice seedlings is constitutively expressed as well as regulated by different abiotic stresses including high temperature stress. Ectopic over-expression of *Osgr-rbp4* cDNA imparts

high temperature stress tolerance to wild type yeast cells. It is shown that OsGR-RBP4 in rice leaf cells and its immunologically homologous protein in tobacco BY2 protoplasts are nuclear proteins. Upon heat shock, bulk of these proteins appears to be localized in the cytoplasm. We suggest that OsGR-RBP4 probably bind and stabilize the stress-inducible transcripts under HS conditions.

Keywords: Glycine rich-RNA binding protein (GR-RBP); High temperature; Rice; Stress response; Yeast

Stephen G. Cessna, Tracie K. Matsumoto, Gregory N. Lamb, Shawn J. Rice, Wendy Wenger Hochstedler, The externally derived portion of the hyperosmotic shock-activated cytosolic calcium pulse mediates adaptation to ionic stress in suspension-cultured tobacco cells, *Journal of Plant Physiology*, Volume 164, Issue 7, 26 July 2007, Pages 815-823, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.11.002.

(<http://www.sciencedirect.com/science/article/B7GJ7-4MW900N-1/2/b3dd3a41e343e38ce74cd94238fd5004>)

Abstract: Summary

The influx of Ca²⁺ into the cytosol has long been suggested to serve as a signaling intermediate in the acquisition of tolerance to hyperosmotic and/or salinity stresses. Here we use aequorin-transformed suspension-cultured tobacco cells to directly assess the role of cytosolic calcium (Ca²⁺+cyt) signaling in salinity tolerance acquisition. Aequorin luminescence recordings and ⁴⁵Ca influx measurements using inhibitors of Ca²⁺ influx (Gd³⁺ and the Ca²⁺-selective chelator EGTA), and modulators of organellar Ca²⁺ release (phospholipase C inhibitors U73122 or neomycin) demonstrate that hyperosmolarity, whether imposed by NaCl or by a non-ionic molecule sorbitol, induces a rapid (returning to baseline levels of Ca²⁺ within 10 min) and complex Ca²⁺+cyt pulse in tobacco cells, deriving both from Gd³⁺-sensitive externally derived Ca²⁺ influx and from U73122- and neomycin-sensitive Ca²⁺ release from an organelle. To determine whether each of the two components of this brief Ca²⁺ signal regulate adaptation to hyperosmotic shock, the Ca²⁺ pulse was modified by the addition of Gd³⁺, U73122, neomycin, or excess Ca²⁺, and then cells were treated with salt or sorbitol. After 10 min the cell culture medias were diluted with additional hyperosmotic media to reduce the toxic affects of the modulators, and the growth of cells was measured after 1 week. Gd³⁺ treatment reduced growth in salt relative to control cells but not in sorbitol, and exposure to excess Ca²⁺ increased growth in salt but not in sorbitol. In contrast, exposure to inhibitors of IP₃ formation had no effect on growth in salt or sorbitol. Therefore, although hyperosmotic treatment stimulates both Ca²⁺ influx and Ca²⁺ release from an internal Ca²⁺ depot, only Ca²⁺ influx has a measurable impact on ionic stress tolerance acquisition in tobacco cell suspensions. In contrast, osmoadaptation in these cells appears to occur independent of Ca²⁺ signaling.

Keywords: Aequorin; Calcium; Inositol-1,4,5-trisphosphate; Salinity; Signal transduction

Yoshimi Inaba, Wei Qun Zhong, Xing-Hai Zhang, Jack M. Widholm, Specificity of expression of the GUS reporter gene (*uidA*) driven by the tobacco ASA2 promoter in soybean plants and tissue cultures, *Journal of Plant Physiology*, Volume 164, Issue 7, 26 July 2007, Pages 824-834, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.10.009.

(<http://www.sciencedirect.com/science/article/B7GJ7-4MV19M3-4/2/5571a4f955bfd2f4809e8568afd5f582>)

Abstract: Summary

Twelve independent lines were transformed by particle bombardment of soybean embryogenic suspension cultures with the tobacco anthranilate synthase (ASA2) promoter driving the *uidA* ([beta]-glucuronidase, GUS) reporter gene. ASA2 appears to be expressed in a tissue culture specific manner in tobacco (Song H-S, Brotherton JE, Gonzales RA, Widholm JM. Tissue culture specific expression of a naturally occurring tobacco feedback-insensitive anthranilate synthase. *Plant Physiol* 1998;117:533-43). The transgenic lines also contained the hygromycin

phosphotransferase (hpt) gene and were selected using hygromycin. All the selected cultures or the embryos that were induced from these cultures expressed GUS measured histochemically. However, no histochemical GUS expression could be found in leaves, stems, roots, pods and root nodules of the plants formed from the embryos and their progeny. Pollen from some of the plants and immature and mature seeds and embryogenic cultures initiated from immature cotyledons did show GUS activity. Quantitative 4-methylumbelliferyl-glucuronide (MUG) assays of the GUS activity in various tissues showed that all with observable histochemical GUS activity contained easily measurable activities and leaves and stems that showed no observable histochemical GUS staining did contain very low but measurable MUG activity above that of the untransformed control but orders of magnitude lower than the constitutive 35S-uidA controls used. Low but clearly above background levels of boiling sensitive GUS activity could be observed in the untransformed control immature seeds and embryogenic cultures using the MUG assay. Thus in soybean the ASA2 promoter drives readily observable GUS expression in tissue cultures, pollen and seeds, with only extremely low levels seen in vegetative tissues of the plants. The ASA2 driven expression seen in mature seed was, however, much lower than that seen with the constitutive 35S promoter; less than 2% in seed coats and less than 0.13% in cotyledons and embryo axes. The predominate tissue culture specific expression pattern of the ASA2 promoter may be useful for genetic transformation of crops.

Keywords: Anthranilate synthase; ASA2 promoter; [β]-glucuronidase assays; Glycine max; Tissue-specific expression

R. Kavitha, S. Umesha, Prevalence of bacterial spot in tomato fields of Karnataka and effect of biological seed treatment on disease incidence, *Crop Protection*, Volume 26, Issue 7, July 2007, Pages 991-997, ISSN 0261-2194, DOI: 10.1016/j.cropro.2006.09.007.

(<http://www.sciencedirect.com/science/article/B6T5T-4M7CM8X-7/2/a46cd1cbc37210de1bb98a558a1e889d>)

Abstract:

Bacterial spot disease of tomato caused by *Xanthomonas vesicatoria* was studied during the field survey in the Karnataka state, India. The disease incidence ranged from 22% to 50%. The pathogen was isolated from the infected plant material and seed samples. In the laboratory the pathogen was isolated following the routine laboratory assay method i.e. direct plating method using Tween B medium. Further the pathogen was confirmed by biochemical, physiological, hypersensitivity in tobacco (*Nicotiana tabacum*) plants and finally the pathogenicity tests. Biological seed treatment with antagonistic *Pseudomonas fluorescens* improved the seed quality ($p=0.05$) under laboratory conditions and tremendously decreased the bacterial spot disease incidence in field.

Keywords: Bacterial spot; Tomato; *Xanthomonas vesicatoria*; *Pseudomonas fluorescens*; Seed treatment

Martina Mackova, Blanka Vrchotova, Katerina Francova, Michel Sylvestre, Monika Tomaniova, Petra Lovecka, Katerina Demnerova, Tomas Macek, Biotransformation of PCBs by plants and bacteria - consequences of plant-microbe interactions, *European Journal of Soil Biology*, Volume 43, Issue 4, Phytoremediation, Third European Bioremediation Conference, July-August 2007, Pages 233-241, ISSN 1164-5563, DOI: 10.1016/j.ejsobi.2007.02.006.

(<http://www.sciencedirect.com/science/article/B6VR7-4N8K59J-1/2/3bfe46311b7a0a45177e9a8482b5ef82>)

Abstract:

Plant-microbial interactions within rhizosphere can evolve beneficial effect on degradation or increased accumulation of organic and inorganic contaminants. Our study shows the possibility of additional metabolic interactions between bacteria and plants in contaminated environment on level of their intermediates and their transformation. On example of model contaminants - PCBs,

we give more detailed information about abilities of biological systems to metabolise original xenobiotics and also their intermediates and products. The enzymes of bacterial biphenyl operon were able to metabolise intermediates of plant PCB transformation namely 2-chloro-4-hydroxy biphenyl (2Cl-4OHBP) and 3-chloro-4-hydroxybiphenyl (3Cl-4OHBP). In case of other tested hydroxylated chlorobiphenyls the mode of oxygenation as well as the stability of the metabolites produced, greatly differed depending on the type of substituents. Similarly plant cells were able to metabolize, in limited extent, bacterial products of PCB degradation - chlorobenzoates (CBAs). From the four tested plant species tobacco, horseradish, nightshade and alfalfa showed significant transformation abilities only horseradish and black nightshade. Both metabolized in 2 weeks more than 90% of 2-chlorobenzoate and 20-40% of 2,3-; 2,4-; 2,5- and 2,6-dichlorobenzoates.

Keywords: Plant-microbe interactions; Polychlorinated biphenyls; Chlorobenzoic acids; Degradation; Hydroxychlorobiphenyls

Sakuntala Sivasupramaniam, Graham P. Head, Leigh English, Yue Jin Li, Ty T. Vaughn, A global approach to resistance monitoring, *Journal of Invertebrate Pathology*, Volume 95, Issue 3, Special Issue for SIP 2007, SIP 2007, July 2007, Pages 224-226, ISSN 0022-2011, DOI: 10.1016/j.jip.2007.03.013.

(<http://www.sciencedirect.com/science/article/B6WJV-4NBBYYG-2/2/e023985055808460e0e4604aeda8f952>)

Abstract:

Transgenic crops producing insecticidal toxins from the bacterium *Bacillus thuringiensis* (Bt) have been grown in many parts of the world since 1996. In the United States, the Environmental Protection Agency (EPA) has required that industry submit insect resistance management (IRM) plans for each Bt corn and cotton product commercialized. A coalition of stakeholders including the EPA, USDA, academic scientists, industry, and grower organizations have cooperated in developing specific IRM strategies. Resistance monitoring (requiring submission of annual reports to the EPA), and a remedial action plan addressing any contingency if resistance should occur, are important elements of these strategies. At a global level, Monsanto conducts baseline susceptibility studies (prior to commercialization), followed by monitoring studies on target pest populations, for all of its commercialized Bt crop products. To date, Monsanto has conducted baseline/monitoring studies in Argentina, Australia, Brazil, Canada, China, Colombia, India, Mexico, the Philippines, South Africa, Spain, and the United States. Examples of pests on which resistance monitoring has been conducted include cotton bollworm, *Helicoverpa zea*, European corn borer, *Ostrinia nubilalis*, pink bollworm, *Pectinophora gossypiella*, Southwestern corn borer, *Diatraea grandiosella*, tobacco budworm, *Heliothis virescens*, and western corn rootworm, *Diabrotica virgifera virgifera*, in the United States, cotton bollworm, *Helicoverpa armigera*, in China, India and Australia, and *H. virescens* and *H. zea* in Mexico. No field-selected resistance to Bt crops has been documented.

Keywords: Monsanto; *Bacillus thuringiensis*; Resistance monitoring; Bollgard(R); Bollgard(R) II; Yieldgard(R); Cry1Ab, Cry1Ac; Cry2Ab2

Ryan W. Kurtz, Alan McCaffery, David O'Reilly, Insect resistance management for Syngenta's VipCot(TM) transgenic cotton, *Journal of Invertebrate Pathology*, Volume 95, Issue 3, Special Issue for SIP 2007, SIP 2007, July 2007, Pages 227-230, ISSN 0022-2011, DOI: 10.1016/j.jip.2007.03.014.

(<http://www.sciencedirect.com/science/article/B6WJV-4NBH239-7/2/465bfcbf13e05b53e62857481f5d02d1>)

Abstract:

Syngenta is seeking commercial registration for VipCot(TM) cotton, a pyramided transgenic cotton trait that expresses two insecticidal proteins derived from *Bacillus thuringiensis* Vip3A and Cry1Ab. Both proteins are highly effective against two key cotton pests, *Helicoverpa zea* cotton bollworm;

and *Heliothis virescens*, tobacco budworm. To investigate the role of VipCot(TM) cotton in delaying the development of resistance in these pests to transgenic Bt traits, Syngenta has performed studies to determine the dose of proteins expressed in VipCot(TM) and evaluate the potential for cross-resistance between the component proteins. Following United States Environmental Protection Agency (US EPA) high dose methods 1 and 4, VipCot(TM) was shown to express a high dose of proteins for *H. zea* and *H. virescens*. VipCot(TM) was also confirmed to express a high dose of proteins for *H. zea* through US EPA Method 5. Additionally, all the data collected to date verify a lack of cross-resistance between Vip3A and Cry proteins. These two key pieces of information indicate that VipCot(TM) cotton should be very durable under the currently mandated high dose plus refuge insect resistance management strategy.

Keywords: *Bacillus thuringiensis*; Insect resistance management; Vip3A; *Helicoverpa zea*; *Heliothis virescens*

F.M. Alves-Santos, D. Martinez-Bermejo, M.C. Rodriguez-Molina, J.J. Diez, Cultural characteristics, pathogenicity and genetic diversity of *Fusarium oxysporum* isolates from tobacco fields in Spain, *Physiological and Molecular Plant Pathology*, Volume 71, Issues 1-3, July-September 2007, Pages 26-32, ISSN 0885-5765, DOI: 10.1016/j.pmpp.2007.09.007.

(<http://www.sciencedirect.com/science/article/B6WPC-4PT7X0F-3/2/75152f1c8bf74ecbafb62985fc1d2c52>)

Abstract:

Several formae speciales of *Fusarium oxysporum* are capable to produce disease in tobacco plants. Different authors have classified those isolates as a forma specialis or a race within on the basis of the severity of disease and host specificity. *Fusarium* wilt of tobacco plant in Extremadura (central Spain) tobacco fields have been recorded in the last years and *F. oxysporum* was isolated from symptomatic plants. The aim of our study was to characterize these *F. oxysporum* populations. For this purpose, the in vitro spore production and growth and the virulence (severity of disease) have been tested. Although all isolates behaved as pathogen, the virulence of isolates was different. The differences in growth could not be correlated with other characteristics but the two isolates with scarce spore production have also behaved as the weakest pathogen. We have analyzed intergenic spacer (IGS) region polymorphism of ribosomal DNA and random amplified polymorphic DNA (RAPD) markers to assess the genetic diversity within *F. oxysporum* isolates. These molecular analyses showed two major groups with different physiological capabilities that could reflect two different lineages. One group was characterized by medium-high sporulation, high virulence and the same IGS-RFLP pattern. The other group was more heterogeneous featuring low-medium sporulation and variable virulence and growth. This first experimental approach to pathogen population could be a good starting point for further studies including non-pathogenic isolates and a larger number of pathogen that could clarify if there are two or more genetic lineages.

Keywords: *Fusarium oxysporum*; Tobacco; Pathogenicity; Genetic diversity; RAPD; ITS; Sporulation

Jun-Shan Gao, Nobumitsu Sasaki, Hiromi Kanegae, Ken-ichi Konagaya, Kaori Takizawa, Naomi Hayashi, Yosuke Okano, Masahiro Kasahara, Yasuhiko Matsushita, Hiroshi Nyunoya, The TIR-NBS but not LRR domains of two novel N-like proteins are functionally competent to induce the elicitor p50-dependent hypersensitive response, *Physiological and Molecular Plant Pathology*, Volume 71, Issues 1-3, July-September 2007, Pages 78-87, ISSN 0885-5765, DOI: 10.1016/j.pmpp.2007.11.002.

(<http://www.sciencedirect.com/science/article/B6WPC-4R8HHRH-1/2/e3a85e610540bb6b23b317112bf17fe7>)

Abstract:

The tobacco N protein recognizes the helicase domain (p50) of the Tobacco mosaic virus (TMV) replicase as an elicitor and mediates hypersensitive response (HR). We obtained two cDNA clones encoding novel N-like (NL) proteins NL-C26 and NL-B69 from *Nicotiana tabacum* cv. Samsun NN. NL-C26 and NL-B69 had a Toll-interleukin-1 receptor/nucleotide-binding site/leucine-rich repeat (TIR-NBS-LRR) structure and showed 78% and 73% identities to N, respectively. The NL-C26 and NL-B69 genes were also expressed in *N. tabacum* cv. Samsun nn, which lacks the N gene. Unlike N, NL-C26 and NL-B69, when coexpressed with p50, failed to induce HR on the sites of agroinfiltration in Samsun nn leaves. However, the elicitor-dependent HR in Samsun nn was induced efficiently by chimeric N proteins with the continuous TIR-NBS domains of NL-C26 and NL-B69. On the other hand, the efficiency of HR induction varied significantly among chimeric N proteins with either of the TIR and NBS domains of the NL proteins. In contrast, chimeras carrying the LRR domains of the NL proteins did not induce HR. Thus, the TIR-NBS domains of NL-C26 and NL-B69 could functionally adapt to the LRR domain of N, which may determine the specificity for the elicitor. We speculate that the NL genes are potential HR-inducing resistance genes for undetermined pathogens other than TMV.

Keywords: N gene; N homolog; *Nicotiana tabacum*; Samsun; Elicitor; Hypersensitive response; HR

Davide Gobbin, Fabio Rezzonico, Cesare Gessler, Quantification of the biocontrol agent *Pseudomonas fluorescens* Pf153 in soil using a quantitative competitive PCR assay unaffected by variability in cell lysis- and DNA-extraction efficiency, *Soil Biology and Biochemistry*, Volume 39, Issue 7, July 2007, Pages 1609-1619, ISSN 0038-0717, DOI: 10.1016/j.soilbio.2007.01.015.

(<http://www.sciencedirect.com/science/article/B6TC7-4N3X61P-1/2/451ab113c181555167099de4e6e5c066>)

Abstract:

Although often neglected, variability in cell lysis efficiency and DNA extraction yield represents the major hurdles of any polymerase chain reaction (PCR)-based quantification protocol in soil and other natural environments. In this study we developed a technique that minimizes the effects of these constraints, providing at the same time a reliable internal control to distinguish between PCR-inhibition and negative results. We used *Pseudomonas fluorescens* Pf153, a root-colonizing bacterium that shows biocontrol activity against tobacco and cucumber black root rot, as the target organism for PCR quantification. Prior to DNA extraction, the genetically engineered, cognate reference strain *P. fluorescens* CHA0/c2 was inoculated in a reference soil. CHA0/c2 in the reference soil and Pf153 in the soil sample were lysed in parallel and afterward the lysates were mixed in known proportions. CHA0/c2 carries the plasmid pME6031-cmp2 that contains an allelic variant (competitor) of the Pf153 specific sequence Pf153_2. In a quantitative competitive PCR (QC-PCR) assay the competitor allows the quantification of the target strain down to 0.66 Pf153 CFU/mg soil. Processing the reference strain in the same way as Pf153 enables the exact quantification of the target strain in biocontrol assays performed in natural soil, overcoming differences in DNA extraction efficiency and PCR amplification from different soil environments. This technique is easily adaptable to other *Pseudomonas* strains simply by replacing the competitor used here with one derived from a SCAR-marker which is specific for the strain of choice.

Keywords: Biocontrol; DNA extraction internal control; Monitoring; Quantitative competitive PCR; *Pseudomonas fluorescens*; Risk assessment

Sayaka Masada, Kazuyoshi Terasaka, Hajime Mizukami, A single amino acid in the PSPG-box plays an important role in the catalytic function of CaUGT2 (Curcumin glucosyltransferase), a Group D Family 1 glucosyltransferase from *Catharanthus roseus*, *FEBS Letters*, Volume 581, Issue 14, 12 June 2007, Pages 2605-2610, ISSN 0014-5793, DOI: 10.1016/j.febslet.2007.05.002.

(<http://www.sciencedirect.com/science/article/B6T36-4NPHHMF-2/2/fba1eee39fca8e2735269a04ab9f9d07>)

Abstract:

Curcumin glucosyltransferase (CaUGT2) isolated from cell cultures of *Catharanthus roseus* exhibits unique substrate specificity. To identify amino acids involved in substrate recognition and catalytic activity of CaUGT2, a combination of domain swapping and site-directed mutagenesis was carried out. Exchange of the PSPG-box of CaUGT2 with that of NtGT1b (a phenolic glucosyltransferase from tobacco) led to complete loss of enzyme activity in the resulting recombinant protein. However, replacement of Arg378 of the NtGT1b PSPG-box with cysteine, the corresponding amino acid in CaUGT2, restored the catalytic activity of the chimeric enzyme. Further site-directed mutagenesis revealed that the size of the amino acid side-chain in that particular site is critical to the catalytic activity of CaUGT2.

Keywords: Glucosyltransferase; Curcumin; PSPG-box; Catalytic function; Domain swapping; Site-directed mutagenesis

B.G. Xiao, J. Zhu, X.P. Lu, Y.F. Bai, Y.P. Li, Analysis on genetic contribution of agronomic traits to total sugar in flue-cured tobacco (*Nicotiana tabacum* L.), *Field Crops Research*, Volume 102, Issue 2, 5 June 2007, Pages 98-103, ISSN 0378-4290, DOI: 10.1016/j.fcr.2007.03.002.

(<http://www.sciencedirect.com/science/article/B6T6M-4NF2HF4-1/2/ce256116a9ba50fe036fb6c33e95e485>)

Abstract:

To uncover the genetic contributions of agronomic traits to content of total sugar (TS) and find indicator traits for indirect selection on TS in the flue-cured tobacco (*Nicotiana tabacum* L.), multivariable conditional analysis was conducted based on a genetic model containing additive-dominance and their interactions with environments. Fourteen cultivars (or breeding lines) and derived 41 F1 crosses were grown at four locations in Yunnan province, China. Significant phenotypic contribution to TS was detected for six agronomic traits, plant height (PH), girth of stem (GS), internode length (INL), number of leaves (NL), length of middle leaves (LML) and width of middle leaves (WML). There was large contribution of additive effects due to each of the five agronomic traits (PH, GS, INL, LML and WML). The contribution ratio of dominance effect was high due to PH. By serving as high contributor of additive effects to TS and having high ratios of additive variance to phenotypic variance, INL and PH could be used as indicative agronomic traits for selecting breeding lines with suitable TS. Among the six agronomic traits, PH had the highest contribution to dominance effects of TS for most F1 crosses, and could be used for selecting the crosses with suitable TS.

Keywords: Flue-cured tobacco; *Nicotiana tabacum* L.; Diallel analysis; Genetic correlation; Conditional analysis

Yoshiko Mitsuya, Yoshihiro Takahashi, Yukiko Uehara, Thomas Berberich, Atsushi Miyazaki, Hideki Takahashi, Tomonobu Kusano, Identification of a novel Cys2/His2-type zinc-finger protein as a component of a spermine-signaling pathway in tobacco, *Journal of Plant Physiology*, Volume 164, Issue 6, 4 June 2007, Pages 785-793, ISSN 0176-1617, DOI: 10.1016/j.jpjph.2006.05.011.

(<http://www.sciencedirect.com/science/article/B7GJ7-4KJ5T2F-1/2/5bcfcf1a384ca285d422e10acd690d93>)

Abstract: Summary

In a previous work, we identified a Cys2/His2-type zinc-finger transcription repressor, (ZFT1), that functions in a spermine-mediated signal transduction pathway in tobacco plants. Database search disclosed the presence of another Cys2/His2-type zinc-finger protein ZFP1 (accession number AAC06243) in tobacco plants. In this work, we characterized ZFP1 and investigated whether this protein is also involved in a Spm-signaling pathway. This factor showed the highest identity to petunia ZPT2-2 and higher similarity to petunia ZPT2-3, Arabidopsis STZ/ZAT10, soybean SCOF-

1, red pepper CAZFP1/CaPIF1 as well as to tobacco ZFT1. ZFP1 localized to the nucleus and had a specific DNA-binding activity, supportive to be a transcription factor. Furthermore, the protein had a mild repression activity on transcription in plant cells. The expression of ZFP1, encoding ZFP1, was upregulated during tobacco mosaic virus-induced hypersensitive response. ZFP1 expression was also induced by exogenously applied spermine and its induction was repressed by inhibitors of amine oxidase/polyamine oxidase. Collectively, our data indicate that ZFP1 is a new transcription factor which functions in a spermine-signaling pathway in tobacco.

Keywords: Biotic stress; Polyamine catabolism; Spermine; Tobacco; Transcription factor

Xiang-yang LI, Guo-shun LIU, Yong-feng YANG, Chun-hua ZHAO, Qi-wei YU, Shi-xu SONG, Relationship Between Hyperspectral Parameters and Physiological and Biochemical Indexes of Flue-Cured Tobacco Leaves, *Agricultural Sciences in China*, Volume 6, Issue 6, June 2007, Pages 665-672, ISSN 1671-2927, DOI: 10.1016/S1671-2927(07)60098-4.

(<http://www.sciencedirect.com/science/article/B82XG-4P48RF7-5/2/d2e3bd0fba73787b6e89e6e4a3c5a494>)

Abstract: Abstract

The experiment was set up for examining the physiological and biological indexes quickly and exactly, for obtaining information of tobacco-field fertilizing and tobacco growing. The ASD Field spec FR 2500 was used to measure spectra reflectance of flue-cured tobacco and the relationship between hyperspectral parameters and biochemical contents (total nitrogen, chlorophyll, carotenoid), and physiological indexes (fresh weight, dry weight, moisture content) of flue-cured tobacco leaves was studied by correlation and stepwise regression statistic methods at different nitrogen and potassium levels. The results indicated that the spectra curves of different treatments had obvious rules and great diversities. There were high correlations between different types of spectra parameters and ten physiological and biochemical indexes of flue-cured tobacco leaves. Hyperspectral characteristic variables of ten physiological and biochemical indexes were found through stepwise regression, and SDr/SDb was the characteristic variable closest to seven biochemical contents. Simultaneously, the R² and regression coefficient of equations reached 0.05 significant level and the equations had good estimating effects through the examination of other samples. Accordingly, this study suggested that the ten physiological and biochemical indexes could be estimated quickly by the estimating models, at the same time nitrogen-potassium fertilization and growth condition of flue-cured tobacco could be inspected.

Keywords: flue-cured tobacco leaves; hyperspectral parameters; physiological and biochemical indexes

Joachim Thiede, Sabrina A. Schmidt, Barbara Rudolph, Phylogenetic implication of the chloroplast rpoC1 intron loss in the Aizoaceae (Caryophyllales), *Biochemical Systematics and Ecology*, Volume 35, Issue 6, June 2007, Pages 372-380, ISSN 0305-1978, DOI: 10.1016/j.bse.2006.12.010.

(<http://www.sciencedirect.com/science/article/B6T4R-4N2DRRC-1/2/a6774b0cc56427df0180e7299bf9e39d>)

Abstract:

A representative sample of 69 species from all recognized infrafamilial taxa of the family Aizoaceae (angiosperms, eudicotyledons, Caryophyllales) was surveyed for the presence/absence of the rpoC1 intron. PCR fragments of the samples fall into two size classes: a long fragment of approximately 1200 bp, and a short fragment of approximately 500 bp which was found in all samples from the tribes Drosanthemeae and Ruschieae of subfamily Ruschioideae. The length difference of about 700 bp corresponds to the length of the intron (738 bp in tobacco). Sequencing of the short fragment from *Monilaria moniliformis* revealed the precise excision of the intron as found in a previous study of the cactus family. It is concluded that the intron lacks in all samples from the clade including the tribes Drosanthemeae and Ruschieae of subfamily Ruschioideae,

thus providing valuable PCR-based, sequence- and morphology-independent evidence for the monophyly of this lineage.

Keywords: Aizoaceae; Caryophyllales; Intron loss; *Monilaria moniliformis*; rpoC1 intron; Subfamily Ruschioideae tribe Drosanthemeae; Subfamily Ruschioideae tribe Ruschieae

J. Yang, Y. Hu, J.B. Cai, X.L. Zhu, Q.D. Su, Y.Q. Hu, F.X. Liang, Selective hair analysis of nicotine by molecular imprinted solid-phase extraction: An application for evaluating tobacco smoke exposure, *Food and Chemical Toxicology*, Volume 45, Issue 6, June 2007, Pages 896-903, ISSN 0278-6915, DOI: 10.1016/j.fct.2006.11.010.

(<http://www.sciencedirect.com/science/article/B6T6P-4MF3Y1Y-3/2/63beef054daae669bf842bb149b6b0a4>)

Abstract:

A method using a molecularly imprinted polymer (MIP) as the selective sorbent for solid-phase extraction (SPE) has been developed. Its application to the assay of hairy nicotine level among smokers and non-smokers with high-performance liquid chromatography (HPLC) and evaluation of exposures to the environmental tobacco smoke (ETS) were validated. The MIP was synthesized using nicotine as the template molecule and methacrylic acid (MAA) as the functional monomer. This MIP-SPE method provided inherent selectivity and a sensitive response to nicotine with a detection limit of 0.2 ng/ml hair at a signal-to-noise ratio of 3:1 and the limit of quantification was 0.5 ng/ml. The linearity was assessed in the range of 0.5-80 ng/ml hair, with a coefficient (r^2) greater than 0.987. The amounts of nicotine determined in smokers and non-smokers hair were in the range of 5.1-69.5 ng/mg hair and 0.50-9.3 ng/mg hair, respectively. The reported measures of ETS exposure were significantly associated with hairy nicotine levels. This assay of nicotine in hair using MISPE provided a very selective and reliable method for the evaluation of the exposure to tobacco smoke.

Keywords: Nicotine; Molecularly imprinted polymer; Solid-phase extraction; Hair; Tobacco smoke exposure; High-performance liquid chromatography

Thomas E. McGrath, Jan B. Wooten, W. Geoffrey Chan, Mohammad R. Hajaligol, Formation of polycyclic aromatic hydrocarbons from tobacco: The link between low temperature residual solid (char) and PAH formation, *Food and Chemical Toxicology*, Volume 45, Issue 6, June 2007, Pages 1039-1050, ISSN 0278-6915, DOI: 10.1016/j.fct.2006.12.010.

(<http://www.sciencedirect.com/science/article/B6T6P-4MN3VB3-2/2/fb120e38d92dc857d16beceb5ef205c4>)

Abstract:

The formation of condensed ring polycyclic aromatic hydrocarbons (PAHs) from the pyrolysis of ground tobacco in helium over the temperature range of 350-600 [degree sign]C was investigated. PAH yields in the ng/g range were detected and the maximum yields of all PAHs studied including benzo[a]pyrene (B[a]P) and benzo[a]anthracene (B[a]A) occurred between 500 and 550 [degree sign]C. The pathway to PAH formation in the 350-600 [degree sign]C temperature range is believed to proceed via a carbonization process where the residual solid (char) undergoes a chemical transformation and rearrangement to give a more condensed polycyclic aromatic structure that upon further heating evolves PAH moieties. Extraction of tobacco with water led to a two fold increase in the yields of most PAHs studied. The extraction process removed low temperature non-PAH-forming components, such as alkaloids, organic acids and inorganic salts, and concentrated instead (on a per unit weight basis) tobacco components such as cell wall biopolymers and lipids. Hexane extraction of the tobacco removed lipophilic components, previously identified as the main source of PAH precursors, but no change in PAH yields was observed from the hexane-extracted tobacco. Tobacco cell wall components such as cellulose, hemicellulose, and lignin are identified as major low temperature PAH precursors. A link between the formation of a low temperature char that evolves PAHs upon heating is established and the observed ng/g

yields of PAHs from tobacco highlights a low temperature solid phase formation mechanism that may be operable in a burning cigarette.

Keywords: Low temperature pyrolysis; Polycyclic aromatic hydrocarbons; Tobacco

Petra Matouskova, Iva Pichova, Ales Svatos, Functional characterization of a desaturase from the tobacco hornworm moth (*Manduca sexta*) with bifunctional Z11- and 10,12-desaturase activity, *Insect Biochemistry and Molecular Biology*, Volume 37, Issue 6, June 2007, Pages 601-610, ISSN 0965-1748, DOI: 10.1016/j.ibmb.2007.03.004.

(<http://www.sciencedirect.com/science/article/B6T79-4N7S535-4/2/1f73cc126ad616dd8603f23eca68b89f>)

Abstract:

The pheromone blend produced by the tobacco hornworm moth (*Manduca sexta*) (L.) female is unusually complex and contains two conjugated dienals and trienals together with two monounsaturated alkenals. Here, we describe the identification and construction of two genes encoding MsexKPSE and MsexAPTQ desaturases from a cDNA library prepared from the total RNA of the *M. sexta* pheromone gland. The MsexKPSE desaturase shares a high degree of similarity with [Δ]9-desaturases from different moth species. The functional expression of MsexAPTQ desaturase in *Saccharomyces cerevisiae* followed by a detailed GC-MS analysis of fatty acid methyl esters (FAME) and their derivatized products and gas-phase Fourier transform infrared (FTIR) spectroscopy of the extracted FAME confirms that this enzyme is a bifunctional Z-[Δ]11-desaturase. MsexAPTQ desaturase catalyses the production of Z11-hexadecenoate (Z11-16) and Z10E12- and E10E12-hexadecadienoates (Z10E12-16) via 1,4-desaturation of the Z11-16 substrate. The stereochemistry of 1,4-desaturation and formation of isomers is discussed.

Keywords: Fatty acid; Sex pheromone; Desaturase; Lepidoptera; Conjugated dienes; 1,4-desaturation

Kent S. Shelby, Holly J.R. Popham, Increased plasma selenium levels correlate with elevated resistance of *Heliothis virescens* larvae against baculovirus infection, *Journal of Invertebrate Pathology*, Volume 95, Issue 2, June 2007, Pages 77-83, ISSN 0022-2011, DOI: 10.1016/j.jip.2007.01.001.

(<http://www.sciencedirect.com/science/article/B6WJV-4MVN16J-1/2/027fca832a5a211b8a87b54ce655153b>)

Abstract:

We reported that dietary selenium (Se) impacted the growth and development of *Trichoplusia ni* reared for many generations on diet containing extremely low levels of Se. Larvae had an elevated resistance to per os infection with a baculovirus. In this study, we examine how dietary Se (in the form of selenite) affects the growth, development, and Se content of *Heliothis virescens* that have been laboratory reared for less than two years. Larvae fed a commercial tobacco budworm diet supplemented with greater than 20 ppm Se grew at a slower rate than insects fed lower levels of Se and had an increase in the amount of Se sequestered in pupae. Larvae fed diets containing from 10-60 ppm Se exhibited elevated plasma concentrations of the micronutrient and increased plasma virucidal activity against *Helicoverpa zea* single nucleopolyhedrovirus (HzSNPV). Larvae reared on diet supplemented with 10 or 60 ppm Se until the onset of the penultimate instar were then infected per os or by injection with increasing concentrations of the baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). Larvae fed dietary Se and infected with occluded virus per os displayed a significantly lower mortality compared with infected larvae not fed Se. Our results suggest that dietary Se levels are directly correlated with plasma Se levels, and that plasma Se levels are in turn correlated with baculovirus resistance.

Keywords: *Heliothis virescens*; Selenium; Biological control; Resistance; Nutritional immunology; *Autographa californica* multiple nucleopolyhedrovirus; *Helicoverpa zea* single nucleopolyhedrovirus

David J. Millar, Marianne Long, Georgina Donovan, Paul D. Fraser, Alain-Michel Boudet, Saida Danoun, Peter M. Bramley, G. Paul Bolwell, Introduction of sense constructs of cinnamate 4-hydroxylase (CYP73A24) in transgenic tomato plants shows opposite effects on flux into stem lignin and fruit flavonoids, *Phytochemistry*, Volume 68, Issue 11, June 2007, Pages 1497-1509, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2007.03.018.

(<http://www.sciencedirect.com/science/article/B6TH7-4NRMD96-1/2/99e70a1dfc01a7ff8c55f63fdd7da83c>)

Abstract:

Understanding regulation of phenolic metabolism underpins attempts to engineer plants for diverse properties such as increased levels of antioxidant flavonoids for dietary improvements or reduction of lignin for improvements to fibre resources for industrial use. Previous attempts to alter phenolic metabolism at the level of the second enzyme of the pathway, cinnamate 4-hydroxylase have employed antisense expression of heterologous sequences in tobacco. The present study describes the consequences of homologous sense expression of tomato CYP73A24 on the lignin content of stems and the flavonoid content of fruits. An extensive number of lines were produced and displayed four developmental variants besides a normal phenotype. These aberrant phenotypes were classified as dwarf plants, plants with distorted (curly) leaves, plants with long internodes and plants with thickened waxy leaves. Nevertheless, some of the lines showed the desired increase in the level of rutin and naringenin in fruit in a normal phenotype background. However this could not be correlated directly to increased levels of PAL and C4H expression as other lines showed less accumulation, although all lines tested showed increases in leaf chlorogenic acid which is typical of Solanaceous plants when engineered in the phenylpropanoid pathway. Almost all transgenic lines analysed showed a considerable reduction in stem lignin and in the lines that were specifically examined, this was correlated with partial sense suppression of C4H. Although not the primary purpose of the study, these reductions in lignin were amongst the greatest seen in plants modified for lignin by manipulation of structural genes. The lignin showed higher syringyl to coniferyl monomeric content contrary to that previously seen in tobacco engineered for downregulation of cinnamate 4-hydroxylase. These outcomes are consistent with placing CYP73A24 more in the lignin pathway and having a role in flux control, while more complex regulatory processes are likely to be involved in flavonoid and chlorogenic acid accumulation.

Keywords: *Lycopersicon esculentum*; Solanaceae; Tomato; Flavonoids; Lignin; Cinnamate 4-hydroxylase; Sense expression; Carotenoids

N. Sabharwal, I. Icoz, D. Saxena, G. Stotzky, Release of the recombinant proteins, human serum albumin, [beta]-glucuronidase, glycoprotein B from human cytomegalovirus, and green fluorescent protein, in root exudates from transgenic tobacco and their effects on microbes and enzymatic activities in soil, *Plant Physiology and Biochemistry*, Volume 45, Issues 6-7, June-July 2007, Pages 464-469, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2007.03.009.

(<http://www.sciencedirect.com/science/article/B6VRD-4N85B0N-6/2/18b57130d32826ed3c9cb72840876f81>)

Abstract:

We determined the release in root exudates of human serum albumin (HSA), [beta]-glucuronidase (GUS), glycoprotein B (gB) from human cytomegalovirus, and green fluorescent protein (GFP) from genetically modified transgenic tobacco expressing the genes for these proteins in hydroponic culture and non-sterile soil. GUS, gB, and GFP were expressed in the plant but were not released in root exudates, whereas HSA was both expressed in the plant and released in root exudates, as shown by a 66.5-kDa band on SDS-PAGE and Western blot and confirmed by ELISA. Root exudates from GUS and gB plants showed no bands that could be attributed to these proteins on SDS-PAGE, and root exudates from GFP plants showed no fluorescence. The

concentration of HSA in root exudates was estimated to be 0.021 ng ml⁻¹, whereas that in the plant biomass was estimated to be 0.087 ng ml⁻¹. The concentration of HSA in soil was estimated to be 0.049 ng g⁻¹. No significant differences in the number of microorganisms and the activity of selected enzymes were observed between rhizosphere soil of non-modified and HSA tobacco.

Keywords: Transgenic tobacco; Human serum albumin; [beta]-Glucuronidase; Glycoprotein B; Green fluorescent protein; Root exudates; Soil microbes; Soil enzymes

Shu Wei, Yaniv Semel, Ben-Ami Bravdo, Henryk Czosnek, Oded Shoseyov, Expression and subcellular compartmentation of *Aspergillus niger* [beta]-glucosidase in transgenic tobacco result in an increased insecticidal activity on whiteflies (*Bemisia tabaci*), *Plant Science*, Volume 172, Issue 6, June 2007, Pages 1175-1181, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2007.02.018.

(<http://www.sciencedirect.com/science/article/B6TBH-4N7SBJ9-1/2/06037bc0de218db439d48dd11dc23ced>)

Abstract:

Transgenic *Nicotiana tabacum* plants expressing *Aspergillus niger* [beta]-glucosidase (EC 3.2.1.21) gene (BGL1) in different subcellular compartments [cell wall (Tcw), endoplasmic reticulum (Ter), and vacuole (Tvc)] were analyzed to study the effects of BGL1 localization on plant growth and plant-insect interaction. Transgenic and non-transgenic plants were grown and characterized in a greenhouse with 25/16 [degree sign]C day/night temperatures and natural sunlight. Plant insecticidal activity was analyzed with adult whiteflies (*Bemisia tabaci*) in vial and cage experiments. Compared with wild-type controls, Ter and Tvc transgenic plants did not differ significantly in seed germination, plant growth rate, plant height, or flowering time. However, in Tcw seed germination and beginning of flowering were significantly delayed, and leaf area and plant fresh weight were significantly reduced. Transgenic plants had a marked insecticidal effect on whiteflies (*Bemisia tabaci*) and on Diptera spp. flies. The density of secretory glandular trichomes was significantly greater in transgenic than in wild-type leaves. This work indicates that hydrolysis of yet to be identified glycosides, may play an important role in plant insect resistance mechanism and plant trichome development.

Keywords: *Bemisia tabaci*; [beta]-Glucosidase; *Nicotiana tabacum*; Plant-insect interactions; Trichome

Angelika Mustroph, Uwe Sonnewald, Sophia Biemelt, Characterisation of the ATP-dependent phosphofructokinase gene family from *Arabidopsis thaliana*, *FEBS Letters*, Volume 581, Issue 13, 29 May 2007, Pages 2401-2410, ISSN 0014-5793, DOI: 10.1016/j.febslet.2007.04.060.

(<http://www.sciencedirect.com/science/article/B6T36-4NM6DP9-1/2/631f536aab9391648196c87362c53ad3>)

Abstract:

Plants possess two different types of phosphofructokinases, an ATP-dependent (PFK) and a pyrophosphate-dependent form (PFP). While plant PFPs have been investigated in detail, cDNA clones coding for PFK have not been identified in *Arabidopsis thaliana*. Searching the *A. thaliana* genome revealed 11 putative members of a phosphofructokinase gene family. Among those, four sequences showed high homology to the alpha- or beta-subunits of plant PFPs. Seven cDNAs resulted in elevated PFK, but not PFP activity after transient expression in tobacco leaves suggesting that they encode *Arabidopsis* PFKs. RT-PCR revealed different tissue-specific expression of the individual forms. Furthermore, analysis of GFP fusion proteins indicated their presence in different sub-cellular compartments.

Keywords: Phosphofructokinase; *Agrobacterium* infiltration; *Arabidopsis thaliana*

Huijun Liu, Chunhong Wei, Yongwang Zhong, Yi Li, Rice black-streaked dwarf virus minor core protein P8 is a nuclear dimeric protein and represses transcription in tobacco protoplasts, *FEBS*

Letters, Volume 581, Issue 13, 29 May 2007, Pages 2534-2540, ISSN 0014-5793, DOI: 10.1016/j.febslet.2007.04.071.

(<http://www.sciencedirect.com/science/article/B6T36-4NN1PY7-6/2/839c9836347f87bf302b8cb620ef06e7>)

Abstract:

Virus-encoding nuclear transcriptional regulators play important roles in the viral life cycle. Most of these proteins exhibit intrinsic transcriptional activation or repression activity, and are involved in the regulation of the expression of virus genome itself or important cellular genes to facilitate viral replication and inhibit antiviral responses. Here, we report that the minor core protein P8 of Rice black-streaked dwarf virus, a dsRNA virus infecting host plants and insects, is targeted to the nucleus of insect and plant cells via its N-terminal 1-40 amino acids and possesses potent active transcriptional repression activity in Bright Yellow-2 tobacco suspension cells. Moreover, P8, like many transcriptional regulatory proteins, is capable of forming homo-dimers within insect cells and in vitro. All these data suggest that P8 is likely to enter the nucleus of host cell and play an important role as a negative transcriptional regulator of host gene expression during the process of virus-host interaction.

Keywords: RBSDV P8; Minor core protein; Nuclear targeting; Transcriptional repression; Dimer

Md. Anamul Hoque, Eiji Okuma, Mst. Nasrin Akhter Banu, Yoshimasa Nakamura, Yasuaki Shimoishi, Yoshiyuki Murata, Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities, *Journal of Plant Physiology*, Volume 164, Issue 5, 3 May 2007, Pages 553-561, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.03.010.

(<http://www.sciencedirect.com/science/article/B7GJ7-4JVT1G5-1/2/ed5b4b6e60c4a7fb0c6f648304852e6c>)

Abstract: Summary

Proline and betaine accumulate in plant cells under environmental stresses including salt stress. Here, we investigated effects of proline and betaine on the growth and activities of antioxidant enzymes in tobacco Bright Yellow-2 (BY-2) culture cells in suspension under salt stress. Both proline and betaine mitigated the inhibition of growth of BY-2 cells under salt stress and the mitigating effect of proline was more than that of betaine. Salt stress significantly decreased the activities of superoxide dismutase (SOD), catalase and peroxidase in BY-2 cells. Exogenous application of proline or betaine alleviated the reduction in catalase and peroxidase activities but not SOD activity under salt stress. In addition, proline was found to be effective in alleviating the inhibition of salt stress-induced catalase and peroxidase activities in BY-2 cells. Neither proline nor betaine directly scavenged superoxide (O₂⁻) or hydrogen peroxide (H₂O₂). It is concluded that exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine because of its superior ability to increase the activities of antioxidant enzymes.

Keywords: Antioxidant enzymes; Betaine; Proline; Reactive oxygen species; Salt stress

Takanori Kobayashi, Toshihiro Yoshihara, Reiko Nakanishi Itai, Hiromi Nakanishi, Michiko Takahashi, Satoshi Mori, Naoko K. Nishizawa, Promoter analysis of iron-deficiency-inducible barley IDS3 gene in Arabidopsis and tobacco plants, *Plant Physiology and Biochemistry*, Volume 45, Issue 5, Iron nutrition and Interactions in Plants, XIII International Symposium on Iron Nutrition and Interactions in Plants (ISINIP), May 2007, Pages 262-269, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2007.03.007.

(<http://www.sciencedirect.com/science/article/B6VRD-4N85B0N-3/2/20561be726d6aebbf88d2681678d785b>)

Abstract:

Under conditions of iron deficiency, graminaceous plants induce the expression of genes involved in the biosynthesis of mugineic acid family phytosiderophores. We previously identified the novel

cis-acting elements IDE1 and IDE2 (iron-deficiency-responsive element 1 and 2) through promoter analysis of the barley (*Hordeum vulgare* L.) iron-deficiency-inducible IDS2 gene in tobacco (*Nicotiana tabacum* L.). To gain further insight into plant gene regulation under iron deficiency, we analyzed the barley iron-deficiency-inducible IDS3 gene, which encodes mugineic acid synthase. IDS3 promoter fragments were fused to the [beta]-glucuronidase (GUS) gene, and this construct was introduced into *Arabidopsis thaliana* L. and tobacco plants. In both *Arabidopsis* and tobacco, GUS activity driven by the IDS3 promoter showed strongly iron-deficiency-inducible and root-specific expression. Expression occurred mainly in the epidermis of *Arabidopsis* roots, whereas expression was dominant in the pericycle, endodermis, and cortex of tobacco roots, resembling the expression pattern conferred by IDE1 and IDE2. Deletion analysis revealed that a sequence within -305 nucleotides from the translation start site was sufficient for specific expression in both *Arabidopsis* and tobacco roots. Gain-of-function analysis revealed functional regions at -305/-169 and -168/-93, whose coexistence was required for the induction activity in *Arabidopsis* roots. Multiple IDE-like sequences were distributed in the IDS3 promoter and were especially abundant within the functional region at -305/-169. A sequence moderately homologous to that of IDE1 was also present within the -168/-93 region. These IDE-like sequences would be the first candidates for the functional iron-deficiency-responsive elements in the IDS3 promoter.

Keywords: cis-acting elements; IDE; IDS3; Inducible gene expression; Iron deficiency; Promoter analysis; Root-specific expression

Satoshi Ito, Haruhiko Inoue, Takanori Kobayashi, Masaaki Yoshida, Satoshi Mori, Naoko Nishizawa, Kyoko Higuchi, Interspecies compatibility of NAS1 gene promoters, *Plant Physiology and Biochemistry*, Volume 45, Issue 5, Iron nutrition and Interactions in Plants, XIII International Symposium on Iron Nutrition and Interactions in Plants (ISINIP), May 2007, Pages 270-276, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2007.04.001.

(<http://www.sciencedirect.com/science/article/B6VRD-4NF4F15-3/2/70964a4e2411e2436358223538d4e774>)

Abstract:

Nicotianamine and nicotianamine synthase (NAS) play key roles in iron nutrition in all higher plants. However, the mechanism underlying the regulation of NAS expression differs among plant species. Sequences homologous to iron deficiency-responsive elements (IDEs), i.e., cis-acting elements, are found on the promoters of these genes. We aimed to verify the interspecies compatibility of the Fe-deficiency response of NAS1 genes and understand the universal mechanisms that regulate their expression patterns in higher plants. Therefore, we introduced the graminaceous (*Hordeum vulgare* L. and *Oryza sativa* L.) NAS1 promoter::GUS into dicots (*Nicotiana tabacum* L. and *Arabidopsis thaliana* L.). Fe deficiency induced HvNAS1 expression in the shoots and roots when introduced into rice. HvNAS1 promoter::GUS and OsNAS1 promoter::GUS induced strong expression of GUS under Fe-deficient conditions in transformed tobacco. In contrast, these promoters only definitely functioned in *Arabidopsis* transformants. These results suggest that some Fe nutrition-related trans-factors are not compatible between graminaceous plants and *Arabidopsis*. HvNAS1 promoter::GUS induced GUS activity only in the roots of transformed tobacco under Fe-deficient conditions. On the other hand, OsNAS1 promoter::GUS induced GUS activity in both the roots and shoots of transformed tobacco under conditions of Fe deficiency. In tobacco transformants, the induction of GUS activity was induced earlier in the shoots than roots. These results suggest that the HvNAS1 and OsNAS1 promoters are compatible with Fe-acquisition-related trans-factors in the roots of tobacco and that the OsNAS1 promoter is also compatible with some shoot-specific Fe deficiency-related trans-factors in tobacco.

Keywords: Dicotyledonous plants; Fe deficiency; Graminaceous plants; Heterologous expression; Nicotianamine synthase

Magdalena Arasimowicz, Jolanta Floryszak-Wieczorek, Nitric oxide as a bioactive signalling molecule in plant stress responses, *Plant Science*, Volume 172, Issue 5, May 2007, Pages 876-887, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2007.02.005.

(<http://www.sciencedirect.com/science/article/B6TBH-4N25VP7-1/2/4012168189de55bf8ce9e0d916e3f236>)

Abstract:

Nitric oxide (NO) is an important signalling molecule with diverse physiological functions in plants. It was found to play a crucial role in plant growth and development, starting from germination to flowering, ripening of fruit and senescence of organs. Also in case of environmental stress hazard, caused by both abiotic and biotic factors, enhanced NO generation is observed in different plant species and organs. This review is focused mainly on the essential role of NO in plant signalling network, leading to the expression of defence response genes under various stress conditions. NO can provoke both beneficial and harmful effects in plant cells. This dual role probably depends on the local concentration of NO as an effect of the rate of synthesis, translocation, effectiveness of removal of this reactive nitrogen species, as well as its ability to directly interact with other molecules and signals.

Keywords: Nitric oxide; Biotic and abiotic stress; cGMP-dependent and -independent signalling; Programmed cell death

Keita Takada, Shin Watanabe, Tsunenori Sano, Biao Ma, Hiroshi Kamada, Hiroshi Ezura, Heterologous expression of the mutated melon ethylene receptor gene Cm-ERS1/H70A produces stable sterility in transgenic lettuce (*Lactuca sativa*), *Journal of Plant Physiology*, Volume 164, Issue 4, 5 April 2007, Pages 514-520, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.10.003.

(<http://www.sciencedirect.com/science/article/B7GJ7-4MRFC2S-4/2/c5e038e3194858c0a447de6863d0fe29>)

Abstract: Summary

The mutated melon ethylene receptor gene Cm-ERS1/H70A was introduced into tobacco and induced stable sterility in transgenic lines. This gene contains a missense mutation that converts the His70 residue to Ala in the melon ethylene receptor gene Cm-ERS1. To test the applicability of this inducible sterility system to other plants, lettuce (*Lactuca sativa*) was transformed with the gene using *Agrobacterium*, and putative transformants containing Cm-ERS1/H70A were obtained. Thirteen randomly selected putative transformants were grown in a growth room under constant conditions, and seven of the lines showed sterility or significantly reduced fertility. DNA gel blot analysis confirmed the integration of the Cm-ERS1/H70A gene into the genomes of the putative transformants, and RT-PCR and protein gel blot analysis confirmed the expression of Cm-ERS1/H70A mRNA and protein in all of the transformants. Five transformants showing sterility or reduced fertility when grown in a growth room under constant conditions were randomly selected to be grown in an open-air greenhouse under various environmental conditions. All five showed stable sterility under the various conditions. These results suggest that Cm-ERS1/H70A can induce sterility in heterologous transgenic plants.

Keywords: Lettuce transformation; Melon ethylene receptor gene (Cm-ERS1); Mutated Cm-ERS1 gene (H70A); Sterility

Elisabeth Moyano, Javier Palazon, Mercedes Bonfill, Lidia Osuna, Rosa M. Cusido, Kirsi-Marja Oksman-Caldentey, M. Teresa Pinol, Biotransformation of hyoscyamine into scopolamine in transgenic tobacco cell cultures, *Journal of Plant Physiology*, Volume 164, Issue 4, 5 April 2007, Pages 521-524, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.06.012.

(<http://www.sciencedirect.com/science/article/B7GJ7-4KM46M6-7/2/4a975affede4db53ed5ae843daad081e>)

Abstract: Summary

Hyoscyamine-6[β]-hydroxylase (H6H) catalyses the conversion of hyoscyamine into its epoxide scopolamine, a compound with a higher added value in the pharmaceutical market than hyoscyamine. We report the establishment of tobacco cell cultures carrying the *Hyoscyamus muticus* h6h gene under the control of the promoter CAMV 35S. The cell cultures were derived from hairy roots obtained via genetically modified *Agrobacterium rhizogenes* carrying the pRi and pLAL21 plasmids. The cultures were fed with hyoscyamine, and 4 weeks later the amount of scopolamine produced was quantified by HPLC. The transgenic cell suspension cultures showed a considerable capacity for the bioconversion of hyoscyamine into scopolamine, and released it to the culture medium. Although the scale-up from shake-flask to bioreactor culture usually results in reduced productivities, our transgenic cells grown in a 5-L turbine stirred tank reactor in a batch mode significantly increased the scopolamine accumulation.

Keywords: Biotransformation; Hyoscyamine-6[β]-hydroxylase; Hyoscyamine; Scopolamine; Tobacco cell cultures

Deying Ma, Kevin Gorman, Greg Devine, Wanchun Luo, Ian Denholm, The biotype and insecticide-resistance status of whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae), invading cropping systems in Xinjiang Uygur Autonomous Region, northwestern China, *Crop Protection*, Volume 26, Issue 4, April 2007, Pages 612-617, ISSN 0261-2194, DOI: 10.1016/j.cropro.2006.04.027.

(<http://www.sciencedirect.com/science/article/B6T5T-4KJ5T26-1/2/7b892ac16ae640f45e43959ab463cd90>)

Abstract:

Xinjiang Uygur Autonomous Region in northwestern China is undergoing rapid development of its agricultural industries. Areas planted with cotton, grapes and vegetables have expanded dramatically in recent years. The tobacco whitefly, *Bemisia tabaci*, was first found in Xinjiang in 1998 on poinsettias (*Euphorbia pulcherrima*) and may have been imported to the region on that crop. Analysis of non-specific esterases using native polyacrylamide gel electrophoresis showed six samples of *B. tabaci* collected within a 200 km radius of Urumqi (Xinjiang's capital city) to belong to the highly invasive B biotype. The samples showed very similar profiles of insecticide resistance with very strong (>1000-fold) resistance to pyrethroids, low to moderate resistance to imidacloprid and pyriproxyfen, and no resistance to abamectin. The implications for resistance management and contending with further invasions of aggressive *B. tabaci* biotypes are discussed.

Keywords: *Bemisia tabaci*; Biotype; Insecticide resistance; Cotton; Xinjiang; China

Odalys Garcia, Joci A.N. Macedo, Ricardo Tiburcio, Gustavo Zaparoli, Johana Rincones, Livia M.C. Bittencourt, Geruza O. Ceita, Fabienne Micheli, Abelmon Gesteira, Andrea C. Mariano, Marlene A. Schiavinato, Francisco J. Medrano, Lyndel W. Meinhardt, Goncalo A.G. Pereira, Julio C.M. Cascardo, Characterization of necrosis and ethylene-inducing proteins (NEP) in the basidiomycete *Moniliophthora perniciosa*, the causal agent of witches' broom in *Theobroma cacao*, *Mycological Research*, Volume 111, Issue 4, April 2007, Pages 443-455, ISSN 0953-7562, DOI: 10.1016/j.mycres.2007.01.017.

(<http://www.sciencedirect.com/science/article/B7XMR-4N1JRXT-2/2/e2c1d321cc5f9afc1b08169129ad60c3>)

Abstract:

The hemibiotrophic basidiomycete *Moniliophthora perniciosa* causes witches' broom disease of *Theobroma cacao*. Analysis of the *M. perniciosa* draft genome led to the identification of three putative genes encoding necrosis and ethylene-inducing proteins (MpNEPs), which are apparently located on the same chromosome. MpNEP1 and 2 have highly similar sequences and are able to induce necrosis and ethylene emission in tobacco and cacao leaves. MpNEP1 is expressed in both biotrophic and saprotrophic mycelia, the protein behaves as an oligomer in solution and is

very sensitive to temperature. MpNEP2 is expressed mainly in biotrophic mycelia, is present as a monomer in solution at low concentrations (<40 [μ]m) and is able to recover necrosis activity after boiling. These differences indicate that similar NEPs can have distinct physical characteristics and suggest possible complementary roles during the disease development for both proteins. This is the first report of NEP1-like proteins in a basidiomycete.

Keywords: Basidiomycota; Cacao; Plant pathology; WBD

Trabelsi Darine, M.B. Allagui, M. Rouaissi, A. Boudabbous, Pathogenicity and RAPD analysis of *Phytophthora nicotianae* pathogenic to pepper in Tunisia, *Physiological and Molecular Plant Pathology*, Volume 70, Issues 4-6, April-June 2007, Pages 142-148, ISSN 0885-5765, DOI: 10.1016/j.pmpp.2007.08.002.

(<http://www.sciencedirect.com/science/article/B6WPC-4PJCYB4-1/2/90be7a02f811a93eacf4ae80f7afd756>)

Abstract:

Nine isolates of *Phytophthora nicotianae* were isolated from infected pepper plants. Their pathogenicity was studied in *Capsicum annuum* in comparison with *P. nicotianae* isolates from tomato and tobacco. The pathogenicity test showed that pepper isolates of *P. nicotianae* are adapted to their host. Banding patterns obtained by RAPD analysis with six oligonucleotide primers revealed polymorphism that grouped the isolates independently of the plant host. The polygenic dendrogram showed that pepper isolates were more similar to tomato isolates than to tobacco isolates. The RAPD bands of 1300 and 1500 bp, detected with primers OPD-01 and OPD-10, respectively, appeared specific to the most pathogenic pepper isolates. The OPK-08-1950 seems specific to the isolates of *P. nicotianae* from tomato. These results suggest that host specified might occur in *P. nicotianae* and that may be due to interspecific hybridization events resulting in novel pathogenic behavior.

Keywords: *Phytophthora nicotianae*; RAPD pathogenicity

Vera Quecini, Mario L. Lopes, Flavia T.H. Pacheco, Maria das G. Ongarelli, Tomato spotted wilt virus triggers specific and shared defense mechanisms in hypersensitive and susceptible Solanaceae hosts, *Physiological and Molecular Plant Pathology*, Volume 70, Issues 4-6, April-June 2007, Pages 189-197, ISSN 0885-5765, DOI: 10.1016/j.pmpp.2007.09.006.

(<http://www.sciencedirect.com/science/article/B6WPC-4PT7X0F-2/2/699d2306c719f104b7b780b8e552ac65>)

Abstract:

In situ and in vitro techniques were employed to investigate the metabolic changes caused by Tomato spotted wilt virus in hypersensitive and susceptible hosts; *Petunia hybrida* and *Nicotiana tabacum*, respectively. In petunia, H₂O₂ accumulation preceded increased peroxidase and shikimate dehydrogenase activity at local lesion sites. In systemic tobacco plants, peroxidase activity was induced prior to symptom onset and the activity of shikimate dehydrogenase was disrupted upon viral infection. Taken together, our data suggest that reactive oxygen species-based mechanisms of defense are shared by hypersensitive and susceptible hosts, although downstream components and regulatory mechanisms are distinct.

Keywords: Hypersensitive response; *Nicotiana tabacum*; Pathogenesis-related proteins; Peroxidase; *Petunia hybrida*; Reactive oxygen species; Shikimate dehydrogenase; Susceptibility; Tissue print; Tospovirus

Joon Ho Park, Mohammed Oufattole, John C. Rogers, Golgi-mediated vacuolar sorting in plant cells: RMR proteins are sorting receptors for the protein aggregation/membrane internalization pathway, *Plant Science*, Volume 172, Issue 4, April 2007, Pages 728-745, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.12.008.

(<http://www.sciencedirect.com/science/article/B6TBH-4MMPNDR-1/2/bc9113d2e01f38cac871ef0ae71ca24d>)

Abstract:

The mechanism by which storage proteins carrying C-terminal vacuolar sorting determinants (ctVSDs) are sorted to vacuoles in plant cells has been controversial, and some reports suggest that receptors of the BP80/AtVSR family are responsible. Here we show that an Arabidopsis receptor homology-transmembrane-RING H2 (RMR) protein binds specifically the tobacco chitinase ctVSD both in vitro and in vivo, but not the proaleurain sequence-specific vacuolar sorting determinant (ssVSD). In contrast, BP80 binds the proaleurain ssVSD but not the chitinase ctVSD in vivo. In co-expression studies with red- and green-tagged forms of reporter proteins carrying the RMR or BP80 cytoplasmic tails, RMR protein and BP80 do not co-localize, as compared to BP80-AtVSR1 or RMR-RMR co-expressions. RMR protein co-localizes with the green fluorescent protein-chitinase ctVSD reporter while AtVSR1 does not. In transient expression experiments, reporters with the RMR protein cytoplasmic tail are internalized into vacuoles while those carrying the BP80 or AtVSR1 cytoplasmic tails are not. We propose that aggregation of soluble proteins carrying a ctVSD is accompanied by RMR binding and membrane internalization. Binding of aggregates would explain how the RMR protein, which does not recycle back to the Golgi, could serve as an efficient sorting receptor.

Keywords: RMR protein; Vacuolar sorting receptor; BP80; Dense vesicles

, Use of cell morphology to evaluate the effect of a peroxidase gene on cell death induction thresholds in tobacco, *Plant Science*, Volume 172, Issue 4, April 2007, Page 852, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.03.023.

(<http://www.sciencedirect.com/science/article/B6TBH-4MR88WT-1/2/c5c1303cc0fb3f7f70ed13d3ee58e6e7>)

Emma Burbridge, Mark Diamond, Philip J. Dix, Paul F. McCabe, Use of cell morphology to evaluate the effect of a peroxidase gene on cell death induction thresholds in tobacco, *Plant Science*, Volume 172, Issue 4, April 2007, Pages 853-860, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.03.024.

(<http://www.sciencedirect.com/science/article/B6TBH-4MP055Y-2/2/7089065151d5fc9216ab8eb4dbec6470>)

Abstract:

Tobacco suspension cultures were subjected to a range of heat stresses and used to compare morphological aspects of programmed cell death (PCD) and necrosis. Cells undergoing PCD were found to display characteristic death morphology, caused by cytoplasmic retraction of the protoplast, and to have cleaved DNA. We evaluated if the morphological characteristics of PCD could be used to monitor changes in cell death induction thresholds in transgenic cell cultures with high levels of peroxidase activity. Again, using a heat shock assay, we show that tobacco cell cultures with elevated levels of peroxidase have higher cell death induction threshold levels than wild type tobacco cell cultures. Thus, assessing PCD associated morphological changes can report on the effect of altering peroxidase genes on cell death activation in tobacco. This study demonstrates that PCD morphology could routinely be used to monitor the effects of introduced genes on programmed cell death induction thresholds in plants.

Keywords: Programmed cell death; Heat-shock; Peroxidase

Sally L. Letsinger, Greg A. Olyphant, Distributed energy-balance modeling of snow-cover evolution and melt in rugged terrain: Tobacco Root Mountains, Montana, USA, *Journal of Hydrology*, Volume 336, Issues 1-2, 30 March 2007, Pages 48-60, ISSN 0022-1694, DOI: 10.1016/j.jhydro.2006.12.012.

(<http://www.sciencedirect.com/science/article/B6V6C-4MP56D9-1/2/ad4b93c6678c6abfa53bc68299fca820>)

Abstract: Summary

A distributed energy-balance model was developed for simulating snowpack evolution and melt in rugged terrain. The model, which was applied to a 43-km² watershed in the Tobacco Root Mountains, Montana, USA, used measured ambient data from nearby weather stations to drive energy-balance calculations and to constrain the model of Liston and Sturm [Liston, G.E., Sturm, M., 1998. A snow-transport model for complex terrain. *Journal of Glaciology* 44 (148), 498-516] for calculating the initial snowpack thickness. Simulated initial snow-water equivalent ranged between 1 cm and 385 cm w.e. (water equivalent) with high values concentrated on east-facing slopes below tall summits. An interpreted satellite image of the snowcover distribution on May 6, 1998, closely matched the simulated distribution with the greatest discrepancy occurring in the floor of the main trunk valley. Model simulations indicated that snowmelt commenced early in the melt season, but rapid meltout of snow cover did not occur until after the average energy balance of the entire watershed became positive about 45 days into the melt season. Meltout was fastest in the lower part of the watershed where warmer temperatures and tree cover enhanced the energy income of the underlying snow. An interpreted satellite image of the snowcover distribution on July 9, 1998 compared favorably with the simulated distribution, and melt curves for modeled canopy-covered cells mimicked the trends measured at nearby snow pillow stations. By the end of the simulation period (August 3), 28% of the watershed remained snow covered, most of which was concentrated in the highest parts of the watershed where initially thick accumulations had been shaded by surrounding summits. The results of this study provide further demonstration of the critical role that topography plays in the timing and magnitude of snowmelt from high mountain watersheds.

Keywords: Snowmelt; Distributed modeling; Energy budget; Runoff generation; Mountainous terrain

Laury Chaerle, Sandor Lenk, Dik Hagenbeek, Claus Buschmann, Dominique Van Der Straeten, Multicolor fluorescence imaging for early detection of the hypersensitive reaction to tobacco mosaic virus, *Journal of Plant Physiology*, Volume 164, Issue 3, 7 March 2007, Pages 253-262, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.01.011.

(<http://www.sciencedirect.com/science/article/B7GJ7-4JHMF9K-1/2/ea2690c8984c3dcbe29a3f56c44359de>)

Abstract: Summary

The physiological status of plants can nowadays be promptly monitored with non-invasive methods. This opens the possibility to continuously follow-up plant performance and permits to detect stress-induced deviations presymptomatically. Upon stress, plants may synthesize specific compounds, depending on the causal agent. Such compounds may alter the absorption of the light impinging on plant leaves, hence the spectrum of reflected, re-emitted, and transmitted light changes. UV-excited fluorescence imaging specifically allows visualization of the accumulation of phenolic compounds, e.g. those associated with the hypersensitive response to pathogens. By using imaging at regular intervals (time-lapse series) of tobacco mosaic virus (TMV) infection in resistant tobacco we aimed at the description and quantification of the kinetics of blue-green fluorescence compared to the visual development of the disease. Presymptomatic responses to TMV infection were observed with a multicolor fluorescence and reflectance imaging setup. The onset of increases in blue-green and chlorophyll fluorescence were comparable in timing, although further symptom development was strikingly different. Compounds known to accumulate during the hypersensitive response and displaying blue-green fluorescence revealed different dynamics of fluorescence evolution in time. The multichannel imaging system permitted to discern the key components salicylic acid and scopoletin. In contrast, for the compatible interaction between TMV and non-resistant tobacco, no presymptomatic responses were detected on inoculated leaves.

This work proves the potential of multispectral imaging to unveil stress-associated signatures, and the power of blue-green fluorescence imaging to monitor accumulation of secondary compounds.

Keywords: Blue-green fluorescence; Chlorophyll fluorescence; Plant stress; Salicylic acid; Scopoletin

Rosa A. Vacca, Daniela Valenti, Antonella Bobba, Maria C. de Pinto, Riccardo S. Merafina, Laura De Gara, Salvatore Passarella, Ersilia Marra, Proteasome function is required for activation of programmed cell death in heat shocked tobacco Bright-Yellow 2 cells, *FEBS Letters*, Volume 581, Issue 5, 6 March 2007, Pages 917-922, ISSN 0014-5793, DOI: 10.1016/j.febslet.2007.01.071.

(<http://www.sciencedirect.com/science/article/B6T36-4N0HSDS-4/2/00a9ff684724def3c1b4bcf1246d4475>)

Abstract:

To find out whether and how proteasome is involved in plant programmed cell death (PCD) we measured proteasome function in tobacco cells undergoing PCD as a result of heat shock (HS-PCD). Reactive oxygen species (ROS) production, cytochrome c levels and caspase-3-like protease activation were also measured in the absence or presence of MG132, a proteasome inhibitor. We show that proteasome activation occurs in early phase of HS-PCD upstream of the caspase-like proteases activation; moreover inhibition of proteasome function by MG132 results in prevention of PCD perhaps due to the prevention of ROS production, cytochrome c release and caspase-3-like protease activation.

Keywords: Plant programmed cell death; Proteasome; Reactive oxygen species; Cytosolic ascorbate peroxidase; Cytochrome c; Caspase-like proteases

Evan L. Preisser, Sarah E. Gibson, Lynn S. Adler, Edwin E. Lewis, Underground herbivory and the costs of constitutive defense in tobacco, *Acta Oecologica*, Volume 31, Issue 2, March-April 2007, Pages 210-215, ISSN 1146-609X, DOI: 10.1016/j.actao.2006.09.004.

(<http://www.sciencedirect.com/science/article/B6VVR3-4MV1FCW-1/2/749bc79c20a7c1c62eef7ec07ba5e451>)

Abstract:

Nicotine is both a constitutive and induced defense in cultivated tobacco (*Nicotiana tabacum*). Nicotine is thought primarily to defend against above-ground herbivory; however, below-ground herbivores like the nematode *Meloidogyne incognita* can also damage plants. We evaluated the costs and benefits of constitutive nicotine production in four near-isogenic lines of *N. tabacum* differing in nicotine content. We exposed the four lines to levels of nematode infection below that found to induce nicotine synthesis, and measured nematode density and each line's response to nematode presence. Nematode density did not differ among lines and was not related to leaf nicotine content in any of the lines, suggesting that constitutive nicotine content did not affect nematode survival or reproduction. Most measures of plant performance were unaffected by nematodes; however, nematode infection decreased flowering in the high nicotine line relative to the other lines. Lines with less constitutive nicotine did not incur similar costs, suggesting a tradeoff between nicotine production and tolerance of low levels of herbivory. A cost of nicotine production is also suggested by the fact that flowering was inversely correlated with leaf nicotine content in all four lines. Although nicotine conferred no resistance to nematodes, high nicotine content reduced the plant's tolerance of low levels of nematode infection and was correlated with reduced flowering. In examining the costs and benefits of a constitutive plant defense, this work complements and extends previous research addressing the relationship between plant tolerance and induced defenses.

Keywords: Constitutive defense; Tolerance; *Meloidogyne incognita*; *Nicotiana tabacum*; Nicotine

M. Janouskova, M. Vosatka, L. Rossi, N. Lugon-Moulin, Effects of arbuscular mycorrhizal inoculation on cadmium accumulation by different tobacco (*Nicotiana tabacum* L.) types, *Applied*

Soil Ecology, Volume 35, Issue 3, March 2007, Pages 502-510, ISSN 0929-1393, DOI: 10.1016/j.apsoil.2006.10.002.

(<http://www.sciencedirect.com/science/article/B6T4B-4MCWMC5-2/2/bf443ae0be468f0797e72ef5bf9c35e5>)

Abstract:

The effect of arbuscular mycorrhiza (AM) on cadmium (Cd) uptake by tobacco (*Nicotiana tabacum* L.) was studied in a pot experiment. Three commercial varieties, Basma BEK, K326 and TN90, representing three distinct tobacco types, were each grown in a different soil with nutritional conditions matching as closely as possible their requirements for field production. Cd concentrations in these soils were within the background range. Each variety was either non-mycorrhizal or inoculated with one of five AM fungal isolates. Cd concentration in leaves was decreased by inoculation with selected isolates in the K326 and TN90 variety grown in acidic soils. In contrast, it was increased by inoculation with most isolates in the Basma BEK variety grown in a basic soil with low Cd availability. Besides, plants of all three varieties had significantly higher leaf concentrations of phosphorus and nitrogen in some inoculated treatments. The percentage of root colonisation was mostly low in the inoculated treatments. In the Basma BEK and TN90 variety, the tested AM fungal isolates differed in their ability to colonise roots, but no correlation was found between the root colonisation of an isolate and its effects on the Cd concentrations in tobacco leaves. One isolate influenced most pronouncedly Cd concentrations and improved mineral nutrition in all the three combinations of variety and soil despite its low colonisation levels. AM symbiosis probably affected Cd uptake of tobacco by indirect mechanisms such as stimulation of root growth or mycorrhizal plant mediated changes in chemical or biological soil properties.

Keywords: Agriculture; Glomus; Heavy metals; Nitrogen; Phosphorus

J.R. Hayes, D.R. Meckley, M.S. Stavanja, P.R. Nelson, K.R. Van Kampen, J.E. Swauger, Effect of a flue-curing process that reduces tobacco specific nitrosamines on the tumor promotion in SENCAR mice by cigarette smoke condensate, *Food and Chemical Toxicology*, Volume 45, Issue 3, March 2007, Pages 419-430, ISSN 0278-6915, DOI: 10.1016/j.fct.2006.08.024.

(<http://www.sciencedirect.com/science/article/B6T6P-4KWJY1H-3/2/baeca4f7496a443d03f1ac5a98025db9>)

Abstract:

A 30-week dermal tumor promotion study was conducted to evaluate the dermal tumor-promoting potential of cigarette smoke condensate (CSC) collected from cigarettes containing flue-cured tobacco cured by a heat-exchange process (HE) relative to that of cigarettes containing flue-cured tobacco cured by the traditional direct-fire process (DF). Heat-exchange process cured tobacco contains significantly lower concentrations of tobacco specific nitrosamines (TSNAs) compared to traditional direct-fire cured tobacco. Mainstream CSCs were collected by cold trap from smoke generators using the Federal Trade Commission puffing regimen. Groups of 40 female SENCAR mice were initiated by a single application of 75 [μ]g 7,12-dimethylbenz[a]anthracene (DMBA) to the shaved dorsal skin. CSCs were then applied to the skin three times/week for 29 weeks at 9, 18, or 36 mg tar/application. End-points included body weights, clinical observations, organ weights, dermal tumor development and histopathology. The numbers of dermal tumors and the numbers of tumor-bearing mice for each CSC were statistically different from the DMBA/acetone control group and increased with increasing dose. When corresponding doses of each CSC were compared, only the DMBA/mid-dose HE CSC group was statistically significantly different (lower) from the corresponding DMBA/mid-dose DF CSC group. In this assay, the dermal tumor-promotion potential of CSC from heat-exchange flue-cured tobacco did not differ from that of traditional direct-fire flue-cured tobacco CSC.

Keywords: Tobacco specific nitrosamines; Dermal tumor promotion; Two-stage carcinogenesis; Cigarette smoke condensates

Eric Claeysen, Jean Rivoal, Isozymes of plant hexokinase: Occurrence, properties and functions, *Phytochemistry*, Volume 68, Issue 6, March 2007, Pages 709-731, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2006.12.001.

(<http://www.sciencedirect.com/science/article/B6TH7-4MV71MW-3/2/4a2375ba085c698a76307d057474c443>)

Abstract:

Hexokinase (HK) occurs in all phyla, as an enzyme of the glycolytic pathway. Its importance in plant metabolism has emerged with compelling evidence that its preferential substrate, glucose, is both a nutrient and a signal molecule that controls development and expression of different classes of genes. A variety of plant tissues and organs have been shown to express multiple HK isoforms with different kinetic properties and subcellular localizations. Although plant HK is known to fulfill a catalytic function and act as a glucose sensor, the physiological relevance of plural isoforms and their contribution to either function are still poorly understood. We review here the current knowledge and hypotheses on the physiological roles of plant HK isoforms that have been identified and characterized. Recent findings provide hints on how the expression patterns, biochemical properties and subcellular localizations of HK isoforms may relate to their modes of action. Special attention is devoted to kinetic, mutant and transgenic data on HKs from *Arabidopsis thaliana* and the Solanaceae potato, tobacco, and tomato, as well as HK gene expression data from *Arabidopsis* public DNA microarray resources. Similarities and differences to known properties of animal and yeast HKs are also discussed as they may help to gain further insight into the functional adaptations of plant HKs.

Keywords: *Arabidopsis*; Carbon metabolism; Cruciferae; Glucose; Glycolysis; Hexokinase; Hexose sensing; Microarray; Sequence analysis; Solanaceae

Apurva Bhargava, Milan Osusky, Robert E. Hancock, Benjamin S. Forward, William W. Kay, Santosh Misra, Antiviral indolicidin variant peptides: Evaluation for broad-spectrum disease resistance in transgenic *Nicotiana tabacum*, *Plant Science*, Volume 172, Issue 3, March 2007, Pages 515-523, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.10.016.

(<http://www.sciencedirect.com/science/article/B6TBH-4MHP944-1/2/65f59d955714d31276e0cba8d7c56637>)

Abstract:

Cationic peptides play an important role in the natural defenses against microbial infections of most of the living organisms. Indolicidin, a cationic peptide derived from bovine neutrophils, demonstrates *in vitro* antibacterial, antifungal and antiviral activity against animal pathogens. To evaluate efficacy of this peptide against plant pathogens, especially viruses, here we describe engineering transgenic plants, expressing indolicidin variants (10R and 11R). The variants were tested *in vitro*, for their effectiveness against plant pathogenic bacteria, fungi and TMV. Genes encoding these peptides were introduced into the tobacco cultivar (*Nicotiana tabacum* var. Xanthi). Leaf assays of transgenic plants showed enhanced resistance against several pathogens including significant resistance against TMV challenge. This is the first report of heterologous cationic peptide mediated antiviral resistance in engineered plants.

Keywords: Indolicidin; Tobacco; Antiviral peptide; Transgenic

Yoichi Ogawa, Masahiro Mii, Meropenem and moxalactam: Novel [beta]-lactam antibiotics for efficient *Agrobacterium*-mediated transformation, *Plant Science*, Volume 172, Issue 3, March 2007, Pages 564-572, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.11.003.

(<http://www.sciencedirect.com/science/article/B6TBH-4MHP944-4/2/cb4276ff1b5038546769f781be794e1a>)

Abstract:

We evaluated the usefulness of novel [beta]-lactam antibiotics, meropenem (MEPM) and moxalactam (LMOX) for *Agrobacterium*-mediated transformation of tobacco, tomato and rice in

comparison with commonly used [beta]-lactams, carbenicillin (CBPC) and cefotaxime (CTX). Although antibacterial activities of [beta]-lactams varied among transformation systems, MEPM at 25 mg l⁻¹ completely suppressed overgrowth of *Agrobacterium* in all systems. In transformation of tobacco, tomato and rice, MEPM at 6.25-25 mg l⁻¹ and LMOX at 6.25-50 mg l⁻¹ led to a higher transformation efficiency than CBPC and CTX. We concluded that introduction of MEPM or LMOX at 25 mg l⁻¹ for non-[beta]-lactamase-producing strains and MEPM at 25 mg l⁻¹ or LMOX at 50 mg l⁻¹ for [beta]-lactamase-producing strains, respectively, to *Agrobacterium*-mediated transformation may be a simple and efficient strategy to improve transformation efficiency of many plant species without modification of existing protocols.

Keywords: *Agrobacterium*-mediated transformation; [beta]-Lactam antibiotics; Meropenem; Moxalactam; Overgrowth of *Agrobacterium*; Transformation efficiency

Sonia Herrero, Margaret E. Daub, Genetic manipulation of Vitamin B-6 biosynthesis in tobacco and fungi uncovers limitations to up-regulation of the pathway, *Plant Science*, Volume 172, Issue 3, March 2007, Pages 609-620, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.11.011.

(<http://www.sciencedirect.com/science/article/B6TBH-4MK0XWG-1/2/418991fae518030cc66802ad2385cf07>)

Abstract:

Transgenic expression of vitamin biosynthetic genes has been investigated for over-production of these dietary supplements in microorganisms and plants. In plants, successful efforts have been reported with Vitamins A, C, E and B-9, however information is lacking for other vitamins. Vitamin B-6 is an essential cofactor for numerous enzymatic reactions, and has also been shown to be a potent antioxidant involved in protecting phytopathogenic *Cercospora* fungi from their own toxin, cercosporin. In this report, we transformed and expressed two Vitamin B-6 biosynthetic genes (PDX1 and PDX2) isolated from *Cercospora nicotianae* in cercosporin-sensitive organisms, tobacco and the fungal species *Aspergillus flavus* and *Neurospora crassa*. Our goal was to determine if Vitamin B-6 levels could be increased by constitutive expression of these genes, and if over-production confers resistance to oxidative stresses induced by cercosporin and salinity stress. Elevated Vitamin B-6 levels were observed in one tobacco line. For other lines evaluated in this work, expression of PDX1 and PDX2 in transgenic organisms did not result in a significant increase in Vitamin B-6 content over controls. Analysis of gene expression in tobacco indicated that the lack of elevated B-6 content was not due to lack of enzymatic activity, but to down-regulation of the endogenous tobacco genes compounded with limited transgene expression. The single line with elevated B-6 levels had higher expression of both the PDX1 and PDX2 transgenes compared to the other lines, and the observed increase on Vitamin B-6 was correlated with higher enzyme activity. Consistent with our inability to elevate cellular B-6 levels, only small changes were observed in the response to either cercosporin or high salt, and most transgenic individuals were as susceptible as controls. Compared to tobacco lines transformed to express either PDX1 or PDX2 alone, half of the transgenic tobacco lines expressing both genes were impaired in seed germination and initial growth. However no correlation was observed between the observed phenotype and Vitamin B-6 levels in seeds. This is the first report on genetic engineering to manipulate the Vitamin B-6 pathway in plants. Our results suggest that genetic manipulation of the Vitamin B-6 biosynthetic pathway is possible but is limited by regulation of endogenous genes.

Keywords: Genetic engineering; Pyridoxine; Pyridoxal phosphate; SNZ; YaaD

Shang-Jing Guo, Hai-Yan Zhou, Xian-Sheng Zhang, Xin-Guo Li, Qing-Wei Meng, Overexpression of CaHSP26 in transgenic tobacco alleviates photoinhibition of PSII and PSI during chilling stress under low irradiance, *Journal of Plant Physiology*, Volume 164, Issue 2, 23 February 2007, Pages 126-136, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.01.004.

(<http://www.sciencedirect.com/science/article/B7GJ7-4JCSJVD-1/2/47d8cdf23f38cddd2746e3114edd4f9a>)

Abstract: Summary

A sweet pepper cDNA clone, CaHSP26 encoding the chloroplast (CP)-localized small heat shock protein (sHSP), was isolated and characterized with regard to its sequence, response to various temperatures and function in transgenic tobacco plants. The deduced amino acid sequence contained three highly conserved regions, showing high identities with other plant sHSPs. Expression of the CaHSP26 gene showed that the mRNA accumulation of CaHSP26 was induced by heat stress. Higher transcript levels were observed when sweet pepper leaves were treated at 42 [degree sign]C for 3 h. However, the expression of the CaHSP26 gene was not induced by chilling stress (4 [degree sign]C) in the absence of heat shock (HS). But the transcripts were still detected at 48 h at 4 [degree sign]C after HS while not at 25 [degree sign]C. The photochemical efficiency of PSII (Fv/Fm) and the oxidizable P700 in transgenic tobacco overexpressing CaHSP26 were higher than that in wild type tobacco during chilling stress under low irradiance. These results suggest that CP sHSP protein plays an important role in protection of PSII and PSI during chilling stress under low irradiance.

Keywords: Chilling stress under low irradiance; Chloroplast small heat shock protein; Photoinhibition; Sweet pepper; Transgenic tobacco

Changfu Zhu, Friedrich Kauder, Susanne Romer, Gerhard Sandmann, Cloning of two individual cDNAs encoding 9-cis-epoxycarotenoid dioxygenase from *Gentiana lutea*, their tissue-specific expression and physiological effect in transgenic tobacco, *Journal of Plant Physiology*, Volume 164, Issue 2, 23 February 2007, Pages 195-204, ISSN 0176-1617, DOI: 10.1016/j.jplph.2006.02.010.

(<http://www.sciencedirect.com/science/article/B7GJ7-4JRM019-4/2/c3beb60cd0a0810ed8de325c3c093bb7>)

Abstract: Summary

Two 9-cis-epoxycarotenoid dioxygenase (NCED) cDNAs have been cloned from a petal library of *Gentiana lutea*. Both cDNAs carry a putative transit sequence for chloroplast import and differ mainly in their length and the 5'-flanking regions. GINCED1 was evolutionary closely related to *Arabidopsis thaliana* NCED6 whereas GINCED2 showed highest homology to tomato NCED1 and *A. thaliana* NCED3. The amounts of GINCED2 transcript were below Northern detection in *G. lutea*. In contrast, GINCED1 was specifically expressed at higher levels in developing flowers when petals start appearing. By genetic engineering of tobacco with coding regions of either gene under a constitutive promoter, their function was further analyzed. Although mRNA of both genes was detectable in the corresponding transgenic plants, a physiological effect was only found for GINCED1 but not for GINCED2. In germination experiments of GINCED1 transgenic lines, delayed radicle formation and cotyledon appearance were observed. However, the transformants exhibited no improved tolerance against desiccation stress. In contrast to other plants with over-expressed NCEDs, prolonged delay of seed germination is the only abscisic-acid-related phenotypic effect in the GINCED1 transgenic lines.

Keywords: Effect in transgenic tobacco; Flower developmental stages; Flower-specific 9-cis-epoxycarotenoid dioxygenase; *Gentiana lutea*; Tissue-specific expression

Evah W. Murage, Paul Voroney, Ronald P. Beyaert, Turnover of carbon in the free light fraction with and without charcoal as determined using the ¹³C natural abundance method, *Geoderma*, Volume 138, Issues 1-2, 15 February 2007, Pages 133-143, ISSN 0016-7061, DOI: 10.1016/j.geoderma.2006.11.002.

(<http://www.sciencedirect.com/science/article/B6V67-4MKTY01-1/2/ed4995727a88d8ffff8bf034e1171b4a>)

Abstract:

Charcoal fragments have been reported frequently in the light fraction (LF) and this suggests that charcoal C is an important constituent of LF of North American soils. The LF is considered to have

a rapid turnover but charcoal is highly resistant to biological degradation, hence it will have significant implications for studies of LF composition, dynamics and modeling exercises. This study aimed to quantify the contribution of charcoal to free LF (density $\leq 1.8 \text{ g cm}^{-3}$) C, and its effect on the turnover of C in that fraction using the $\delta^{13}\text{C}$ technique. Duplicate free LF samples were obtained from the 0-20 cm depth of no-tilled and conventionally tilled soils, each under corn and tobacco/rye cropping. Based on morphological properties, charcoal and plant fragments were handpicked under a light microscope from one set of free LF samples and their $\delta^{13}\text{C}$ were measured. The $\delta^{13}\text{C}$ of whole free LF samples were also measured. The chemical properties of charcoal were characterized using solid ^{13}C NMR spectroscopy technique, and these were compared with those documented for thermally generated charcoal. A two end-member mixing model was used to estimate the proportion of free LF C derived from non-charcoal and charcoal residues in continuous corn plots.

Light microscopy of charcoal fragments showed a particulate morphology consistent with charcoal and charred plant residues. The chemical properties of charcoal fragments were also consistent with charcoal. The $\delta^{13}\text{C}$ of charcoal (-26 to -25 ‰) showed that charcoal C was derived entirely from C_3 vegetation. Charcoal and non-charcoal $\text{C}_3\text{-C}$ accounted for between 72 to 75% and 25 to 28%, respectively, of the total $\text{C}_3\text{-C}$ in the free LF, indicating that charcoal C was responsible for the persistence of a sizable portion of native C in free LF. The turnover of $\text{C}_3\text{-C}$ in free LF with charcoal was slower than that of $\text{C}_3\text{-C}$ in free LF without charcoal by 2.5 times. These results provide supporting evidence that charcoal C is likely to lead to misinterpretation of dynamics of the LF and suggest that concepts about soil C dynamics and LF turnover may have to be adjusted to account for charred C, and that charring may be an under-appreciated stabilization mechanism.

Keywords: Free light fraction; Charcoal; Plant residues; ^{13}C natural abundance; ^{13}C NMR; Turnover

Renate Gehwolf, Richard Weiss, Maximilian Gabler, Annette C. Hurst, Adam Bertl, Josef Thalhamer, Gerhard Obermeyer, From sequence to antibody: Genetic immunisation is suitable to generate antibodies against a rare plant membrane protein, the KAT 1 channel, FEBS Letters, Volume 581, Issue 3, 6 February 2007, Pages 448-452, ISSN 0014-5793, DOI: 10.1016/j.febslet.2007.01.004.

(<http://www.sciencedirect.com/science/article/B6T36-4MV17FD-1/2/4762b64367f1d7223d4eb9191e31b377>)

Abstract:

Monoclonal antibodies against the K^+ channel KAT1 of *Arabidopsis thaliana*, a low abundance, plant plasma membrane protein, were generated by genetic immunisation to avoid the time and labour consuming purification of native or recombinant proteins and peptides usually necessary for conventional immunisation techniques. The resulting polyclonal and monoclonal antibody sera recognised a single protein band in a microsomal fraction of wild-type *A. thaliana* leaves and in membrane fractions of transgenic yeast cells and tobacco plants expressing the KAT1 protein. Therefore, genetic immunisation is suitable for generating monoclonal antibodies against plant proteins and particularly, against plant membrane proteins of low abundance.

Keywords: Genetic immunisation; K^+ channel; Monoclonal antibody; Plasma membrane protein

Mathias Choquer, Miin-Huey Lee, Huey-Jiunn Bau, Kuang-Ren Chung, Deletion of a MFS transporter-like gene in *Cercospora nicotianae* reduces cercosporin toxin accumulation and fungal virulence, FEBS Letters, Volume 581, Issue 3, 6 February 2007, Pages 489-494, ISSN 0014-5793, DOI: 10.1016/j.febslet.2007.01.011.

(<http://www.sciencedirect.com/science/article/B6T36-4MV1652-8/2/e42d10a06345f3145497209a0c050cad>)

Abstract:

Many phytopathogenic *Cercospora* species produce a host-nonspecific polyketide toxin, called cercosporin, whose toxicity exclusively relies on the generation of reactive oxygen species. Here, we describe a *Cercospora nicotianae* CTB4 gene that encodes a putative membrane transporter and provide genetic evidence to support its role in cercosporin accumulation. The predicted CTB4 polypeptide has 12 transmembrane segments with four conserved motifs and has considerable similarity to a wide range of transporters belonging to the major facilitator superfamily (MFS). Disruption of the CTB4 gene resulted in a mutant that displayed a drastic reduction of cercosporin production and accumulation of an unknown brown pigment. Cercosporin was detected largely from fungal hyphae of *ctb4* disruptants, but not from the surrounding medium, suggesting that the mutants were defective in both cercosporin biosynthesis and secretion. Cercosporin purified from the *ctb4* disruptants exhibited toxicity to tobacco suspension cells, insignificantly different from wild-type, whereas the disruptants formed fewer lesions on tobacco leaves. The *ctb4* null mutants retained normal resistance to cercosporin and other singlet oxygen-generating photosensitizers, indistinguishable from the parental strain. Transformation of a functional CTB4 clone into a *ctb4* null mutant fully revived cercosporin production. Thus, we propose that the CTB4 gene encodes a putative MFS transporter responsible for secretion and accumulation of cercosporin.

Keywords: Gene cluster; Polyketide; Split marker

QIN Song, WANG Zheng-yin, SHI Jun-xiong, Quality Characteristics of Tobacco Leaves with Different Aromatic Styles from Guizhou Province, China, *Agricultural Sciences in China*, Volume 6, Issue 2, February 2007, Pages 220-226, ISSN 1671-2927, DOI: 10.1016/S1671-2927(07)60038-8. (<http://www.sciencedirect.com/science/article/B82XG-4N7RX4X-F/2/12795382574ad39dbdb3751c9186532e>)

Abstract:

The relationships between chemical components and quality indexes were studied in the tobacco leaves with different aromatic styles. A total of 16 chemical components, 4 quality indexes, and 6 smoking quality indexes from 366 tobacco leaf samples with 4 different types of aroma from Guizhou Province, China, were subjected to principal component analysis and stepwise regression analysis. The tobacco leaves with different types of aroma showed remarkable difference in the contents of chemical components, quality indexes, and smoking quality indexes. The first principal factors (carbohydrates and nitrogen-containing compounds) of the chemical composition of the leaf were similar among different types of aroma, which showed that the quality of the leaf was mainly influenced by carbohydrates and nitrogen-containing compounds and their ratios. The factors for the second through the seventh principal components varied largely among various aromatic types, suggesting the contribution of other chemical components to the leaf quality. In addition, the smoking quality of four different aromatic leaves showed significant correlation with the different chemical components. The quality of tobacco leaves with different types of aroma was influenced by multiple factors, especially ecological conditions and culture techniques, which may provide guidance for directive cultivation of high-quality tobacco leaves.

Keywords: tobacco (*Nicotiana tabacum* L.); Guizhou Province; type of aroma; quality

R.S. Pappas, G.M. Polzin, C.H. Watson, D.L. Ashley, Cadmium, lead, and thallium in smoke particulate from counterfeit cigarettes compared to authentic US brands, *Food and Chemical Toxicology*, Volume 45, Issue 2, February 2007, Pages 202-209, ISSN 0278-6915, DOI: 10.1016/j.fct.2006.08.001.

(<http://www.sciencedirect.com/science/article/B6T6P-4KSHB9D-9/2/c22ff94d5c1c5597ae9631b2157f49ef>)

Abstract:

Smoking remains the leading cause of preventable disease in the United States. Exposure to tobacco smoke leads to cancer, heart and lung disease, and addiction. The origin of the tobacco and cigarette manufacturing practices of counterfeit cigarettes are unknown. Because toxic metals

are incorporated into the tobacco lamina during cultivation, the ambient metal content of the soil could produce significant differences in metal levels in both the tobacco and smoke of counterfeit cigarettes. We compared mainstream smoke cadmium, thallium, and lead deliveries from counterfeit and authentic brands. Mainstream smoke levels of all three metals were far greater for counterfeit than the authentic brands, in some cases by an order of magnitude. Significant differences still existed even after normalizing mainstream smoke metal levels with nicotine delivery; the counterfeits typically delivered much higher levels of all three analytes. Our findings, based on 21 different counterfeit samples, suggest that counterfeit cigarettes potentially result in a markedly greater exposure to toxic heavy metals than authentic brands, even after correcting for differences in nicotine intake. In view of the unknown health risks associated with inhaling higher levels of toxic metals, it is prudent to minimize exposure to toxic substances whenever possible.

Keywords: Tobacco; Cigarettes; Smoke; Lead; Cadmium; Thallium

Zhiyan Liu, Linda Ho, Bryony Bonning, Localization of a *Drosophila melanogaster* homolog of the putative juvenile hormone esterase binding protein of *Manduca sexta*, *Insect Biochemistry and Molecular Biology*, Volume 37, Issue 2, February 2007, Pages 155-163, ISSN 0965-1748, DOI: 10.1016/j.ibmb.2006.11.003.

(<http://www.sciencedirect.com/science/article/B6T79-4MBT1CM-1/2/345c9488dabfb1846302cabeada2bda1>)

Abstract:

A putative juvenile hormone esterase (JHE) binding protein, P29, was isolated from the tobacco hornworm *Manduca sexta* [*J. Biol. Chem.* 275(3), 1802-1806]. A homolog of P29 was identified in *Drosophila melanogaster* by sequence alignment. This gene, CG3776 was cloned, recombinant DmP29 expressed in *Escherichia coli* and two anti-DmP29 antisera raised. In vitro binding of the P29 homolog to *Drosophila* JHE was confirmed. P29 mRNA and an immunoreactive protein of 25 kDa were detected in *Drosophila* larvae, pupae and adults. The predicted size of the protein is 30 kDa. *Drosophila* P29 is predicted to localize to mitochondria (MitoProt; 93% probability) and has a 6 kDa N-terminal targeting sequence. Subcellular organelle fractionation and confocal microscopy of *Drosophila* S2 cells confirmed that the immunoreactive 25 kDa protein is present in mitochondria but not in the cytosol. Expression of P29 without the predicted N-terminal targeting sequence in High Five™ cells showed that the N-terminal targeting sequence is shorter than predicted, and that a second, internal mitochondrial targeting signal is also present. An immunoreactive protein of 50 kDa in the hemolymph does not result from alternative splicing of CG3776 but may result from dimerization of P29. The function of P29 in mitochondria and the possible interaction with JHE are discussed.

Keywords: *Drosophila melanogaster*; Juvenile hormone esterase binding protein; P29; *Manduca sexta*; Mitochondria

William G. Heim, Katie A. Sykes, Sherry B. Hildreth, Jian Sun, Rong-He Lu, John G. Jelesko, Cloning and characterization of a *Nicotiana tabacum* methylputrescine oxidase transcript, *Phytochemistry*, Volume 68, Issue 4, February 2007, Pages 454-463, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2006.11.003.

(<http://www.sciencedirect.com/science/article/B6TH7-4MK6148-2/2/2e7015f1f8bba953edcd8b12f528351c>)

Abstract:

The oxidative deamination of N-methylputrescine is an essential step in both pyridine and tropane alkaloid biosynthesis. Reverse genetic approaches have not resulted in the cloning of a methylputrescine oxidase gene (MPO). However, we have used a homology-based approach to clone a full-length tobacco MPO1 cDNA. The MPO1 gene is part of a small multigene family comprised of approximately six members. MPO1-like transcript levels increased in roots that were either deprived of auxin or treated with methyl jasmonic acid. Similar to other known nicotine

biosynthetic genes in domesticated tobacco, MPO1-like mRNA levels were lower in roots with the mutant a and b alleles. The MPO1 protein was expressed in bacteria as a recombinant Thioredoxin-His6-MPO1 fusion protein. The recombinant MPO1 protein utilized N-methylputrescine more efficiently than other diamines. Therefore, the kinetic properties of the MPO1 enzyme may play an important role in determining the pyridine alkaloid profiles observed in tobacco roots.

Keywords: Amine oxidase; Burley 21; Cadaverine; Methylputrescine oxidase; N-methylputrescine; Nicotine; *Nicotiana tabacum*; Tobacco; Solanaceae; Putrescine methyltransferase; Pyridine alkaloid; Quinolinate phosphoribosyl transferase; Tropane alkaloid

Jin-Li Huang, Lai-Liang Cheng, Zhen-Xian Zhang, Molecular cloning and characterization of violaxanthin de-epoxidase (VDE) in *Zingiber officinale*, *Plant Science*, Volume 172, Issue 2, February 2007, Pages 228-235, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.08.013.

(<http://www.sciencedirect.com/science/article/B6TBH-4KXVB5H-2/2/e8685c63e5dd161f5c487b8ac81060e9>)

Abstract:

Ginger (*Zingiber officinale* Rosc.), an important horticultural crop in tropical Southeast Asia, is prone to photoinhibition under intense sunlight and grows well at low light intensity. Violaxanthin de-epoxidase (VDE) as the key enzyme of xanthophyll cycle plays an important role in protecting photosynthesis apparatus from the damage of excessive light. In this study, a full length (2000 bp) cDNA encoding violaxanthin de-epoxidase (GVDE) (GenBank accession no. AY876286) was cloned from ginger using RT-PCR and 5', 3' rapid amplification of cDNA ends (RACE). The expression patterns of GVDE in response to light were characterized. GVDE has a 1431 bp open reading frame and the predicted polypeptide contains 476 amino acids with the molecular mass of 53.7 kDa. Northern blot analysis showed that the GVDE was mainly expressed in leaves. GVDE mRNA level increased as the illumination time prolonged under high light. For determining the GVDE function, its antisense sequence was inserted into tobacco plants via EHA105. PCR-Southern blot analysis confirmed the integration of antisense GVDE in the tobacco genome. Chlorophyll fluorescence measurements showed that, transgenic plants had lower values of non-photochemical quenching (NPQ) and the maximum efficiency of PSII photochemistry (Fv/Fm) compared with the untransformed controls under high light. The size of xanthophyll cycle pigment pool (V + A + Z) and the ratio of (A + Z)/(V + A + Z) were lower in T-VDE tobacco plants than in control, indicating that GVDE was suppressed in antisense T-VDE tobacco. These results showed that VDE plays a major role in alleviating photoinhibition.

Keywords: Ginger; VDE; Antisense vector; Northern blot; NPQ; (A + Z)/(V + A + Z)

Sung Chul Lee, Dae Sung Kim, Nak Hyun Kim, Byung Kook Hwang, Functional analysis of the promoter of the pepper pathogen-induced gene, CAIP2, during bacterial infection and abiotic stresses, *Plant Science*, Volume 172, Issue 2, February 2007, Pages 236-245, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.08.015.

(<http://www.sciencedirect.com/science/article/B6TBH-4M0J32W-1/2/383eb07a515aa8564bc24adf66ba6341>)

Abstract:

Promoter is a region of DNA to which transcription factor binds before initiating the transcription of DNA into RNA. The pepper pathogen-induced protein gene, CAIP2, was locally or systemically induced in pepper plants infected by *Xanthomonas campestris* pv. *vesicatoria*. In this study, we isolated and transiently characterized the CAIP2 promoter in tobacco leaves to identify the cis-acting regulatory sequences involved in CAIP2 gene expression. The 991-bp DNA sequence upstream of the CAIP2 gene was assessed for the activity of the CAIP2 promoter fused to the [β]-glucuronidase (GUS) reporter gene, via an *Agrobacterium*-mediated transient expression assay. Several cis-acting elements, including GT1, MYB, RAV, and W-box, resided within the

genomic sequence upstream of the CAPIP2 gene. The activation of the CAPIP2 promoter was induced by *Pseudomonas syringae* pv. *tabaci*, salicylic acid, methyl jasmonate and abscisic acid, NaCl and cold stress. The expression of the pepper transcription factors, CARAV1 and CAZFP1, was shown to activate the CAPIP2 promoter. Analysis of a series of 5'-deletions of the CAPIP2 promoter suggests that novel cis-acting elements necessary to induce gene expression by pathogens and environmental stresses are specifically localized in the CAPIP2 promoter region.

Keywords: Cis-acting elements; Environmental stress; Pathogenesis-related gene; Promoter analysis; Systemic acquired resistance; Transient assay

Mingfeng Yang, Yinong Xu, Oleate accumulation, induced by silencing of microsomal omega-6 desaturase, declines with leaf expansion in transgenic tobacco, *Journal of Plant Physiology*, Volume 164, Issue 1, 22 January 2007, Pages 23-30, ISSN 0176-1617, DOI: 10.1016/j.jplph.2005.11.002.

(<http://www.sciencedirect.com/science/article/B7GJ7-4HSXVR0-1/2/4b7e17dd9072facc5fbfb011f8e1d26b>)

Abstract: Summary

All higher plants contain at least one microsomal omega-6 desaturase (FAD2), which inserts a double bond between the carbons 12 and 13 of monounsaturated oleic acid to generate polyunsaturated linoleic acid and controls most of the polyunsaturated lipid synthesis in plant cells. RNA interference can be used to silence endogenous genes by effective degradation of target transcripts. To investigate development-related silencing of the FAD2, fatty acid composition was analyzed in the context of leaf expansion in FAD2-silenced tobacco lines obtained by RNA interference technology. We observed that the increased oleate level in unexpanded leaves due to FAD2-silencing receded significantly in fully expanded leaves. The mechanism involved in this interesting phenomenon was investigated by analyses of individual lipid proportion, fatty acid composition of individual lipids, and FAD2 transcript level in the transgenic leaves at different expansion stages. Data revealed that the expansion-related FAD2-silencing effect was not due to rebound of FAD2 transcript, but rather probably due to chloroplast development with leaf expansion.

Keywords: Expansion; Lipid; Microsomal omega-6 desaturase; RNA interference; Tobacco

N.A. Karaivazoglou, N.C. Tsotsolis, C.D. Tsadilas, Influence of liming and form of nitrogen fertilizer on nutrient uptake, growth, yield, and quality of Virginia (flue-cured) tobacco, *Field Crops Research*, Volume 100, Issue 1, 4 January 2007, Pages 52-60, ISSN 0378-4290, DOI: 10.1016/j.fcr.2006.05.006.

(<http://www.sciencedirect.com/science/article/B6T6M-4K7WJ6J-3/2/2c76162b42b50c5bd0417837e40a3373>)

Abstract:

Soil acidity is a limiting factor affecting the growth and yield of many crops all over the world. It is recognized that liming is the most common management practice of profitable crop production on acid soils. On the other hand, it is well-known that the form of nitrogen may affect tobacco yield and quality. In this work, the impact of the interaction between three hydrated lime (HL, Ca(OH)₂) rates (0, 1.5 and 3 t HL ha⁻¹) and three nitrogen fertilizer forms (NO₃-N 100%, NH₄-N 100% and NO₃-N 50% plus NH₄-N 50%) on growth, yield and quality characteristics of Virginia (flue-cured) tobacco was investigated in a 4-year (1995-1998) field experiment established in an acid soil (pH_{water} 1:1 5.3) located in Northern Greece. Lime was applied only once in December 1994, while nitrogen fertilizer was applied annually before transplanting. The results showed that the effect of liming on tobacco growth was not dependent on time, weather conditions and form of nitrogen fertilizer. Liming increased soil pH, enhanced the early growth of tobacco (within 30 days after transplanting (DAT)) and finally increased the total gross and trade yield of tobacco proportionally to the amount of HL added. However, the quality index (organoleptic characteristics)

of the cured product was improved only at the HL application rate of 3 t HL ha⁻¹. Furthermore, liming significantly increased Ca and P concentrations but decreased K concentration in cured tobacco leaves. Tobacco yield increase was attributed to the increase of P uptake. Liming also increased the ash content of cured leaves, whereas it did not significantly affect nicotine, total nitrogen and reducing sugars. The use of ammonium N in fertilizer delayed the early growth of tobacco, reduced the nicotine concentration and increased the reducing sugars concentration of the cured product. Total-N, P, K and Mg concentrations of cured leaves were not significantly affected by the form of nitrogen fertilizer used. The results suggested that an initial application of hydrated lime at a rate of 3 t HL ha⁻¹ may ameliorate soil acidity and increase the yield and quality characteristics of Virginia tobacco at least over a 4-year period after application, independent of the form of N fertilizer used.

Keywords: Virginia (flue-cured) tobacco; Liming; Nitrogen fertilizers; Acid soils; Cured leaves yield; Chemical characteristics

Duong Tan Nhut, Truong Thi Thuy An, Nguyen Thi Dieu Huong, Nguyen Trinh Don, Nguyen Thanh Hai, Nguyen Quoc Thien, Nguyen Hong Vu, Effect of genotype, explant size, position, and culture medium on shoot generation of *Gerbera jamesonii* by receptacle transverse thin cell layer culture, *Scientia Horticulturae*, Volume 111, Issue 2, 4 January 2007, Pages 146-151, ISSN 0304-4238, DOI: 10.1016/j.scienta.2006.10.008.

(<http://www.sciencedirect.com/science/article/B6TC3-4MFJTMH-1/2/4063de56dd43b301db58a53fe3718f73>)

Abstract:

An unique procedure for the mass shoot propagation of *Gerbera* using receptacle transverse thin cell layer (tTCL) culture procedure was developed. Genotype, flower bud age, explant size, position of receptacle tTCLs and culture media were found to affect the success of culture. Ten interspecific crosses of *Gerbera* showed different shoot regeneration rates and callus induction via receptacle tTCL culture, all of which had shoot regeneration rates higher than 57%. Flower buds collected on the 10th day resulted in 91% shoot regeneration after 6 weeks of culture on basal MS medium [Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.* 15, 475-497] supplemented with 0.02 mg l⁻¹ thidiazuron (TDZ), 0.8 mg l⁻¹ adenine and 10% (v/v) coconut water (CW). This was significantly higher than those from flower buds on the 7th and 14th days (22% and 54%), respectively. Shoot regeneration rate was the highest (94-100%) in the middle layers of the receptacle. For mass shoot propagation, shoot clusters were subcultured on half-strength MS medium supplemented with 0.5 mg l⁻¹ indole-3-butyric acid (IBA), 0.5 mg l⁻¹ 6-benzyladenine (BA) and 2.0 mg l⁻¹ kinetin after every 4 weeks. Plantlets formed when single shoots were cultured on half-strength MS medium containing 1 mg l⁻¹ IBA. All plantlets acclimatized well in the greenhouse.

Keywords: *Gerbera jamesonii*; Genotype; Receptacle; TDZ; TCL

Wim Verbeke, Ilse De Bourdeaudhuij, Dietary behaviour of pregnant versus non-pregnant women, *Appetite*, Volume 48, Issue 1, January 2007, Pages 78-86, ISSN 0195-6663, DOI: 10.1016/j.appet.2006.07.078.

(<http://www.sciencedirect.com/science/article/B6WB2-4M04HVH-1/2/20c9f71d23d2c600b5870d7982c82a4b>)

Abstract:

This study investigates dietary behaviour and the perceived role of food for health of pregnant versus non-pregnant women. Data were collected between 15 January 2003 and 15 March 2003 in Belgium. One hundred and forty-eight pregnant and 130 non-pregnant women aged between 20 and 40 years completed a self-administered questionnaire about their dietary behaviour and nutritional attitudes. Both sub-samples match with respect to individual factors such as relevant socio-demographics and general food perceptions. Pregnant women report higher consumption of

fruits, which results in a better score for fibre intake. They also report higher consumption of beef and dairy products, as well as a higher fat intake. No difference in fish consumption between pregnant and non-pregnant women is observed. In line with recommendations, pregnant women report reduced consumption of food products with heightened safety-related risks, lower use of alcohol and tobacco, and safer food handling practices. Reduced intake of raw vegetables for food safety reasons is not compensated by higher intake of cooked vegetables. Pregnant women also report a lower frequency of moderate physical activity. Most differences in food choice by pregnant versus non-pregnant women pertain to the avoidance of specific, potentially harmful food groups. A substantial share of pregnant women does not follow upon recommendations with respect to alcohol use and exposure to tobacco. Personal medical sources for pregnant women and personal social sources for non-pregnant women are reported as the most attended sources of diet-related information. The perceived role of food for health is not different between pregnant and non-pregnant women, and there were no significant interaction effects between pregnancy and presence of children, which indicates that the observed differences in dietary behaviour can be attributed to the state of being pregnant.

Keywords: Attitude; Belgium; Consumer; Diet; Food; Health; Safety; Pregnancy

Radka Sudova, Daniela Pavlikova, Tomas Macek, Miroslav Vosatka, The effect of EDDS chelate and inoculation with the arbuscular mycorrhizal fungus *Glomus intraradices* on the efficacy of lead phytoextraction by two tobacco clones, *Applied Soil Ecology*, Volume 35, Issue 1, January 2007, Pages 163-173, ISSN 0929-1393, DOI: 10.1016/j.apsoil.2006.04.004.

(<http://www.sciencedirect.com/science/article/B6T4B-4K1HDPW-1/2/18a9b96e8900cc5698b0d8696334313a>)

Abstract:

Two pot experiments were conducted to investigate the effect of inoculation with the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* on Pb uptake by two clones of *Nicotiana tabacum* plants. Non-transgenic tobacco plants, variety Wisconsin 38, were compared in terms of Pb uptake with transgenic plants of the same variety with inserted gene coding for polyhistidine anchor in fusion with yeast metallothionein. Bioavailability of Pb in experimentally contaminated soil was enhanced by the application of a biodegradable chelate ethylenediaminedissuccinate (EDDS).

EDDS addition (2.5 and 5.0 mmol kg⁻¹ substrate) increased Pb uptake from the substrate and enhanced Pb translocation from the roots to the shoots, with shoot Pb concentrations reaching up to 800 mg kg⁻¹ at the higher chelate dose. Application of a single dose of 5 mmol kg⁻¹ proved to be more efficient at increasing shoot Pb concentrations than two successive doses of 2.5 mmol kg⁻¹, in spite of a marked negative effect on plant growth and phytotoxicity symptoms. Pb amendment (1.4 g kg⁻¹ substrate) connected with either dose of EDDS decreased significantly plant biomass as well as reduced the development of AM fungi. AM inoculation promoted the growth of tobacco plants and partly alleviated the negative effect of Pb contamination, mainly in the case of root biomass.

No consistent difference in Pb uptake was found between transgenic and non-transgenic tobacco plants. The effect of AM inoculation on Pb concentrations in plant biomass varied between experiments, with no effect observed in the first experiment and significantly higher root Pb concentrations and increased root-shoot ratio of Pb concentrations in the biomass of inoculated plants in the second experiment. Due to probable retention of Pb in fungal mycelium, the potential of AM for phytoremediation resides rather in Pb stabilisation than in phytoextraction.

Keywords: *Nicotiana tabacum*; Transgenic plants; Yeast metallothionein; Pb; Arbuscular mycorrhizal symbiosis; Ethylenediaminedissuccinate

Johannes Sauer, Jumanne M. Abdallah, Forest diversity, tobacco production and resource management in Tanzania, *Forest Policy and Economics*, Volume 9, Issue 5, January 2007, Pages 421-439, ISSN 1389-9341, DOI: 10.1016/j.forpol.2005.10.007.

(<http://www.sciencedirect.com/science/article/B6VT4-4J2M0S6-1/2/dfab957624b5b0c0c0ac8a93a31dd18c>)

Abstract:

This paper aims to deliver empirical evidence on the links between production efficiency, biodiversity, and resource management by analysing a case study on small-scale tobacco production in the Miombo woodlands in Tanzania. The subsistence nature of tobacco production in Tanzania suggests that most power-driven equipments, fertilizers and sustainable crop processing technologies are beyond the reach of most small-scale tobacco growers. The consequence is that in order to expand their production, tobacco farmers heavily substitute such inputs by an increasing use of wood. Hence, an increasing amount of forest land is cleared by the farmers resulting in forest degradation and a loss of biodiversity. This study determines in a first step the efficiency of tobacco production bordering the Miombo woodlands in Tanzania as well as investigates factors for the relative inefficiency on farm level. In a second step, the relation between forest species diversity in the surrounding woodlands and tobacco production efficiency as well as between diversity and the type of institutional arrangement with respect to forest management are empirically analysed. The results indicate that the different efficiency measures vary widely over the sample, showing a significant positive effect of the curing technology-i.e., the design of the barn-and the source of the firewood. The majority of farmers produce with increasing returns to scale. A strong positive correlation between the tobacco production efficiency and forest diversity as well as between community-based arrangements and forest diversity is revealed. This suggests that an increase in agricultural production efficiency with respect to tobacco is conducive for environmental sustainability in Tanzania. It finally supports property rights-based institutional arrangements for the management of forest resources as such motivate the sustainable management of unreserved forest resources.

Keywords: Forest diversity; Efficiency analysis; Resource management; DEA; 2SLS

Cindy E. Morris, Linda L. Kinkel, Kun Xiao, Philippe Prior, David C. Sands, Surprising niche for the plant pathogen *Pseudomonas syringae*, *Infection, Genetics and Evolution*, Volume 7, Issue 1, January 2007, Pages 84-92, ISSN 1567-1348, DOI: 10.1016/j.meegid.2006.05.002.

(<http://www.sciencedirect.com/science/article/B6W8B-4K8SC1X-2/2/80ff9b27f1c1ed5b148b3971372e688f>)

Abstract:

The biology and ecology of plant pathogenic bacteria have been studied almost exclusively in agricultural contexts. In contrast, for numerous human pathogens their biological activity in niches outside of medical contexts is well-known. Whereas there is increasing evidence that traits fostering survival in 'environmental' niches can be the basis for virulence factors of human pathogens, niches for plant pathogenic bacteria outside of plants or of agricultural settings have not been elucidated. Most phytopathogenic bacteria are not obligate parasites, some of them can be transported to altitudes of several kilometres, they are scrubbed from the atmosphere by rainfall, and thus they are presumably transported to and might survive in a wide range of habitats. We isolated *Pseudomonas syringae* from river epilithon (rock-attached biofilms composed of algae, diatoms, rotifers, bacteria and nematodes) at densities up to 6000 cells g⁻¹ in France and the USA, some in pristine settings where waters flowed directly from snow melt and had not passed through agricultural zones. These strains induced hypersensitivity in indicator plants (tobacco) suggesting the presence of functional pathogenicity systems, and many induced disease in 1-7 of the plant species tested and produced a syringomycin-like toxin. Strains also were resistant to some antibiotics used to control plant diseases but not to copper sulphate. Sequencing of the 16S rDNA of epilithon strains and of reference strains of *P. syringae* revealed that a genetic lineage containing the strains with the broadest host range was distributed across several continents. Is it likely that wide spread dissemination of *P. syringae* occurs via aerosols and precipitation. This work highlights our limited understanding of non-agricultural niches in the

ecology and evolution of plant pathogenic bacteria, of their role in the development of agricultural epidemics both as sources of inoculum and as sources of novel traits that may enhance bacterial pathogenicity and fitness.

Keywords: Plant pathogens; Ecology; *Pseudomonas syringae*; Biofilms; Epilithon; Dissemination; Sources of inoculum

Allen S. Farnham, Jason W. Flora, Sandra S. Ingram, Daryl L. Faustini, No evidence of substantial nicotine metabolism by *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) reared on tobacco, *Journal of Stored Products Research*, Volume 43, Issue 2, 2007, Pages 171-176, ISSN 0022-474X, DOI: 10.1016/j.jspr.2006.04.003.

(<http://www.sciencedirect.com/science/article/B6T8Y-4MD469M-1/2/cf297e288332649a50ed6ad816cf55fd>)

Abstract:

The cigarette beetle, *Lasioderma serricorne* (Fabricius), is the most prevalent pest of stored tobacco and is responsible for substantial economic damage. Other than *L. serricorne*, few insects have been found to infest tobacco due to its low nutritional value and nicotine toxicity. Self, L.S., Guthrie, F.E., Hodgson, E. [1964a. Metabolism of nicotine by tobacco-feeding insects. *Nature* 204, 300-301] reported that *L. serricorne* metabolizes at least 70% of ingested nicotine to cotinine. This study re-examined nicotine metabolism by the *L. serricorne* using gas chromatography/flame ionization detection (GC/FID) and gas chromatography/thermal desorption with time-of-flight mass spectrometry (GC/TDS/ToF). Cigarette beetles reared on whole-wheat flour were compared with those reared on tobacco. Larvae, depurated larvae, frass, and both diets were analyzed to determine if nicotine was assimilated, sequestered, metabolized, and/or excreted. Contrary to previous findings, these data indicate that *L. serricorne* does not metabolize a significant amount of nicotine into cotinine. Nicotine is excreted unmodified. Older research involving nicotine metabolism by other insects should be reviewed in the light of these findings.

Keywords: Tobacco; Frass; Tobacco beetle; Stored product; Nicotine metabolism; Cotinine

Xiaoming Zhao, Xiaoping She, Yuguang Du, Xinmiao Liang, Induction of antiviral resistance and stimulatory effect by oligochitosan in tobacco, *Pesticide Biochemistry and Physiology*, Volume 87, Issue 1, January 2007, Pages 78-84, ISSN 0048-3575, DOI: 10.1016/j.pestbp.2006.06.006.

(<http://www.sciencedirect.com/science/article/B6WP8-4K96S9S-1/2/2a77c1f4c5ead30daeb551c7934b66d9>)

Abstract:

Oligochitosan was applied by spraying it on tobacco leaves for inhibition of tobacco mosaic virus (TMV). The maximum inhibition of TMV by oligochitosan was observed when inoculation occurred at 24 h after spraying 50 [μ]g ml⁻¹ oligochitosan. The production of H₂O₂ and NO in epidermal tobacco cells induced by oligochitosan was investigated by epidermal strip bioassay and LSCM, using cell permeable fluorophore diaminofluorescein diacetate (DAF-2D) and 2',7'-dichlorofluorescein diacetate (H₂DCF-DA), respectively. Epidermal tobacco cells treated with oligochitosan resulted in a strong increase of intracellular NO and H₂O₂. Oligochitosan and NO donor sodium nitroprusside (SNP) induced the defense reaction against tobacco mosaic virus (TMV), and increased phenylalanine ammonia-lyase (PAL) activity. Co-treatment of the tobacco cells with oligochitosan and NO scavenger CPTIO blocked the inducing resistance. The results indicated that the defense response induced by oligochitosan was connected with NO pathway.

Keywords: Oligochitosan; Tobacco mosaic virus; Plant protection; Nitric oxide; Hydrogen peroxide

Bart P.J. Geraats, Peter A.H.M. Bakker, Huub J.M. Linthorst, Jan Hoekstra, L.C. van Loon, The enhanced disease susceptibility phenotype of ethylene-insensitive tobacco cannot be counteracted by inducing resistance or application of bacterial antagonists, *Physiological and*

Molecular Plant Pathology, Volume 70, Issues 1-3, January-March 2007, Pages 77-87, ISSN 0885-5765, DOI: 10.1016/j.pmpp.2007.07.003.

(<http://www.sciencedirect.com/science/article/B6WPC-4P59X6H-2/2/e54fffd9c5ab357b5972b2679353c427>)

Abstract:

In an attempt to overcome the enhanced disease susceptibility phenotype that is typical for transgenic ethylene-insensitive tobacco (Tetr), Tetr plants were treated with chemical agents that induce resistance or with antagonistic rhizobacteria. Treatments with [beta]-aminobutyric acid (BABA), benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), methyl jasmonate (MeJA), or salicylic acid (SA) induced PR-genes generally to a lesser extent than in non-transformed plants and did not reduce wilting symptoms upon infection with *Pythium* sp., except for a marginal effect of SA. In Tetr lines overexpressing PR-1g, PR-5c, or both, no significant reduction in disease development was apparent. Also treatment of Tetr plants with the antagonistic rhizobacteria *Bacillus cereus* UW85, *Pseudomonas aeruginosa* 7NSK2, *Pseudomonas fluorescens* WCS417r or Q8r-196, *Pseudomonas putida* WCS358r, or antibiotic-producing derivatives of WCS358r, did not reduce symptoms caused by *Pythium*.

Keywords: [ss]-aminobutyric acid; *Bacillus cereus*; Benzothiadiazole; Biocontrol; Induced systemic resistance; Methyl jasmonate; *Nicotiana tabacum*; Pathogenesis-related proteins; *Pseudomonas aeruginosa*; *Pseudomonas fluorescens*; *Pseudomonas putida*; *Pythium* sp.; Salicylic acid

Zhiqiang Lin, Kangquan Yin, Xiaoxiao Wang, Meihua Liu, Zhangliang Chen, Hongya Gu, Li-Jia Qu, Virus induced gene silencing of AtCDC5 results in accelerated cell death in *Arabidopsis* leaves, *Plant Physiology and Biochemistry*, Volume 45, Issue 1, January 2007, Pages 87-94, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2006.12.003.

(<http://www.sciencedirect.com/science/article/B6VRD-4MNR0H4-1/2/fcf18dd4d82bc21e6ce59ba0a46ac00a>)

Abstract:

CDC5, a Myb-related protein, is reported to be essential for the G2 phase of cell cycle in yeast and animals, but little is known about its function in plants. In this study, *Arabidopsis thaliana* CDC5 (AtCDC5) is found to be nuclear localized, and the C-terminus of this protein is of transcriptional activation activity in yeast. By taking advantage of the virus induced gene silencing (VIGS) technique, we analyzed the phenotypes of the plants in which AtCDC5 is specifically silenced. The AtCDC5 VIGS plants died before bolting, in which accelerated cell death was detected. Further analysis showed that the transcripts of AtSPT and SAG13, but not SAG12, accumulated in these AtCDC5 VIGS plants, suggesting that the accelerated cell death is different from that occurred during leaf senescence. Furthermore, silencing of AtCDC5 by VIGS in either wild-type, *npr1* or *nahG* plants all induces cell death, suggesting that SA is not crucial for the AtCDC5-associated cell death.

Keywords: Virus-induced gene silencing (VIGS); AtCDC5; Transcriptional factor; Cell death; Salicylic acid

M.B. Kirkham, Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation, and amendments, *Geoderma*, Volume 137, Issues 1-2, 31 December 2006, Pages 19-32, ISSN 0016-7061, DOI: 10.1016/j.geoderma.2006.08.024.

(<http://www.sciencedirect.com/science/article/B6V67-4M0BHNJ-5/2/bdd941fd1edd523974f602328d2c16c7>)

Abstract:

Cadmium (Cd) is a heavy metal that is of great concern in the environment, because of its toxicity to animals and humans. This article reviews recent papers showing how soil factors (such as pH, phosphate, zinc, and organic matter), Cd hyperaccumulation, and soil amendments affect Cd availability. The studies confirm that the pH of the soil is usually the most important factor that

controls uptake, with low pH favoring Cd accumulation, and that phosphate and zinc decrease Cd uptake. The work reveals that the availability of Cd is increased by the application of chloride and reduced by application of silicon. The most striking result of this review is the elevated levels of Cd in plants that are being reported in recent studies. Data for concentrations of Cd in soils and plants under variously polluted conditions are presented in a table and show that all plants have Cd concentrations ≥ 0.1 mg/kg, the normal concentration in plants. Concentrations ranged from two low concentrations of 0.1 mg/kg Cd (in grain of corn, *Zea mays*, on an abandoned sludge disposal site that had not received sludge for 10 years, and in roots of hybrid poplar, *Populus deltoides* x *P. nigra*, at a 25-year old active sludge farm) to 380 mg/kg Cd in leaves of penny-cress (*Thlaspi caerulescens*). Plants that hyperaccumulate Cd (i.e., have 100 mg/kg Cd in the tissue or more) belong to the genus *Thlaspi*, the only known Cd hyperaccumulator. Of particular concern for humans are the high concentrations of Cd in rice grain and tobacco leaves. Even if Cd availability is decreased by adding amendments, it is still in the soil and a potential hazard. The best solution for maintaining non-contaminated soils and plants is to remove the sources of Cd in the environment. Given that that is essentially impossible at this time, further research needs to determine how soil and plant factors affect Cd availability on polluted soils.

Keywords: Cadmium; Phytoremediation; Bioavailability; Soil pH; Amendments; Chelators; Hyperaccumulators

Juan M. Ruiz, Juan J. Rios, Miguel A. Rosales, Rosa M. Rivero, Luis Romero, Grafting between tobacco plants to enhance salinity tolerance, *Journal of Plant Physiology*, Volume 163, Issue 12, 7 December 2006, Pages 1229-1237, ISSN 0176-1617, DOI: 10.1016/j.jplph.2005.09.013.

(<http://www.sciencedirect.com/science/article/B7GJ7-4HPKBK6-2/2/25109c5d0db8736a7c9f05b79c5407cc>)

Abstract: Summary

We analysed the technique of grafting as a tool to increase salt-stress resistance in tobacco plants. With this aim, we performed two experiments. First, we selected, from among 6 commercial tobacco cultivars (cv. BB-162, cv. H-20, cv. Jarandilla, cv. ZB-3, cv. Havana II and cv. Havana 307) those most tolerant and sensitive to salinity, studying the response of certain nutritional and biochemical indicators of resistance in these plants. In the second experiment, we analysed the response to salinity in grafted tobacco plants using the rootstock of the most tolerant plants, and the scion of the most sensitive ones. In addition, these plants were subjected to salinity to test the viability and efficiency of this grafting technique, assessing the production of foliar biomass and the different quality parameters in this crop. In the first experiment, we found that the most tolerant tobacco cultivars were cv. BB-162 and cv. H-20, which were characterized by reduced uptake and foliar accumulation of Na^+ and Cl^- , together with greater synthesis of sucrose and proline, thereby reducing lipid peroxidation and thus oxidative damage, reflected in higher foliar biomass with respect to the other cultivars studied (primarily cv. Jarandilla, defined as the most salt-sensitive). In the second, we demonstrated that the grafting of salt-sensitive tobacco scions to salt-tolerant rootstocks improves the production and quality of tobacco leaves under conditions of saline stress. Our results show that the rootstocks cv. BB-162 and cv. H-20 best induced salt resistance in tobacco cv. Jarandilla, registering the lowest foliar concentrations of Na^+ and Cl^+ , the lowest lipid peroxidation, and the highest proline and sugar concentrations. Overall, this is reflected in better biomass production and quality of the aerial part of the plant.

Keywords: Foliar biomass; Grafting; *Nicotiana tabacum*; Nicotine; Salt-stress

Chang-rong GONG, Ai-hua WANG, Song-feng WANG, Changes of Polyphenols in Tobacco Leaves During the Flue-Curing Process and Correlation Analysis on Some Chemical Components, *Agricultural Sciences in China*, Volume 5, Issue 12, December 2006, Pages 928-932, ISSN 1671-2927, DOI: 10.1016/S1671-2927(07)60006-6.

(<http://www.sciencedirect.com/science/article/B82XG-4MRXBCR-6/2/e710d00f9700c7ad39cfbf79b28d2076>)

Abstract:

The changes of polyphenols in tobacco leaves during the flue-curing process and correlation analysis on some chemical components were studied. Leaf samples were taken from different tobacco-producing regions in Henan Province, China. The results indicated that the content of total phenols increased during the first 24 h of curing, and then decreased. It reached the lowest value at 72 h of curing and increased rapidly after that. The content of chlorogenic acid also increased during 0-24 h of curing. But the lowest point occurred at 60 or 72 h of curing and then it increased till the end of the curing process. The content of rutin generally increased with curing, and showed little fluctuations. The changes of PPO and POD activity were the opposite. Rutin was found to have a highly significant positive correlation with total sugar ($r = 0.822'$), but a highly significant negative correlation with starch, nicotine, and protein.

Keywords: tobacco (*Nicotiana tabacum* L); flue-curing; total phenols; chlorogenic acid; rutin; chemical components

Bjorn Vandekerkhove, Elmer Van Baal, Karel Bolckmans, Patrick De Clercq, Effect of diet and mating status on ovarian development and oviposition in the polyphagous predator *Macrolophus caliginosus* (Heteroptera: Miridae), *Biological Control*, Volume 39, Issue 3, December 2006, Pages 532-538, ISSN 1049-9644, DOI: 10.1016/j.biocontrol.2006.06.002.

(<http://www.sciencedirect.com/science/article/B6WBP-4K544M3-3/2/b7697c6f81c81b42874c1db6dc77d64a>)

Abstract:

The mirid bug *Macrolophus caliginosus* is commercially reared on eggs of *Ephestia kuehniella*, constituting an effective but expensive factitious food. Artificial diets can decrease the rearing costs of this natural enemy, but developing and evaluating an artificial diet is a very time-consuming activity. In the current study, development and reproduction of *M. caliginosus* on two artificial diets based on egg yolk were investigated. The artificial diets resulted in longer development and lower adult weights, but survival was comparable with that of control insects fed *E. kuehniella* eggs. Reproductive potential of the predator reared on factitious and artificial foods was assessed using a dissection method. The influence of nymphal food on fecundity was less important than that of adult food. Adults fed *E. kuehniella* eggs had a preoviposition period of about 4 days, whereas adults offered only plant material started laying eggs about 7 days after emergence. Ovarian scores at day 7 were higher for females fed *E. kuehniella* eggs than for those given access only to a tobacco leaf. Ovarian scores were not significantly affected by mating status. In a final test, a parallel comparison of two methods for assessing reproductive response to diet was made. Here, adult couples were offered one of four diets: *E. kuehniella* eggs, one of two artificial diets or no food. Half of the females were dissected and the other half was held for determining lifetime oviposition. Females fed *E. kuehniella* eggs had superior ovarian scores and laid more eggs than those fed either artificial diet or those given no extra food. A good correlation ($r = 0.97$) was obtained between ovarian scores and oviposition data, indicating that dissecting females after 1 week provides a reliable estimate of fecundity as affected by diet quality. Rapid reproductive assessments as used in the current study will help to increase the rate of development of artificial diets and may contribute to more cost effective production methods for augmentative biological control agents.

Keywords: *Macrolophus caliginosus*; Fecundity; Artificial diet; Predator; *Ephestia kuehniella*; Mass rearing

Yucheng Wang, Jing Jiang, Xin Zhao, Guifeng Liu, Chuanping Yang, Liping Zhan, A novel LEA gene from *Tamarix androssowii* confers drought tolerance in transgenic tobacco, *Plant Science*,

Volume 171, Issue 6, December 2006, Pages 655-662, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.06.011.

(<http://www.sciencedirect.com/science/article/B6TBH-4KD5831-1/2/671407f7ba35df72c023a088edd8cbb3>)

Abstract:

A novel LEA gene (DQ663481) was cloned from *Tamarix androssowii*. The cDNA sequence of this LEA gene is 676 bp in length, including 157 bp of the 5' untranslated region and 207 bp of the 3' untranslated region. The open reading frame (ORF) is 312 bp in length, encoding a deduced amino acid sequence of 103 residues with a molecular weight of 11.257 kDa and an isoelectric point of 7.93. BLASTP analysis showed that the cloned LEA gene shares the highest identity in amino acid sequence (47%) with LEA5 from *Nicotiana tabacum*. The cloned LEA gene was transformed into tobacco, after which 10 transgenic lines were selected for drought tolerance testing. The malondialdehyde (MDA) content, relative electrical conductivity, percentage of wilted leaves and relative rate of height growth were compared between transgenic and nontransgenic tobacco. The results showed that both the MDA content and relative electrical conductivity are lower significantly in transgenic plants under drought stress, implying that the LEA gene may enhance drought tolerance by protecting cell membranes from damage. The comparison of the percentage of wilted leaves and relative rate of height growth between transgenic and nontransgenic tobacco showed that, under drought stress, the presence of the LEA gene can induce an increase in height growth rate and a reduction in the number of wilted leaves. These results indicate that the *T. androssowii* LEA gene is an excellent drought tolerance gene and may have potential usage in the genetic improvement of drought tolerance in plants.

Keywords: LEA gene; *Tamarix androssowii*; Drought; Stress tolerance; Tobacco

Nausicaa Lannoo, Willy J. Peumans, Els Van Pamel, Rick Alvarez, Tou-Cheu Xiong, Gerd Hause, Christian Mazars, Els J.M. Van Damme, Localization and in vitro binding studies suggest that the cytoplasmic/nuclear tobacco lectin can interact in situ with high-mannose and complex N-glycans, *FEBS Letters*, Volume 580, Issue 27, 27 November 2006, Pages 6329-6337, ISSN 0014-5793, DOI: 10.1016/j.febslet.2006.10.044.

(<http://www.sciencedirect.com/science/article/B6T36-4M7CK3C-3/2/0a6301b58a21fbf4fad3352585c90c60>)

Abstract:

The possible in vivo interaction of the *Nicotiana tabacum* agglutinin (Nictaba) with endogenous glycoproteins was corroborated using a combination of confocal/electron microscopy of an EGFP-Nictaba fusion protein expressed in tobacco Bright Yellow-2 (BY-2) cells and biochemical analyses. In vitro binding studies demonstrated that the expressed EGFP-Nictaba possesses carbohydrate-binding activity. Microscopic analyses confirmed the previously reported cytoplasmic/nuclear location of Nictaba in jasmonate-treated tobacco leaves and provided evidence for the involvement of a nuclear localization signal-dependent transport mechanism. In addition, it became evident that the lectin is not uniformly distributed over the nucleus and the cytoplasm of BY-2 cells. Far Western blot analysis of extracts from whole BY-2 cells and purified nuclei revealed that Nictaba interacts in a glycan inhibitable way with numerous proteins including many nuclear proteins. Enzymatic deglycosylation with PNGase F indicated that the observed interaction depends on the presence of N-glycans. Glycan array screening, which showed that Nictaba exhibits a strong affinity for high-mannose and complex N-glycans, provided a reasonable explanation for this observation. The cytoplasmic/nuclear localization of a plant lectin that has a high affinity for high-mannose and complex N-glycans and specifically interacts with conspecific glycoproteins suggests that N-glycosylated proteins might be more important in the cytoplasm and nucleus than is currently believed.

Keywords: Bright Yellow-2 cells; Glycan array; Lectin; Localization; *Nicotiana tabacum* agglutinin

Omid Karami, Ali Deljou, Mahmoud Esna-Ashari, Prisa Ostad-Ahmadi, Effect of sucrose concentrations on somatic embryogenesis in carnation (*Dianthus caryophyllus* L.), *Scientia Horticulturae*, Volume 110, Issue 4, 27 November 2006, Pages 340-344, ISSN 0304-4238, DOI: 10.1016/j.scienta.2006.07.029.

(<http://www.sciencedirect.com/science/article/B6TC3-4KYY42X-1/2/17f8ccde027ee47a5ca1fa173edcc10e>)

Abstract:

The effect of sucrose concentration on callus induction followed by differentiation of embryogenic callus derived from petal explants of four carnation cultivars (Nelson, Sagres, Spirit and Impulse) was investigated. Embryogenic calli were produced on Murashige and Skoog [Murashige, T., Skoog, F.A., 1962. Revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* 154, 73-479] basal medium (MS) culture medium containing six concentrations of sucrose (3, 6, 9, 12, 15 and 18%, w/v) all supplemented with 9 [μ]M 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.8 [μ]M 6-benzyladenine (BA). Maximum frequency of embryogenic callus was obtained from the media containing 9 and 12% sucrose. Somatic embryos were induced on a hormone-free MS media containing the seven concentrations of sucrose. Development of somatic embryos was enhanced by increasing sucrose concentration from 1.5 to 12%, while it was reduced in higher concentrations of 15 and 18%. However, normal embryos were not developed in the media containing 1.5 and 3% sucrose. Ninety-five percent of somatic embryos were regenerated to form the entire plantlets when they transferred onto the half-strength hormone-free MS culture medium containing 3% sucrose. Plantlets were also continued to grow normally under greenhouse condition.

Keywords: Carnation; Embryogenic callus; Somatic embryos; Sucrose

H.N. Opolot, A. Agona, S. Kyamanywa, G.N. Mbata, E. Adipala, Integrated field management of cowpea pests using selected synthetic and botanical pesticides, *Crop Protection*, Volume 25, Issue 11, November 2006, Pages 1145-1152, ISSN 0261-2194, DOI: 10.1016/j.cropro.2005.03.019.

(<http://www.sciencedirect.com/science/article/B6T5T-4KV2R6X-1/2/4b64e4714a25de1c2b21413fc142e009>)

Abstract:

This study evaluated the effect of integrating synthetic and botanical pesticide sprays in the management of cowpea (*Vigna unguiculata*) field and storage pests. Cypermethrin (Ambush CY 5% EC) and unitary crude concoctions of tobacco (*Nicotiana tabacum*) were used as the synthetic and botanical pesticides, respectively. Treatments were applied at budding, flowering, pod formation, pod filling and pod physiological maturity. The control was untreated cowpea. Cypermethrin was more effective than tobacco and resulted in decreased pest densities and better yields. Tobacco was beneficial only when the latter was applied at the podding stages, tobacco being ineffective against the flower thrips. Applying tobacco at the podding stages significantly reduced pod pests and *Callosobruchus maculatus* infestation in storage. Use of synthetics and tobacco was more economically beneficial than using synthetics alone. Since the yield difference between the cypermethrin treated plots and those treated with cypermethrin followed by tobacco did not offset the expense of cypermethrin, botanical pesticides could substitute for synthetic pesticides at the podding stages for control of pod pests when absolute pest control is not important.

Keywords: Aphids; Flower thrips; Pod borer; Pod sucking bugs; Pod fly; Bruchids; *Vigna unguiculata*

Reinskje Talhout, Antoon Opperhuizen, Jan G.C. van Amsterdam, Sugars as tobacco ingredient: Effects on mainstream smoke composition, *Food and Chemical Toxicology*, Volume 44, Issue 11, November 2006, Pages 1789-1798, ISSN 0278-6915, DOI: 10.1016/j.fct.2006.06.016.

(<http://www.sciencedirect.com/science/article/B6T6P-4KC2J9W-3/2/7e76996aae9786fd098360cf966bd9c8>)

Abstract:

Sugars are natural tobacco components, and are also frequently added to tobacco during the manufacturing process. This review describes the fate of sugars during tobacco smoking, in particular the effect of tobacco sugars on mainstream smoke composition. In natural tobacco, sugars can be present in levels up to 20 wt%. In addition, various sugars are added in tobacco manufacturing in amounts up to 4 wt% per sugar. The added sugars are usually reported to serve as flavour/casing and humectant. However, sugars also promote tobacco smoking, because they generate acids that neutralize the harsh taste and throat impact of tobacco smoke. Moreover, the sweet taste and the agreeable smell of caramelized sugar flavors are appreciated in particular by starting adolescent smokers. Finally, sugars generate acetaldehyde, which has addictive properties and acts synergistically with nicotine in rodents. Apart from these consumption-enhancing pyrolysis products, many toxic (including carcinogenic) smoke compounds are generated from sugars. In particular, sugars increase the level of formaldehyde, acetaldehyde, acetone, acrolein, and 2-furfural in tobacco smoke. It is concluded that sugars in tobacco significantly contribute to the adverse health effects of tobacco smoking.

Keywords: Tobacco; Additives; Sugar; Pyrolysis; Smoke; Addiction

Richard R. Baker, The generation of formaldehyde in cigarettes--Overview and recent experiments, *Food and Chemical Toxicology*, Volume 44, Issue 11, November 2006, Pages 1799-1822, ISSN 0278-6915, DOI: 10.1016/j.fct.2006.05.017.

(<http://www.sciencedirect.com/science/article/B6T6P-4K4DH6C-2/2/2903692c8077c74c3fb4ad64e1775af7>)

Abstract:

In recent years much effort has been devoted to assessing the influence of tobacco ingredients on the chemistry and toxicity of cigarette mainstream smoke. All of the studies have indicated that commonly used tobacco ingredients do not change the toxicity of smoke as measured in specified assays. Also, the ingredients have little effect on the levels of most smoke constituents that may be relevant to smoking-related diseases. One exception to this generalisation is formaldehyde, which is generated from saccharides used as tobacco ingredients. However, the past studies have generally used mixtures of ingredients added to the tobacco so that the exact effect of each saccharide in turn could not be precisely determined. This is addressed in the present study.

Many diverse studies over the last 30 years have examined particular aspects of formaldehyde in smoke and its generation although no attempt has been made to draw the various aspects together. This has also been addressed in the present paper and an overview is developed on the subject. The experimental results of the present study are rationalised within the framework of this previous knowledge. In the present experimental study, several individual saccharides commonly used as tobacco ingredients have been added to cigarettes, the cigarettes have been machine-smoked and the yields of formaldehyde in the resultant smoke have been compared to those from a control (no ingredient) cigarette. Using four series of cigarettes made on different occasions, the results indicate that all tested sugars added to tobacco increase the yield of formaldehyde in mainstream cigarette smoke under ISO standard smoking machine conditions. Increases up to 60% are observed at maximum sugar levels used on cigarettes. The increases are mostly statistically significant although their magnitudes are variable. These results with formaldehyde are consistent with all previously published studies on the subject.

The increases in mainstream formaldehyde are also observed using smoking machine conditions that are more intense than the standard ISO conditions. Different sugars increase mainstream formaldehyde to different extents, which may be due at least partially to the presence of varying amounts of amino compounds in some of the sugars, such as honey and maple syrup. The presence of such compounds has been shown to inhibit the generation of formaldehyde from

sugars. In general, the first puff of the cigarette generates abnormally high yields of formaldehyde, and this effect has been shown to persist in the presence of added sugars. In contrast to the situation with mainstream smoke, the levels of formaldehyde in sidestream smoke are not affected by the presence of sugars.

The addition of the various saccharides to tobacco also produced some statistically significant effects in the cigarette mainstream yields of six other carbonyl smoke constituents that were analysed at the same time as formaldehyde. These effects were generally small, less than 16%, were not consistent amongst the various cigarette series and lost their significance when the long-term analytical variability was taken into account.

Keywords: Burning; Cigarette; Combustion; Formaldehyde; Hoffmann analytes; Ingredient; Saccharide; Cellulose; Sugars; Pyrolysis; Smoke; Tobacco

J. Jablonski, E. Jablonska, J. Moniuszko-Jakoniuk, Pentobarbital in tobacco, *Food and Chemical Toxicology*, Volume 44, Issue 11, November 2006, Pages 1948-1951, ISSN 0278-6915, DOI: 10.1016/j.fct.2006.06.028.

(<http://www.sciencedirect.com/science/article/B6T6P-4KF74SB-3/2/81628023b6c9d92c2159a377f7be0478e>)

Abstract:

The spectrometric analysis of extracts from tobacco and tobacco smoke revealed the presence of pentobarbital in the analyzed substances. Tobacco samples and tobacco smoke were extracted with chloroform, determinations were performed with the Perkin-Elmer Autosystem XL system, on a Turbo Mass spectrometer.

Subject to analysis were 4 cigarette brands manufactured in Poland and raw, unprocessed tobacco. The presence of pentobarbital in the analyzed samples was confirmed by the analysis of the mass spectrum of the substance, as well as by comparison of retention time with standard of pentobarbital.

The determined pentobarbital concentrations in tobacco amounted to 3-6 [μ g]/cigarette, and in tobacco smoke they were approximately 45% lower.

In case of tobacco extracts it can with high probability be excluded that pentobarbital is synthesized during chromatographical analysis. The presence of pentobarbital in tobacco is thus beyond question.

Keywords: Tobacco; Pentobarbital; Concentration

Stephanie E. Sen, Jeffrey R. Hitchcock, Jessica L. Jordan, Thenesha Richard, Juvenile hormone biosynthesis in *M. sexta*: Substrate specificity of insect prenyltransferase utilizing homologous diphosphate analogs, *Insect Biochemistry and Molecular Biology*, Volume 36, Issue 11, November 2006, Pages 827-834, ISSN 0965-1748, DOI: 10.1016/j.ibmb.2006.08.003.

(<http://www.sciencedirect.com/science/article/B6T79-4KSHB7X-2/2/c5b13c8faf124b77225efb557b67ed0b>)

Abstract:

Analogues of dimethylallyl diphosphate (DMAPP) and geranyl diphosphate (GPP) were prepared and tested as potential substrates of prenyltransferase of the tobacco hornworm, *Manduca sexta*, and of a sesquiterpene synthase derived from pig liver. Enzyme derived from *corpora allata* homogenates of both the larval and adult stage of *M. sexta* coupled each of the DMAPP analogues to produce homologous geranyl and farnesyl diphosphate products in the order (Z)-3-ethyl>(Z)-3-n-propyl>(Z)-3-methyl (DMAPP)>(Z)-3-i-propyl[greater-or-equal, slanted](Z)-3-n-butyl. In competition studies, the ethyl and n-propyl analogues either enhanced or had no effect on DMAPP coupling, whereas the larger analogues were inhibitors. (Z)-7-ethyl and (2Z,6Z)-3,7-diethyl analogues of GPP were as good, if not better substrates of larval prenyltransferase, while the C-3 ethyl analogue of GPP, which is precursor to an isomeric form of juvenile hormone (JH) that is not typically found in insects, was poorly coupled by the enzyme. While similarities were seen for whole-cell extracts

derived from adult and larval *M. sexta*, adult prenyltransferase derived from cytosolic and 16,000xg pellet fractions displayed distinct competitive coupling of GPP and its homologs, suggesting differences in substrate specificity as a result of enzyme localization. In contrast to *M. sexta*, the pig liver enzyme poorly coupled each of the homologous DMAPP derivatives, and the homologous derivatives of GPP were less efficiently coupled than GPP. These results indicate that prenyltransferase in *M. sexta* possesses high steric latitude at the (Z)-C-3 and C-7 alkyl positions of DMAPP and GPP, respectively, in contrast to other animal prenyltransferases but in keeping with the enzyme's presumptive role in homologous JH metabolism.

Keywords: Juvenile hormone biosynthesis; Prenyltransferase; Homolog; Isoprenoid coupling

Mehbuba Begam, Sushil Kumar, Sribash Roy, James J. Campanella, H.C. Kapoor, Molecular cloning and functional identification of a ribosome inactivating/antiviral protein from leaves of post-flowering stage of *Celosia cristata* and its expression in *E. coli*, *Phytochemistry*, Volume 67, Issue 22, November 2006, Pages 2441-2449, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2006.08.015. (<http://www.sciencedirect.com/science/article/B6TH7-4KYXHR-1/2/9f0f13a933b94b644906850f88526c9e>)

Abstract:

A full-length cDNA clone, encoding a ribosome inactivating/antiviral protein (RIP/AVP) was isolated from the cDNA library of post-flowering stage of *Celosia cristata* leaves. The full-length cDNA consisted of 1015 nucleotides, with an open reading frame encoding 283 amino acids. The deduced amino acid sequence had a putative active site domain conserved in other ribosome inactivating/antiviral proteins (RIPs/AVPs). The coding region of the cDNA was amplified by polymerase chain reaction (PCR), cloned and expressed in *Escherichia coli* as recombinant protein of 72 kDa. The expressed fusion product was confirmed by Western analysis and purification by affinity chromatography. Both the recombinant protein (reCCP-27) and purified expressed protein (eCCP-27) inhibited translation in rabbit reticulocytes showing IC₅₀ values at 95 ng and 45 ng, respectively. The native purified nCCP-27 has IC₅₀ at 25 ng. The purified product also showed N-glycosidase activity towards tobacco ribosomes and antiviral activity towards tobacco mosaic virus (TMV) and sunnhemp rosette virus (SRV).

Keywords: Antiviral; cDNA; *Celosia cristata*; Expression; Ribosome inactivating

J.-Y. Chiang, N. Balic, S.-W. Hsu, C.-Y. Yang, C.-W. Ko, Y.-F. Hsu, I. Swoboda, C.-S. Wang, A pollen-specific polygalacturonase from lily is related to major grass pollen allergens, *Plant Physiology and Biochemistry*, Volume 44, Issues 11-12, November-December 2006, Pages 743-751, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2006.10.005. (<http://www.sciencedirect.com/science/article/B6VRD-4M4KJ0G-1/2/44a1d48bd69524025d42ff078426fa75>)

Abstract:

A pollen-specific gene from lily (*Lilium longiflorum* Thunb. cv. Snow Queen), designated LLP-PG, was characterized. Southern blots of lily genomic DNA indicated that LLP-PG is a member of a small gene family. A thorough sequence analysis revealed that the LLP-PG gene is interrupted by two introns and encodes a protein of 413 amino acids, with a calculated molecular mass of 44 kDa, and a pI of 8.1. Evaluation of the hydropathy profile showed that the protein has a hydrophobic segment at the N-terminus, indicating the presence of a putative signal peptide. A sequence similarity search showed a significant homology of the encoded protein to pollen polygalacturonases (PGs) from various plant species and to an important group (group 13) of grass pollen allergens. The LLP-PG transcript is pollen-specific and it accumulates only at the latest stage during pollen development, in the mature pollen. In contrast to other "late genes" LLP-PG transcript can neither be induced by abscisic acid (ABA) nor by dehydration. Immunoblot analyses of pollen protein extracts from lily, timothy grass and tobacco with IgG antibodies directed against LLP-PG and against the timothy grass pollen allergen, Phl p 13, indicated that lily

LLP-PG shares surface-exposed epitopes with pollen PGs from monocotyledonous and dicotyledonous plants. Enzyme-linked immunosorbent assay (ELISA) analyses and inhibition ELISA assays with patients' IgE demonstrated a very low IgE reactivity of lily rLLP-PG and a lack of cross-reactivity between rLLP-PG and the timothy grass pollen allergen, rPhl p 13. These data demonstrated that despite the significant sequence homology and the conserved surface-exposed epitopes LLP-PG represents a low-allergenic member of pollen PGs.

Keywords: Allergen; Gene expression; IgE cross-reactivity; *Lilium longiflorum*; Pollen-specific; Polygalacturonase

M. Iriti, M. Sironi, S. Gomarasca, A.P. Casazza, C. Soave, F. Faoro, Cell death-mediated antiviral effect of chitosan in tobacco, *Plant Physiology and Biochemistry*, Volume 44, Issues 11-12, November-December 2006, Pages 893-900, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2006.10.009.

(<http://www.sciencedirect.com/science/article/B6VVD-4M6S921-3/2/62820bc8e66516644322d675149f5361>)

Abstract:

The antiviral activity induced by chitosan (CHT), and the mechanisms underlying it, were studied in a tobacco-tobacco necrosis necrovirus (TNV) pathosystem. Treatments with 0.1% CHT enhanced tobacco inducible defenses against TNV, reducing significantly the virus-induced necrotic lesions (in a range from 32% to 83%). In planta, this resistance was associated with a network of callose deposits, micro-oxidative bursts and micro-hypersensitive responses (micro-HRs), as assessed, respectively, by aniline blue, 3,3'-diaminobenzidine (DAB) and Evans blue staining. In order to verify if CHT-elicited cell death could be regarded as an apoptotic process, tobacco bright yellow 2 (BY2) cell cultures were treated with different CHT concentrations, ranging from 0.01% to 0.1%. After 6 h about half of the cultured cells incubated in 0.05% CHT were Evans blue positive, showing some typical morphological features of apoptosis, such as cytoplasm shrinkage and nuclear chromatin condensation. The latter was checked by 4',6-diamino-2-phenylindole (DAPI) and ethidium bromide nuclear staining and was visible already at 2 h after treatment. Moreover, the cell death kinetic induced by CHT was delayed by Verapamil(R), a calcium channel blocker. Finally, electrophoresis of genomic DNA extracted from cultured cell after 48 h treatment showed internucleosomal fragmentation, visualized as a distinct ladder of DNA bands corresponding to oligonucleosomal units.

Keywords: Chitosan; Induced resistance; TNV; Tobacco; BY2; Antiviral activity

Vipaporn Phuntumart, Pascal Marro, Jean-Pierre Metraux, Liliane Sticher, A novel cucumber gene associated with systemic acquired resistance, *Plant Science*, Volume 171, Issue 5, November 2006, Pages 555-564, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.05.014.

(<http://www.sciencedirect.com/science/article/B6TBH-4KBF195-1/2/558cf33792143dab9520e20be7b85a87>)

Abstract:

Several genes were isolated by differential display of mRNAs from cucumber leaves inoculated with the bacterium, *Pseudomonas syringae* pv. *lachrymans*. A full-length cDNA encoding a novel pathogen-induced gene, Cupi4, was cloned and characterized in detail. While Cupi4 did not share evident homology with known sequences in the database at the nucleotide level, the predicted amino acid sequence of Cupi4 shared homology with the pathogen-inducible proteins, pMB57-10G 5' of *Brassica napus* (21%) and CXc750/ESC1 of *Arabidopsis thaliana* (16%). Cupi4 transcripts accumulated after 12 h in leaves inoculated with *P. s. lachrymans* and after 48 h in the systemic upper leaves of the inoculated plants. Treatment with the chemical inducers of systemic acquired resistance (SAR), salicylic acid, 2,6-dichloroisonicotinic acid and benzothiadiazole as well as inoculation with different pathogens, *P. s. syringae*, *Colletotrichum lagenarium* and tobacco necrosis virus also led to the accumulation of Cupi4 transcripts. The increase of Cupi4 transcripts

in both the inoculated first leaf and in systemic upper leaves suggested that the Cupi4 gene product is associated with systemic acquired resistance in cucumber. Induced expression of CUPi4 in different host strains of a bacterium, *Escherichia coli*, led to death of bacterial host cells, suggesting that CUPi4 might have antibacterial properties.

Keywords: Class III chitinase; Cucumber; Cupi4; *Pseudomonas syringae* pv. *lachrymans*; Pathogen-induced proteins; Systemic acquired resistance

Wendy Van Hemelrijck, Piet F.W. Wouters, Margreet Brouwer, An Windelinckx, Inge J.W.M. Goderis, Miguel F.C. De Bolle, Bart P.H.J. Thomma, Bruno P.A. Cammue, Stijn L. Delaure, The *Arabidopsis* defense response mutant *esa1* as a model to discover novel resistance traits against *Fusarium* diseases, *Plant Science*, Volume 171, Issue 5, November 2006, Pages 585-595, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.06.013.

(<http://www.sciencedirect.com/science/article/B6TBH-4KCH9VM-1/2/6e900bffd77abdc84f7588d66022be4b>)

Abstract:

The *Arabidopsis thaliana* mutant *esa1* was previously shown to exhibit enhanced susceptibility to the necrotrophic fungal pathogens *Alternaria brassicicola*, *Botrytis cinerea* and *Plectosphaerella cucumerina*. In this work, we tried to elaborate on this susceptibility by investigating whether the *esa1* phenotype can be extended to *Fusarium* species, a genus that includes several economically relevant pathogens. We show that the *esa1* mutant exhibits increased susceptibility to several *Fusarium* species, including *Fusarium oxysporum* f. sp. *matthioli*, *F. solani*, and *F. culmorum*. Furthermore, we show that the causal agent of the Panama disease on banana, *F. oxysporum* f. sp. *cubense*, a pathogen for which wild-type *A. thaliana* shows non-host resistance, causes enhanced lesion formation on *esa1* as compared to wild-type plants, suggesting that *esa1* is more sensitive to *F. oxysporum* f. sp. *cubense*. In addition, we were able to show that the *A. thaliana* wild-type resistance phenotype towards the latter pathogen can be partially restored by expression of the pathogenesis-related proteins PR1 or PR5 from tobacco in *esa1*, suggesting that PR1 and/or PR5 expression may be useful traits to obtain enhanced resistance to *F. oxysporum* f. sp. *cubense* in banana. As such, *esa1* proves to be an ideal model system for research on the plant's defense response against fungal pathogens in general and *Fusarium* species in particular.

Keywords: *Arabidopsis*; *Fusarium* spp.; *Fusarium oxysporum* f. sp. *cubense*; Disease resistance; *esa1*

Helena Synkova, Sarka Semoradova, Renata Schnablova, Karel Muller, Jana Pospisilova, Helena Ryslava, Jiri Malbeck, Noemi Cerovska, Effects of biotic stress caused by Potato virus Y on photosynthesis in *ipt* transgenic and control *Nicotiana tabacum* L., *Plant Science*, Volume 171, Issue 5, November 2006, Pages 607-616, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.06.002.

(<http://www.sciencedirect.com/science/article/B6TBH-4K9C399-1/2/483629d5a357016387c98974facf2abe>)

Abstract:

We studied the effect of biotic stress caused by Potato virus YNTN (PVY) on photosynthesis in transgenic *Pssu-ipt* tobacco overproducing endogenous cytokinins (CK) in comparison with control (non-transformed) plants. Both control and transgenic tobacco were grown as rooted or grafted plants. Content of viral protein increased significantly in control tobacco within ca. 18 days after inoculation, whereas transgenic plants exhibited much lower accumulation. This corresponded also with the presence of visible symptoms of PVY infection; while they were always present in control, rooted tobacco, they never developed in transgenic grafts. Contents of CKs (mostly in the forms of N- and/or O-glucosides) increased in all infected plants except transgenic grafts, where the highest amount of CKs was found already prior the inoculation. The photosynthetic rate (PN) was significantly inhibited by PVY infection in control and transgenic rooted plants, while both grafted types were less affected. Reduction of PN was caused not only by stomata closure, but

also by the decrease of ribulose-1,5-bisphosphate carboxylase/oxygenase activity, contents of chlorophylls and xanthophyll cycle pigments, and activity of photosystem II (PSII). The negative effect on PS II was promoted by high irradiance treatment particularly in both rooted types infected by PVY.

Keywords: Potato virus Y; Photosynthesis; Transgenic tobacco; ipt; Cytokinins

Gadab C. Ghosh Biswas, Callista Ransom, Mariam Sticklen, Expression of biologically active *Acidothermus cellulolyticus* endoglucanase in transgenic maize plants, *Plant Science*, Volume 171, Issue 5, November 2006, Pages 617-623, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.06.004.

(<http://www.sciencedirect.com/science/article/B6TBH-4K9C2RK-2/2/7dbae5d73e33aad59a53f13d50f46c04>)

Abstract:

Commercial production of ethanol from plant biomass sources employs enzymatic hydrolysis of cellulose to glucose. Transgenic plants that can produce their own hydrolysis enzymes may offer an inexpensive and convenient system for the large-scale production of these enzymes. The catalytic domain of an endo-1,4-[beta]-d-glucanase gene from the eubacterium, *Acidothermus cellulolyticus*, was transferred to maize using particle bombardment, and transgenic plants were regenerated from five independent experiments containing E1 catalytic domain (E1-cd). Several of these plants grown in the greenhouse reached maturity and set seeds. Stable integration of the transgene in the genome of these plants was confirmed by Southern blot and expression of the transgene in plants by Western blot analysis. Expression of the recombinant E1-cd varied in different transgenic plants and the protein was enzymatically active. The activity-based assays indicate that the enzyme accumulated to concentrations up to 2.1% of plant total soluble proteins with enzymatic activity of 0.845 nmol/[mu]g/min in leaf, and 2.08% with enzymatic activity of 0.835 nmol/[mu]g/min in root tissues. The present data demonstrate the feasibility to produce a bacterial cellulase within the maize biomass crop for possible biomass conversion into fermentable sugars.

Keywords: Particle bombardment; Plant biomass; Transgenic maize plants; E1 endoglucanase; *Acidothermus cellulolyticus*

Cleberon F. Fernandes, Vadjah C.P. Moraes, Ilka M. Vasconcelos, Joaquim A.G. Silveira, Jose T.A. Oliveira, Induction of an anionic peroxidase in cowpea leaves by exogenous salicylic acid, *Journal of Plant Physiology*, Volume 163, Issue 10, 5 October 2006, Pages 1040-1048, ISSN 0176-1617, DOI: 10.1016/j.jplph.2005.06.021.

(<http://www.sciencedirect.com/science/article/B7GJ7-4HDG93S-4/2/768235d8226c6428855d07c479eb2ecc>)

Abstract: Summary

Two isoperoxidases were detected in cowpea (*Vigna unguiculata*) leaves. Treatment of the primary leaves with 10 mM salicylic acid increased the total peroxidase activity contributed by the anionic isoform. To isolate both the anionic and cationic peroxidases the leaf crude extract was loaded on a Superose 12 HR 10/30 column followed by chromatography on Mono-Q HR 5/5. Both enzymes were stable in a pH range from 5 to 7. The optimum-temperatures for the cationic and anionic peroxidase isoforms were, respectively, 20-30 [degree sign]C and 30 [degree sign]C. The dependence of guaiacol oxidation rate varying its concentration at constant H₂O₂ concentration showed, for both enzymes, Michaelis-Menten-type kinetic. Apparent K_ms were 0.8 and 4.8 [mu]M for the cationic and anionic isoperoxidases, respectively.

Keywords: Induction; Kinetic properties; Peroxidase; Salicylic acid; *Vigna unguiculata*

Frederic Lincker, Melanie Messmer, Guy Houlne, Martine Devic, Marie-Edith Chaboute, E2F factors rate controls the dual role of CDE/E2F composite element: A model of E2F-regulated gene

expression in plant development, FEBS Letters, Volume 580, Issue 22, 2 October 2006, Pages 5167-5171, ISSN 0014-5793, DOI: 10.1016/j.febslet.2006.08.067.

(<http://www.sciencedirect.com/science/article/B6T36-4KTMSN1-9/2/50e375fe51ca391d37550cde418e8b78>)

Abstract:

The promoters of several E2F-regulated genes identified in plants contain a variety of E2F motifs, notably a composite element consisting of a 'CDE-like element' C/GGCGG on one strand, described as repressor in animals, associated with an E2F element on the complementary strand. This detailed study throughout plant development using ribonucleotide reductase promoters, allows us to propose a model, where E2F and composite elements play a dual role. Such regulation is mainly conditioned by the availability of E2F factors in tissues and during the cell cycle in tobacco.

Keywords: Cell cycle-dependent element; E2F; Ribonucleotide reductase; Plants; Cell cycle; Development

S. Senthil Nathan, K. Kalaivani, Combined effects of azadirachtin and nucleopolyhedrovirus (SpliNPV) on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) larvae, Biological Control, Volume 39, Issue 1, October 2006, Pages 96-104, ISSN 1049-9644, DOI: 10.1016/j.biocontrol.2006.06.013.

(<http://www.sciencedirect.com/science/article/B6WBP-4KCH735-1/2/fabd06cddf772bbceb031633db5b9a87>)

Abstract:

The effects of azadirachtin (AZA) and *Spodoptera* nucleopolyhedrovirus (SpliNPV) on development and mortality of *Spodoptera litura* Fabricius (tobacco cutworm) were evaluated in the laboratory. The effective concentrations for AZA and SpliNPV were determined and tested as single and combination treatments. AZA and SpliNPV produced synergistic effects on tobacco cutworm mortality in higher dose combination treatment. Combinations of AZA + SpliNPV at 0.25 ppm + 1 x 10³ OB and 0.50 ppm + 1 x 10⁶ OB resulted in a significantly higher larval mortality than treatment with either virus/botanical insecticide alone at the corresponding concentrations. When consumed together (AZA and SpliNPV) larvae died significantly faster compared with larvae consuming SpliNPV or AZA. These results suggest that treatments with AZA and SpliNPV at appropriate combinations of concentration levels may result in improved control of tobacco cutworm compared with treatment with either AZA or virus alone.

Keywords: Tobacco cutworm; *Spodoptera*; Nucleopolyhedroviruses; Azadirachtin; Biology; Development; Synergism; Mortality

Dirk Weihrauch, Active ammonia absorption in the midgut of the Tobacco hornworm *Manduca sexta* L.: Transport studies and mRNA expression analysis of a Rhesus-like ammonia transporter, Insect Biochemistry and Molecular Biology, Volume 36, Issue 10, October 2006, Pages 808-821, ISSN 0965-1748, DOI: 10.1016/j.ibmb.2006.08.002.

(<http://www.sciencedirect.com/science/article/B6T79-4KPNVS9-2/2/b8e61fe1a19a977a3b531188ba478f66>)

Abstract:

In this study the mid- and hindgut of *Manduca sexta* larvae were tested for their ammonia transport properties using a custom-made Ussing chamber. In the presence of 0.1 mmol l⁻¹ ammonia on both sides of the isolated epithelium, active transepithelial ammonia absorption (aTEPA) was observed in all midgut sections, with greatest transport rates (ca. 140 nmol cm⁻² h⁻¹) detected in the median midgut. The hindgut showed no aTEPA. In the median midgut inhibition of energy metabolism by azide blocked aTEPA completely, whereas inhibition of vacuolar H⁺-ATPase by bafilomycin A1 reduced the active transport by 50%. The imposition of a luminal-directed NH₃-gradient (pH 6.5 apical, pH 8.5 basal) lowered the aTEPA by approximately 50% but did not

reverse its direction. Apical addition of amiloride reduced aTEPA by 90%, suggesting a role of carrier-mediated ammonia transport across the apical membrane via a member of the NHE family. Inhibition of the microtubule network by colchicine reduced aTEPA by ca. 50%. In contrast, blocking basal K⁺ channels by Ba²⁺ had no effect on aTEPA. Using molecular methods, evidence for intestinal expression of a Rhesus-like ammonia transporter (RhMS) was found with low mRNA expression in midgut tissues, but high expression levels in the hindgut, Malpighian tubules and ganglia.

Keywords: *Manduca sexta*; Active ammonia transport; Rhesus-like protein; Amiloride; Bafilomycin A1; Colchicine; Ba²⁺; Ussing chamber

Renata Schnablova, Helena Synkova, Anna Vicankova, Lenka Burketova, Josef Eder, Milena Cvikrova, Transgenic ipt tobacco overproducing cytokinins overaccumulates phenolic compounds during in vitro growth, *Plant Physiology and Biochemistry*, Volume 44, Issue 10, October 2006, Pages 526-534, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2006.09.004.

(<http://www.sciencedirect.com/science/article/B6VVD-4M0S211-2/2/7b25c4c5a052d0b031ffd3e1ef8c3b7c>)

Abstract:

We present evidence that overproduction of endogenous cytokinins (CK) caused stress response in non-rooting Pssu-ipt transgenic tobacco (*Nicotiana tabacum* L.) grown in vitro. It was demonstrated by overaccumulation of phenolic compounds, synthesis of pathogenesis related proteins (PR proteins), and increase in peroxidase (POD) activities. Immunolocalization of zeatin and also PR-1b protein on leaf cryo-sections proved their accumulation in all mesophyll cells of transgenic tobacco contrary to control non-transgenic plants. Intensive blue autofluorescence of phenolic compounds induced by UV in cross-sections of leaf midrib showed enhanced contents of phenolics in transgenic tobacco compared with controls, nevertheless, no significant difference between both plant types was found in leaf total lignin content. Transgenic plantlets exhibited higher peroxidase activities of both soluble and ionically bound fractions compared with controls. HPLC analysis of phenolic acids confirmed the increase in all phenolic acids in transgenic tobacco except for salicylic acid (SA). The effect of high phenolic content on rooting of transgenic tobacco is discussed.

Keywords: Pssu-ipt tobacco; Phenolic acids; Cytokinins; In vitro cultivation; Peroxidases

M. Onishi, H. Tachi, T. Kojima, M. Shiraiwa, H. Takahara, Molecular cloning and characterization of a novel salt-inducible gene encoding an acidic isoform of PR-5 protein in soybean (*Glycine max* [L.] Merr.), *Plant Physiology and Biochemistry*, Volume 44, Issue 10, October 2006, Pages 574-580, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2006.09.009.

(<http://www.sciencedirect.com/science/article/B6VVD-4M2WV9H-1/2/a67e323a283e1145d0fd500bf86efbd9>)

Abstract:

We identified a novel salt-inducible soybean gene encoding an acidic-isoform of pathogenesis-related protein group 5 (PR-5 protein). The soybean PR-5-homologous gene, designated as *Glycine max* osmotin-like protein, acidic isoform (GmOLPa); accession no, AB116251), encodes a putative polypeptide having an N-terminal signal peptide. The mature GmOLPa protein without the signal peptide has a calculated molecular mass of 21.5 kDa and a pI value of 4.4, and was distinguishable from a known PR-5-homologous gene of soybean (namely P21 protein) through examination of the structural features. A comparison with two intracellular salt-inducible PR-5 proteins, tobacco osmotin and tomato NP24, revealed that GmOLPa did not have a C-terminal extension sequence functioning as a vacuole-targeting motif. The GmOLPa gene was transcribed constitutively in the soybean root and was induced almost exclusively in the root during 24 h of high-salt stress (300 mM NaCl). Interestingly, GmOLPa gene expression in the stem and leaf, not observed until 24 h, was markedly induced at 48 and 72 h after commencement of the high-salt

stress. Abscisic acid (ABA) and dehydration also induced expression of the GmOLPa gene in the root; additionally, dehydration slightly induced expression in the stem and leaf. In fact, the 5'-upstream sequence of the GmOLPa gene contained several putative cis-elements known to be involved in responsiveness to ABA and dehydration, e.g. ABA-responsive element (ABRE), MYB/MYC, and low temperature-responsive element (LTRE). These results suggested that GmOLPa may function as a protective PR-5 protein in the extracellular space of the soybean root in response to high-salt stress and dehydration.

Keywords: Gene expression; GmTDF; Pathogenesis-related protein; Soil salinity; Soybean (*Glycine max* [L.] Merr.)

B. Feng, Y. Chen, C. Zhao, X. Zhao, X. Bai, Y. Du, Isolation of a novel Ser/Thr protein kinase gene from oligochitosan-induced tobacco and its role in resistance against tobacco mosaic virus, *Plant Physiology and Biochemistry*, Volume 44, Issue 10, October 2006, Pages 596-603, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2006.10.003.

(<http://www.sciencedirect.com/science/article/B6VVD-4M4CTKC-2/2/c3582515712e5c80ad10919a49ef160d>)

Abstract:

Oligochitosan induces defense responses to pathogenic microbes in a wide variety of plants by acting as an elicitor. In the present study, mRNA differential display was used to investigate oligochitosan-induced transcriptional activation of defense-related genes. Accordingly, a novel Ser/Thr protein kinase gene was isolated and designated as oligochitosan-induced protein kinase (oipk). Molecular cloning showed that oipk contains six introns interrupted by seven exons. The open reading frame (ORF) of the gene is 1848 bp, which encodes a putative protein of 615 amino acids with the predicted molecular mass of 70.96 kDa and a pI of 6.32. A plant oipk antisense expression vector was constructed and transformed into tobacco by *Agrobacterium tumefaciens*. Decreased phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity and decreased resistance to tobacco mosaic virus (TMV) were observed in transgenic tobacco. RT-PCR analysis revealed that oipk was expressed at high levels after oligochitosan induction in wild-type tobacco, but not in transgenic tobacco. These results indicated that oipk is involved in the signal pathway of oligochitosan-induced resistance in tobacco.

Keywords: Oligochitosan; Ser/Thr protein kinase; PAL; TMV resistance; Transgenic tobacco

P. Priya, P. Venkatachalam, A. Thulaseedharan, Molecular cloning and characterization of the rubber elongation factor gene and its promoter sequence from rubber tree (*Hevea brasiliensis*): A gene involved in rubber biosynthesis, *Plant Science*, Volume 171, Issue 4, October 2006, Pages 470-480, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.05.009.

(<http://www.sciencedirect.com/science/article/B6TBH-4K5SS0V-1/2/29be0834ab127b37b0709c40f5a23f62>)

Abstract:

Hevea rubber tree (*Hevea brasiliensis*) is the only plant species being cultivated for commercial production of rubber in the world. In order to meet ever increasing rubber demand, it is a prerequisite to identify and characterize a key gene involved in rubber biosynthesis and over-expression of rubber biosynthesis gene will eventually lead to enhance the latex (rubber) production in transgenic *Hevea* plants. Rubber elongation factor (REF) is a major protein located on the surface of large rubber particles in latex and is involved which is involved in rubber biosynthesis in *H. brasiliensis*. We report here cloning and characterization of REF gene as well as its 5' promoter region from *Hevea*. REF gene (1367 bp) has three exons interrupted by two introns and encoded a 138 amino acid peptide containing an open reading frame of 414 bp with a calculated MW of 14,700 Da. Nucleotide sequence analysis showed that 1.3 kb genomic DNA showed 100% homology to REF cDNA from *Hevea*. Southern blot hybridization of genomic DNA with REF gene probe revealed that REF gene is encoded by a small gene family consisting of two

members. RNA blot analysis indicated that REF transcript is highly expressed in high yielding clone than in low yielder. The cloned 5' promoter region has a putative TATA element at -150 and CAAT box at -221 position. To identify the regulatory role of REF promoter, chimaeric fusion between REF promoter sequence and the [beta]-glucuronidase (GUS) coding, uidA gene was constructed and used to transform tobacco and Arabidopsis. Expression of the uidA reporter gene was detected histochemically in the transformed tobacco plants where, GUS activity was detected in the leaf and petiole of transformed plants. The stable integration of REF:uidA fusion into the tobacco genome was further confirmed by PCR amplification and Southern blot analysis. A histochemical study of stable transformants demonstrated that the 5' upstream region of REF can drive strong GUS gene expression specifically in the vascular tissues (xylem and phloem) of leaf, stem and midribs of transgenic Arabidopsis. GUS staining revealed that REF:GUS expression was also induced by wounding. The results suggested that the cloned REF promoter is capable of directing gene expression. Our ultimate goal is to produce transgenic Hevea plants with enhanced latex yield by over expression of REF protein.

Keywords: Hevea brasiliensis (Hb); Rubber elongation factor (REF) gene; Cloning; Promoter; Tobacco; Arabidopsis; Transformation

YuanHong Xie, BenZhong Zhu, XiangLong Yang, HongXing Zhang, DaQi Fu, HongLiang Zhu, Yi Shao, YingCong Li, HongYan Gao, YunBo Luo, Delay of postharvest ripening and senescence of tomato fruit through virus-induced LeACS2 gene silencing, *Postharvest Biology and Technology*, Volume 42, Issue 1, October 2006, Pages 8-15, ISSN 0925-5214, DOI: 10.1016/j.postharvbio.2006.04.016.

(<http://www.sciencedirect.com/science/article/B6TBJ-4KXF2T0-1/2/6bdd4b4a697369ae97edfb77eee058fb>)

Abstract:

Plant virus-induced gene silencing (VIGS) is currently a powerful tool for the study of gene function in plants. Here we report the silencing of LeACS2 by vacuum-infiltration and the tobacco rattle virus (TRV)-based VIGS method, which leads to a significant delay of the postharvest ripening and senescence of tomato fruit. Harvested mature green tomato fruit were vacuum-infiltrated with *Agrobacterium* strain GV3101 containing pTRV1 and pTRV2-LeACS2. Because of the silencing of LeACS2, the ethylene climacteric and pigment changes were clearly delayed. The onset of fruit ripening and senescence was significantly postponed, and transcription of LeACS2 and ACC synthase activity were also suppressed in treated tomato fruit during storage. The silencing of LeACS2 by vacuum infiltration, however, did not alter the contents assayed at the end of storage. Our results indicate that vacuum infiltration is a highly efficient TRV-based VIGS method to silence LeACS2 in harvested tomato fruit. It can obviously delay ripening and senescence, and is a potential method for postharvest preservation of tomato fruit.

Keywords: VIGS; Vacuum infiltration; *Lycopersicon esculentum*; Fruit ripening

Andrew M. Dacks, Joel B. Dacks, Thomas A. Christensen, Alan J. Nighorn, The cloning of one putative octopamine receptor and two putative serotonin receptors from the tobacco hawkmoth, *Manduca sexta*, *Insect Biochemistry and Molecular Biology*, Volume 36, Issue 9, September 2006, Pages 741-747, ISSN 0965-1748, DOI: 10.1016/j.ibmb.2006.07.002.

(<http://www.sciencedirect.com/science/article/B6T79-4KKFXKR-1/2/4b5d7031104e513fd89c1b2d775242d3>)

Abstract:

Serotonin and octopamine (OA) are biogenic amines that are active throughout the nervous systems of insects, affecting sensory processing, information coding and behavior. As an initial step towards understanding the modulatory roles of these amines in olfactory processing we cloned two putative serotonin receptors (Ms5HT1A and Ms5HT1B) and one putative OA (MsOAR) receptor from the moth *Manduca sexta*. Ms5HT1A and Ms5HT1B were both similar to 5HT1-type

receptors but differed from each other in their N-terminus and 3rd cytoplasmic loop. Ms5HT1A was nearly identical to a serotonin receptor from *Heliothis virescens* and Ms5HT1B was almost identical to a serotonin receptor from *Bombyx mori*. The sequences for homologs of Ms5HT1A from *B. mori* and Ms5HT1B from *H. virescens* were also obtained, suggesting that the Lepidoptera likely have at least two serotonin receptors. The MsOAR shares significant sequence homology with pharmacologically characterized OA receptors, but less similarity to putative OA/tyramine receptors from the moths *B. mori* and *H. virescens*. Using the MsOAR sequence, fragments encoding putative OA receptors were obtained from *B. mori* and *H. virescens*, suggesting that MsOAR is the first OA receptor cloned from a lepidopteran.

Keywords: Serotonin; Octopamine; Manduca; Receptors; Modulation

S. Jung, Y. Lee, K. Back, A tobacco plastidal transit sequence cannot override the dual targeting capacity of *Myxococcus xanthus* protoporphyrinogen oxidase in transgenic rice, *Pesticide Biochemistry and Physiology*, Volume 86, Issue 1, September 2006, Pages 49-56, ISSN 0048-3575, DOI: 10.1016/j.pestbp.2005.11.010.

(<http://www.sciencedirect.com/science/article/B6WP8-4JF8H6D-1/2/ddb05ab5a499091dcc86369829bfca2b>)

Abstract:

The effect of a plastidal transit sequence in *Myxococcus xanthus* protoporphyrinogen oxidase (Protox) on gene targeting ability was investigated by generating transgenic rice that overexpressed *M. xanthus* Protox with the additional plastidal transit sequence (TTS line). In transgenic lines TTS3 and TTS4, the Protox antibody cross-reacted with the mature *M. xanthus* Protox protein of 50 kDa. In an in vitro import system using the *M. xanthus* Protox gene with the plastidal transit sequence, *M. xanthus* protein was detected in both chloroplasts and mitochondria, confirming that it was targeted into both organelles, as in transgenic rice line, M4, that overexpressed *M. xanthus* Protox lacking the plastidal transit sequence. A prominent increase in chloroplastic and mitochondrial Protox activity was observed in TTS3 and TTS4 relative to the wild type. However, the increase was lower than that in transgenic line M4. Seeds from all transgenic lines (TTS3, TTS4, and M4) were able to germinate when treated with up to 500 [μ]M of the Protox-inhibiting herbicide, oxyfluorfen, whereas seeds from the wild type failed to germinate even when treated at levels as low as 1 [μ]M. After foliar application of oxyfluorfen, TTS3 and TTS4 exhibited a reduced Protox activity, however, it was much greater than uninhibited Protox activity of wild type. The great increase in conductivity was followed by the great accumulation of photodynamic protoporphyrin IX only in oxyfluorfen-treated wild-type plants, not in oxyfluorfen-treated TTS lines. The presence of the plastidal transit sequence neither excludes the intrinsic ability of subcellular translocation of *M. xanthus* Protox nor changes herbicide resistance in TTS lines.

Keywords: Protoporphyrin IX; Protoporphyrinogen oxidase; Herbicide resistance; Oxyfluorfen; Transgenic rice

Railene de Azevedo Pereira, Joao Aguiar Nogueira Batista, Maria Cristina Mattar da Silva, Osmundo Brilhante de Oliveira Neto, Edson Luiz Zangrando Figueira, Arnubio Valencia Jimenez, Maria Fatima Grossi-de-Sa, An [α]-amylase inhibitor gene from *Phaseolus coccineus* encodes a protein with potential for control of coffee berry borer (*Hypothenemus hampei*), *Phytochemistry*, Volume 67, Issue 18, September 2006, Pages 2009-2016, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2006.06.029.

(<http://www.sciencedirect.com/science/article/B6TH7-4KKWVX2-1/2/4887e70690a40a341dcef807bbd1b395>)

Abstract:

Plant [α]-amylase inhibitors are proteins found in several plants, and play a key role in natural defenses. In this study, a gene encoding an [α]-amylase inhibitor, named [α]AI-Pc1, was

isolated from cotyledons of *Phaseolus coccineus*. This inhibitor has an enhanced primary structure to *P. vulgaris* [α]-amylase inhibitors ([α]-AI1 and [α]-AI2). The [α]AI-Pc1 gene, constructed with the PHA-L phytohemagglutinin promoter, was introduced into tobacco plants, with its expression in regenerated (T0) and progeny (T1) transformant plants monitored by PCR amplification, enzyme-linked immunosorbent assay (ELISA) and immunoblot analysis, respectively. Seed protein extracts from selected transformants reacted positively with a polyclonal antibody raised against [α]AI-1, while no reaction was observed with untransformed tobacco plants. Immunological assays showed that the [α]AI-Pc1 gene product represented up to 0.05% of total soluble proteins in T0 plants seeds. Furthermore, recombinant [α]AI-Pc1 expressed in tobacco plants was able to inhibit 65% of digestive *H. hampei* [α]-amylases. The data herein suggest that the protein encoded by the [α]AI-Pc1 gene has potential to be introduced into coffee plants in order to increase their resistance to the coffee berry borer.

Keywords: Coffee berry borer; *Phaseolus coccineus*; Digestive enzymes; Insect

Toshiyuki Ohnishi, Takahito Nomura, Bunta Watanabe, Daisaku Ohta, Takao Yokota, Hisashi Miyagawa, Kanzo Sakata, Masaharu Mizutani, Tomato cytochrome P450 CYP734A7 functions in brassinosteroid catabolism, *Phytochemistry*, Volume 67, Issue 17, September 2006, Pages 1895-1906, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2006.05.042.

(<http://www.sciencedirect.com/science/article/B6TH7-4KGPPHY-1/2/fbbf2942074369ce9d0daa08fc01e442>)

Abstract:

Several cytochrome P450 monooxygenases (P450s) catalyze essential oxidative reactions in brassinosteroid (BR) biosynthesis as well as in BR catabolism; however, only limited information exists on the P450s involved in the BR catabolic pathway. Here, we report the characterization of two P450 mRNAs, CYP734A7 and CYP734A8, from *Lycopersicon esculentum*. These P450s show high homology with *Arabidopsis* CYP734A1/BAS1 (formerly CYP72B1), which inactivates BRs via C-26 hydroxylation. Transgenic tobacco plants that constitutively overexpressed CYP734A7 showed an extreme dwarf phenotype similar to BR deficiency. Quantitative gas chromatography-mass spectrometry analysis of endogenous BRs in the transgenic plants showed that the levels of castasterone and 6-deoxocastasterone significantly decreased in comparison with those in wild-type plants. By measuring the Type I substrate-binding spectra using recombinant CYP734A7, the dissociation constants for castasterone, brassinolide, and 6-deoxocastasterone were determined to be 6.7, 12, and 12 [μ]M, respectively. In an *in vitro* assay, CYP734A7 was confirmed to metabolize castasterone to 26-hydroxycastasterone. In addition, 28-norcastasterone and brassinolide were converted to the hydroxylated products. The expression of CYP734A7 and CYP734A8 genes in tomato seedlings was upregulated by exogenous application of bioactive BRs. These results indicated that CYP734A7 is a C-26 hydroxylase of BRs and is likely involved in BR catabolism in tomato. The presence of the CYP734A subfamily in various plant species suggests that oxidative inactivation of BRs by these proteins is a widespread phenomenon in plants.

Keywords: *Lycopersicon esculentum*; Solanaceae; Brassinosteroid; Brassinolide; Castasterone; Brassinosteroid catabolism; Cytochrome P450; CYP734A7; CYP734A8

Robert A. Raguso, Boris O. Schlumpberger, Rainee L. Kaczorowski, Timothy P. Holtsford, Phylogenetic fragrance patterns in *Nicotiana* sections *Alatae* and *Suaveolentes*, *Phytochemistry*, Volume 67, Issue 17, September 2006, Pages 1931-1942, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2006.05.038.

(<http://www.sciencedirect.com/science/article/B6TH7-4KDBM68-1/2/c44a46419bcec3fd733a900a87eb83f6>)

Abstract:

We analyzed floral volatiles from eight tobacco species (*Nicotiana*; Solanaceae) including newly discovered Brazilian taxa (*Nicotiana mutabilis* and '*Rastroensis*') in section *Alatae*. Eighty-four compounds were found, including mono- and sesquiterpenoids, nitrogenous compounds, benzenoid and aliphatic alcohols, aldehydes and esters. Floral scent from recent accessions of *Nicotiana alata*, *Nicotiana bonariensis* and *Nicotiana langsdorffii* differed from previously published data, suggesting intraspecific variation in scent composition at the level of biosynthetic class. Newly discovered taxa in *Alatae*, like their relatives, emit large amounts of 1,8-cineole and smaller amounts of monoterpenes on a nocturnal rhythm, constituting a chemical synapomorphy for this lineage. Fragrance data from three species of *Nicotiana* sect. *Suaveolentes*, the sister group of *Alatae*, (two Australian species: *N. cavicola*, *N. ingulba*; one African species: *N. africana*), were compared to previously reported data from their close relative, *N. suaveolens*. Like *N. suaveolens*, *N. cavicola* and *N. ingulba* emit fragrances dominated by benzenoids and phenylpropanoids, whereas the flowers of *N. africana* lacked a distinct floral scent and instead emitted only small amounts of an aliphatic methyl ester from foliage. Interestingly, this ester also is emitted from foliage of *N. longiflora* and *N. plumbaginifolia* (both in section *Alatae* s.l.), which share a common ancestor with *N. africana*. This result, combined with the synapomorphic pattern of 1,8 cineole emission in *Alatae* s.s., suggests that phylogenetic signal explains a major component of fragrance composition among tobacco species in sections *Alatae* and *Suaveolentes*. At the intraspecific level, interpopulational scent variation is widespread in sect. *Alatae*, and may reflect edaphic specialization, introgression, local pollinator shifts, genetic drift or artificial selection in cultivation. Further studies with genetically and geographically well-defined populations are needed to distinguish between these possibilities.

Keywords: *Nicotiana* sp.; Solanaceae; GC-MS; Hawkmoths; Headspace; Hummingbirds; Pollination

Jeremy N. Friedberg, Stephen R. Bowley, Bryan D. McKersie, William B. Gurley, Eva Czarnicka-Verner, Isolation and characterization of class A4 heat shock transcription factor from alfalfa, *Plant Science*, Volume 171, Issue 3, September 2006, Pages 332-344, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.04.007.

(<http://www.sciencedirect.com/science/article/B6TBH-4K00C3R-1/2/3fc3a2c6867c5b340d434829cf4f7662>)

Abstract:

Plant heat shock transcription factors (HSFs) regulate transcription of heat shock (HS) genes. In *Arabidopsis thaliana*, 21 HSFs have been classified into groups A-C. Members of class A act as typical transcriptional activators, whereas B HSFs function as coactivators or repressors depending on promoter context. The function of class C HSFs is still unclear. Here, we present the isolation and characterization of the first HSF from alfalfa (*Medicago sativa* L.) and designate it MsHSFA4 based on amino acid sequence analysis. The MsHSFA4 gene was determined to be single copy and was detected at two separate genetic loci in the tetraploid *Medicago sativa*. Overexpression of MsHSFA4 in tobacco mesophyll protoplasts resulted in weak transcriptional activity, similar to that exhibited by *Arabidopsis* AtHSFA4a. The MsHSFA4 proximal promoter contains three putative HSE elements, and the gene itself is activated both by heat and cold stress.

Keywords: Heat shock factor; C-terminal region; Promoter; Chromosomal localization

Kentaro Ishimaru, Keita Takada, Shin Watanabe, Hiroshi Kamada, Hiroshi Ezura, Stable male sterility induced by the expression of mutated melon ethylene receptor genes in *Nicotiana tabacum*, *Plant Science*, Volume 171, Issue 3, September 2006, Pages 355-359, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.04.006.

(<http://www.sciencedirect.com/science/article/B6TBH-4JYKHMN-2/2/516a57b9d065d2017cf362c9ebc63ab3>)

Abstract:

A major concern about genetically modified crops is transgene flow through pollen dispersal. We previously demonstrated that overexpression of the mutated melon ethylene receptor genes Cm-ETR1/H69A or Cm-ERS1/H70A induces pollen abortion and altered flower architecture, resulting in sterility or reduced fertility in transgenic tobacco plants. To investigate the stability of these traits, three transgenic tobacco lines in which Cm-ETR1/H69A or Cm-ERS1/H70A confer sterility or reduced fertility were grown in a greenhouse with environmental conditions that changed, depending on the outside conditions. During the growth of the plants, the temperature ranged from 31 [degree sign]C at the beginning of September to 17 [degree sign]C at the beginning of November. The light provided was natural sunlight. The first group of plants flowered in late September, and the second group flowered in late October. The wild-type plants showed the homostyly type of floral architecture, whereas, three transgenic lines showed the heterostyly type. The floral architecture was stable during the different flowering periods. Pollen production was significantly reduced in two transgenic lines and completely aborted in one transgenic line, and these traits were also stable during the different flowering periods. These results suggest that the sterility or reduced fertility induced by the expression of mutated melon ethylene receptor genes in transgenic tobacco plants is stable under varying environmental conditions.

Keywords: *Nicotiana tabacum*; Ethylene receptor gene; Melon; Sterility; Pollen abortion; Floral architecture; Heterostyly

Imke Ortmann, Uwe Conrath, Bruno M. Moerschbacher, Exopolysaccharides of *Pantoea* agglomerans have different priming and eliciting activities in suspension-cultured cells of monocots and dicots, *FEBS Letters*, Volume 580, Issue 18, 7 August 2006, Pages 4491-4494, ISSN 0014-5793, DOI: 10.1016/j.febslet.2006.07.025.

(<http://www.sciencedirect.com/science/article/B6T36-4KF1G1B-4/2/c438afc904d47e27469807b0ee9087d3>)

Abstract:

Induced disease resistance of plants is often associated with an enhanced capacity to activate cellular defense responses to pathogen attack, named the 'primed' state of the plant. Exopolysaccharides of *Pantoea* agglomerans have recently been reported as the first priming active component of bacterial origin in wheat cells. We now show that *Pantoea* exopolysaccharides also prime rice cells for better elicitation of a rapid oxidative burst. In contrast, in tobacco and parsley cell cultures *Pantoea* exopolysaccharides activate the oxidative burst response directly. Our results point to a different recognition and/or mode of action of *Pantoea* exopolysaccharides in monocot and dicot plants.

Keywords: Extracellular polysaccharides; Induced disease resistance; Monocots; Oxidative burst; Priming; *Erwinia herbicola*

Maciej A. Pszczolkowski, Angela Tucker, Asoka Srinivasan, Sonny B. Ramaswamy, On the functional significance of juvenile hormone in the accessory sex glands of male *Heliothis virescens*, *Journal of Insect Physiology*, Volume 52, Issue 8, August 2006, Pages 786-794, ISSN 0022-1910, DOI: 10.1016/j.jinsphys.2006.03.005.

(<http://www.sciencedirect.com/science/article/B6T3F-4JH6C7S-2/2/f6a114df60c9d4e2b4b7ec5d059e6a23>)

Abstract:

The storage of large quantities of juvenile hormone (JH) in male abdomens is a phenomenon known from some species of moths. Juvenile hormone, stored in male accessory sex glands (ASG), may be transferred to the female during copulation, but the physiological significance of the JH transfer remains unclear. Here, using the moth *Heliothis virescens* as a model, we show that JH transferred from male to the promiscuous female promotes JH synthesis and egg development in the female. We propose that this explains the functional significance of JH transfer in species

that exhibit last male sperm precedence, and that this hormone acts as a bioactive substance which the first male to mate uses for co-opting and regulating the female's gonadotropic mechanisms, thereby ensuring that despite last male sperm precedence he will sire a significant number of viable offspring.

Keywords: Oocyte maturation; Tobacco budworm; Polyandry; Post-coital sexual selection

Ajay Amar Vashisht, Narendra Tuteja, Stress responsive DEAD-box helicases: A new pathway to engineer plant stress tolerance, *Journal of Photochemistry and Photobiology B: Biology*, Volume 84, Issue 2, 1 August 2006, Pages 150-160, ISSN 1011-1344, DOI: 10.1016/j.jphotobiol.2006.02.010.

(<http://www.sciencedirect.com/science/article/B6TH0-4JS1MWK-1/2/16fcd0246f0777bc9b3d27eaec07dfda>)

Abstract:

Abiotic stresses including various environmental factors adversely affect plant growth and limit agricultural production worldwide. Minimizing these losses is a major area of concern for all countries. Therefore, it is desirable to develop multi-stress tolerant varieties. Salinity, drought, and cold are among the major environmental stresses that greatly influence the growth, development, survival, and yield of plants. UV-B radiation of sunlight, which damages the cellular genomes, is another growth-retarding factor. Several genes are induced under the influence of various abiotic stresses. Among these are DNA repair genes, which are induced in response to the DNA damage. Since the stresses affect the cellular gene expression machinery, it is possible that molecules involved in nucleic acid metabolism including helicases are likely to be affected. The light-driven shifts in redox-potential can also initiate the helicase gene expression. Helicases are ubiquitous enzymes that catalyse the unwinding of energetically stable duplex DNA (DNA helicases) or duplex RNA secondary structures (RNA helicases). Most helicases are members of DEAD-box protein superfamily and play essential roles in basic cellular processes such as DNA replication, repair, recombination, transcription, ribosome biogenesis and translation initiation. Therefore, helicases might be playing an important role in regulating plant growth and development under stress conditions by regulating some stress-induced pathways. There are now few reports on the up-regulation of DEAD-box helicases in response to abiotic stresses. Recently, salinity-stress tolerant tobacco plants have already been raised by overexpressing a helicase gene, which suggests a new pathway to engineer plant stress tolerance [N. Sanan-Mishra, X.H. Pham, S.K. Sopory, N. Tuteja, Pea DNA helicase 45 overexpression in tobacco confers high salinity tolerance without affecting yield. *Proc. Natl. Acad. Sci. USA* 102 (2005) 509-514]. Presently the exact mechanism of helicase-mediated stress tolerance is not understood. In this review we have described all the reported stress-induced helicases and also discussed the possible mechanisms by which they can provide stress tolerance.

Keywords: ABA; Abiotic stress; Biotic stress; Cold shock proteins; DEAD-box protein; DNA helicase; Plant helicases; RNA helicase; Ribosome; Signal transduction; Stress tolerance; UV radiation

Dae-Kyun Ro, Jorg Bohlmann, Diterpene resin acid biosynthesis in loblolly pine (*Pinus taeda*): Functional characterization of abietadiene/levopimaradiene synthase (PtTPS-LAS) cDNA and subcellular targeting of PtTPS-LAS and abietadienol/abietadienal oxidase (PtAO, CYP720B1), *Phytochemistry*, Volume 67, Issue 15, Rod Croteau Special Issue, Part 1, August 2006, Pages 1572-1578, ISSN 0031-9422, DOI: 10.1016/j.phytochem.2006.01.011.

(<http://www.sciencedirect.com/science/article/B6TH7-4JB9N1D-4/2/647827c48f17252fa58a1589d6b6d96d>)

Abstract:

Diterpene resin acids are prominent defense compounds against insect pests and pathogens in conifers. Biochemical and molecular analyses in grand fir (*Abies grandis*), Norway spruce (*Picea*

abies), and loblolly pine (*Pinus taeda*) have identified two classes of genes and enzymes that generate much of the structural diversity of terpenoid defense compounds: The terpenoid synthases (TPS) and cytochrome P450 monooxygenases (P450). Using a single substrate, geranylgeranyl diphosphate, families of single-product and multi-product diterpene synthases generate an array of cyclic diterpene olefins. These diterpenes are converted to diterpene resin acids by activity of one or more P450 enzymes. A few conifer diterpene synthases have previously been cloned and characterized in grand fir and in Norway spruce. We have also previously shown that the loblolly pine P450 abietadienol/abietadienal oxidase (PtAO) catalyzes multiple oxidations of several diterpene alcohols and aldehydes. Conifer diterpene synthases are thought to function in plastids while P450s can also be localized to plastids or to the endoplasmic reticulum (ER). Here, we show that a loblolly pine cDNA (PtTPS-LAS) encodes a typical multi-product conifer diterpene synthase that forms levopimaradiene, abietadiene, palustradiene, and neoabietadiene similar to the grand fir abietadiene synthase and Norway spruce levopimaradiene/abietadiene synthase. Subcellular targeting of PtTPS-LAS and PtAO to plastids and ER, respectively, was shown with green fluorescent fusion protein expression in tobacco cells. These data suggest that enzymes for conifer diterpene resin acid biosynthesis are localized to at least two different subcellular compartments, plastids and ER, requiring efficient transport of intermediates and secretion of diterpene resin acids into the extracellular space.

Keywords: Terpene synthase; Cytochrome P450 monooxygenase; Abietic acid; Conifer defense; Oleoresin

Feng Ren, Ying-Tang Lu, Overexpression of tobacco hydroxyproline-rich glycopeptide systemin precursor A gene in transgenic tobacco enhances resistance against *Helicoverpa armigera* larvae, *Plant Science*, Volume 171, Issue 2, August 2006, Pages 286-292, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.04.001.

(<http://www.sciencedirect.com/science/article/B6TBH-4JVSSJG-1/2/d7687f5ca1f3c5cbbd87f1d16ad0272c>)

Abstract:

Two tobacco hydroxyproline-rich glycopeptide systemin precursor proteins (TobpreproHypSys-A and -B) have been discovered recently. The TobpreproHypSys-A, from which TobHypSys I and TobHypSys II were released, has been reported to be induced by mechanical and herbivorous wounding systemically. Here our experiments by overexpressing TobpreproHypSys-A in transgenic plants indicated the overexpression of TobpreproHypSys-A enhances the resistance against *Helicoverpa armigera* larvae. The growth and development of *H. armigera* larvae were reduced dramatically feeding on transgenic tobacco plants. To determine the causes of the increased resistance, both PIs and PPO as defense proteins were investigated by Northern blot and activity analysis. PIs expression and activity were dramatically induced in the TobpreproHypSys-A overexpressed plants. PPO activity was also induced substantially in transgenic plants. These data suggest that the enhanced resistance to insect larvae could result from the accumulation of PIs and PPO induced by the TobpreproHypSys-A expression in transgenic plants.

Keywords: *Helicoverpa armigera*; *Nicotiana tabacum*; Polyphenol oxidase; Proteinase inhibitor; Resistance; Systemin

E.N. Matu, K.L. Lindsey, J. van Staden, Micropropagation of *Maytenus senegalensis* (Lam.) Exell, *South African Journal of Botany*, Volume 72, Issue 3, August 2006, Pages 409-415, ISSN 0254-6299, DOI: 10.1016/j.sajb.2005.11.005.

(<http://www.sciencedirect.com/science/article/B7XN9-4JS1TN9-2/2/9d866930dec0f6224bd5ab43f0fe6efb>)

Abstract:

A micropropagation protocol for *Maytenus senegalensis* was established using in vitro germinated 6-week-old seedlings as a source of explants. Shoot proliferation was only achieved using nodal explants. The presence of cytokinins was necessary for shoot induction and growth. While benzyl-6-aminopurine (BA) generally promoted the average number of shoots produced per explant, kinetin promoted the mean length of shoots. Inclusion of auxin (IAA and IBA) in the shoot induction and growth medium did not have significant effects on either the average number of roots produced per shoot or the mean length of shoots. Low levels of BA (0.5 or 1.0 mg l⁻¹) were optimal for shoot induction and growth. As a result, shoot multiplication was done on a medium supplemented with BA (0.5 mg l⁻¹) alone. Rooting was achieved in 2 stages. In the first stage, 8-week-old shoots (2-3 cm long) were pulse-treated in the dark using 1/2 strength, MS [Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* 15, 473-497] liquid medium containing auxins and then transferred to a solid hormone-free medium at a 12/12 h photoperiod. The shoots pulse-treated with 25 mg l⁻¹ IBA for 120 h produced the highest number of roots per shoot. IBA concentration and the period of pulse treatment had significant effects on the average number of roots produced per shoot. The rooted plantlets were successfully acclimatized.

Chikako Honda, Takaya Moriguchi, High GUS expression in protoplasts isolated from immature peach fruits, *Scientia Horticulturae*, Volume 109, Issue 3, 21 July 2006, Pages 244-247, ISSN 0304-4238, DOI: 10.1016/j.scienta.2006.04.014.

(<http://www.sciencedirect.com/science/article/B6TC3-4K2SKF1-2/2/f07db992330f067bf0b016e3a3f9d5fd>)

Abstract:

A protocol for transient expression analysis was developed using protoplast isolated from immature peach fruits. The uid A gene for [beta]-glucuronidase (GUS) was introduced into the protoplast as a reporter gene to evaluate the protoplast activity. Protoplasts isolated from immature fruits at 28-32 days after full bloom (DAFB) showed high GUS activity under the cauliflower mosaic virus 35S promoter. The highest GUS activity was obtained from the protoplasts isolated at 32 DAFB, but it was difficult to isolate protoplasts showing high GUS activity from 34 DAFB. A comparison of the effect of promoter cassette on gene expression showed that the promoter containing the tobacco mosaic virus [Omega] sequence enhanced GUS activity by at least 10-fold in the peach protoplasts relative to the 35S promoter. The level of GUS activity under the 35S promoter in the peach protoplasts was 0.47-fold as that obtained from maize protoplasts isolated from young greening leaves, indicating that the GUS activity of immature peach protoplast is sufficient for gene expression analysis. Since stable transformation and evaluation of fruit traits in peach transformants are difficult, this transient expression system could be useful for the characterization of genes expressed in peach and other Rosaceae fruit species.

Keywords: Electroporation; Fruit trees; Maize; Peach; Transient gene expression

Yuri L. Dorokhov, Olga Y. Frolova, Eugene V. Skurat, Peter A. Ivanov, Tatjana V. Gasanova, Anna A. Sheveleva, Nikolay V. Ravin, Kristiina M. Makinen, Victor I. Klimyuk, Konstantin G. Skryabin, Yuri Y. Gleba, Joseph G. Atabekov, A novel function for a ubiquitous plant enzyme pectin methylesterase: The enhancer of RNA silencing, *FEBS Letters*, Volume 580, Issue 16, 10 July 2006, Pages 3872-3878, ISSN 0014-5793, DOI: 10.1016/j.febslet.2006.06.013.

(<http://www.sciencedirect.com/science/article/B6T36-4K6CN8H-4/2/8d7f0387a9b874a686041975afe2c03a>)

Abstract:

Co-agroinjection of *Nicotiana benthamiana* leaves with the pectin methylesterase (proPME) gene and the TMV:GFP vector resulted in a stimulation of virus-induced RNA silencing (inhibition of GFP production, virus RNA degradation, stimulation of siRNAs production). Conversely, co-expression of TMV:GFP with either antisense PME construct or with enzymatically inactive

proPME restored synthesis of viral RNA. Furthermore, expression of proPME enhanced the GFP transgene-induced gene silencing accompanied by relocation of the DCL1 protein from nucleus to the cytoplasm and activation of siRNAs and miRNAs production. It was hypothesized that DCL1 relocated to the cytoplasm may use as substrates both miRNA precursor and viral RNA. The capacity for enhancing the RNA silencing is a novel function for the polyfunctional PME.

Keywords: Pectin methylesterase; Tobacco mosaic virus; RNA silencing

Aparajita Das-Chatterjee, Lily Goswami, Susmita Maitra, Krishnarup Ghosh Dastidar, Sudipta Ray, Arun Lahiri Majumder, Introgression of a novel salt-tolerant L-myo-inositol 1-phosphate synthase from *Porteresia coarctata* (Roxb.) Tateoka (PcINO1) confers salt tolerance to evolutionary diverse organisms, *FEBS Letters*, Volume 580, Issue 16, 10 July 2006, Pages 3980-3988, ISSN 0014-5793, DOI: 10.1016/j.febslet.2006.06.033.

(<http://www.sciencedirect.com/science/article/B6T36-4K7F88D-C/2/c1bfab73e70b21c27e54856a51479870>)

Abstract:

We have previously demonstrated that introgression of PcINO1 gene from *Porteresia coarctata* (Roxb.) Tateoka, coding for a novel salt-tolerant L-myo-inositol 1-phosphate synthase (MIPS) protein, confers salt tolerance to transgenic tobacco plants (Majee, M., Maitra, S., Dastidar, K.G., Pattnaik, S., Chatterjee, A., Hait, N.C., Das, K.P. and Majumder, A.L. (2004) A novel salt-tolerant L-myo-inositol-1-phosphate synthase from *Porteresia coarctata* (Roxb.) Tateoka, a halophytic wild rice: molecular cloning, bacterial overexpression, characterization, and functional introgression into tobacco-conferring salt-tolerance phenotype. *J. Biol. Chem.* 279, 28539-28552). In this communication we have shown that functional introgression of the PcINO1 gene confers salt-tolerance to evolutionary diverse organisms from prokaryotes to eukaryotes including crop plants albeit to a variable extent. A direct correlation between unabated increased synthesis of inositol under salinity stress by the PcINO1 gene product and salt tolerance has been demonstrated for all the systems pointing towards the universality of the application across evolutionary divergent taxa.

Keywords: Salinity stress; MIPS; Inositol; Transgenics; PcINO1 gene

Johannes A. van der Merwe, Ian A. Dubery, Benzothiadiazole inhibits mitochondrial NADH:ubiquinone oxidoreductase in tobacco, *Journal of Plant Physiology*, Volume 163, Issue 8, 3 July 2006, Pages 877-882, ISSN 0176-1617, DOI: 10.1016/j.jplph.2005.08.016.

(<http://www.sciencedirect.com/science/article/B7GJ7-4HHP36H-1/2/eaea87087482f4028a59820c03637d73>)

Abstract: Summary

An inducer of acquired disease resistance in plants, benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester, exhibited direct, concentration-dependent inhibition of the NADH:ubiquinone oxidoreductase activity of complex I of the mitochondrial electron transport chain of cultured tobacco cells. The complex I activity was less sensitive to inhibition by salicylic acid, an endogenous activator of acquired disease resistance. Using a dichlorodihydrofluorescein assay, it was found that benzothiadiazole, salicylic acid and the complex I inhibitor rotenone, increased reactive oxygen species production within cells in a concentration-dependent manner. The results indicate that both benzothiadiazole and salicylic acid affect the mitochondria of treated plant cells and result in increased production of reactive oxygen species. The biochemical basis of this response could be related to the inhibition of the NADH:ubiquinone oxidoreductase activity of complex I that results in channelling of electrons via complex II, with concomitant higher levels of superoxide production.

Keywords: Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester; Complex I; Mitochondria; NADH:Ubiquinone oxidoreductase; Reactive oxygen species; Salicylic acid; Systemic acquired resistance

Candida Vannini, Marcello Iriti, Marcella Bracale, Franca Locatelli, Franco Faoro, Paolo Croce, Raul Pirona, Antimo Di Maro, Immacolata Coraggio, Annamaria Genga, The ectopic expression of the rice *Osmyb4* gene in *Arabidopsis* increases tolerance to abiotic, environmental and biotic stresses, *Physiological and Molecular Plant Pathology*, Volume 69, Issues 1-3, July-September 2006, Pages 26-42, ISSN 0885-5765, DOI: 10.1016/j.pmpp.2006.12.005.

(<http://www.sciencedirect.com/science/article/B6WPC-4MVN107-1/2/2540165a112701b706920438ebd261e4>)

Abstract:

The *Osmyb4* rice gene encodes a Myb transcription factor involved in cold acclimation. Its constitutive expression in *Arabidopsis thaliana* results in improved cold and freezing tolerance. *Osmyb4* up-regulated 254 genes, 22% of which encode proteins involved in gene expression regulation and signal transduction, suggesting an upstream role of Myb4 in stress response. Most of the up-regulated genes are known to be involved in tolerance not only to cold, but also to other abiotic and environmental stresses (drought, salt, oxidative stresses). Moreover, a high proportion has known functions in resistance to pathogen attacks.

Therefore, we analyzed the biochemical and physiological differences between *Osmyb4*-expressing and wild-type plants and found increased levels of several amino acids that are involved in stress adaptation, acting as osmolytes, scavengers and/or metabolite precursors.

When exposed to different adverse conditions, namely drought, salt, u.v., ozone, viruses, bacteria and fungi, transgenic plants effectively demonstrated improved tolerance/resistance to all these stress conditions, suggesting that *Osmyb4* represents a crucial knot in the cross-talk of stress signalling cascades through the activation of multiple components.

Keywords: *Arabidopsis thaliana*; *Botrytis cinerea*; Microarray analysis; *Osmyb4*; *Pseudomonas syringae* pv. tomato; Stress tolerance; Tobacco necrosis virus (TNV); Transcription factor

F. Li, Z. Jin, W. Qu, D. Zhao, F. Ma, Cloning of a cDNA encoding the *Saussurea medusa* chalcone isomerase and its expression in transgenic tobacco, *Plant Physiology and Biochemistry*, Volume 44, Issues 7-9, July-September 2006, Pages 455-461, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2006.08.006.

(<http://www.sciencedirect.com/science/article/B6VRD-4KSSV3N-1/2/82c3c7f9ef12a49e1b8fca591ebb1054>)

Abstract:

Chalcone isomerase (CHI; EC 5.5.1.6) is a key enzyme in the flavonoid biosynthesis pathway. We isolated a CHI gene (*SmCHI*) from a cDNA library derived from *Saussurea medusa* (Asteraceae) cell cultures. The cDNA and genomic sequences of *SmCHI* are the same; in other words, this gene is intronless. The coding region of the gene is 699 bp long, and its deduced protein consists of 232 amino acids with a predicted molecular mass of 24 kDa and a pI of 4.7. The deduced amino acid sequence of *SmCHI* shares 79.3% identity with CHI from *Callistephus chinensis*, a familial relative to *S. medusa*; this homology is higher than those with CHI's from any other plant species. A functional bioassay for *SmCHI* was performed by transforming *Nicotiana tabacum* plants in the sense or antisense orientation under the regulation of the cauliflower mosaic virus (CaMV) 35S promoter. Transgenic tobacco plants overexpressing sense *SmCHI* produced up to fivefold total flavonoids over wild-type tobacco plants, mainly due to an enhanced accumulation of rutin. Transgenic tobacco plants with antisense *SmCHI* accumulated smaller amounts of flavonoids; this is apparently brought about by suppressed expression of the endogenous CHI gene. CHI activities also positively correlated with the amounts of total flavonoids accumulated in the transgenic plants. It is concluded that overexpression of *SmCHI* can be used as a useful approach to increase flavonoid production in transgenic plants.

Keywords: Anthocyanin; Chalcone isomerase; Flavonoids; Rutin; *Saussurea medusa*; Tobacco

Hsiang-En Huang, Mang-Jye Ger, Chao-Ying Chen, Mei-Kuen Yip, Mei-Chu Chung, Teng-Yung Feng, Plant ferredoxin-like protein (PFLP) exhibits an anti-microbial ability against soft-rot pathogen *Erwinia carotovora* subsp. *carotovora* in vitro and in vivo, *Plant Science*, Volume 171, Issue 1, July 2006, Pages 17-23, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.01.007.

(<http://www.sciencedirect.com/science/article/B6TBH-4J6NGJ1-1/2/a8857127fe0aef9fe4acf2c6e16fc815>)

Abstract:

The anti-microbial protein was frequently used to control the plant bacterial disease. In this study, it was investigated that the anti-microbial activity of recombinant plant ferredoxin-like protein (PFLP) both in vitro and in vivo. The in vitro assay demonstrated that PFLP produced by transformed *Escherichia coli* exhibited an anti-microbial activity against several bacteria strain including *E. coli*, *Erwinia carotovora* and *Pseudomonas syringae*. The effectiveness of this anti-microbial activity was depending on the FeSO₄ that was applied in the cultivated medium. PFLP lost its anti-microbial activity when it was mutated in the 86th cysteine residue that responding for iron binding. Besides, soft-rot symptom of tobacco plants infected by *E. carotovora* was reduced by application of recombinant PFLP. Transgenic tobacco ectopically over expressing PFLP in the cytoplasm protected plant from the infection of *E. carotovora* during the initial stage. These results indicate that PFLP is an anti-microbial protein that might be able to control the plant diseases via reducing the growth of bacterial pathogen.

Keywords: Plant ferredoxin-like protein (PFLP); Iron competition; *Erwinia carotovora* subsp. *carotovora*; Anti-microbial ability

Elena Marin, Fanchon Divol, Nicole Bechtold, Alain Vavasseur, Laurent Nussaume, Cyrille Forestier, Molecular characterization of three *Arabidopsis* soluble ABC proteins which expression is induced by sugars, *Plant Science*, Volume 171, Issue 1, July 2006, Pages 84-90, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.02.014.

(<http://www.sciencedirect.com/science/article/B6TBH-4JJ27XP-1/2/f35e291cd89e9f9a7850b0e8e58632b5>)

Abstract:

Among the complete set of *Arabidopsis* ABC proteins, 15 correspond to the soluble or NBD-type (nucleotide binding domains). We have cloned cDNAs coding for three closely homologous NBD-like proteins, AtNAP2, AtNAP4 and AtNAP9 that display a characteristic ABC signature motif. In transitory expression assays, the three AtNAP proteins fused to the green-fluorescent protein (GFP) were localized in the cytosol of tobacco and *Arabidopsis* cell cultures. The expression of these genes is enhanced by 1% sucrose in the culture medium but not by non-assimilable sugars. This result was confirmed with an AtNAP2::GUS translational fusion derived from a promoter trap line in which the T-DNA is inserted near the 3' end of the AtNAP2 coding sequence. According to the GUS staining, AtNAP2 is expressed in the elongation zone and meristem of roots of white light-grown seedlings and in the hypocotyl of dark-grown seedlings. We suggest that AtNAP2 might be involved in cell elongation processes.

Keywords: ABC protein; *Arabidopsis thaliana*; Cell elongation; Reporter gene; NBD domain

Emma Burbridge, Mark Diamond, Philip J. Dix, Paul F. McCabe, Use of cell morphology to evaluate the effect of a peroxidase gene on cell death induction thresholds in tobacco, *Plant Science*, Volume 171, Issue 1, July 2006, Pages 139-146, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.03.004.

(<http://www.sciencedirect.com/science/article/B6TBH-4JMKP4F-2/2/b20a9be9033a85185e033d1f90b640d1>)

Abstract:

Tobacco suspension cultures were subjected to a range of heat stresses and used to compare morphological aspects of programmed cell death (PCD) and necrosis. Cells undergoing PCD were

found to display characteristic death morphology, caused by cytoplasmic retraction of the protoplast, and to have cleaved DNA. We evaluated if the morphological characteristics of PCD could be used to monitor changes in cell death induction thresholds in transgenic cell cultures with high levels of peroxidase activity. Again, using a heat shock assay, we show that tobacco cell cultures with elevated levels of peroxidase have higher cell death induction threshold levels than wild type tobacco cell cultures. Thus, assessing PCD associated morphological changes can report on the effect of altering peroxidase genes on cell death activation in tobacco. This study demonstrates that PCD morphology could routinely be used to monitor the effects of introduced genes on programmed cell death induction thresholds in plants.

Keywords: Programmed cell death; Heat-shock; Peroxidase

Ryo Moriguchi, Koki Kanahama, Yoshinori Kanayama, Characterization and expression analysis of the tomato telomere-binding protein LeTBP1, *Plant Science*, Volume 171, Issue 1, July 2006, Pages 166-174, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.03.010.

(<http://www.sciencedirect.com/science/article/B6TBH-4JRV768-4/2/5070704407c1feb19a85dd5c03f366e6>)

Abstract:

We cloned LeTBP1 cDNA that encoded a putative tomato telomere-binding protein. Sequence analysis revealed that LeTBP1 contained an open reading frame of 2067 bp and encoded a protein consisting of 689 amino acids with a predicted molecular mass of 77.0 kDa. The deduced amino sequence of LeTBP1 indicated that a myb-like motif, a structure particular to double-stranded telomere-binding proteins found in various organisms, existed in the C-terminal region. Southern blot analysis revealed that LeTBP1 was a single-copy gene in the tomato genome. Northern blot analyses showed that LeTBP1 was highly expressed in tissues with high cell division capacities as well as in fully differentiated tissues. The high level of expression of LeTBP1 in inflorescences was independent of flower formation, as shown with mutant inflorescences lacking flowers. However, the level of LeTBP1 mRNA was greatly reduced in fruit. Gel shift assay revealed that LeTBP1509-689, which contained Gly509 to Ala689 of LeTBP1, including a myb-like motif, bound specifically to the double-stranded telomeric sequence, indicating that LeTBP1 is a double-stranded telomeric DNA-binding protein. We then transformed tobacco BY-2 cells with LeTBP1 to analyze the effect of LeTBP1 expression on telomere lengths in vivo. Telomere lengths decreased from 15-55 to 15-35 kbp with expression of LeTBP1. Therefore it is most likely that LeTBP1 negatively controls the extension of telomere length in tissues with high cell division capacities although its function is unknown in fully differentiated tissues.

Keywords: BY-2 cell; LeTBP1; Telomere; Telomere-binding protein; Tomato

M. Thiruvengadam, S. Varisai Mohamed, C.H. Yang, N. Jayabalan, Development of an embryogenic suspension culture of bitter melon (*Momordica charantia* L.), *Scientia Horticulturae*, Volume 109, Issue 2, 29 June 2006, Pages 123-129, ISSN 0304-4238, DOI: 10.1016/j.scienta.2006.03.012.

(<http://www.sciencedirect.com/science/article/B6TC3-4JVTC0H-1/2/bf9ae1f8f38e28297faf1b23127f1d3f>)

Abstract:

We have optimized a system for the somatic embryogenesis via embryogenic suspension cultures in bitter melon (*Momordica charantia* L.). Friable calli could be induced in 30-day-old leaves on semi-solid MS [Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15, 473-497.] medium supplemented with 1.0 mg/l 2,4-D. Large number of globular embryos (24.6%) were noticed when the calli was subcultured in liquid medium containing 1.5 mg/l 2,4-D. The complete removal of 2,4-D in the later stages of culture, stimulated their further development to heart and torpedo stages. Microscopic examination revealed the ontogeny of somatic cell development via the formation of cell clusters, which then

enlarged to pro-embryos, and gave rise to heart and torpedo stages within a period of 2 weeks. Somatic embryos successfully germinated on agarified MS medium with no additional growth regulators. An effect of media, other components and stimulating factors such as carbohydrates, amino acids has also been evaluated for somatic embryogenesis. The full strength MS medium containing 50 mg/l PVP and 40 mg/l glutamine was effective to achieve a high frequency of somatic embryo induction, maturation and further development. An average of 6.2% young plants was achieved from friable callus and was phenotypically normal. To our knowledge, there is no published report on somatic embryogenesis of bitter melon (*M. charantia* L.) via embryogenic callus or cell suspension cultures. These results are likely to facilitate genetic transformation of bitter melon.

Keywords: Embryogenic callus; Cell suspension culture; Somatic embryos; Growth regulators; *Momordica charantia* L

Xiao-Qiang Yu, Yukun Ma, Calcium is not required for Immulectin-2 binding, but protects the protein from proteinase digestion, *Insect Biochemistry and Molecular Biology*, Volume 36, Issue 6, June 2006, Pages 505-516, ISSN 0965-1748, DOI: 10.1016/j.ibmb.2006.03.010.

(<http://www.sciencedirect.com/science/article/B6T79-4JNF060-2/2/1b004b6982902130cd4808ff7626adc8>)

Abstract:

Mammalian C-type lectins are calcium-dependent carbohydrate-binding proteins. They serve as cell adhesion molecules in cell-cell interactions, or function as pattern-recognition receptors in innate immunity. Calcium is a direct ligand for carbohydrate binding in mammalian C-type lectins such as mannose-binding proteins and macrophage mannose receptor. In the tobacco hornworm *Manduca sexta*, a group of lectins named immulectins have been discovered. Each immulectin contains dual carbohydrate-recognition domains. Previously, we showed that immulectin-2 (IML-2) binds to a bacterial lipopolysaccharide, and agglutination of *Escherichia coli* cells by IML-2 is calcium dependent. In this study, we demonstrated that IML-2 bound to bacterial lipid A, smooth and rough mutants of lipopolysaccharide, lipoteichoic acid and peptidoglycan, as well as to fungal mannan and [β]-1, 3-glucan (laminarin and curdlan). Binding of IML-2 to microbial components was calcium independent, and was increased by addition of spermine, a polyamine. In addition, plasma IML-2 bound to mannan-agarose independent of calcium. But trypsin digestion of IML-2 was inhibited in the presence of calcium. Our results suggest that calcium is not required for IML-2 binding but protects IML-2 from trypsin digestion.

Keywords: C-type lectin; Carbohydrate-recognition domain; Immulectin-2; Lipopolysaccharide; Lipoteichoic acid; Calcium independent; *Manduca Sexta*

Coralie E. Halls, Sally W. Rogers, Mohammed Oufattole, Ole Ostergard, Birte Svensson, John C. Rogers, A Kunitz-type cysteine protease inhibitor from cauliflower and *Arabidopsis*, *Plant Science*, Volume 170, Issue 6, June 2006, Pages 1102-1110, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.01.018.

(<http://www.sciencedirect.com/science/article/B6TBH-4JCC4RJ-1/2/2a3fd66d4024a3f90cf1edba93e48949>)

Abstract:

A Kunitz-type protease inhibitor co-purified from cauliflower florets with a granulin domain cysteine protease that cleaved barley proaleurain to yield a molecular form the same size as that for mature aleurain. The purified cauliflower protease required treatment with SDS detergent to become active. This observation raised the question of whether the protease inhibitor might have the ability to interact with the granulin domain protease. Here we express an *Arabidopsis* homolog of the protease inhibitor as a recombinant protein and demonstrate that it is a potent inhibitor of the recombinant proaleurain maturation protease and of papain when assayed at pH 4.5 but not at pH 6.3. In a pull-down assay, the inhibitor bound tightly to papain, but only weakly to the aspartate

protease pepsin. When the cauliflower protease inhibitor was transiently expressed in tobacco suspension culture protoplasts, it colocalized with BP-80, a vacuolar sorting receptor that interacts with proaleurain and traffics to prevacuolar compartments for lytic vacuoles. Our results indicate that the cauliflower and Arabidopsis protease inhibitors would traffic through cellular compartments where proaleurain also traffics. Their ability to inhibit a cysteine protease implicated in maturation of proaleurain to active form at the acidic pH found in vacuoles raises the possibility that they could participate in regulating activation of aleurain.

Keywords: Granulin domain; Vacuole; Prevacuolar compartment; BP80; Proaleurain

Yuri L. Dorokhov, Eugene V. Skurat, Olga Yu. Frolova, Tatjana V. Gasanova, Peter A. Ivanov, Nikolay V. Ravin, Konstantin G. Skryabin, Kristiina M. Makinen, Viktor I. Klimyuk, Yuri Yu. Gleba, Joseph G. Atabekov, Role of the leader sequence in tobacco pectin methylesterase secretion, *FEBS Letters*, Volume 580, Issue 13, 29 May 2006, Pages 3329-3334, ISSN 0014-5793, DOI: 10.1016/j.febslet.2006.04.090.

(<http://www.sciencedirect.com/science/article/B6T36-4JX3B3G-7/2/1ccda1a6a4af3ce09ffcc5f315afbb8f>)

Abstract:

We report that unprocessed tobacco pectin methylesterase (PME) contains N-terminal pro-sequence including the transmembrane (TM) domain and spacer segment preceding the mature PME. The mature portion of PME was replaced by green fluorescent protein (GFP) gene and various deletion mutants of pro-sequence fused to GFP were cloned into binary vectors and agroinjected in *Nicotiana benthamiana* leaves. The PME pro-sequence delivered GFP to the cell wall (CW). We showed that a transient binding of PME TM domain to endoplasmic reticulum membranes occurs upon its transport to CW. The CW targeting was abolished by various deletions in the TM domain, i.e., anchor domain was essential for secretion of GFP to CW. By contrast, even entire deletion of the spacer segment had no influence on GFP targeting.

Keywords: Pectin methylesterase; Transmembrane domain; Cell wall

Hamid Abdollahi, Rosario Muleo, Eddo Rugini, Optimisation of regeneration and maintenance of morphogenic callus in pear (*Pyrus communis* L.) by simple and double regeneration techniques, *Scientia Horticulturae*, Volume 108, Issue 4, 25 May 2006, Pages 352-358, ISSN 0304-4238, DOI: 10.1016/j.scienta.2006.02.007.

(<http://www.sciencedirect.com/science/article/B6TC3-4JRKD59-1/2/e04ce7e4e3b61979268f4a01e8888992>)

Abstract:

The purpose of our work was to improve the regeneration capacity of leaf explants and the maintenance of shoot morphogenesis in callus of six pear cultivars: Abate Fetel, Conference, Dar Gazi, Harrow Sweet, Kaiser and Williams, by altering the composition of both regeneration and proliferation media of explant donor shoots, and choosing the right type of explant. Regeneration capacity of leaf explants collected from in vitro shoots has been improved in the majority of cultivars also due to shoot preconditioning. For the first time, long term morphogenic callus production and maintenance have been established in some cultivars by a 'double regeneration'. Using this technique, morphogenic callus of two cultivars, 'Dar Gazi' and 'Conference', was maintained for several subcultures but only when they were initiated from small leaflets - less than 2-3 mm long - which had been collected from the neofomed adventitious buds. MS medium [Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant.* 15, 473-497] proved to be an efficient regeneration medium by stimulating adventitious buds, while the explants of all cultivars, except for Kaiser, showed a high regeneration capacity when they were collected from shoots proliferated on modified QL medium [Quoirin, M, Lepoivre, P., Boxus, P., 1977. Un premier bilan de dix annees de recherche sur les cultures de meristemes et la multiplication in vitro de fruitiers ligneux. *Compte rendu des*

recherches, Station des Cultures Fruitières et Maraîchères de Gembloux (1976-1977), 93-117]. This medium conferred leaf expansion, overcoming 90% of regeneration in explants of cv Dar Gazi and Williams. Well expanded leaves were obtained and collected by rooting the shoots, while regeneration percentage was not improved and the number of adventitious shoots was increased in most cultivars, reaching up to 10 shoots per explant. When cefotaxime at 200 mg/l, which is normally effective in controlling *Agrobacterium*, was used for genetic transformation, regeneration percentage and number of shoots per explant (in leaf explants collected from rooted shoots) were increased and a uniform bud regeneration on all the leaf surface was promoted.

Keywords: Adventitious bud; Cefotaxime; Morphogenesis; $\text{NH}_4^+/\text{NO}_3^-$ ratio; Vancomycin; Double regeneration system

Soumendra K. Naik, Pradeep K. Chand, Nutrient-alginate encapsulation of in vitro nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange, *Scientia Horticulturae*, Volume 108, Issue 3, 8 May 2006, Pages 247-252, ISSN 0304-4238, DOI: 10.1016/j.scienta.2006.01.030.

(<http://www.sciencedirect.com/science/article/B6TC3-4JF97TK-2/2/6b4a0e9c8b97cdc7f14ee8c4d76f56a2>)

Abstract:

The present paper demonstrates the potential of nutrient-alginate encapsulation of axenic nodal segments of pomegranate for synthetic seed technology, which could be useful in germplasm distribution and exchange. Nodal segments from in vitro shoot cultures derived from mature nodal explants (source A) or axenic cotyledonary nodes (source B) were encapsulated in calcium alginate hydrogel containing Murashige and Skoog's [Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* 15, 473-497] medium (MS) supplemented with 4.44 μM benzyladenine (BA) and 0.54 μM naphthalene acetic acid (NAA). Of various concentrations of sodium alginate (1-6%) and the complexation solution of calcium chloride (50-125 mM), a combination of 3% sodium alginate and 100 mM calcium chloride was most suitable for formation of ideal synthetic seeds. Morphogenic response of encapsulated nodal segments to seven different planting media was evaluated. Encapsulated nodal segments of both the sources exhibited shoot development only in four selected media. Of the planting media evaluated, % sprouting (shoot development) was the highest in MS medium augmented with 4.44 μM BA and 0.54 μM NAA and lowest in (1/2) MSS medium. One step germination i.e. both shoot and root formation was possible only with encapsulated nodal segments of source B in MS, (1/2) MSS and natural soil + (1/2) MSS, with MS being most effective. Encapsulated nodal segments stored up to 30 days at 4 $^{\circ}\text{C}$ were capable of sprouting. Plants regenerated from the encapsulated nodal segments were hardened off and transferred to soil.

Keywords: Fruit tree; *Punica granatum* L.; Sodium alginate; Synthetic seed

M Angeles Sanchez-Zamora, Jose Cos-Terrer, Diego Frutos-Tomas, Roberto Garcia-Lopez, Embryo germination and proliferation in vitro of *Juglans regia* L., *Scientia Horticulturae*, Volume 108, Issue 3, 8 May 2006, Pages 317-321, ISSN 0304-4238, DOI: 10.1016/j.scienta.2006.01.041.

(<http://www.sciencedirect.com/science/article/B6TC3-4JHMY7S-1/2/10f190e54e53e36f5c5655b874efb2ef>)

Abstract:

This paper presents the optimal culture conditions for the in vitro embryo germination and proliferation of *Juglans regia* L. rootstock cv. Peralta, selected by the IMIDA fruticulture team. *J. regia* L. rootstock cv. Peralta is characterised by its resistance to salinity, lime and by its vigour. The first experiment determined the best culture medium for in vitro embryo germination. The Murashige and Skoog medium (MS; Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissues cultures. *Physiol. Plant.* 15, 473-497), the medium

developed by our team for walnut (NGE), the Lloyd and McCown medium (WPM; Lloyd, G., McCown, B., 1981. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by the use of shoot tip culture. *Proc. Plant Prop. Soc.* 30, 421-427) and that of Driver and Kuniyuki (DKW; Driver, J.A., Kuniyuki, A.M., 1984. In vitro propagation of Paradox walnut rootstock. *HortScience* 19 (4), 507-509), were compared, all without growth regulators. The best germination percentage was obtained in WPM (81%) with significant differences between the different media. In the second experiment, the optimal benzylaminopurine and indole butyric acid concentrations were determined for the proliferation stage of the explants obtained in the first experiment. The proliferation rates obtained varied from 0 in the medium without cytokinins to 6 in the medium with 0.5 mg l⁻¹ BAP. The cluster proliferation quality and other parameters studied indicate that the optimal treatment was 0.5 mg l⁻¹ BAP.

Keywords: Germination; Proliferation; Micropropagation; In vitro culture; Juglans; Walnut

Jiusheng Lin, Yuan Wang, Genxuan Wang, Salt stress-induced programmed cell death in tobacco protoplasts is mediated by reactive oxygen species and mitochondrial permeability transition pore status, *Journal of Plant Physiology*, Volume 163, Issue 7, 3 May 2006, Pages 731-739, ISSN 0176-1617, DOI: 10.1016/j.jplph.2005.06.016.

(<http://www.sciencedirect.com/science/article/B7GJ7-4H392SF-7/2/8026ffe808d641daac42dd1c0d895f14>)

Abstract: Summary

The status of mitochondrial permeability transition pore (PTP) and levels of reactive oxygen species (ROS) play key roles in regulating apoptosis in animal cells. To investigate if the PTP and cellular oxidation-reduction state are also involved in salt stress-induced programmed cell death (PCD) in tobacco (*Nicotiana tabacum*, cultivar BY-2) protoplasts, flow cytometry was used to simultaneously monitor ROS levels, PTP status and PCD. Increased ROS and decreased mitochondrial membrane potential ($[\Delta][\Psi]m$) were observed before the appearance of PCD. Pre-treatment with an inhibitor of the PTP opening, cyclosporin A (CsA), effectively retarded the onset of PCD, the $[\Delta][\Psi]m$ decrease and the ROS content increase. Addition of ascorbic acid (AsA) during the salt stress significantly decreased the percentage of protoplasts undergoing PCD and ROS levels but increased $[\Delta][\Psi]m$. Hydrogen peroxide effectively induced the appearance of PCD and caused an increase in ROS and a decrease in $[\Delta][\Psi]m$. Pre-treatment of protoplasts with CsA weakened the effects of H₂O₂. All these results suggest that the open state of PTP and ROS are necessary elements for salt stress-induced PCD in tobacco protoplasts. The open states of PTP and ROS could promote each other suggesting that ROS could lead to a self-amplifying process. This positive feedback loop may act as an all-or-nothing switch, which is in good accordance with the hypothesis that PTP is an important coordinator and executioner of PCD in both animals and plants.

Keywords: Permeability transition pore; Programmed cell death; Reactive oxygen species; Salt stress; Tobacco protoplasts

Lee Robertson, Jose A. Lopez-Perez, Antonio Bello, Miguel A. Diez-Rojo, Miguel Escuer, Ana Piedra-Buena, Caridad Ros, Casimiro Martinez, Characterization of *Meloidogyne incognita*, *M. arenaria* and *M. hapla* populations from Spain and Uruguay parasitizing pepper (*Capsicum annum* L.), *Crop Protection*, Volume 25, Issue 5, May 2006, Pages 440-445, ISSN 0261-2194, DOI: 10.1016/j.cropro.2005.07.008.

(<http://www.sciencedirect.com/science/article/B6T5T-4H3JJ3K-1/2/05c5ad75ac247fec43688e30ddad7e7d>)

Abstract:

A total of 136 populations of *Meloidogyne arenaria*, *M. hapla*, *M. incognita* and *M. javanica* were collected from infected soil from representative horticultural regions of Spain and Uruguay, and evaluated in a bioassay designed to characterize the virulence on cultivars of pepper, tomato,

cotton, tobacco and watermelon. None of the of *M. arenaria* race 2 or *M. javanica* populations parasitized any of the resistant pepper cultivars used, but all of the *M. hapla* populations reproduced on resistant peppers. Forty-three populations were found to parasitize both susceptible and resistant pepper cultivars, of those, 37 populations belonged to *M. incognita* (all races), one to *M. arenaria* (new race 3), and five to *M. hapla* races A and B. Seventeen of the *M. incognita* populations that were virulent on resistant pepper did not parasitize the resistant tomato cv. Nikita containing the Mi gene. The results obtained have important implications for the design of alternative nematode management strategies using resistant cultivars.

E.W. Willems, B. Rambali, W. Vleeming, A. Opperhuizen, J.G.C. van Amsterdam, Significance of ammonium compounds on nicotine exposure to cigarette smokers, *Food and Chemical Toxicology*, Volume 44, Issue 5, May 2006, Pages 678-688, ISSN 0278-6915, DOI: 10.1016/j.fct.2005.09.007.

(<http://www.sciencedirect.com/science/article/B6T6P-4HHP58W-1/2/1f510659136ba17bcdb3236fb29f89e5>)

Abstract:

The tobacco industry publicly contends that ammonia compounds are solely used as tobacco additive for purposes of tobacco flavoring, process conditioning and reduction of its subjective harshness and irritation. However, neither objective scientific reports, nor the contents of a large number of internal tobacco company documents support this contention.

The present review focuses on the hypothesis that addition of ammonium compounds to tobacco enhances global tobacco use due to smoke alkalization and enhanced free-nicotine nicotine exposure. Obviously, ammonia enhances the alkalinity of tobacco smoke. Consequently, the equilibrium shifts from non-volatile nicotine salts to the volatile free base that is more readily absorbed from the airways. The observed change in the kinetics of nicotine (i.e., shorter t_{1/2} and higher c_{max}) after ammoniation is, however, predominantly due to the higher concentration of nicotine in the smoke, rather than to an increase in the absorption rate of free-base nicotine in the respiratory tract.

Although several findings support the hypothesis, additional studies are required and suggested to provide a proper, objective and independent scientific judgment about the effect of tobacco ammoniation on nicotine bioavailability. Scientific and public awareness of the effects of tobacco-specific ammonia compounds may stimulate global control, legislation and restriction of their use in cigarette manufacture.

Keywords: Additives; Ammonia; Nicotine exposure and tobacco

R.S. Pappas, G.M. Polzin, L. Zhang, C.H. Watson, D.C. Paschal, D.L. Ashley, Cadmium, lead, and thallium in mainstream tobacco smoke particulate, *Food and Chemical Toxicology*, Volume 44, Issue 5, May 2006, Pages 714-723, ISSN 0278-6915, DOI: 10.1016/j.fct.2005.10.004.

(<http://www.sciencedirect.com/science/article/B6T6P-4HMNG92-1/2/4602f75fda680cffdb5059445f38fe78>)

Abstract:

The deliveries of cadmium, thallium, and lead in mainstream smoke particulate from cigarettes with different smoke delivery designs were determined by inductively coupled plasma-mass spectrometry in order to investigate their impact on the delivery of these known toxic compounds. Analyses showed that the levels of all three metals in smoke particulate were associated with their tar delivery category. After normalizing the metal concentrations to tar, there were no longer any statistically significant delivery differences between full-flavor, light or ultra-light cigarettes. When the concentrations were normalized to nicotine, the mean levels from the three delivery groups were much smaller than before normalization. But unlike the case using tar to normalize, in some of the cases, there were still some statistically significant differences in the nicotine-normalized results. These findings suggest that if smokers compensate for differences in nicotine intake, they

receive exposures to toxic heavy metals from ultra-light, light and full-flavor cigarettes that are more similar than results would suggest from using the Federal Trade Commission method alone.
Keywords: Tobacco; Cigarettes; Smoke; Lead; Cadmium; Thallium

David Prefontaine, Andre Morin, Catherine Jumarie, Andrew Porter, In vitro bioactivity of combustion products from 12 tobacco constituents, *Food and Chemical Toxicology*, Volume 44, Issue 5, May 2006, Pages 724-738, ISSN 0278-6915, DOI: 10.1016/j.fct.2005.10.005.
(<http://www.sciencedirect.com/science/article/B6T6P-4HP6GFG-1/2/9128a33540533f70121d0c01de87c7a9>)

Abstract:

Twelve chemical components of tobacco leaf, representing 50% of its dry weight, were individually combusted and the bioactivities of their combustion products i.e. total particulate matter (TPM) were assayed using three in vitro tests. These components included carbohydrates, amino acids, proteins, polyphenols and carboxylic acids. The mutagenic potencies were assessed with the *Salmonella* mutagenicity assay (*S. typhimurium* TA98 and TA100). The induction of chromosomal damage, determined with the micronucleus test (IVMNT), and the neutral red uptake cytotoxicity test (NRU), were conducted on V79 hamster lung fibroblast cells. The *Salmonella* mutagenicity test and IVMNT were conducted with and without rat liver microsomal S9 fraction. *Salmonella* mutagenicity data confirmed the mutagenicity of TPM samples obtained from nitrogenous compounds (amino acids and proteins). The IVMNT showed that precursors of phenols in smoke (i.e. polyphenols) exhibited significantly higher levels of toxicity compared to other tobacco components. While S9 activation amplified the *Salmonella* mutagenicity response to combustion products, it significantly inhibited the toxicity measured with the IVMNT. NRU data demonstrated the increasing cytotoxicity induced following longer exposure time to TPM samples from nitrogenous and phenolic components. This study is the first to characterize the toxicity of the combustion products of major tobacco constituents. Our data suggest different mechanisms of toxicity and underline the relevance of using various bioassays.

Keywords: Combustion; Tobacco constituents; In vitro toxicity; Micronucleus assay; *Salmonella* mutagenicity; Neutral red uptake

Flavio Antonio Blanco, Maria Eugenia Zanetti, Claudia Anahi Casalongue, Gustavo Raul Daleo, Molecular characterization of a potato MAP kinase transcriptionally regulated by multiple environmental stresses, *Plant Physiology and Biochemistry*, Volume 44, Issues 5-6, May-June 2006, Pages 315-322, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2006.05.005.
(<http://www.sciencedirect.com/science/article/B6VVRD-4K6CN7S-1/2/d909332b2eb5b484022be88fb4205c0c>)

Abstract:

The MAPK cascade is an evolutionary conserved signaling pathway that links external stimuli with cellular responses. Using polymerase chain reaction (PCR), a DNA fragment corresponding to a *Solanum tuberosum* MAPK, StMPK1, was isolated. StMPK1 amino acid sequence displayed over 90% identity with tomato MPK1 (LeMPK1) and tobacco SIPK. Southern blot analysis indicated that the gene encoding StMPK1 is present in a single copy in the potato genome. StMPK1 mRNA levels differentially accumulated in potato tuber in response to wounding and to wounding plus *Fusarium solani* f. sp. *eumartii*. Transcript accumulation after infection was transient and started earlier than what was observed in wounded tubers. StMPK1 mRNA levels also increased in potato tuber after 24 h of treatment with jasmonic acid (JA) and abscisic acid (ABA), but not in response to ethylene or salicylic acid. In addition, StMPK1 transcript levels increased after a heat-shock treatment at 42 [degree sign]C. The results suggest that StMPK1 may participate in the cellular responses against multiple environmental stimuli in potato tubers.

Keywords: Abscisic acid; Fungal infection; *Fusarium eumartii*; Heat shock; Jasmonic acid; MAP kinase, *Solanum tuberosum*; Wounding

Dali Liu, Xinxin Zhang, Yuxiang Cheng, Tetsuo Takano, Shenkui Liu, rHsp90 gene expression in response to several environmental stresses in rice (*Oryza sativa* L.), *Plant Physiology and Biochemistry*, Volume 44, Issues 5-6, May-June 2006, Pages 380-386, ISSN 0981-9428, DOI: 10.1016/j.plaphy.2006.06.011.

(<http://www.sciencedirect.com/science/article/B6VVRD-4K7NG5K-1/2/58ee2caae9e0eb88e9e561ebe0013f96>)

Abstract:

In this study, the gene for a rice (*Oryza sativa* L.) 90 kDa heat shock protein (rHsp90, GenBank accession no. AB037681) was identified by screening rice root cDNAs that were up-regulated under carbonate (NaHCO₃) stress using the method of differential display, and cloned. The open-reading-frame of rHsp90-cDNA was predicted to encode a protein containing 810 amino acids, which showed high similarity to proteins in *Hordeum vulgare* (accession no. X67960) and *Catharatus roseus* (accession no. L14594). Further studies showed that rHsp90 mRNA accumulated following exposure to several abiotic stresses, including salts (NaCl, NaHCO₃ and Na₂CO₃), desiccation (using polyethylene glycol), high pH (8.0 and 11.0) and high temperature (42 and 50 [degree sign]C). Yeast (*Saccharomyces cerevisiae*) over-expressing rHsp90 exhibited greater tolerance to NaCl, Na₂CO₃ and NaHCO₃ and tobacco seedlings over-expressing rHsp90 could tolerate salt concentrations as high as 200 mM NaCl, whereas untransformed control seedlings couldn't. These results suggest that rHsp90 plays an important role in multiple environmental stresses.

Keywords: rHsp90; Rice (*Oryza sativa* L.); Stress; Tolerance; Transgenic tobacco; Yeast

Hani Al-Ahmad, Shmuel Galili, Jonathan Gressel, Infertile interspecific hybrids between transgenically mitigated *Nicotiana tabacum* and *Nicotiana sylvestris* did not backcross to *N. sylvestris*, *Plant Science*, Volume 170, Issue 5, May 2006, Pages 953-961, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.01.005.

(<http://www.sciencedirect.com/science/article/B6TBH-4J5T4WF-1/2/ce7c389340d14de5aba169c570128a75>)

Abstract:

Genes may introgress from transgenic crops into sexually compatible wild relatives via pollen flow. This could potentially enhance the ecological expansion of the introgressed hybrids and their progeny at the cost of other plant species, or affect health of humans and animals, depending on the novel trait engineered into the crop. To prevent generating such competitive transgenic progeny, we previously used tobacco (*Nicotiana tabacum* L.) as a model for validating a transgenic mitigation (TM) mechanism using tandem constructs where a gene of choice is linked to mitigating genes that are positive or neutral to the crop, but deleterious to a recipient when in competition with the wild type. In the present study, attempts were made to achieve interspecific sexual hybridization between transgenic TM allotetraploid *N. tabacum* (pollen donor, representing a crop bearing novel traits) into one of its progenitors, diploid wild type *Nicotiana sylvestris* (representing a wild relative as well as a progenitor). *N. sylvestris* plants were manually pollinated by transgenic tobacco. The F1 interspecific sexual hybrids had >75% pollen sterility and produced no seeds. When the F1 was backcrossed as the pollen donor to *N. sylvestris*, the progeny produced almost no germinable seeds. With such low risk of gene flow, transgenic tobacco bearing novel traits could be cultivated with minimal concern where *N. sylvestris* is a native or ornamental species.

Keywords: Gene flow; Infertility; Interspecific sexual hybridization; *Nicotiana tabacum*; *Nicotiana sylvestris*; Transgenic mitigation

L. Hao, T. Hsiang, P.H. Goodwin, Role of two cysteine proteinases in the susceptible response of *Nicotiana benthamiana* to *Colletotrichum destructivum* and the hypersensitive response to

Pseudomonas syringae pv. *tomato*, Plant Science, Volume 170, Issue 5, May 2006, Pages 1001-1009, ISSN 0168-9452, DOI: 10.1016/j.plantsci.2006.01.011.

(<http://www.sciencedirect.com/science/article/B6TBH-4J91N42-1/2/b28b73d9f0ed1bdcc9de9fcbb685b256>)

Abstract:

Two cysteine proteinase genes of the papain family, NbCYP1 and NbCYP2, were amplified from cDNA of *Nicotiana benthamiana* leaves infected with the hemibiotrophic fungus *Colletotrichum destructivum*. Both genes showed peak expression corresponding with the switch from biotrophic to necrotrophic growth by *C. destructivum* at 72 h post-inoculation (HPI). For *N. benthamiana* inoculated with the incompatible bacterium, *Pseudomonas syringae* pv. *tomato*, expression of NbCYP1 significantly decreased at 12 HPI, whereas NbCYP2 expression increased at 3 HPI. Expression of both genes then returned to near pre-inoculation levels a