1

BULG. J. PLANT PHYSIOL., 2002, 28(1–2), 59–65

CHANGES IN LIPID COMPOSITION OF *Phaseolus vulgaris* LEAVES AFTER SIMULATING ACID RAIN TREATMENT

Violeta Velikova*, Albena Ivanova, Ivan Yordanov

Acad. M Popov Institute of Plant Physiology, Acad. G. Bonchev Str.,, Sofia 1113, Bulgaria

Received April 25, 2002

Summary. In the present study we have examined the influence of single spraying with simulated acid rain (pH 1.8) on the content of main lipid classes and their fatty acid composition in the thylakoid membranes of bean plants. Acid stress caused considerable changes in the investigated parameters. A decline in the content of linolenic acid, a highly unsatureted fatty acid which is an indicator of lipid peroxidation was observed. This decrease should result in decreased fluidity of the membrane lipid bilayer. The MGDG/DGDG ratio considerably increased at the 3rd hour after acid spraying, which is an indicator of membrane injury and of the lower packing properties.

PHOTOSYNTHETICA 43 (2): 313-316, 2005

2

BRIEF COMMUNICATION

Effect of reddening of cotton (*Gossypium hirsutum* L.) leaves on the ultrastructure of mesophyll cells

D. STOYANOVA-KOLEVA[*], A. EDREVA[**], V. VELIKOVA[***], and A. GÜREL[+]

Department of Botany, Faculty of Biology, Sofia University "St. Kl. Ohridski", Bd. D. Tzankov 8, 1164 Sofia, Bulgaria[*]
D. Kostoff Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria[**]
Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bl. 21, 1113 Sofia, Bulgaria[***]
Department of Bioengineering, Faculty of Engineering, Aegean University, 35100 Bornova-Izmir, Turkey[+]

Abstract

The ultrastructure of cotton leaves, exhibiting reddening as symptom of physiological disorder, was examined by means of transmission electron microscopy. Osmiophilisation of the membrane compartment was established. Massive agglomerations on the tonoplast in the vacuole of cells under the adaxial epidermis were observed, and were referred to as electron-dense osmiophilic substance, most probably of anthocyanin nature. In chloroplast stroma a zone of low electron density enclosing numerous osmiophilic aggregations of unclear chemical character was differentiated. Fragmentation and severe destruction of thylakoids in chloroplasts of reddening cotton leaves was not detected.

3

The Changing Chemical Climate of the Atmosphere

First Accent Symposium

Urbino,
September 12-16
2005

ISOPRENE IN A CHANGING ENVIRONMENT – EFFECT OF HIGH TEMPERATURE AND OZONE ON ISOPRENE EMISSION

Violeta Velikova (1), Csengele Barta (2), Isabel Nogues (2), Federico Brilli (2), Silvano Fares (2), Francesco Loreto (2)

(1) Institute of Plant Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria,
(2) Istituto di Biologia Agroambientale e Forestale, CNR, Monterotondo Scalo (Roma) Italy

Corresponding author: Violeta Velikova, violet@obzor.bio21.bas.bg

Summary

The effect of two environmental stresses (high temperature and ozone) on isoprene emission was studied. *Phragmites australis* – a strong isoprene emitter – was used as a model plant. Moderately high temperature treatment (38°C for 1.5h) increased isoprene emission approximately two-fold over the initial rate. Significant increase of isoprene emission was also observed after exposure to ozone (3h / 300 ppb). Leaves in which isoprene emission was previously inhibited developed stronger oxidative stress, producing more hydrogen peroxide (H_2O_2) and malonyldialdehyde (MDA) during and after high temperature episodes and after ozone treatments, with respect to isoprene-emitting leaves. Thermal stress was exacerbated by simultaneous exposure to high light intensity, but to a much lower extent in isoprene-emitting leaves. Our results confirm a general effect of volatile isoprene against abiotic stresses although the mechanism of action remains ambiguous. It is suggested that isoprene may contribute to stabilize membranes, to scavenge reactive oxygen species, or may indirectly contribute to protection by forming other isoprenoids (carotenoids) exerting antioxidant actions.

CSIRO PUBLISHING

www.publish.csiro.au/journals/fpb *Functional Plant Biology*, 2006, **33**, 931–940

4

Isoprene prevents the negative consequences of high temperature stress in *Platanus orientalis* leaves

Violeta Velikova[A,D], *Francesco Loreto*[B], *Tsonko Tsonev*[A], *Federico Brilli*[B] *and Aglika Edreva*[C]

[A]Institute of Plant Physiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria.
[B]CNR-Istituto di Biologia Agroambientale e Forestale, Monterotondo Scalo, Rome, Italy.
[C]Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria.
[D]Corresponding author. Email: violet@obzor.bio21.bas.bg

Abstract. The phenomenon of enhanced plant thermotolerance by isoprene was studied in leaves of the same age of 1- or 2-year-old *Platanus orientalis* plants. Our goals were to determine whether the isoprene emission depends on the age of the plant, and whether different emission rates can influence heat resistance in plants of different age. Two-year-old plants emit greater amounts of isoprene and possess better capacity to cope with heat stress than 1-year-old plants. After a high temperature treatment (38°C for 4 h), photosynthetic activity, hydrogen peroxide content, lipid peroxidation and antiradical activity were preserved in isoprene emitting leaves of 1- and 2-year-old plants. However, heat inhibited photosynthesis and PSII efficiency, caused accumulation of H_2O_2, and increased all indices of membrane damage and antioxidant capacity in leaves of plants of both ages in which isoprene was inhibited by fosmidomycin. In isoprene-inhibited leaves fumigated with exogenous isoprene during the heat treatment, the negative effects on photosynthetic capacity were reduced. These results further support the notion that isoprene plays an important role in protecting photosynthesis against damage at high temperature. It is suggested that isoprene is an important compound of the non-enzymatic defence of plants against thermal stress, possibly contributing to scavenging of reactive oxygen species (ROS) and membrane stabilising capacity, especially in developed plants.

5

Secondary metabolites: tools for stress protection in plants

Aglika M. Edreva[1], Violeta B. Velikova[2], Tsonko D. Tsonev[2], Emilia Z. Gesheva[1], Soleia Z. Dagnon[3], Francesco Loreto[4], Aynur Gürel[5], Hüseyin Hakerlerler[6], Bülent Yağmur[6] & Hüseyin Akdemir[7]

[1] Acad. D. Kostoff Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria, e-mail: edreva5@yahoo.com (corresponding author);

[2] Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev St., Bl. 21, 1113 Sofia, Bulgaria;

[3] Tobacco and Tobacco Products Institute, 4108 Plovdiv, Bulgaria;

[4] Istituto di Biologia Agroambientale e Forestale, CNR, Via Salaria Km 29,300 – 00016 Monterotondo Scalo (Roma), Italy;

[5] Department of Bioengineering, Faculty of Engineering, Aegean University, 35100 Bornova – Izmir, Turkey;

[6] Department of Soil Science, Faculty of Agriculture, Aegean University, 35100 Bornova – Izmir, Turkey;

[7] Department of Crop Science, Faculty of Agriculture, Aegean University, 35100 Bornova – Izmir, Turkey

Abstract. The protective role of secondary metabolites against environmental constraints is substantiated by studying various plant – abiotic stress stimuli systems. It was shown that phenylamides, conjugates of hydroxycinnamic acids and polyamines, are induced in tobacco and bean leaves, subjected to water excess and heat shock, respectively. ROS-scavenging ability of phenylamides was evidenced. Anthocyanins were found to dramatically accumulate in cotton leaves due to Na/K unbalance; shifts from mono- to orthodihydroxy substitution in the B-ring of anthocyanin aglycone was established, with this conferring a higher ROS-scavenging capacity of the molecule. The protective effect of a gaseous secondary metabolite, isoprene, against ozone fumigation and heat shock was shown. The ROS-scavenging ability of isoprene was demonstrated. Altogether, the data provide evidence that secondary metabolites can be involved in the non-enzymatic plant defence strategy by their antioxidant and antiradical properties.

ISSN 1021-4437, Russian Journal of Plant Physiology, 2007, Vol. 54, No. 3, pp. 287–301.
Published in Russian in Fiziologiya Rastenii, 2007, Vol. 54, No. 3, pp. 325–341.

REVIEWS

6

Phenylamides in Plants[1]

A. M. Edreva[a], V. B. Velikova[b], and T. D. Tsonev[b]

[a] *Acad. D. Kostoff Institute of Genetics, Bulgarian Academy of Sciences, Tsarigradsko sh. 13 km, Sofia, 1113 Bulgaria; e-mail: edreva5@yahoo.com*

[b] *Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Received July 20, 2006

Abstract—Phenylamides, secondary metabolites, conjugates of aliphatic polyamines or arylmonoamines and hydroxycinnamic acids, combining the properties of both parent compounds, are a subject of increasing interest, as follows from their ubiquitous distribution and multiplicity of functions. In this paper the occurrence, distribution, localization, metabolism, as well as chemical structure and properties of phenylamides underlying their physiological functions are briefly reviewed, with an emphasis on their involvement in the developmental and stress-defense phenomena of plants. Basic knowledge on phenylamides is supplemented with recent data pointing to the structural similarity of plant phenylamides with those found in insect toxins. This finding challenges the view of phenylamides as plant-specific molecules and suggests their role as a common defense tool in various organisms.

Photosynth Res (2007) 94:321–332
DOI 10.1007/s11120-007-9216-7

7

REGULAR PAPER

Trapping of the quenched conformation associated with non-photochemical quenching of chlorophyll fluorescence at low temperature

Petar H. Lambrev · Tsonko Tsonev · Violeta Velikova · Katya Georgieva · Maya D. Lambreva · Ivan Yordanov · László Kovács · Győző Garab

Abstract The kinetics of non-photochemical quenching (NPQ) of chlorophyll fluorescence was studied in pea leaves at different temperatures between 5 and 25°C and during rapid jumps of the leaf temperature. At 5°C, NPQ relaxed very slowly in the dark and was sustained for up to 30 min. This was independent of the temperature at which quenching was induced. Upon raising the temperature to 25°C, the quenched state relaxed within 1 min, characteristic for qE, the energy-dependent component of NPQ. Measurements of the membrane permeability (ΔA_{515}) in dark-adapted and preilluminated leaves and NPQ in the presence of dithiothreitol strongly suggest that the effect of low temperature on NPQ was not because of limitation by the lumenal pH or the de-epoxidation state of the xanthophylls. These data are consistent with the notion that the transition from the quenched to the unquenched state and vice versa involves a structural reorganization in the photosynthetic apparatus. An eight-state reaction scheme for NPQ is proposed, extending the model of Horton and co-workers (FEBS Lett 579:4201–4206, 2005), and a hypothesis is put forward concerning the nature of conformational changes associated with qE.

8

Singlet Oxygen Quenching by Phenylamides and their Parent Compounds

Violeta B. Velikova[a,*], Aglika M. Edreva[b], Tsonko D. Tsonev[a], and Hamlyn G. Jones[c]

[a] Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bl. 21, 1113 Sofia, Bulgaria. Fax: +359-2-8739952. E-mail: violet@bio21.bas.bg

[b] Acad. D. Kostoff Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

[c] Division of Environmental and Applied Biology, Biological Sciences Institute, University of Dundee, Dundee DD1 4HN, UK

* Author for correspondence and reprint requests

Z. Naturforsch. **62 c**, 833–838 (2007); received February 21/May 18, 2007

This paper demonstrates for the first time that plant metabolites of the phenylamide type, conjugates of putrescine with hydroxycinnamic acids (*p*-coumaric, caffeic and ferulic), possess $^1O_2$ quenching properties. Data were obtained confirming that their acidic parent compounds were also able to quench $^1O_2$, as did polyamines (putrescine, spermidine and spermine), and that this ability depends on the number of amino groups. Potentiation of the $^1O_2$ quenching ability of the conjugates relative to both parent components was established. The importance of polyamines and phenylamides in the plant non-enzymatic antioxidant defence at sites of intensive $^1O_2$ generation, such as the photosynthetic centers, was suggested.

Plant Biology ISSN 1435-8603

9

RESEARCH PAPER

Characterization of juvenile and adult leaves of *Eucalyptus globulus* showing distinct heteroblastic development: photosynthesis and volatile isoprenoids

V. Velikova[1], F. Loreto[2], F. Brilli[2], D. Stefanov[1] & I. Yordanov[1]

1 Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bl. 21, 1113 Sofia, Bulgaria

2 Consiglio Nazionale delle Ricerche (CNR) – Istituto di Biologia Agroambientale e Forestale (IBAF), Via Salaria Km. 29, 300-00016 Monterotondo Scalo (Roma), Italy

ABSTRACT

Heteroblastic *Eucalyptus* (*Eucalyptus globulus* L.) leaves were characterized for their functional diversity examining photosynthesis and photosynthesis limitations, transpiration, and the emission of isoprene and monoterpenes. *In vivo* and combined analyses of gas-exchange, chlorophyll fluorescence, and light absorbance at 830 nm were made on the adaxial and abaxial sides of juvenile and adult leaves. When adult leaves were reversed to illuminate the abaxial side, photosynthesis and isoprene emission were significantly lower than when the adaxial side was illuminated. Monoterpene emission, however, was independent on the side illuminated and similarly partitioned between the two leaf sides. The abaxial side of adult leaves showed less diffusive resistance to CO_2 acquisition by chloroplasts, but also lower ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity, than the adaxial leaf side. In juvenile leaves, photosynthesis, isoprene, and monoterpene emissions were similar when the adaxial or abaxial side was directly illuminated. In the abaxial side of juvenile leaves, photosynthesis did not match the rates attained by the other leaf types when exposed to elevated CO_2, which suggests the occurrence of a limitation of photosynthesis by ribulose bisphosphate (RuBP) regeneration. Accordingly, a reduced efficiency of both photosystems and a high non-radiative dissipation of energy was observed in the abaxial side of juvenile leaves. During light induction, the adaxial side of juvenile leaves also showed a reduced efficiency of photosystem II and a large non-radiative energy dissipation. Our report reveals distinct functional properties in *Eucalyptus* leaves. Juvenile leaves invest more carbon in isoprene, but not in monoterpenes, and have a lower water use efficiency than adult leaves. Under steady-state conditions, in adult leaves the isobilateral anatomy does not correspond to an equal functionality of the two sides, while in juvenile leaves the dorsiventral anatomy does not result in functional differences in primary or secondary metabolism in the two sides. However, photochemical limitations may reduce the efficiency of carbon fixation in the light, especially in the abaxial side of juvenile leaves.

10

Plant Biology ISSN 1435-8603

RESEARCH PAPER

Isoprene emission and primary metabolism in *Phragmites australis* grown under different phosphorus levels

S. Fares[1], F. Brilli[1], I. Noguès[1], V. Velikova[2], T. Tsonev[2], S. Dagli[3] & F. Loreto[1]

1 Istituto di Biologia Agroambientale e Forestale (IBAF), Via Salaria km. 29,300, 00016 Monterotondo Scalo (Roma), Italy
2 Institute of Plant Physiology ''M. Popov'', Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
3 Scientific and Technical Research Council of Turkey, Marmara Research Center Energy Systems and Environmental Research Institute, 41470 Gebze Kocaeli, Turkey

ABSTRACT

Aquatic plants are generally used for wastewater purification and phytoremediation, but some of them also emit large amounts of isoprene, the most abundant biogenic volatile organic compound. Since isoprenoid biosynthesis requires high amounts of phosphorylated intermediates, the emission may also be controlled by inorganic phosphorus concentration (Pi) in leaves. We carried out experiments to determine the emission of isoprene from *Phragmites australis* plants used in reconstructed wetlands to phytoremediate elevated levels of phosphorus contributed by urban wastes. Four groups of plants were grown hydroponically in water containing different levels of KH_2PO_4. High levels of phosphorus in the water resulted in high Pi in the leaves. High Pi stimulated photosynthesis at intercellular CO_2 concentrations lower and higher than ambient, implying higher ribulose 1,5-bisphosphate carboxylase (Rubisco) activity and higher ribulose 1,5-bisphosphate regeneration rates, respectively. However, isoprene emission was substantially lower at high Pi than at low Pi, and was not associated to photosynthesis rates at high Pi. This surprising result suggests that isoprene is limited by processes other than photosynthetic intermediate availability or by energetic (ATP) requirements under high Pi levels. Irrespective of the mechanism responsible for the observed reduction of isoprene emission, our results show that *Phragmites* plants may effectively remove phosphorus from water without concurrently increase isoprene emission, at least on a leaf area basis. Thus, *Phragmites* used in reconstructed wetlands for phytoremediation of urban wastes rich of phosphates will not contribute high loads of hydrocarbons which may influence air quality over urban and peri-urban areas.

Journal of Plant Interactions
Vol. 3, No. 1, March 2008, 1–15

Taylor & Francis
Taylor & Francis Group

11

REVIEW

Isoprene as a tool for plant protection against abiotic stresses

Violeta B. Velikova*

Institute of Plant Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

(Received 28 September 2007; final version received 11 December 2007)

Isoprene is emitted from numerous plant species of different genera. Due to its high volatility it is rapidly lost from plant tissue into the atmosphere where it undergoes chemical reactions. Since isoprene synthesis takes place in chloroplasts, it was suggested that a link may exist between isoprene production and environmental stresses affecting the photosynthetic apparatus. Isoprene synthesis is therefore of interest to the study of both atmospheric chemistry and plant biology. This review presents a brief overview of studies on the role of isoprene in atmospheric chemistry and the environmental control of isoprene emission. A special emphasis is placed on the physiological role of isoprene in plant defense against abiotic constrains. The physiological function of isoprene is still matter of debates. It has been hypothesized that this compound may stabilize thylakoid membranes and/or may exert antioxidant properties thus increasing plant tolerance to environmental stresses. The involvement of isoprene in non-enzymatic plant defense strategy is suggested.

Plant, Cell and Environment (2008) **31**, 1882–1894

12

Isoprene and nitric oxide reduce damages in leaves exposed to oxidative stress

VIOLETA VELIKOVA[1], SILVANO FARES[2] & FRANCESCO LORETO[2]

[1]*Bulgarian Academy of Sciences – Institute of Plant Physiology, Sofia, Bulgaria and* [2]*Consiglio Nazionale delle Ricerche (CNR) – Istituto di Biologia Agroambientale e Forestale, Monterotondo Scalo, Rome, Italy*

ABSTRACT

Isoprene and nitric oxide (NO) are two volatile molecules that are produced in leaves. Both compounds were suggested to have an important protective role against stresses. We tested, in two isoprene-emitting species, *Populus nigra* and *Phragmites australis*, whether: (1) NO emission outside leaves is measurable and is affected by oxidative stresses; and (2) isoprene and NO protect leaves against oxidative stresses, both singularly and in combination. The emission of NO was undetectable, and the compensation point was very low in control poplar leaves. Both emission and compensation point increased dramatically in stressed leaves. NO emission was inversely associated with stomatal conductance. More NO was emitted in leaves that were isoprene-inhibited, and more isoprene was emitted when NO was reduced by NO scavenger c-PTIO. Both isoprene and NO reduced oxidative damages. Isoprene-emitting leaves which were also fumigated with NO, or treated with NO donor, showed low damage to photosynthesis, a reduced accumulation of H_2O_2 and a reduced membrane denaturation. We conclude that measurable amounts of NO are only produced and emitted by stressed leaves, that both isoprene and NO are effective antioxidant molecules and that an additional protection is achieved when both molecules are released.

GEN. APPL. PLANT PHYSIOLOGY, 2008, SPECIAL ISSUE, 34 (1-2), 67-78

13

STRESS-PROTECTIVE ROLE OF SECONDARY METABOLITES: DIVERSITY OF FUNCTIONS AND MECHANISMS

A. Edreva[1*], V. Velikova[2], T. Tsonev[2], S. Dagnon[3], A. Gürel[4], L. Aktaş[4], E. Gesheva[1]

[1]Acad. D. Kostoff Institute of Genetics, BAS, 1113 Sofia, Bulgaria
[2]Acad. M. Popov Institute of Plant Physiology, BAS, 1113 Sofia, Bulgaria
[3]Tobacco and Tobacco Products Institute, 4108 Plovdiv, Bulgaria
[4]Ege University, 35100 Bornova – Izmir, Turkey

Summary. Secondary metabolites play a major role in the adaptation of plants to the changing environment and in overcoming stress constraints. This flows from the large complexity of chemical types and interactions underlying various functions: structure stabilizing, determined by polymerisation and condensation of phenols and quinones, or by electrostatic interactions of polyamines with negatively charged loci in cell components; photoprotective, related to absorbance of visible light and UV radiation due to the presence of conjugated double bonds; antioxidant and antiradical, governed by the availability of –OH, $-NH_2$, and –SH groupings, as well as aromatic nuclei and unsaturated aliphatic chains; signal transducing. In our research embracing several plant-abiotic stress stimuli systems we evidenced the multiplicity of biochemical mechanisms involved in the protective role of secondary metabolites: condensation of chlorogenoquinone with proteins yielding brown pigments limiting the spread of stress-induced tissue damage in tobacco; accumulation of polyamines and formation of phenylamides in tobacco and bean subjected to water stress and heat shock, respectively, with phenylamides performing ROS-scavenging ability; accumulation of anthocyanins in leaves of cotton suffering Na/K imbalance, and shift from mono- to orthodihydroxy substitution in the B-ring of anthocyanin aglycone, with this conferring a higher ROS-scavenging capacity; relation of drought tolerance in cotton to the level of ROS-scavenging polyphenol compounds. The protective effect of a gaseous secondary metabolite, isoprene, against ozone fumigation and heat shock was shown, and the ability of isoprene to scavenge singlet oxygen was demonstrated. Altogether, the data provided evidence that secondary metabolites through their diversity of functions can be involved in the non-enzymatic plant defense strategy.

Plant, Cell and Environment (2009) **32**, 520–531

Isoprene synthesis protects transgenic tobacco plants from oxidative stress

CLAUDIA E. VICKERS[1]*, MALCOLM POSSELL[2], CRISTIAN I. COJOCARIU[2], VIOLETA B. VELIKOVA[3], JULLADA LAOTHAWORNKITKUL[2], ANNETTE RYAN[2], PHILIP M. MULLINEAUX[1] & C. NICHOLAS HEWITT[2]

[1]*Department of Biological Sciences, Essex University, Wivenhoe Park, Colchester, England C04 3SQ, UK,* [2]*Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK and* [3]*Bulgarian Academy of Sciences, Sofia 1113, Bulgaria*

ABSTRACT

Isoprene emission represents a significant loss of carbon to those plant species that synthesize this highly volatile and reactive compound. As a tool for studying the role of isoprene in plant physiology and biochemistry, we developed transgenic tobacco plants capable of emitting isoprene in a similar manner to and at rates comparable to a naturally emitting species. Thermotolerance of photosynthesis against transient high-temperature episodes could only be observed in lines emitting high levels of isoprene; the effect was very mild and could only be identified over repetitive stress events. However, isoprene-emitting plants were highly resistant to ozone-induced oxidative damage compared with their non-emitting azygous controls. In ozone-treated plants, accumulation of toxic reactive oxygen species (ROS) was inhibited, and antioxidant levels were higher. Isoprene-emitting plants showed remarkably decreased foliar damage and higher rates of photosynthesis compared to non-emitting plants immediately following oxidative stress events. An inhibition of hydrogen peroxide accumulation in isoprene-emitting plants may stall the programmed cell death response which would otherwise lead to foliar necrosis. These results demonstrate that endogenously produced isoprene provides protection from oxidative damage.

15

Environmental Pollution 157 (2009) 2629–2637

BVOC emissions, photosynthetic characteristics and changes in chloroplast ultrastructure of *Platanus orientalis* L. exposed to elevated CO_2 and high temperature

Violeta Velikova [a,*], Tsonko Tsonev [a], Csengele Barta [b], Mauro Centritto [c], Dimitrina Koleva [d], Miroslava Stefanova [d], Mira Busheva [e], Francesco Loreto [c]

[a] *Bulgarian Academy of Sciences, Institute of Plant Physiology, Acad. G. Bonchev, Bl. 21, 1113 Sofia, Bulgaria*
[b] *US Department of Agriculture, Agricultural Research Center, Arid-Land Agricultural Research Center (USDA, ARS) Maricopa, AZ, USA*
[c] *Consiglio Nazionale delle Ricerche, Istituto di Biologia Agroambientale e Forestale, 00016 Monterotondo Scalo (RM), Italy*
[d] *Sofia University, Faculty of Biology, 1000 Sofia, Bulgaria*
[e] *Bulgarian Academy of Sciences, Institute of Biophysics, 1113 Sofia, Bulgaria*

A B S T R A C T

To investigate the interactive effects of increasing [CO_2] and heat wave occurrence on isoprene (IE) and methanol (ME) emissions, *Platanus orientalis* was grown for one month in ambient (380 μmol mol^{-1}) or elevated (800 μmol mol^{-1}) [CO_2] and exposed to high temperature (HT) (38 °C/4 h). In pre-existing leaves, IE emissions were always higher but ME emissions lower as compared to newly-emerged leaves. They were both stimulated by HT. Elevated [CO_2] significantly reduced IE in both leaf types, whereas it increased ME in newly-emerged leaves only. In newly-emerged leaves, elevated [CO_2] decreased photosynthesis and altered the chloroplast ultrastructure and membrane integrity. These harmful effects were amplified by HT. HT did not cause any unfavorable effects in pre-existing leaves, which were characterized by inherently higher IE rates. We conclude that: (1) these results further prove the isoprene's putative thermo-protective role of membranes; (2) HT may likely outweigh the inhibitory effects of elevated [CO_2] on IE in the future.

16

J Chem Ecol (2010) 36:629–641
DOI 10.1007/s10886-010-9801-7

Influence of Feeding and Oviposition by Phytophagous Pentatomids on Photosynthesis of Herbaceous Plants

Violeta Velikova · Gianandrea Salerno · Francesca Frati · Ezio Peri · Eric Conti · Stefano Colazza · Francesco Loreto

Abstract Feeding by herbivorous insects may change photosynthetic activity of host plants. We studied how feeding and oviposition by herbivorous stink bugs, *Murgantia histrionica* and *Nezara viridula* (Heteroptera: Pentatomidae), affect photosynthetic parameters of *Brassica oleracea* (savoy cabbage) and *Phaseolus vulgaris* (French bean). First, we measured photosynthetic gas exchange, chlorophyll fluorescence imaging, and emission of induced volatile organic compounds (VOC) immediately after feeding and during a post-feeding period. Photosynthesis decreased rapidly and substantially in *B. oleracea* and *P. vulgaris* infested by feeding bugs. Stomatal conductance did not decrease proportionally with photosynthesis; instead, inhibition of photosynthesis likely was due to a reduced diffusion of CO_2 in the mesophyll. We also measured the impact of oviposition *per se* and oviposition associated with feeding on photosynthetic parameters. A surprisingly large inhibition of photosynthesis was detected in cabbage leaves in response to oviposition by *M. histrionica*, even when oviposition was not associated with feeding activity. High resolution chlorophyll fluorescence imaging revealed that the damage to photochemistry caused by feeding and oviposition was restricted to the attacked areas. By contrast, the photochemical yield increased temporarily in the unaffected areas of the attacked leaves, indicating the onset of a compensatory response. Measurement of volatile organic compounds (VOC) revealed that feeding-damaged plants did not emit detectable amounts of VOC, indicating cellular damage (methanol and green leaf volatiles). However, feeding by *M. histrionica* induced emission of mono- and sesquiterpenes in savoy cabbage leaves. The different time-course of the induction of these two classes of terpenes may reflect the induction of two different biosynthetic pathways and indicate different roles of these terpenoids in tritrophic interactions.

17

Journal of Environmental Protection and Ecology 11, No 1, 122–129 (2010)

Ecology

STRUCTURAL RESPONSES OF *Platanus orientalis* L. LEAVES TO ELEVATED CO_2 CONCENTRATION AND HIGH TEMPERATURE

D. KOLEVA[a]*, M. STEFANOVA[a], TS. GANEVA[a], V. VELIKOVA[b], TS. TSONEV[b], FR. LORETO[c]

[a]*Faculty of Biology, Sofia University 'St. Kliment Ohridski', 1000 Sofia, Bulgaria*

E-mail: koleva@biofac.uni-sofia.bg

[b]*Institute of Plant Physiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

[c]*CNR – Istituto di Biologia Agroambientale e Forestale, 00 016 Monterotondo Scalo (RM), Italy*

Abstract. Anatomical structure of the leaves and the leaf surface of two-year-old *Platanus orientalis* L. plants after treatment with elevated CO_2 (800 ppm) and high temperature (38°C for 4 h) were studied. Light microscopy analysis (LM) revealed that the examined stress factors have the strongest effect on the photosynthesising parenchyma of the leaves. They cause changes of the shape and structure of the photosynthetic cells and increase the apoplast volume in the spongy tissue. The most prominent effect on the tissue structure of expanding leaves has the combined treatment with stress factors. Scanning electron microscopy (SEM) analysis showed that elevated CO_2 stimulates wax formation in expanding leaves as epidermal reaction in stress conditions.

18

Plant Biosystems, Vol. 145, No 4, December 2011, pp. 841-8447

Taylor & Francis
Taylor & Francis Group

Effect of water deficit and potassium fertilization on photosynthetic activity in cotton plants

T. TSONEV[1], V. VELIKOVA[1], L. YILDIZ-AKTAS[2], A. GÜREL[3], & A. EDREVA[4]

[1]*Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria,* [2]*Department of Biology, Faculty of Science, Ege University, 35100 Bornova-Izmir, Turkey,* [3]*Department of Bioengineering, Faculty of Engineering, Ege University, 35100 Bornova-Izmir, Turkey, and* [4]*Acad. D. Kostoff Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

Abstract

Physiological mechanisms that can contribute to drought tolerance and the role of potassium fertilization in cotton were studied by evaluation of parameters describing photosynthetic performance. Gas-exchange and chlorophyll fluorescence characteristics were measured on leaves of two cotton genotypes, one drought sensitive (Nazilli 84-S) and the other drought tolerant (Sahin 2000), grown in field conditions in the Aegean region of Turkey under different regimes of water and potassium supply. It was shown that under drought conditions without potassium fertilization Sahin 2000 had a higher photosynthetic rate and stomatal conductance than Nazilli 84-S. Potassium fertilization to a great extent compensated for the inhibitory effect of drought on photosynthesis. Application of the JIP test by using chlorophyll fluorescence data revealed that the drought sensitive Nazilli 84-S was more responsive to potassium fertilization than Sahin 2000, as judged by a number of parameters representing quantum efficiency of the processes and energy fluxes in photosystem (PS) II. The observed decrease in photosynthetic CO_2 assimilation in both cotton cultivars under drought conditions was not accompanied by any significant decrease in the electron transport flux in PSII and maximum quantum yield of primary photochemistry.

19

Environmental Pollution 159 (2011) 1058–1066

Changes in photosynthesis, mesophyll conductance to CO_2, and isoprenoid emissions in *Populus nigra* plants exposed to excess nickel

Violeta Velikova[a,*], Tsonko Tsonev[a], Francesco Loreto[b], Mauro Centritto[c]

[a] Bulgarian Academy of Sciences, Acad. M. Popov Institute of Plant Physiology, Acad. G. Bonchev, Bl. 21, 1113 Sofia, Bulgaria
[b] Consiglio Nazionale delle Ricerche, Istituto per la Protezione delle Piante, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Firenze, Italy
[c] Consiglio Nazionale delle Ricerche, Istituto di Biologia Agroambientale e Forestale, 00015 Monterotondo Scalo (RM), Italy

A B S T R A C T

Poplar (*Populus nigra*) plants were grown hydroponically with 30 and 200 μM Ni (Ni_{30} and Ni_{200}). Photosynthesis limitations and isoprenoid emissions were investigated in two leaf types (mature and developing). Ni stress significantly decreased photosynthesis, and this effect depended on the leaf Ni content, which was lower in mature than in developing leaves. The main limitations to photosynthesis were attributed to mesophyll conductance and metabolism impairment. In Ni-stressed developing leaves, isoprene emission was significantly stimulated. We attribute such stimulation to the lower chloroplastic [CO_2] than in control leaves. However chloroplastic [CO_2] did not control isoprene emission in mature leaves. Ni stress induced the emission of cis-β-ocimene in mature leaves, and of linalool in both leaf types. Induced biosynthesis and emission of isoprenoids reveal the onset of antioxidant processes that may also contribute to reduce Ni stress, especially in mature poplar leaves.

20

Increased Thermostability of Thylakoid Membranes in Isoprene-Emitting Leaves Probed with Three Biophysical Techniques[1[W][OA]]

Violeta Velikova, Zsuzsanna Várkonyi, Milán Szabó, Liliana Maslenkova, Isabel Nogues, László Kovács, Violeta Peeva, Mira Busheva, Győző Garab, Thomas D. Sharkey, and Francesco Loreto*

Institute of Plant Physiology and Genetics (V.V., L.M., V.P.) and Institute of Biophysics and Biomedical Engineering (M.B.), Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria; Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, 6726 Szeged, Hungary (Z.V., M.S., L.K., G.G.); Institute of Agroenvironmental and Forest Biology, National Research Council, 00015 Monterotondo, Rome, Italy (I.N.); Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824 (T.D.S.); and Institute for Plant Protection, National Research Council, 50019 Sesto Fiorentino, Florence, Italy (F.L.)

Three biophysical approaches were used to get insight into increased thermostability of thylakoid membranes in isoprene-emittingplants.Arabidopsis (*Arabidopsis thaliana*) plants genetically modified to make isoprene and *Platanus orientalis* leaves, in which isoprene emission was chemically inhibited, were used. First, in the circular dichroism spectrum the transition temperature of the main band at 694 nm was higher in the presence of isoprene, indicating that the heat stability of chiral macrodomains of chloroplast membranes, and specifically the stability of ordered arrays of light-harvesting complex II-photosystem II in the stacked region of the thylakoid grana, was improved in the presence of isoprene. Second, the decay of electrochromic absorbance changes resulting from the electric field component of the proton motive force (ΔA_{515}) was evaluated following single-turnover saturating flashes. The decay of ΔA_{515} was faster in the absence of isoprene when leaves of Arabidopsis and *Platanus* were exposed to high temperature, indicating that isoprene protects the thylakoid membranes against leakiness at elevated temperature. Finally, thermoluminescence measurements revealed that $S_2Q_B^-$ charge recombination was shifted to higher temperature in Arabidopsis and *Platanus* plants in the presence of isoprene, indicating higher activation energy for $S_2Q_B^-$ redox pair, which enables isoprene-emitting plants to perform efficient primary photochemistry of photosystem II even at higher temperatures. The data provide biophysical evidence that isoprene improves the integrity and functionality of the thylakoid membranes at high temperature. These results contribute to our understanding of isoprene mechanism of action in plant protection against environmental stresses.

Plant Physiology®, October 2011, Vol. 157, pp. 905–916, www.plantphysiol.org

21

Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 64, No 7, 2011

EFFECTS OF ENHANCED BRASSINOSTEROID PERCEPTION ON PHOTOSYNTHESIS IN *ARABIDOPSIS THALIANA* LINE *BRIOE*

Sashka Krumova, Miroslava Zhiponova*, Kolyo Dankov,
Georgi Rashkov, Tsonko Tsonev**, Eugenia Russinova*,
Violeta Velikova**, Mira Busheva

Abstract

The relation between brassinosteroid signalling and photosynthesis in *Arabidopsis thaliana* is studied by comparing the photosynthetic performance of wild-type plants and *BRIOE* line with increased level of the brassinosteroid receptor BRI1. The data reveal that enhanced brassinosteroid perception does not influence the net photosynthetic rate but leads to lower stomatal conductance and transpiration rate. Furthermore, the results presented demonstrate that *BRIOE* plants are characterized by lower oxygen evolution yield and alterations of the energy coupling of photosystem II core complex. While the photochemistry of photosystem II in *BRIOE* is not modified, the photochemical efficiency of photosystem I is reduced.

Plant Signaling & Behavior 7:1, 1–3; January 2012; © 2012 Landes Bioscience

Stabilization of thylakoid membranes in isoprene-emitting plants reduces formation of reactive oxygen species

Violeta Velikova,[1] Thomas D. Sharkey[2] and Francesco Loreto[3,*]

[1]Institute of Plant Physiology and Genetics; Bulgarian Academy of Sciences; Sofia, Bulgaria; [2]Department of Biochemistry and Molecular Biology; Michigan State University; East Lansing, Michigan USA; [3]Institute for Plant Protection; National Research Council; Florence, Italy

Isoprene is emitted by a significant fraction of the world's vegetation. Isoprene makes leaves more thermotolerant, yet we do not fully understand how. We have recently shown that isoprene stabilizes thylakoid membranes under heat stress. Here we show that heat-stressed, isoprene-emitting transgenic Arabidopsis plants also produce a lower pool of reactive oxygen and reactive nitrogen species, and that this was especially due to a lower accumulation of H_2O_2 in isoprene emitting plants. It remains difficult to disentangle whether in heat stressed plants isoprene also directly reacts with and quenches reactive oxygen species (ROS), or reduces ROS formation by stabilizing thylakoids. We present considerations that make the latter a more likely mechanism, under our experimental circumstances.

FUNCTIONAL PLANT BIOLOGY

CSIRO PUBLISHING

The impact of winter flooding with saline water on foliar carbon uptake and the volatile fraction of leaves and fruits of lemon (*Citrus x limon* L. (Burm. f.)) trees

Violeta Velikova[A,B], *Tommaso La Mantia*[C], *Marco Lauteri*[A], *Marco Michelozzi*[D], *Isabel Nogues*[A] *and Francesco Loreto*[E,F]

[A]CNR – Istituto di Biologia Agroambientale e Forestale, Porano, Terni, Italy

[B]BAS - Institute of Plant Physiology and Genetics, 1113 Sofia, Bulgaria

[C]Universita' di Palermo - Dipartimento di Colture Arboree, Facoltà di Agraria, Palermo, Italy

[D]CNR – Istituto di Genetica Vegetale, Sesto Fiorentino, Firenze, Italy

[E]CNR – Istituto per la Protezione delle Piante, Sesto Fiorentino, Firenze, Italy

[F]Corresponding author. Email: francesco.loreto@ipp.cnr.it

Abstract. We investigated the consequences of recurrent winter flooding with saline water on a lemon (*Citrus x lemon*) orchard, focusing on photosynthesis limitations and emission of secondary metabolites (isoprenoids) from leaves and fruits. Measurements were carried out immediately after flooding (December), at the end of winter (April) and after a dry summer in which plants were irrigated with optimal quality water (September). Photosynthesis was negatively affected by flooding. The effect was still visible at the end of winter, whereas photosynthetic rate was fully recovered after summer, indicating an unexpected resilience capacity of flooded plants. Photosynthesis inhibition by flooding was not due to diffusive limitations to CO_2 entry into the leaf, as indicated by measurements of stomatal conductance and intercellular CO_2 concentration. Biochemical and photochemical limitations seemed to play a more important role in limiting photosynthesis of flooded plants. In young leaves, characterized by high rates of mitochondrial respiration, respiratory rates were enhanced by flooding. Flooding transiently caused large and rapid emission of several volatile isoprenoids. Emission of limonene, the most abundant compound, was stimulated both in the leaves and in young and mature fruits. Flooding changed the blend of emitted isoprenoids, while only few changes were observed in the stored isoprenoids pool.