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BIOLOGIE
Physiologie des plantes

EFFECT OF HIGH TEMPERATURES ON THE GROWTH, FREE PROLINE CONTENT AND SOME ANTIOXIDANTS IN TOBACCO PLANTS[1]

S. Ivanov, T. Konstantinova*, D. Parvanova*, D. Todorova, D. Djilianov*, V. Alexieva

(Submitted by Corresponding Member E. Karanov on February 19, 2001)

Environmental stresses represent the most limiting factors for agricultural productivity worldwide. These stresses impact not only current crop species, they are also significant barriers to the introduction of crop plants into areas that are not currently used for agriculture. Stresses associated highly with temperature, salinity and drought, singly or in combination, are likely to enhance the severity of problems to which plants will be exposed in the coming decades. These growth conditions, along with the increasingly higher summer temperatures from year to year contribute to the drastic decrease in crop productivity and quality.

It is well known that most of the native and anthropogenic stresses induce similar physiological reactions in higher plants. These primary non-specific events include changes in oxidative enzyme activities, level of stress markers and free proline content [1, 4, 10–12].

The aim of this study is to evaluate the effect of high temperatures and the relatively reduced low air humidity (which mimics the conditions during the hot summer day) on one of the most important cash crop in Bulgaria – tobacco, which is grown in regions with low soil fertility, limited availability of water, and normally high temperatures. The levels of some enzymes (assumed to be a part of a system which renders protection against oxidative burst) and stress markers were determined.

Materials and methods. PLANT MATERIAL, GROWTH CONDITIONS AND TREATMENT. In the experiments tobacco (*Nicotiana tabacum* L.) cv. Nevrokop plants were used. The plants were grown as a soil culture in plastic pods ($d = 20$ cm, $h = 15$ cm, soil:perlite, 2:1, v/v; growth conditions – 16/8 h light/dark photoperiod, 25°/20° C day/night temperatures; air humidity about 80%). At 9–10 leaf stage the plants were subjected to high temperature stress (HTS) for 7 days. During each day the temperature was elevated from 30 to 45° C (from 9:00 a.m. to 12:00 a.m.), till 5:00 p.m. the temperature was kept and after that it dropped again till 30° C. Soil humidity was kept about 60%, and that of the air was — 55–65%. All measurements were made 24, 72 and 168 h after the beginning of HTS.

BIOMETRIC AND BIOCHEMICAL MEASUREMENTS. Fresh weight was estimated before the plants were dried at 105° C to constant weight. For the analyses the following methods were used: Relative leaf water content (RWC), FLETCHER et al. [1]; total phenol content, DUNNING et al. [2]; free proline content, BATES [3]; electrolyte leakage, DORFFLING [4]. Lipid peroxidation (as malondialdehyde, MDA equivalents) was estimated as the thiobarbituric acid reactive material using the molar extinction coefficient

[1]Part of this study was presented as a poster at the XII FESPP Congress, 21-25 July, 2000, Hungary.

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EFFECT OF LOW AND HIGH TEMPERATURE TREATMENT ON THE GLUTATHIONE LEVEL POOL AND ACTIVITY OF GLUTATHIONE-S-TRANSFERASE IN WILD AND ETHYLENE INSENSITIVE MUTANT *eti5* OF *ARABIDOPSIS THALIANA* (L.) HEYNH PLANTS

S. Ivanov, V. Alexieva, I. Sergiev, E. Karanov

(*Submitted on April 8, 2002*)

Abstract

Comparative studies on the effects of temperature treatments on the wild type and an ethylene insensitive mutant (*eti5*) of *Arabidopsis thaliana* (L.) Heynh were performed. Thirty-day-old plants grown on soil/perlite mixture were subjected to low (4 °C) or high (38 °C) temperature for 24 h in darkness. The measurements of glutathione and activity of glutathione-S-transferase were performed 0, 24, 28 and 120 h after the cessation of the stress programme. The temperature treatments provoked a rise both in total and in the oxidized glutathione content. There were no significant differences in these parameters in the mutant plants. Since the changes in the glutathione-S-transferase activity followed similar trends, it was supposed that the increment of the total amounts of glutathione in wild type plants was due mainly to the activation of glutathione/ascorbate cycle detoxifying the hydrogen peroxide excess resulted from temperature induced oxidative events. The data presented demonstrate the previously reported lower susceptibility of the *eti5* mutant than the wild type to extreme temperatures.

Key words: *Arabidopsis thaliana*, glutathione, glutathione-S-transferase, temperature stress, oxidative events, ethylene

Abbreviations: AOS – active oxygen species, GSH – reduced glutathione, GSSG – oxidized glutathione, CDNB – (1-chloro-2,4-dinitrobenzene), GST – glutathione-S-transferase, GR – glutathione reductase; NEM – N-ethylmaleimide

Introduction. Oxygen is essential for the aerobic life, yet reactive oxygen intermediates can be highly toxic to cells. Because of their reactive nature, activated oxygen species (AOS) can lead to DNA, protein, and membrane damage [1]. To protect themselves against AOS, plants have developed a variety of enzymatic and non-enzymatic antioxidant mechanisms. During oxidative stress the balance between the scavenging capacity of the antioxidant systems and production of AOS is disturbed [2]. The potential of AOS production is greatly enhanced by a wide variety of environmental stresses, and it is thought that the ensuing damage results from the accumulation of these AOS to levels exceeding the cell antioxidant capacity [3].

The tripeptide glutathione (GSH; γ-glutamylcysteinylglycine) is an important antioxidant which plays a crucial role in the defence against AOS [4]. GSH is the most abundant non-protein thiol in plant cells and is present at millimolar concentrations [5]. GSH functions both as a scavenger of free radicals, and as a component of the GSH/ascorbate cycle. It also plays a role as a cofactor in GST-mediated detoxification of electrophilic compounds. The ability of cells to withstand an oxidative stimulus depends at least in part on the capacity for de novo GSH synthesis [6], and the frequency

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BIOLOGIE
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EFFECT OF HERBICIDE GLYPHOSATE ON GLUTATHIONE LEVELS, GLUTATHIONE-S-TRANSFERASE AND GLUTATHIONE REDUCTASE ACTIVITIES IN TWO PLANT SPECIES

L. Miteva, S. Ivanov, V. Alexieva, E. Karanov

(*Submitted on October 30, 2002*)

Abstract

The effect of herbicide glyphosate [(N-phosphonomethyl)glycine] on the endogenous level of glutathione (total and oxidized), amount of free thiol groups, and activity of some related to its metabolism enzymes (glutathione reductase and glutathione-S transferase) was studied. As model systems two species of vascular plants, pea (*Pisum sativum* L.), and wheat (*Triticum aestivum* L.) were used. An enhancement of the level of total glutathione and free thiol groups accompanied by augmentation of the activity of glutathione-S-transferase was found. The glyphosate application provoked an increase of the GSSG/TG ratio.

Key words: herbicide stress, glyphosate, glutathione, glutathione-S-transferase, glutathione reductase

Abbreviations: TG – total glutathione; GSSG – oxidized glutathione; GST – glutathione-S transferase; GR – glutathione reductase; DTNB – 5,5′-dithio-bis(2-nitrobenzoic acid); CDNB – 1-chloro-2,4-dinitrobenzene

Introduction. Glutathione (γ-Glu-Cys-Gly) is one of the most spread forms of organic sulfur in plants. It has various functions both in plant and in animal organisms. First of all it is very important part of the protective and defence system. It acts as an antioxidant against active oxygen species. Glutathione takes part in glutathione-ascorbate shuttle (Halliwel-Asada cycle) where it provides electrons for reduction of ascorbate. Reduction of glutathione is completed by the enzyme glutathione reductase (GR, EC 1.6.4.2.). GR is an enzyme found in both photosynthetic and in non-photosynthetic tissues [1] with evidences for its presence in the chloroplasts [2]. With participation of the enzyme glutathione peroxidase (GPox, EC 1.11.1.9) glutathione has direct function as scavenger of hydrogen peroxide. A well-known function of glutathione is detoxifying of different xenobiotics as herbicides and heavy metals by conjugation. The process can be accomplished with or without the participation of the enzyme glutathione-S-transferase (GST, EC 2.5.1.18) [3-5]. This detoxifying process usually has two phases: conjugation and compartmentation of the conjugates, which

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BIOLOGIE
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EFFECTS OF PROLONGED ACTION OF SUB-HERBICIDE CONCENTRATIONS OF ATRAZINE ON THE PHOTOSYNTHETIC FUNCTION OF PEA PLANTS

P. Lambrev, S. Ivanov*, V. Goltsev**

(*Submitted by Corresponding Member E. Karanov on November 27, 2002*)

Abstract

The effect of low atrazine concentrations on young pea plants was monitored during their development by means of fluorescence induction analysis. It was shown that atrazine in concentrations compatible to those found in underground and surface water has a significant gradually increasing impact on the photosynthetic function.

Key words: herbicide residuals, atrazine, chlorophyll fluorescence

Introduction. Triazines are one of the economically most important and widely used groups of selective herbicides. The main representative of this group is atrazine (2-chloro-4-isopropyl-6-ethyl-*s*-triazine). It is well known that the extensive agricultural use of any herbicides has caused their wide accumulation in underground and surface water and soil, as well as their distribution by aerosols [1,2]. Unfortunately, limited information is available about the post-effects of atrazine and other *s*-triazines in relation to their main targets – the plant species susceptible to their action. The herbicide efficiency is most commonly judged by the I_{50} plant growth inhibition [3]. The effect of herbicide residuals on the productivity of crops grown on areas previously treated with atrazine was scarcely studied [4].

Photosynthesis is the primary target of the triazines. Atrazine blocks the photoinduced electron transport by specific binding to the Q_B-site of the D1 protein in the Photosystem 2 (PS 2) reaction centre [5,6]. A popular non-destructive probe of the functional state of the photosynthetic apparatus is the chlorophyll fluorescence [7,8]. Direct registration of fluorescence induction transients with high time resolution has been used to screen the fate of the excitation energy within the photosynthetic apparatus and the electron transport through PS 2 [9].

Materials and methods. As a model system pea (*Pisum sativum* L., cv. Manuela) plants were used. Seeds were soaked on tap water for 4–6 h and put on moisturised filter paper in Petri dishes for germination (25 °C, in the dark, 72 h). Seedlings were grown as a water culture (Hoagland-Arnon nutrient medium, changed every

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COMPARATIVE EFFECT OF 2,4-D ON THE GLUTATHIONE LEVELS, GLUTATHIONE-S-TRANSFERASE AND GLUTATHIONE REDUCTASE ACTIVITIES IN PEA (*PISUM SATIVUM* L.) AND WHEAT (*TRITICUM AESTIVUM* L.)

L. Miteva, S. Ivanov, V. Alexieva

(*Submitted by Corresponding Member E. Karanov on December 12, 2002*)

Abstract

The effect of herbicide 2,4-D [2,4-dichlorophenoxy acetic acid] on the endogenous level of glutathione (total and oxidized), amount of free thiol groups, and activity of some related to its metabolism enzymes (glutathione reductase and glutathione-S transferase) was studied. As model systems two species of vascular plants with different sensitivity to herbicide, pea (*Pisim sativum* L.), and wheat (*Triticum aestivum* L.) were used. An enhancement of the level of total glutathione and free thiol groups accompanied by an augmentation of the activity of glutathione-S-transferase in wheat plants was found. Opposite tendencies were observed in more sensitive pea plants.

Key words: herbicide stress, 2,4-D, glutathione, glutathione-S-transferase, glutathione reductase, thiol groups

Abbreviations: 2,4-D – 2,4-dichlorophenoxy acetic acid; TG – total glutathione; GSSG – oxidized glutathione; GST – glutathione-S-transferase; GR – glutathione reductase; DTNB – 5,5′-dithio-bis(2-nitrobenzoic acid); CDNB – 1-chloro-2,4-dinitrobenzene.

Introduction. Herbicide stress is a situation plants have to deal with frequently. 2,4-D (2,4-dichlorophenoxy acetic acid) is widely used auxin-type herbicide. It is selective herbicide, which is active against dicotyledonous weeds in cereal crops [1]. Pea and wheat are plants with different sensitivity to this xenobiotic so that these plant species were chosen for studying the physiological responses in relation to some aspects of glutathione metabolism.

It is supposed that growth inhibition caused by 2,4-D treatment is due to supra-optimal auxin-induced ethylene evolution. 2,4-D has a hormonal-like physiological activity, and, that is why it is easily taken in the plant. It may cause hormonal imbalance and uncontrolled growth, which might exhaust the plant and be another reason for the plant death [2].

Thiol groups in the cell play extraordinarily important roles in almost all aspects of cellular function [3]. Tripeptide glutathione (γ-L-Glu-L-Cys-Gly) is one of the widely

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BIOLOGIE
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Dedicated to Ivan Katerov

EFFECT OF TWO DAILY AND LOW-INTENSITY UV-B RADIATIONS ON GROWTH AND STRESS MARKERS IN YOUNG PEA (*PISUM SATIVUM* L.) PLANTS

Z. Katerova, V. Alexieva, S. Ivanov, S. Mapelli*, E. Karanov

(*Submitted on March 26, 2003*)

Abstract

The effect of two regimes of UV-B irradiation differing in their duration on the levels of growth and some stress markers in young pea plants was studied. The plants were irradiated daily during 3 weeks for 20 or 60 s. All measurements were made on the 7th, 14th, and 21st day, 20 h after the cessation of the stress programmes. For the experiments leaves from different nodes (2nd, 3rd, 4th and 5th) were separated. Growth in higher UV-B radiation resulted in significant reduction of stems' fresh weight. Both stress regimes dropped MDA content. An increase in amounts of free proline, hydrogen peroxide and electrolyte leakage was observed; the effect was more pronounced in the older leaves when more prolonged UV-B was applied.

Key words: UV-B radiation, stress markers, pea

Abbreviations: UV-B – ultraviolet-B radiation; MDA – malondialdehyde; H_2O_2 – hydrogen peroxide; LP – lipid peroxidation; SH – free thiol groups; TBA – thiobarbituric acid; ROS – reactive oxygen species; FW – fresh weight

Introduction. Stratospheric ozone depletion by anthropogenic halocarbons led to an increase in the flux of UV-B radiation reaching the earth's surface [1]. Although UV-B is only a minor component of solar radiation, due to its high energy, its potential for causing biological damage is exceptionally high and even small increases could lead

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BIOLOGIE

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ENDOGENOUS FREE AND BOUND POLYAMINE CONTENT IN TOBACCO PLANTS SUBJECTED TO HIGH TEMPERATURE STRESS

D. Todorova, D. Parvanova*, T. Konstantinova*, S. Ivanov, D. Djilianov*, V. Alexieva

(*Submitted by Corresponding Member E. Karanov on March 26, 2003*)

Abstract

The levels of free and bound putrescine, spermidine and spermine were measured in leaves of tobacco plants subjected to high temperature treatment for 7 days. Polyamine contents were determined at 24, 74 and 168 h after the beginning of the stress programme. It was established that moderate enduring high temperature stress provoked a rise of the free putrescine as a stress marker, as well as bound to macromolecules polyamines which play a protective role against the damaging oxygen species. However, continuous stress led to a decrease of polyamine levels and diminished possibility for plant survival.

Key words: high temperature stress, *Nicotiana tabacum* L., polyamine, putrescine, spermidine, spermine

Abbreviations: HTS – high temperature stress; PA – polyamines; Put – putrescine, Spd – spermidine, Spm – spermine

Introduction. Aliphatic polyamines are ubiquitous amines synthesized in both prokaryotic and eukaryotic organisms [1]. In plants, they exist in both free and bound forms. There are two classes of polyamines bound either to low-molecular-mass (TSA-soluble) or to high-molecular-mass (TCA-insoluble) compounds [2]. Accumulation of polyamines has been shown under a variety of stress factors, including high salinity,

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PLANT GROWTH REGULATING ACTIVITY OF SOME FLAVONOIDS

I. Sergiev, V. Alexieva, S. Ivanov, V. Bankova*, S. Mapelli**

(*Submitted by Academician E. Karanov on January 21, 2004*)

Abstract

Flavonoids represent a class of plant cell constituents of phenolic nature comprising a large number of compounds with diverse physiological functions. The growth regulating properties of series of flavonoids isolated from natural sources, as well as commercially obtained standards were studied by means of their effect on the enlargement of excised segments of wheat coleoptiles. The influence of the compounds on IAA-oxidase activity was also monitored in a wide concentration range. Correlation was established between the growth regulating effects of the compounds and their influence on IAA-oxidase activity: the compounds stimulating coleoptile elongation acted as inhibitors of IAA-oxidase and vice versa. Some aspects of structure–activity relationships were also outlined.

Key words: flavonoids, plant growth regulators, IAA-oxidase

Introduction. With more than 4500 different representatives known thus far, the flavonoids constitute an enormous class of natural phenolic products [1]. Flavonoids possess a wide range of physiological functions. Hence, many plant–animal interactions are influenced by flavonoids. The colour of flowers and fruits which often functions to attract pollinators and seed dispersers results primary from vacuolar anthocyanins. Related flavonoids, such as flavonols, flavones, chalcones and aurones, also contribute to colour definition [2]. Specific flavonoids can also function to protect plants against UV-B irradiation (kaempferol). Others can act as insect feeding attractants (isoquercetin). In contrast, condensed tannins such as proanthocyanidins add a distinct bitterness or astringency to the taste of plant tissues and function as antifeedants. The flavonoids apigenin and luteolin serve as signal molecules in legume-rhizobium bacteria interactions facilitating nitrogen fixation. In a related function, isoflavonoids are involved in inducible defence against fungal attack in alfalfa and other plant species. Moreover, various flavonoids also have been extensively studied in view of the perspective of health

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Original article

Transgenic tobacco plants accumulating osmolytes show reduced oxidative damage under freezing stress

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Abstract

We studied the reaction to the oxidative component of freezing in several tobacco lines, transformed with genes coding for enzymes involved in the synthesis of osmoprotectants (proline, fructan or glycine betaine) along with their wild type. The levels of some oxidative stress markers (leakage of electrolytes, hydrogen peroxide and malondialdehyde) as well as the activity of antioxidative enzymes catalase (EC 1.11.1.6.) and guaiacol peroxidase (EC 1.11.1.7.) have been followed at acclimation, 12 and 24 h freezing and at recovery. Freezing for 24 h resulted in severe damages for the wild type. A corresponding increase of electrolyte leakage, hydrogen peroxide and malondialdehyde contents, a rise of peroxidase activity and inhibition of catalase activity occurred in the non-transformants. Similar, but significantly lower trend of the same parameters has been found for the transgenic lines. Moreover, the oxidative markers returned to their normal levels when the transformants were able to recover from freezing. It could be speculated that transfer of genes, coding for accumulation of osmoprotectants, is related to reduced intensity of freezing-induced oxidative processes. Our lines and model system could serve as a good prerequisite for additional studies to gain further insights into the complex role of osmoprotectants in freezing tolerance.

Keywords: Freezing; Osmolyte; Oxidative stress; Transgenic tobacco

1. Introduction

Sub-zero temperatures are among the major factors limiting geographical distribution of cultivated plants and their productivity. Freezing acts directly, when ice masses mechanically tear the tissues and indirectly by dehydration resulting from growth of extracellular ice.

Plants have evolved various protective mechanisms that allow acclimation to freezing stress. One of them is the accumulation of low molecular weight metabolites, known as compatible solutes or osmolytes—amino acids (e.g. proline), sugars and sugar alcohols (sucrose, fructan, mannitol) and quaternary ammonium compounds (glycine betaine) [15,30]. It is generally accepted that these compounds serve as osmoprotectants, increasing the ability of cells to retain water not disturbing the normal cellular function [40]. The most obvious function of these compounds is osmotic adjustment. Apart from this role, it is suggested that they have other properties in plants suffering from oxidative stress, protecting cells against the production of hydroxyl radicals [4,34,35].

In common with other abiotic stresses, freezing causes increased production of activated oxygen species able to inactivate enzymes and damage cellular components [22,26]. Oxidative stress occurs when the defense capacity of plants is broken by the formation of free radicals. A relationship between freezing and oxidative stress was originally postu-

Abbreviations: AtPro26, transgenic plants carrying the *P5CS* gene from *Arabidopsis thaliana* for proline accumulation; DTT, dithiotreitol; Fru52, transgenic plants carrying the *SacB* gene for fructan accumulation; GB9, transgenic plants carrying the *codA* gene for glycine betaine accumulation; GDHP, guaiacol dehydrogenation product; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; ROS, reactive oxygen species; VacPro29, transgenic plants carrying the *P5CS* gene form *Vigna aconitifolia* for proline accumulation; WT, wild type plants.

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Alterations in some oxidative parameters in susceptible and resistant wheat plants infected with *Puccinia recondita* f.sp. *tritici*

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KEYWORDS
Antioxidant enzymes;
Hydrogen peroxide;
Hypersensitive response;
Pathogen;
Puccinia recondita f.sp. *tritici*;
Wheat

Summary

We studied the systemic effects after infection of susceptible and resistant (expressing HSR) wheat plants with leaf rust (*Puccinia recondita* f.sp. *tritici*) on the amount of hydrogen peroxide and activity of some ROS scavenging enzymes. Measurements were performed 7 and 21 days after inoculation. In susceptible cultivar (Sadovo 1), an inhibition of activity of catalase and GST was found. By contrast, in resistant cultivar (Kristal), the infection caused an activation of these enzymes. Moreover, it was established that cv. Kristal plants possess constitutive higher levels of hydrogen peroxide, as well as higher superoxide dismutase activity.

Introduction

Rust fungi are obligatory plant pathogens that obtain their nutrients from living host cells. Economically they are one of the most important biological agents that render damages on wheat plants. The most common response of resistant plants to cellular invasion by fungal pathogens is a rapid cell death, which is named hypersensitive response (HSR). The death of infected areas blocks the pathogen's development. HSR is not, however, an obligatory component of the plant's defense—there are also pathosystems in which the resistance is not related to the manifestation of HSR (Heath, 1997).

Abbreviations: GST, glutathione-S-transferase; HSR, hypersensitive response; ROS, reactive oxygen species; SOD, superoxide dismutase

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Cumulative Effect of Low and High Atrazine Concentrations on *Arabidopsis thaliana* Plants[1]

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Abstract—Atrazine belongs to the widely used herbicides blocking the electron transport chain in chloroplasts, thus resulting in the generation of active oxygen species. In the present work, we demonstrated that, at low concentrations mimicking residual amounts, atrazine enhanced the susceptibility of *Arabidopsis* plants to further treatments with the same herbicide applied at the recommended field rate. *Arabidopsis thaliana* plants were treated three times (at five-day intervals) with 1 μM atrazine. Five days after the last treatment, the plants were sprayed with 5 mM atrazine. Atrazine increased the levels of lipid peroxidation products, hydrogen peroxide, and ion leakage, and caused changes in the activities of antioxidant enzymes, such as superoxide dismutase, guaiacol peroxidase, and catalase.

Key words: Arabidopsis thaliana - antioxidant enzymes - residual amounts of atrazine - oxidative stress

INTRODUCTION

Under normal environmental conditions, the plants are exposed to a complex of stress factors which could be formally divided into two major groups, i.e., natural (for example, extreme temperatures, water shortage, or logging) and anthropogenic (pesticides, heavy metals, air pollutants and an increased UV-radiation) [1]. Nowadays, the combination of natural and anthropogenic stressors is a worldwide phenomenon.

Theoretically, two main effects of unfavorable conditions are possible, namely plant death or adaptation to the new environmental situation. Some cases are known when a weak stress factor enhances the plant adapting capacity to a subsequent stress. A typical example is a higher frost resistance after a short exposure to low positive temperatures or water shortage [2]. On the other hand, the combination of acid rain with high temperatures appears to be highly detrimental and provoked the death of entire forests [1].

The presence of high levels of both water and soil pollutants is now a typical situation in most regions. The persistence of most herbicides in the soil varies from several weeks to months (and even years). Although each herbicide is applied for a particular crop–weed situation at limited rate, area, and time period, during the following months some residual amounts of the herbicide are spread usually by rains and underground water over larger areas and contact with other crop plants and ecosystems. Such herbicide spreading cannot be easily followed, and at low concentrations these compounds undoubtedly reach those plant and animal organisms, at which they were not aimed initially. In these cases, they combine their influence with the effects exerted by unfavorable environmental conditions, both natural and anthropogenic. Moreover, in crop fields some additive effects take place due to the subsequent application of herbicides at normal rates.

Atrazine frequently contaminates soil, groundwater, rivers, and ponds [3]. The highest detected residual amounts reach 1mg/kg soil [4]. Usually, in the agricultural areas this herbicide can be detected in 1–100 μg per kg soil or liter water [3, 5–7]. On the other hand, it was reported that in some plants the same atrazine concentrations exhibit cytokinin-like properties [8].

The aim of our experiments was to find how some trace amounts of atrazine could affect the adaptive capacity of the *Arabidopsis thaliana* plants to subsequent acute stress factors. The severe stress was induced by the same herbicide but applied at the lethal dose normally recommended for agricultural practice. Since the primary phytotoxicity of atrazine is due mainly to the overgeneration of active oxygen species (AOS), we considered the amounts of produced hydrogen peroxide and the level of lipid peroxidation measured in malondialdehyde (MDA) equivalents as criteria for the development of free-radical chain reactions causing an oxidative damage to plant tissues.

[1] This article was submitted by the authors in English.

Abbreviations: AOS—active oxygen species; MDA—malondialdehyde; NBT—nitro blue tetrazolium; PSII—photosystem II; SOD—superoxide dismutase.

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EFFECTS OF LONG-TERM TREATMENT WITH LOW CONCENTRATIONS OF HERBICIDES ATRAZINE, GLYPHOSATE AND 2,4D ON IAA OXIDASE ACTIVITY IN YOUNG PEA PLANTS

S. Ivanov, Z. Katerova, E. Ivanova, V. Alexieva

(Submitted by Academician E. Karanov on November 24, 2004)

Abstract

Endogenous IAA content in plant tissues can be modulated via oxidative decarboxylation on the side chain of IAA by IAA oxidase. In plants IAA oxidase activity displays some isoforms of unspecific peroxidases. Usually, stimulation in IAA oxidase activity after treatment with various stress agents corresponded with reduction of endogenous IAA content and growth inhibition. Atrazine, 2,4D and glyphosate have been widely used as herbicides in crop production. The aim of this study was to evaluate the effects of long-term treatment with low concentrations of these herbicides on IAA oxidase activity in young pea plants. Plants were grown hydroponically. Atrazine and 2,4D were added to the nutrition medium in concentration 0.1 μM and 1 μM, and glyphosate in 1 μM and 10 μM, respectively. Leaf material was collected 7 and 14 days after the beginning of the experiment. In general, long-term influence with low concentrations of 2,4D and glyphosate enhanced, and atrazine did not provoke significant changes in IAA oxidase activity. On the basis of our results and previous research in this area we concluded that the changes in IAA oxidase activity strongly correlate with total peroxidase activity in plant cell. Additionally, we speculated that the reduction of IAA content by IAA oxidase activity could be a secondary effect from the activation of some unspecific peroxidases.

Key words: auxin, IAA oxidase, peroxidase, atrazine, 2,4D, glyphosate, pea

Abbreviations: AOS – reactive oxygen species; IAA – indole-3-acetic acid; 2,4D – 2,4-dichlorophenoxyacetic acid

Introduction. Auxins affect numerous processes during plant growth and development including cell division and elongation, differentiation, apical dominance, tropisms, senescence, and flowering. Endogenous IAA content in plant tissues can be modulated via several pathways including synthesis, decomposition and transport of free or conjugated IAA. One well knows that IAA metabolic pathway consists of aerobic oxidative decarboxylation on the side chain of IAA by IAA oxidase leading to formation of either indole-3-methanol or 3-methylene oxindole [1]. It is known since 1955 [2] that IAA oxidation is catalyzed by horseradish peroxidase. In tobacco cell lines BY-2 IAA oxidase activity exhibits only P-type basic guaiacol peroxidase [3]. Subcellular localization of IAA oxidase in etiolated pea epicotyls was studied by Waldhumi and Davies [4]. Their results demonstrated that the enzyme activity is associated most closely with Golgi apparatus and to a lesser degree with lysosomes and endoplasmatic reticulum. In most of the research works in this area the rise of IAA oxidase activity

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BIOLOGIE
Physiologie des plantes

UV-PROTECTING PROPERTIES OF EXOGENOUSLY APPLIED FLAVONOIDS ON EXCISED CUCUMBER COTYLEDONS

I. Sergiev, V. Alexieva, S. Ivanov, V. Bankova*, S. Mapelli, E. Karanov**

(*Submitted on December 22, 2004*)

Abstract

The effects of exogenously applied pectolinarin and its aglycone acetylpectolinarin, and chalcone were studied in relation to some stress markers, endogenous content of total flavonoids and anthocyanins, and the activity of glutathione-S-transferase in UV-irradiated isolated cucumber (*Cucumis sativus* L., cv. Levina) cotyledons. We established that in this model system the flavonoids pectolinarin and acetylpectolinarin rendered protective action against UV stress. The reasons of the observed increase in the glutathione-S-transferase activity after application of these compounds are also discussed.

Key words: UV-irradiation, flavonoids, stress

Abbreviations: GST – glutathione-S-transferase, MDA – malondialdehyde

Introduction. Relatively small part of the total solar irradiation reaches the Earth surface. Passing through the atmosphere the total flux is significantly reduced, and the composition of UV radiation is modified. Shortwave UV-C is completely absorbed by the atmospheric gases, UV-B is additionally absorbed by the stratospheric ozone, while UV-A is hardly absorbed by the ozone. Since in the recent decades the ozone layer has decreased mainly due to the human activity, the impact of UV-B irradiation is becoming of high importance for all living organisms [1]. Elevated UV irradiation induces multiple effects on plant organisms such as DNA damage, photosynthesis reduction, and biomembrane peroxidation, alterations in the secondary metabolism, as well as a number of stress and photomorphogenesis responses [2]. The continuously changing environmental conditions force the plant organisms to elaborate adaptive mechanisms to survive. In general, plants respond in a different manner to UV irradiation either by activating repair mechanisms or by stimulating protection mechanisms to overcome the stress factor. The most spread protective mechanism against damaging irradiation is the biosynthesis of UV-absorbing compounds [3]. In plants, such secondary metabolites, mainly phenolic compounds, flavonoids, and hydroxycinnamate esters accumulate in the vacuoles of epidermal cells where they soothe UV component of sunlight with minimal effect on photosynthetic active radiation and thus decrease the infiltration of UV into the deeper cell layers [2,4,5]. Flavonoids, including anthocyanins, play diverse roles in flowering plants – during reproduction, anthocyanin pigments serve as pollinator attractants [6], flavonoids are found to be induced during various environmental

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BIOLOGIE
Physiologie des plantes

ALTERATIONS OF THE CONTENT OF HYDROGEN PEROXIDE AND MALONDIALDEHYDE AND THE ACTIVITY OF SOME ANTIOXIDANT ENZYMES IN THE ROOTS AND LEAVES OF PEA AND WHEAT PLANTS EXPOSED TO GLYPHOSATE

L. Miteva, J. Tsoneva, S. Ivanov, V. Alexieva

(*Submitted by Academician E. Karanov on February 23, 2005*)

Abstract

The effect of the herbicide glyphosate [(N-phosphonomethyl)glycine] on the endogenous level of hydrogen peroxide and malondialdehyde and on the activity of some antioxidant enzymes (superoxide dismutase, catalase and guaiacol peroxidase) was studied. The herbicide was applied as a nutrient solution as well as by leaf spraying. The leaves and roots of two species of vascular plants – pea (*Pisim sativum* L.), and wheat (*Triticum aestivum* L.) were used as model systems. It was found that glyphosate causes accumulation of hydrogen peroxide and malondialdehyde in both species. In addition, glyphosate application provoked an augmentation of the activity of investigated antioxidant enzymes. The effect of glyphosate was better expresed in wheat plants. The effect of herbicide was less pronounced in the roots of both plants than in their leaves. As a whole, our experiments for the first time unambiguously show that glyphosate causes oxidative events in two different plant species.

Key words: herbicide stress, glyphosate, hydrogen peroxide, antioxidant enzymes

Abbreviations: EPSPS – 5-enolpyruvylshikimic acid-3-phosphate synthase, AOS – active oxygen species, SOD – superoxide dismutase, CAT – catalase, POX – guaiacol peroxidase, MDA – malondialdehyde, DTT – dithiothreitol, PVP – polyvinylpyrrolidone, GST – glutathione-S transferase; GR – glutathione reductase

Introduction. Glyphosate [(N-phosphonomethyl)glycine] is one of the most widely distributed herbicides in the world [1]. It is a very effective herbicide used in both agricultural and nonagricultural lands. It is toxic to practically all plant species, but monocotyledonous are more sensitive to its action [2]. In 1980, STEINRÜCKEN and AMHREIN [3] revealed that the key enzyme inhibited by glyphosate is 5-enolpyruvylshikimic acid-3-phosphate synthase (EC.2.5.1.19). Additional evidences that EPSPS is the target site of its action were provided by STALKER et al. [4]. These authors isolated a mutant form of the enzyme from *Salmonella typhimurium*, which is a unicellular resistant to the action of glyphosate. EPSPS is involved in shikimic acid biosynthetic pathway. Shikimate pathway is an essential part of the plants' biosynthetic cycle and

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EXPLORATIONS COSMIQUES

METHOD FOR DETECTING STRESS INDUCED CHANGES IN LEAF SPECTRAL REFLECTANCE

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(Submitted by Academician S. Panchev on February 15, 2005)

Abstract

A method combining statistical approaches, cluster analysis and discriminant analysis was developed to reveal and assess arising changes in the leaf spectral reflectance characteristics of plants treated with different stressors. Treated and control plant leaves' spectral data in the visible and near infrared ranges and their colour coordinates transformed with affine and perspective transformations were processed. The efficiency of the method was verified on sets of multichannel spectral reflectance data of leaves of tomatoes plants infected with tomato mosaic tobamovirus.

Key words: leaf reflectance, spectral reflectance characteristics, multichannel spectrometer, stress, tomato mosaic tobamovirus

Introduction. Remote sensing techniques are very useful tools in assessing expected changes in the structure and functioning of ecosystems. On ground remote sensing allows for a rapid evaluation of vegetation properties in a nonintrusive way [1,2]. The remote sensing of the optical properties of plants subjected to stress provides the prospect to early detect symptoms below the subjective detection level and timely to mitigate the risk of their action [3–5]. The extent to which the different stress factors can cause correspondingly different changes in the spectral signatures for a given vegetation species is not well established yet. The degree to which the spectral response to a particular stressor may vary among species remains questionable as well [6]. There is increasing number of studies pointing out that leaf reflectance changes due to stress are more consistently detected in the visible range ($400 \div 720$ nm). In the other range of reflected solar spectrum ($730 \div 2500$ nm) the induced changes were found to be rather similar for many common stressors and vascular plant species [7].

A large number of spectral vegetation indices were suggested for on ground and space remote sensing studies aimed at the monitoring of the biomass, phenology and physiological conditions of plants [8–10]. The reflectance characteristics can also provide a rapid and easy alternative means for assessment of the pigment concentrations

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BIOLOGIE
Physiologie des plantes

SALT-INDUCED ALTERATION IN THE LEVELS OF SOME OXIDATIVE PARAMETERS AND UNSPECIFIC DEFENCE COMPOUNDS IN LEAVES OF TWO PLANT SPECIES (COTTON AND BEAN) WITH DIFFERENT SENSITIVITY TO SALINITY

L. Brankova, S. Ivanov, V. Alexieva, E. Karanov

(*Submitted on July 20, 2005*)

This paper is dedicated to Mrs. K. Brankova and Mr. V. Brankov

Abstract

The effect of different NaCl concentration on some oxidative parameters and unspecific defence compounds in two plant species differing in their sensitivity to salt, cotton (*Gossypium hirsutum* L. cv Ogosta) and common bean (*Phaseolus vulgaris* L. cv Dobrujanski 7) was studied. The endogenous content of H_2O_2, proline, phenols as well as the level of lipid peroxidation were measured on the 11th, 17th and 24th day after salt treatment. Regarding the level of lipid peroxidation in salt-stressed cotton and bean plants an enhancement of MDA content was observed which did not differ significantly between the two species. Hydrogen peroxide content increased markedly in salt-treated bean plants, peaking on the 24th day. By contrast, during the whole experiment reduced levels of H_2O_2 in treated cotton plants were measured. Generally, our results showed that more salt-tolerant cotton plants possess constitutive and salt-inducible (only for H_2O_2) lower levels of hydrogen peroxide and MDA. Moreover, by contrast with bean, cotton plants rapidly accumulated proline and phenol compounds in response to salinity stress.

Key words: salinity, cotton, bean, lipid peroxidation, hydrogen peroxide, phenols, proline

Abbreviations: MDA – malondialdehyde, ROS – reactive oxygen species, TCA – trichloracetic acid, FW – fresh weight

Introduction. Excessive soil salinity is among the main abiotic stresses limiting the distribution of plants in natural habitats and is of great significance for agriculture. High levels of salinity caused by elevated salt concentrations in soil solution strongly reduce plant growth and development and result in significant yield losses of a wide variety of crops all over the world. Currently, almost 7% of all land area (1000 million ha) and about 5% of cultivated land (77 million ha) are affected by salinity [1,2]. The high soil salinity is generally caused by NaCl. The detrimental effect of this salt on

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The phenylurea cytokinin 4PU-30 protects maize plants against glyphosate action

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Abstract

The effects of the phenylurea cytokinin 4PU-30 and the herbicide glyphosate, applied alone and in combination on young maize plants were investigated. The influence of the compounds on the changes of growth, chlorophyll content, levels of hydrogen peroxide, and some stress markers, the activities of peroxidase, catalase, and glutathione-*S*-transferase, as well as glutathione amount were measured 3, 6, and 10 days after the treatment. The application of glyphosate increased the levels of lipid peroxidation, glutathione, and free proline content, ion fluxes, and the activity of catalase, guaiacol peroxidase, and glutathione-*S*-transferase, i.e., along with the inhibition of its target enzyme the herbicide induced also an oxidative stress. We found that the phenylurea cytokinin 4PU-30 alleviated in some extent the detrimental effects due to the glyphosate action. Moreover, we speculated that the cytokinin renders its protective action by induction of "hardiness" in the antioxidant defense systems in maize plants similarly to the effects observed after the application of some herbicide safeners.

Keywords: Phenylurea cytokinins; 4PU-30; Herbicide safeners; Glyphosate; Maize; Oxidative stress; Glutathione; Hydrogen peroxide

1. Introduction

Glyphosate [(*N*-phosphonomethyl)glycine] is a highly effective broad-spectrum, non-selective, post-emergence herbicide which is used extensively worldwide. The target site of glyphosate action is the enzyme 5-enoylpyruvylshikimate-3-phosphate synthase [1,2]. The inhibition of this enzyme causes an accumulation of shikimic acid and a consecutive diminish in the biosynthesis of aromatic amino acids, auxins, vitamins, as well as a number of key metabolites produced via the shikimate pathway, all this leads to a suspended plant growth, and in turn to plant death [3]. The glyphosate degradation appears to be very slow or is not taking place in higher plants [4]. Several classes of pesticides (including sim.-triazine and dichloroacetamide herbicides) are metabolized in plants into glutathione conjugates by the action of the enzyme glutathione-*S*-transferase (GST) [5]. Presently, the role of glutathione in glyphosate detoxification (conjugation) has not been exactly elucidated. However, increased levels of non-protein thiols, especially glutathione, as well as glutathione-*S*-transferase activity due to glyphosate action in several plant species were recently reported [6–8]. These observations give a ground for a search of factors which could enhance the plant tolerance to the unfavorable consequences due to the glyphosate action and thus to alleviate the herbicide toxicity.

Cytokinins are the class of plant hormones which were first identified as factors promoting cell division and have been implicated in many aspects of plant growth and development. Proper application of hormone-containing products can not only enhance the plant growth but also can improve the stress tolerance [9]. For example, the treatment with kinetin protects creeping bentgrass subjected to drought [10], and use of zeatin riboside alleviates heat stress injury [11]. Phenylurea cytokinins are compounds evoking growth response comparable to or higher than the adenine derivatives [12,13]. It was found that the application of

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Phytochemical Analysis

Separation and Quantification of the Cellular Thiol Pool of Pea Plants Treated with Heat, Salt and Atrazine

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Abstract: A novel procedure for the separation of the cellular thiol pool according to the molecular weight and localization of compounds with sulphydryl groups is presented. This simple and rapid method allows the differentiation of thiols into three major fractions—low molecular weight (LMT, primarily glutathione and free cysteine), protein-bound (TPT) and pellet-bound (PBT, associated with cell walls and broken organelles). Moreover, determination of the ratio between surface (readily reactive) thiols (ATG) and those that are more or less buried in the protein structure (BTG) can be achieved. In intact pea leaves, the amounts of the total thiols (LMT + PBT + TPT) varies from 2.5 to 4.8 μmol/g of fresh material. The data for LMT, PBT and TPT were related to each other in the approximate ratio 1:2:7. Treatments of pea plants with high temperature, salinity and low amounts of atrazine affect these sulphydryl types differently. For a greater understanding of the applicability of this method to physiological research, the main mechanisms leading to alterations in the cellular thiol pool are discussed. Furthermore, it is suggested that the proportion of available to buried thiols (ATG/BTG) in proteins could be used as a convenient marker for stress impacts. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: atrazine; glutathione; protein-bound; salt/high temperature treatments; thiol groups; sulphydryl types.

INTRODUCTION

Thiol groups play a primary role in the formation of disulphide bonds required for tertiary and quaternary structure of proteins, redox control of enzyme activity and detoxification of reactive oxygen species and xenobiotics (Haugaard, 2000). Generally, according to the molecular weight of the compounds in which thiols are found, they may be divided into two groups—low-molecular-weight (LMT) and high-molecular-weight thiols.

The tripeptide glutathione (γ-glu-cys-gly, or homoglutathione in leguminous species) is the main non-protein, acid-soluble LMT in most organisms (Alsher, 1989). In plants, glutathione (GSH) is thought to be between 3 and 10 mM, and is present in the major cellular compartments (Leustek and Saito, 1999). GSH is involved in the redox regulation of the cell cycle and in the protection of thiol groups in proteins. Furthermore, this tripeptide is a substrate for the glutathione-S-transferases that catalyse the conjugation of GSH with herbicides, xenobiotics and organic hydroperoxides and, most importantly, in the context of oxidative stress resistance, GSH is a donor of reducing equivalents for the scavenging of reactive oxygen species (ROS) (Kranner and Grill, 1996; Foyer *et al.*, 1997).

High-molecular-weight thiols refer to sulphydryl groups located in protein molecules. In proteins different types of cysteine residues are found. Some of them are reactive or available (ATG), and others are more or less buried (BTG) in the protein structure (Haugaard, 2000). A number of biochemical reaction sequences appear to depend on the presence of free -SH groups in enzymes for full activity. In chloroplasts, the major target enzymes with dithiol–disulphide redox regulation are glucose-6-phosphate dehydrogenase, NADP-malate dehydrogenase and fructose-1,6-bisphosphatase (Jacquot *et al.*, 2002). Generally, the dithiol–disulphide reactions, being rapid and readily reversible, are ideally suited to control protein function. This mechanism of redox control is emerging as a major regulatory mechanism in signal transduction.

Several methods are used for the quantitative determination of thiol groups in biological samples—titrimetric, amperometric, spectrophotometric and enzymatic. Most widespread for its simplicity and rapidity is the spectrophotometric method described by Ellman (1959). A number of authors have proposed procedures for the differentiation of thiols according to the molecular weight of the compounds in which they are detected. As early as 1963, Sedlak (1963) investigated changes in protein-bound thiol contents from the liver of rats fed on cabbage. Later Sedlak and Lindsay (1968) developed a procedure for the quantification of -SH groups, dividing them into total, protein-bound and non-protein sulphydryl groups. For the estimation of total and protein-bound thiols the

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Research article

The induction of microsomal NADPH:cytochrome P450 and NADH:cytochrome b_5 reductases by long-term salt treatment of cotton (*Gossypium hirsutum* L.) and bean (*Phaseolus vulgaris* L.) plants

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Abstract

We studied the effect of salinity on the activity of microsomal NADPH:cytochrome P450 reductase (CPR, EC 1.6.2.4) and NADH:ferricytochrome b_5 oxidoreductase (B5R, EC 1.6.2.2) in two dicotyledonous plant species differing in their sensitivity to salt, cotton (*Gossypium hirsutum* L. cv Ogosta) and common bean (*Phaseolus vulgaris* L. cv Dobrujanski 7). A significant inhibition of fresh weight of salt-treated bean plants was observed, while cotton was affected to a much lesser degree. NaCl application resulted in a significant increase in the activity of both reductases, but was more pronounced in salt-tolerant cotton. We suppose that alterations in B5R and CPR activities may be targeted to the maintenance of membrane lipids. Most probably, plants use both enzymes (B5R and CPR) and their respective electron donors (NADH and NADPH) to reduce cytochrome b_5, which can donate reducing equivalents to a series of lipid-modification reactions such as desaturation and hydroxylation.

Keywords: Common bean; Cotton; NADH:ferricytochrome b_5 reductase; NADPH:cytochrome P450 reductase; Salt stress

1. Introduction

Two main electron-transfer systems have been identified in the endoplasmic reticulum (ER) membrane of plant cells. One includes NADH:cytochrome b_5 oxidoreductase and cytochrome b_5 and the other contains the NADPH-dependent NADPH:cytochrome P450 oxidoreductase.

Microsomal NADH:cytochrome b_5 reductase B5R (NADH:ferricytochrome b_5 oxidoreductase, EC 1.6.2.2) is a membrane-bound, FAD-containing flavoprotein. The enzyme is part of an ER associated redox chain that transfers electrons from NADH to cytochrome b_5 which is an intermediate electron donor in various lipid-modification reactions including NADH-dependent fatty acid desaturation, desaturation of sterol precursors and fatty acid hydroxylation [19,21]. NADPH:cytochrome P450 reductase CPR (EC 1.6.2.4) is a membrane-bound flavoprotein. The primary subcellular location of CPR is the ER membrane, but it can also be found in the chloroplast outer membrane, because some typical P450s enzymes are localized in chloroplasts [30]. CPR contains FAD and FMN as prosthetic groups and transfers reducing equivalents from NADPH to diverse P450 monooxygenases that participate in a broad range of reactions in plants, including biosynthesis of secondary metabolites such as phenylpropanoids, terpenoids, alkaloids, lignins and compounds such as signalling molecules, fatty acids, lipids, defense related chemicals and plant hormones [5,12,20]. Plant P450s are also involved in the detoxification of different xenobiotics, including herbicides [2].

Abbreviations: B5R, NADH:ferricytochrome b_5 reductase; CPR, NADPH:cytochrome P450 reductase; ER, endoplasmic reticulum.

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Repetition of Hydrogen Peroxide Treatment Induces a Chilling Tolerance Comparable to Cold Acclimation in Mung Bean

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Additional index words. H_2O_2, calcium, EGTA, electrolyte leakage, glutathione, ruthenium red

Abstract. **Mung bean seedlings (*Vigna radiata* L.) of the cultivar Tainan No. 5 (a chilling-sensitive cultivar) pretreated with multiple sprays of 200 mM H_2O_2 showed a tolerance to chilling at 4 °C for 36 h, measured by electrolyte leakage, that was greater than that induced by a single treatment and similar to that induced by cold-acclimation at 10 °C for 48 h. Two H_2O_2 treatments at an interval of 3 h gave the optimum chilling tolerance. Tolerance induced by H_2O_2 could be distinguished from that induced by acclimation at 10 °C according to length at 4 °C and corresponding electrolyte leakage. Chilling tolerance induced by H_2O_2 depended on accumulation of glutathione (GSH), which could be significantly reversed by pretreatment with buthionine sulfoximine (BSO). In contrast, tolerance induced by incubation at 10 °C for 48 h in light was neither accompanied by accumulation of GSH nor reversed by BSO, suggesting that there are at least two independent mechanisms of developing chilling tolerance. Chilling tolerance of both cold-acclimated and H_2O_2-treated seedlings was decreased by ethyleneglycol-bis(aminoethylether)-*N*,*N*′-tetra-acetic acid (EGTA) but not by ruthenium red, indicating that the influx of Ca^{2+} from extracellular, but not intracellular, pools is an important signal in the induction of tolerance. In confirmation, sprays of Ca^{2+} could be substituted for H_2O_2.**

Environmental stress causes considerable losses in productivity of many crops. Among various stresses, low temperature is one of the most crucial signals affecting plant growth and even leading to death (Sung et al., 2003; Veal et al., 2007). Extensive study on oxidative stress has demonstrated that exposure of plants to low temperature always induces the overproduction of reactive oxygen species (ROS), such as superoxide radical ($O_2^{\cdot-}$), H_2O_2, and hydroxyl radical ($HO^{\cdot}$) in plant cells (Hung et al., 2005). ROS are highly reactive to membrane lipids, protein, and DNA; they are believed to be one of the major contributing factors to chilling injuries (CIs) and to cause rapid cellular damage (Hariyadi and Parkin, 1993; O'Kane et al., 1996; Prasad, 1996). When plants are exposed to low temperature, electron-transport chains tend to form $O_2^{\cdot-}$, which dismutates to form H_2O_2. Furthermore, in chloroplasts, low temperature limits the dark reactions, thus limiting the supply of $NADP^+$ and favoring reduction of O_2 by photosystem II. Therefore, exposure to low temperature in combination with high light intensity leads to more serious damage in plants (Allen and Ort, 2001). In mitochondria, inhibition of ATP formation or electron flow through cytochrome *b* stimulates $O_2^{\cdot-}$ formation by complex I and by ubiquinone (Elstner, 1991).

Plants have evolved both enzymatic and nonenzymatic mechanisms to scavenge the ROS rapidly evolved under low-temperature stress (Apel and Hirt, 2004; Scandalios, 1993). Among the antioxidant mechanisms, the ascorbic acid (AsA)–GSH cycle is a key component for elimination of ROS, especially H_2O_2 (Kingston-Smith and Foyer, 2000; Noctor et al., 2002). In the AsA–GSH cycle, AsA reduces both $O_2^{\cdot-}$ and H_2O_2. In turn, the crucial antioxidant, GSH, reduces dehydroascorbate to regenerate AsA; meanwhile, GSH itself is oxidized to form GSH disulfide (GSSG). NADPH, catalyzed by glutathione reductase (GR), then reduces GSSG to regenerate GSH (Kocsy et al., 2000a, 2000b, 2001). Therefore, inhibition of GSH synthesis by a specific inhibitor, BSO, could dramatically decrease the chilling tolerance of mung bean seedlings and maize (*Zea mays* L.) (Kocsy et al., 2000b; Yu et al., 2002, 2003). Experimental evidence also indicates that the level and redox state of GSH might serve as indicators of plant responses to environmental stresses such as chilling (Foyer et al., 1997; May et al., 1998; Tausz et al., 2004). However, how plants sense low temperature and then transmit a precise signal to eventually elevate the cellular GSH levels is still far from clear.

According to our present understanding of signal transduction in plant cells, $(Ca^{2+})_{cyt}$ plays a pivotal role. The second

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BIOLOGIE
Biochimie

ALTERATION OF ASCORBATE POOL UPON INFLUENCE OF OXIDATIVE STRESS INDUCING HERBICIDES

Lyuba P.-E. Miteva, Sergei V. Ivanov*

(*Submitted by Corresponding Member I. Pashev on May 28, 2007*)

Abstract

Ascorbate is a main antioxidant molecule in plants. In the present study we demonstrate alterations in the endogenous ascorbate content in two plant species: pea and wheat treated with herbicides with different mode of action. All three herbicides used (atrazine, glyphosate, and 2,4-D) induced overproduction of active oxygen species (AOS) in plant tissue. Treatment with 2,4-D increased ascorbate content in wheat (a tolerant species), while in pea (sensitive), a decrease was detected. In both plant species atrazine caused a drop of ascorbate content in the end of the experimental period. Our results demonstrate that the decrease of ascorbate in susceptible to herbicides plants is accompanied with dead of treated plants. On the contrary, in herbicide resistant plants an evaluation of ascorbate levels was detected.

Key words: 2,4-D, antioxidants, atrazine, glyphosate, pea, wheat

Introduction. In modern agriculture, plants are frequently exposed to the impact of different kind of herbicides. Atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine], 2,4-D [2,4-dichlorophenoxy acetic acid], and glyphosate [N-(phosphonomethyl) glycine] are some of the most widely used herbicides for the last 40 years [1]. The main target site of atrazine is the exchangeable quinone (Q_b) in the photosystem II reaction centre. As a result of this inhibition excitation energy generated by photosystem II cannot be dissipated through the normal electron flow beyond Q_a, and singlet oxygen is generated [2]. Treatment of susceptible plant species

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SALICYLIC ACID ALLEVIATES LEAF RUST-INDUCIBLE OXIDATIVE PROCESSES IN WHEAT PLANTS

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ABSTRACT

We studied the effect of salicylic acid (SA) treatment on the amount of some oxidative stress markers as well as the activity of catalase and peroxidase in compatible wheat-rust (*Puccinia recondita* f.sp. *tritici*, race 176) interactions in field experiments. The application of SA was carried out by single and/or triple spraying with Exin R®, containing 4.5% w/v SA as an active ingredient (0.5 mM final concentration). The biochemical determinations were made 61 days after inoculation with leaf rust, 49 days (first), 35 days (second), and 16 days (third) following each treatment with Exin R®. Infection with leaf rust provoked a rise in levels of lipid peroxidation (MDA content) and free proline, enhanced peroxidase and inhibited catalase activities. The application of SA eliminated these effects. Generally, in this study we demonstrated that a compatible leaf wheat-rust interaction caused oxidative stress and the treatment with SA alleviated pathogen-inducible oxidative processes. We suppose that the SA could act as a protector by activation of the antioxidant defense of wheat plants.

Keywords: catalase, peroxidase, *Puccinia recondita*, oxidative damage, salicylic acid, wheat.

AIMS AND BACKGROUND

Plants have developed defense mechanisms against phytophatogens that include preformed physical and chemical barriers, as well as expression of a set of genes. The infection of plants by necrotising pathogens can induce a broad-spectrum resistance to subsequent attack. This non-specific resistance phenomenon has been termed systemic acquired resistance (SAR) (Ref. 1). Salicylic acid (SA) has an important role in the development of SAR. Initially, SA has been proposed as a key signal leading to

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INFLUENCE OF EXTRACTION TECHNIQUES AND SOLVENTS ON THE ANTIOXIDANT CAPACITY OF PLANT MATERIAL

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ABSTRACT

The influence of extraction medium and sample preparation on the total antioxidant capacity (TAC) of pea plants leaves is investigated. Sodium acetate buffer (pH 5), potassium phosphate buffer (pH 7.5), methanol or 0.1% w/v trichloroacetic acid (TCA) were used for homogenization. TAC was measured in the crude supernatants (one-step procedure). The pellets were extracted with acetone and their TAC values were added to those from the previous assay (two-step procedure). The values for TAC of the acetone extract, the low-molecular and protein fractions derived from a partial fractionation of the probes are used in the proposed by us new three-step method. The results indicate that TAC of the probes processed with the two or three-step procedure is significantly higher than that of the crude supernatants (one-step procedure). On the basis of this study we recommend two-step protocol as a minimum and buffer with slightly acid pH or TCA for extraction when plant material is investigated. The highest results were obtained when the new three-step procedure for sample preparation and sodium acetate buffer as an extracting agent were applied. Moreover, the separation of the cellular antioxidants into three different fractions is especially useful for studying the effects of aging, processing and storage on the antioxidant capacity.

Keywords: antioxidant capacity, pea plants, TEAC, FRAP, sample preparation

Introduction

The antioxidant system in plants is very complex, with antioxidants having different targets, sizes and interactions with each other. Halliwell (8) defined biological antioxidants as "molecules which, when present in small concentrations compared to the biomolecules they are supposed to protect, can prevent or reduce the extent of oxidative destruction of biomolecules". They can be divided into enzymatic (e.g superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic (e.g. glutathione, vitamin E, ascorbic acid). Total Antioxidant Capacity (TAC) is a parameter that takes into account all the synergistic and cumulative interactions between the known and unknown antioxidants present in the sample. Several methods have been developed to measure the TAC of biological samples including plants. Some of the most used are "TRAP" (Total Radical-Trapping Antioxidant Parameter), "TEAC" (Trolox Equivalent Antioxidant Capacity), "ORAC" (Oxygen-Radical Absorbance Capacity), "FRAP" (Ferric/ Reducing Antioxidant Power) (24). Sample preparation and extraction solvents are vital (3) but the information on how it affects the subsequent measurements is insufficient. Different authors used various protocols for extraction of antioxidants. Usually, water-soluble and water-insoluble fractions were assessed separately (two-step procedure). For extraction of water-soluble antioxidants water or different buffers were used. Water-insoluble antioxidants were extracted with acetone or chloroform. Numerous authors used solely methanol (one-step procedure) as an extracting agent.

The purpose of this work is to determine how different extraction mediums and sample preparation affect the TAC of plant material. In addition to the one and two steps procedures used by other authors, we developed a three-step method for sample preparation. The new approach shows highest values for TAC in comparison to the standard procedures. Moreover, our method discriminates between water-soluble, water-insoluble lipophilic and protein antioxidants within one sample. For TAC evaluation two widespread assays, TEAC and FRAP were used.

Materials and Methods

In the experiments, pea plants (*Pisim sativum*, L.) cv. Pleven 4 were used. Seeds were soaked in tap water for 6 hours, and germinated for 3 days on a moisturized filter paper in a germination chamber at 25ºC. Plants were grown hydroponically on a half-strength Hoagland's solution with trace microelements in a growth chamber (photoperiod 12/12h, photon flux density 70-90 $\mu mol.s^{-1}.m^{-2}$, temperature 25°C, relative air humidity 60-70%). All samples were collected from second leaf stage.

A scheme describing the procedure for sample preparation is presented in **Fig. 1**. The plant material for homogenizations was gathered at the same time from a random sample. The 0.5 g leaves were ground with a pestle and mortar in sodium acetate buffer (0.1 M, pH 5), potassium phosphate buffer (0.1 M, pH 7.5), methanol or 0.1% (w/v) trichloroacetic acid, respectively. The homogenate were centrifuged at 15 000 g for 30 min and the resulting supernatants (crude fraction) were analyzed for TAC, as reported below (one-step procedure). In the two-step procedure all pellets, except that from the methanol slurry, were extracted with 3 mL of acetone under

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BIOLOGIE
Physiologie des plantes

ALTERATIONS IN ANTIOXIDANT ENZYMES OF PEA PLANTS IN RESPONSE TO PROLONGED INFLUENCE OF SHORT PULSES OF ULTRAVIOLET-C RADIATIONS

Zornitsa Katerova, Elena Shopova, Liliana Brankova, Sergei Ivanov*, Emanuil Karanov

(*Submitted by Corresponding Member I. Pashev on December 12, 2007*)

Abstract

Pea (*Pisum sativum* L., cv. Scinado) seedlings were exposed at midday to short pulses of UV-C radiations for two weeks. Low and moderate regimes were used: C1 (0.01 J cm^{-2} d^{-1}) and C2 (0.03 J cm^{-2} d^{-1}). Hydrogen peroxide (H_2O_2) and major antioxidant enzyme activities were determined in the 2nd pea leaves after 7, 10 or 14 consecutive days of UV-C irradiations. The rise in H_2O_2 levels was incident (7d C1) or constant (after C2 regime). The activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione-reductase (GR) decreased, whereas guaiacol-peroxidase (GPX) and glutathione-S-transferase (GST) were increased. The alterations were expressed primarily in C2-treated plants. In conclusion, nevertheless the activation of GPX and GST the enzymatic antioxidant defence failed under prolonged influence of short pulses of UV-C irradiation

Key words: UV-C radiation, antioxidant enzymes

Abbreviations: APX – ascorbate peroxidase; CAT – catalase; GPX – guaiacol-peroxidase; GST – glutathione-S-transferase; GR – glutathione-reductase; H_2O_2 – hydrogen peroxide; ROS – reactive oxygen species; SOD – superoxide dismutase; UV – ultraviolet radiation

Introduction. Ultraviolet radiation is divided into three classes: UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm). Important, due to its highest energy UV-C quickly creates high levels of damage, more than UV-B [1]. At present, UV-C does not reach the Earth's surface, except high mountain locations due to its absorption in the atmosphere [2]. In the last decades, studies on ultraviolet radiation-induced effects in plants have mainly regarded UV-B exposure, but recently the studies of UV-C effect on plants have been revaluated [3–5]. High levels of UV-C radiations can generate oxidative stress in plants via overproduction of different reactive oxygen species (hydrogen peroxide, superoxide, hydroxyl radicals and singlet oxygen) [1, 4, 5]. Reactive oxygen species

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PROLONGED LOW DOSE OF ULTRAVIOLET-B RADIATION DOES NOT ACTIVATE ANTIOXIDANT DEFENCE IN YOUNG PEA PLANTS

Zornitsa Katerova, Sergei Ivanov*, Sergio Mapelli**

(*Submitted by Academician V. Golemansky on February 15, 2008*)

Abstract

The behaviour of the antioxidant defence system was studied in pea (*Pisum sativum* L., cv. Scinado) leaves after exposure of intact plants to short pulses of ultraviolet-B (UV-B) radiation. Low and moderate regimes were used, B1 (4.4 kJ m^{-2} d^{-1}) and B2 (13.3 kJ m^{-2} d^{-1}), for two weeks. Hydrogen peroxide (H_2O_2) levels increased moderately after 7 and 10 days of B1 and B2 treatments. At the end of the experiment (14d UV-B) accumulation of H_2O_2 was found only in B2-irradiated plants. The activities of superoxide dismutase (SOD) and glutathione-reductase (GR) were not noticeably changed. A substantial increase was found in guaiacol-peroxidase (GPX) and glutathione-S-transferase (GST) activity (only in B2 treated plants), whereas the activity of catalase (CAT) and ascorbate peroxidase (APX) decreased. In conclusion, the activation of enzymatic antioxidant defence system was not noticeable when plants were exposed to prolonged and low levels of UV-B radiation.

Key words: antioxidant enzymes, hydrogen peroxide, UV-B radiation, pea plants

Abbreviations: APX – ascorbate peroxidase; CAT – catalase; GDHP – guaiacol dehydrogenation product; GPX – guaiacol-peroxidase; GST – glutathione-S-transferase; GR – glutathione-reductase; H_2O_2 – hydrogen peroxide; ROS – reactive oxygen species; SOD – superoxide dismutase; UV – ultraviolet radiation

Introduction. Depletion of the ozone layer and changes in climate increased UV-B (280–315 nm) radiation and its negative impact on organisms. UV-B radiation can generate oxidative stress in plants via overproduction of different reactive oxygen species (hydrogen peroxide, singlet oxygen, superoxide and hydroxyl radicals) [1, 2]. Reactive oxygen species (ROS) have capacity to cause damages in proteins, DNA and lipids that led to changes in different plant functions and plant death [3, 4]. Nonenzymatic compounds (e.g. ascorbic acid, glutathione,

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A HOLISTIC APPROACH TO RESURRECTION PLANTS. *HABERLEA RHODOPENSIS* – A CASE STUDY

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ABSTRACT

Recent environmental changes challenge world agriculture and reconfirm the importance of wild flora as useful source of valuable traits. Due to their extreme desiccation tolerance, the so called "Resurrection plants" are extensively studied and characterized. The Bulgarian endemic species Haberlea rhodopensis, apart from its typical resurrection capacity is very interesting also as a potential source of bioactive compounds with putative application in pharmacology, veterinary medicine and cosmetics. Here we discuss our approaches to Haberlea in the frames of the NSF funded project DO02-105 "Centre for sustainable development of plant and animal genomics".

Keywords: resurrection plants, *Haberlea rhodopensis,* molecular analysis

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Background

The recent climatic changes increase the percentage of arid, abandoned regions which until recently were known as crop lands. Global warming and deterioration of agricultural areas are results of many factors including human errors and improper crop management. This worst scenario challenges plant scientists in their efforts to improve the reaction of crop plants to unfavourable environments and to secure sustainable development. Wild flora with its broad biodiversity has always been a source of new ideas and genetic material. On the other hand, the paradigm of sustainable development demands a complex, holistic approach to wild plant species. The search is not only for traits to be transferred to the crops but also for characteristics, if available, that will make the wild plant an attractive producer of valuable bioactive compounds.

In this respect, the so-called resurrection plants can be considered as excellent model systems because of their unique desiccation tolerance (7, 21, 24). Their mature leaves can lose more than 90% of relative water content and survive in such conditions long periods (months, years) of dryness. Upon re-watering, they recover very fast and restore their photosynthetic activity within several hours. These plant species belong to different botanical families; they live in different habitats and under various environmental challenges. Their only common feature that is under investigation so far is the ability of their vegetative tissues to withstand long periods of full desiccation and to recover rapidly upon re-watering (2, 30).

Investigations with various resurrection plants became particularly intensive in the recent 10 years and were extensively reviewed elsewhere (18, 19, 30). Among others, *Craterostigma plantagineum, Myrothamus flabellifolia* and *Xerophyta viscosa* are used not only as models of desiccation tolerance but already for system biology oriented directly to improve the reaction of valuable crops like maize and grapevine to unfavorable environments (19).

The aim of the present mini-review is to highlight the current state of the art in the studies with *Haberlea rhodopensis* – resurrection plant species, endemic for Bulgaria and to outline the investigations planned in the frames of the NSF funded DO02-105 project "Centre for sustainable development of plant and animal genomics".

Object of Studies

Bulgaria is among the few countries in Europe where resurrection plants live in natural habitats. *Haberlea rhodopensis* Friv was documented in the middle of 19th century and was among the first plants recognized as a genuine resurrection plant (8). Both with its relative *Ramonda serbica* Pancic they belong to Gesneriaceae and prefer shady slopes and limestone rocks. It is considered a homoiochlorophyllous plant species, as far as it preserves most of its chlorophyll content during dehydration. Parameters of the reaction to desiccation and recovery have been extensively studied with main target photosynthetic complex and transpiration (9, 10, 11, 13, 14, 22, 23), dynamics

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ORIGINAL PAPER

Phenols, proline and low-molecular thiol levels in pea (*Pisum sativum*) plants respond differently toward prolonged exposure to ultraviolet-B and ultraviolet-C radiations

Zornitsa Katerova · Sergei Ivanov · Sergio Mapelli · Vera Alexieva

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Abstract Pea (*Pisum sativum* L.) seedlings were exposed to low, moderate, and high regimes of ultraviolet-B (UV-B) (ld-B 4.4, md-B 13.3, and hd-B 26.5 kJ m^{-2} day^{-1}), or ultraviolet-C (UV-C) (ld-C 0.1, md-C 0.3, and hd-C 0.6 kJ m^{-2} day^{-1}) radiations. Concentrations of total phenols, free proline, and low-molecular thiol groups were determined in the last formed (young) and older leaves after irradiation for 7, 10 or 14 consecutive days. Shoot length and weight did not change markedly after 14 days of ld-B and ld-C, but reduced substantially after moderate and high regimes of both UV-B and UV-C. Proline decreased upon high doses of irradiation, while in ld-B treated plants, by contrast, an increase was observed. The reduction in total phenols and thiols was stronger after hd-B than after hd-C irradiations, although an induction was found in ld-B treated plants. In contrast to ld-B, ld-C regime led mainly to reductions or insignificant changes in proline, phenols, and thiols. Therefore, the stress-protection mechanisms are different between low UV-B and UV-C irradiation regimes in regard to proline, phenols, and thiols.

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Keywords Free proline · *Pisum sativum* · Thiols · Total phenols · UV radiation

Introduction

The damaging effect of ultraviolet (UV) radiation increases with shorter wavelengths because UV-C (100–280 nm) photons possess more energy than UV-B (280–315 nm). Due to the depletion of the ozone layer, UV-B radiation has an increasingly negative impact on living organisms. At present, UV-C radiation does not reach the Earth's surface, except in high mountain locations, due to its absorption in the atmosphere (Häder et al. 2007). However, direct solar UV-C radiation (0.3 μJ cm^{-2}) was registered by KCl:Eu^{2+} dosimeters at ground level in Madrid on clear-sky days, which was six orders of magnitude lower than UV-B (Córdoba et al. 1997).

In the last few decades, studies on ultraviolet radiation-induced effects in higher plants have mainly considered UV-B exposure. In algae, however, the effect of UV-C attracted more attention (Duval et al. 2000). Recently, studies of UV-C effect on higher plants have been re-evaluated (Casati and Andreo 2001; Shama and Alderson 2005; Procházková and Wilhelmova 2007). In a review Shama and Alderson (2005) considered hormetic (beneficial) effect of low UV-C irradiation doses (0.5–9.0 kJ m^{-2}) in commercial prospects to prevent pathogen disease and delay senescence during fruit storage. UV-B and UV-C photons have enough energy to destroy chemical bonds, causing photochemical reaction in molecules of high biological significance (nucleotides, nucleic acids, proteins, lipids, photosynthetic pigments) (Stapleton 1992; Frohnmeyer and Staiger 2003; Edreva 2005). Thus, they become sensitive targets for UV-damage. UV-B light modifies plant morphology, reduces growth, and

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BRIEF COMMUNICATION

Low doses of ultraviolet-B or ultraviolet-C radiation affect phytohormones in young pea plants

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Abstract

Pea (*Pisum sativum* L., cv. Scinado) seedlings were exposed to low doses of ultraviolet-B (UV-B; 4.4 and 13.3 kJ m^{-2} d^{-1}) or UV-C (0.1 and 0.3 kJ m^{-2} d^{-1}) radiation for 14 d. Aminocyclopropane carboxylic acid (ACC), indoleacetic acid (IAA) and abscisic acid (ABA) contents were quantified by gas chromatography coupled to mass spectrometry (GC-MS). The accumulation of ACC upon irradiation was dose-dependent. ABA content was reduced and IAA content increased upon UV-C treatment whereas the UV-B doses used did not cause significant changes in ABA and IAA contents.

Additional key words: abscisic acid, aminocyclopropane carboxylic acid, indoleacetic acid, *Pisum sativum*, stress.

Due to depletion of the ozone layer, UV-B (280 - 315 nm) radiation has an increasing negative impact on living organisms. At mid latitudes, the biologically effective radiation $UV\text{-}B_{BE}$ may reach up to around 6 kJ m^{-2} d^{-1} but in some regions higher values (about 13 kJ m^{-2} d^{-1}) were detected (Paul 2001). Except for high mountains, UV-C (200 - 280 nm) does not reach the surface of the Earth, due to its absorption in the atmosphere (Häder *et al.* 2007). Nonetheless, Córdoba *et al.* (1997) detected a direct solar UV-C radiation, reaching the ground in Madrid (700 m a.s.l.) at levels of 3×10^{-6} kJ m^{-2} d^{-1} registered by $KCl:Eu^{2+}$ dosimeters.

Abscisic acid (ABA), indole-3-acetic acid (IAA) and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) are important regulators of plant responses to abiotic stresses. ABA is considered as a stress hormone that modulates adaptation to various stresses including UV-B radiation (Albinsky *et al.* 1999). IAA and other auxins are involved in developmental processes like growth, apical dominance and lateral root initiation (Ljung *et al.* 2001). Ethylene is an important element in both stress response and adaptation. Environmental stresses and hormonal signals stimulate the ethylene production through synthesis of ACC (Yu and Yang 1980, Hyodo *et al.* 1985, Nara and Takeuchi 2002, Nakajima *et al.* 2002). There is scarce information regarding the influence of UV-B on IAA (Huang *et al.* 1997, Yang *et al.* 2004), ABA (Rakitina *et al.* 2001, Yang *et al.* 2004) and ACC (Nara and Takeuchi 2002) contents and until now nothing seems to be published on the effect of low UV-C doses. Therefore we concentrated upon the effects of two-week UV-B and UV-C treatments on the ACC, ABA and IAA contents in pea seedlings.

Pea (*Pisum sativum* L., cv. Scinado) seeds were germinated 3 d on moist filter paper in the dark. The seedlings were transferred to Hoagland's solution and grown in a growth chamber (12-h photoperiod, photosynthetic photon flux density of 160 μmol m^{-2} s^{-1}, temperature of 24 ± 2 °C, air humidity of 60 - 70 %). Three days later, seedlings were exposed to UV radiation

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Abbreviations: ABA - abscisic acid; ACC - aminocyclopropane carboxylic acid; B1 - 4.4 kJ m^{-2} d^{-1} UV-B; B2 - 13.3 kJ m^{-2} d^{-1} UV-B; C1 - 0.1 kJ m^{-2} d^{-1} UV-C; C2 - 0.3 kJ m^{-2} d^{-1} UV-C; IAA - indoleacetic acid; PAR - photosynthetically active radiation; PFBBr - pentafluorobenzyl bromide; UV - ultraviolet radiation; $UV\text{-}B_{BE}$ - biologically effective dose of UV-B radiation.
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ORIGINAL PAPER

Effect of exogenous hydrogen peroxide on enzymatic and nonenzymatic antioxidants in leaves of young pea plants treated with paraquat

Irina Moskova · Dessislava Todorova · Vera Alexieva · Sergei Ivanov · Iskren Sergiev

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Abstract The effects of exogenously applied hydrogen peroxide on the antioxidant system of pea plants were investigated. Ten-day-old pea seedlings were sprayed with 2.5 mM H_2O_2 and 24 h later with 0.2 mM PQ. Samples were taken 0, 2 and 5 h after the start of illumination. The protective effect of H_2O_2 was evaluated by monitoring of parameters related to the damage caused by PQ. The treatment with PQ led to a severe leakage of electrolytes from leaf tissues. Malondialdehyde level increased in PQ treated plants, but remained unchanged in H_2O_2 pre-treated ones after 5 h of illumination. Increased catalase and glutathione-S-transferase activity was observed in pea plants treated with H_2O_2 and PQ. Ascorbate peroxidase activity decreased significantly after paraquat application, but pre-treatment with H_2O_2 prevented ascorbate peroxidase inhibition to some extent. Increased guaiacol peroxidase activity was detected after H_2O_2 application. PQ application caused a drastic decline in the levels of thiol-group bearing compounds, reduced glutathione and ascorbate, while the quantity of oxidized glutathione and dehydroascorbate were increased. The results presented on changes in enzymatic and nonenzymatic antioxidants suggest that preliminary H_2O_2 application to pea plants treated with PQ, alleviates the toxic effects of the herbicide.

Keywords Antioxidants · Hydrogen peroxide · Paraquat · *Pisum sativum* · Stress markers

Abbreviations

APX	Ascorbate peroxidase
AsA	Ascorbic acid
DHA	Dehydroascorbic acid
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione-S-transferase
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
PQ	Paraquat
ROS	Reactive oxygen species
SOD	Superoxide dismutase

Introduction

Oxidative stress emerges as a result of unfavorable environmental conditions leading to generation of reactive oxygen species (ROS), such as superoxide radicals (O_2^-), hydroxyl radicals (HO), and hydrogen peroxide (H_2O_2), which are highly detrimental to the cells. Similarly, oxidative stress occurs in response to pathogen attack and during the natural processes of senescence. It is a widespread phenomenon and leads

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RESEARCH PAPERS

Alterations in Glutathione Pool and Some Related Enzymes in Leaves and Roots of Pea Plants Treated with the Herbicide Glyphosate[1]

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Abstract—Our previous studies have demonstrated that application of glyphosate caused oxidative events in young pea and wheat plants. In this work, the changes in the endogenous level of glutathione (total and oxidized) and the activities of glutathione reductase (GR) and glutathione S-transferase (GST) after treatment with glyphosate were studied in pea plants (*Pisum sativum* L., cv. Skinado). Glyphosate was applied in two ways: (1) by leaf spraying with 10 mM solution; and (2) in nutrient medium as 0.01 mM solution. Measurements were made in both leaves and roots. Root and leaf treatments provoked the increase in both total and oxidized glutathione contents. Both types of herbicide application caused activation of GR in treated organs. Slight increase was detected also in untreated roots. It was found that glyphosate application to leaves provoked strong enhancement in the GST activity in leaves, while its root application stimulated the enzyme activity in the roots. We observed the higher GST activity in the organ directly treated with herbicide. Furthermore, we suggested that the activated isoforms of GST(s) participated in detoxification of hydrogen peroxide and lipid peroxides.

Key words: Pisum sativum - glutathione - glutathione reductase - glutathione S-transferase - glyphosate - oxidative stress

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INTRODUCTION

Glutathione ([γ]-Glu-Cys-Gly) is a multifunctional tripeptide found in plants and animals. It is the main nonprotein, low-molecular thiol in most organisms [1, 2]. Glutathione participates in a variety of detoxification, transport, and metabolic processes [3–5]. It is a donor of reducing equivalents in the glutathione–ascorbate shuttle (Halliwell–Asada cycle) [2]. In this process, a reduced form of glutathione becomes oxidized in order to reduce dehydroascorbate (which is transformed into ascorbate). Restoration from the oxidized form of glutathione back to its reduced form is catalyzed by glutathione reductase (GR, EC. 1.8.1.7). Glutathione participates also in direct peroxide detoxification. In this reaction, reduced glutathione (GSH) reacts with hydrogen peroxide (or another organic peroxide) to yield water (or water and alcohol) and glutathione dimer (GSSG). The process is accomplished by glutathione peroxidase (EC 1.11.1.9) or glutathione S-transferase (GST, EC. 2.5.1.18).

Important function of glutathione is its ability to maintain sulfhydryl groups of intracellular proteins in the correct oxidation states [2]. The TG/GSSG ratio is essential for the cell homeostasis and provides information regarding the capability of plants to withstand the oxidative stress [2]. Some authors suggest that the glutathione redox state can be a valuable stress marker in plant ecophysiological studies [6].

Glutathione is responsible for detoxification of potentially harmful molecules, such as pesticides or heavy metals [4]. The process of conjugation can be accomplished spontaneously or in the presence of GST. The important role of GST for detoxification of many herbicides is well known [5, 7]; furthermore, some authors suggest that GST may play a significant role in the process of phytoremediation [8]. Glutathione also participates in the metabolism of various compounds, including the aromatic organic molecules responsible for plant color, flavor, and fragrance, storage form of reduced sulfur, etc. [2, 3, 7].

Glyphosate is a nonselective, post-emergence herbicide, widely used to eliminate unwanted plants both in agricultural and nonagricultural landscapes [9]. It is

[1] This text was submitted by the authors in English.

Abbreviations: CDNB—1-chloro-2,4-dinitrobenzene; DTNB—5,5'-dithio-*bis*(2-nitrobenzoic acid); GR—glutathione reductase; GSH—reduced glutathione; GSSG—oxidized glutathione; GST—glutathione S-transferase; PVP—polyvinylpyrrolidone; SOD—superoxide dismutase; TG—total glutathione.

THE ROLE OF THIOL SPECIES IN THE TOLERANCE OF *Aspergillus niger* B77 TO CADMIUM IONS

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ABSTRACT

In the present study, the level of thiol species and activity of related enzymes were investigated in *Aspergillus niger* B77 to analyse their role in overcoming the stress caused upon exposure to cadmium ions (0–70 mg/l). Significant increases in the levels of low molecular non-protein thiols, total protein thiols, including glutathione (GSH) were observed. In addition, significant increases in the activity of glutathione S-transferase (GST), more clearly expressed in the earlier exponential growth phase (12th h), were noticed in response to Cd(II) ions. The results obtained showed that the elevation of the levels of thiol species and GST activity ceased by Cd(II) ions is a part of the detoxifying system of *Asp. niger* B77.

Keywords: *Aspergillus niger,* thiol species, glutathione, GST, cadmium ions.

AIMS AND BACKGROUND

Heavy metal pollution is an environmental problem of worldwide concern. When they accumulate in living cells, their toxicity markedly inhibits the growth processes[1–3] and can rapidly induce the synthesis of thiol compounds and enzymes, such as glutathione (GSH), phytochelatins, glutathione S-transferase (GST, EC. 2.5.1.18) and glutathione reductase (GR, EC.1.6.4.2.) (Refs 4–6). Cell survival depends on the ability to regulate the intracellular concentration of metal ions. Thus, it is not surprising that microbes have developed heavy metal resistance systems. In some cases, metal resistance has been shown to be due to differences in the levels both of heavy metal uptake and

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GLUTATHIONE INVOLVED IN STRESS RESPONSE DOES NOT DETERMINE THE RESISTANCE AGAINST SINGLET OXYGEN IN PEA (*Pisum sativum* L.) PLANTS

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ABSTRACT

Glutathione (GSH) is a major antioxidant in most aerobic organisms. In this study we investigated the alteration of GSH content in pea plants treated with singlet oxygen producing photosensitiser eosin. Derooted pea plants were infiltrated with three concentrations of eosin (1, 10 and 50 μM) and exposed to continuous light or darkness for 48 h. Eosin treatment combined with continuous light led to significant increase of total GSH content in all experimental groups. The lowest and middle eosin concentrations decreased percent of oxidised glutathione (GSSG), but highest (50 μM) increased it. In parallel dark experiments eosin did not provoke any essential changes in GSH and GSSG amounts.

When the plants were pretreated with GSH synthesis inhibitor buthionine sulfoximine (BSO) the foliar GSH levels were considerably decreased. Separate treatments with eosin reduced fresh weight of pea plants, but BSO alone did not provoke any significant differences. Paradoxically, applied together eosin and BSO caused some rise in the fresh weight compared to the single eosin. Generally, our results showed that plants with blocked GSH synthesis are more resistant to singlet oxygen. Enhancement of GSH level after treatment with eosin is not connected with activation of singlet oxygen detoxification mechanisms.

Keywords: antioxidant defense, BSO inhibitor, glutathione, pea, singlet oxygen.

AIMS AND BACKGROUND

The generation of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide and hydroxyl radicals, and singlet oxygen ($^1O_2$), occurs at all times during plant growth and development. Singlet oxygen is a member of the ROS family. It is

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RESEARCH PAPER

Sugar ratios, glutathione redox status and phenols in the resurrection species *Haberlea rhodopensis* and the closely related non-resurrection species *Chirita eberhardtii*

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Keywords
Antioxidant system; glutathione redox status; *Haberlea rhodopensis*; resurrection plants; soluble sugars; sugar ratios; total phenols.

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ABSTRACT

Because of their unique tolerance to desiccation, the so-called resurrection plants can be considered as excellent models for extensive research on plant reactions to environmental stresses. The vegetative tissues of these species are able to withstand long dry periods and to recover very rapidly upon re-watering. This study follows the dynamics of key components involved in leaf tissue antioxidant systems under desiccation in the resurrection plant *Haberlea rhodopensis* and the related non-resurrection species *Chirita eberhardtii*. In *H. rhodopensis* these parameters were also followed during recovery after full drying. A well-defined test system was developed to characterise the different responses of the two species under drought stress. Results show that levels of H_2O_2 decreased significantly both in *H. rhodopensis* and *C. eberhardtii*, but that accumulation of malondialdehyde was much more pronounced in the desiccation-tolerant *H. rhodopensis* than in the non-resurrection *C. eberhardtii*. A putative protective role could be attributed to accumulation of total phenols in *H. rhodopensis* during the late stages of drying. The total glutathione concentration and GSSG/GSH ratio increased upon complete dehydration of *H. rhodopensis*. Our data on soluble sugars suggest that sugar ratios might be important for plant desiccation tolerance. An array of different adaptations could thus be responsible for the resurrection phenotype of *H. rhodopensis*.

INTRODUCTION

It is expected that a systems-biology approach will lead to a significantly improved understanding of the mechanisms associated with plant desiccation tolerance (Moore *et al.* 2009). In this respect, the so-called resurrection plants can be considered as excellent model systems (Gaff 1971; Oliver *et al.* 2000; Proctor & Tuba 2002; Toldi *et al.* 2006). These species belong to different families, live in different habitats and are exposed to a wide range of environmental challenges. However, they share one common feature – their vegetative tissues are able to withstand long periods of complete desiccation and yet can recover very rapidly upon re-watering (Oliver 1996; Scott 2000; Farrant *et al.* 2003; Bartels 2005; Toldi *et al.* 2009). Acquisition of desiccation tolerance is a complex process that is probably due to an elaborate set of specific adaptations. It has been postulated that different resurrection species may use a mixture of different protective metabolites to allow complete dehydration tolerance (Kranner *et al.* 2002; Kranner & Birtic 2005). In addition, resurrection plants are expected to have superior free radical-scavenging mechanisms, both in their cytoplasm and in vacuoles, to cope with the severe stresses. It has recently been suggested that vacuolar compounds such as fructans might be involved in vacuolar antioxidant mechanisms (Van den Ende & Valluru 2009).

Bulgaria is among the few countries in Europe where resurrection plants grow in the wild. *Haberlea rhodopensis* Friv and *Ramonda serbica* Pancic belong to the Gesneriaceae. Both species prefer shady slopes and limestone rocks and are found at a wide range of altitudes in mountain areas. *H. rhodopensis* was among the first plants recognised as genuine resurrection plants (Ganchev 1950). It is considered a homoiochlorophyllous species, in that it can preserve most of its chlorophyll during dehydration. Photosynthesis and transpiration during desiccation and recovery have been extensively studied in *H. rhodopensis* (Markovska *et al.* 1994, 1995; Peeva & Maslenkova 2004; Georgieva *et al.* 2005, 2007, 2008; Peeva & Cornic 2009). Lipid and sterol changes in leaves (Stefanov *et al.* 1992) and some aspects of compatible solute

IMPROVED PROCEDURE FOR COMET ASSAY ON ACTIVE PHOTOSYNTHETIC CELLS FROM PEA PLANTS

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ABSTRACT

In the recent years the studies of plants on DNA level have become unavoidable approach in almost any research in plant genetics and physiology. Often, the task of an intended research is assessing the influence of different nutrition schemes, chemicals or substances on the molecule of DNA. Therefore, the reliability of the methods revealing damages in DNA molecule is very important for the explanation of the obtained results. One of the most popular methods for detection of DNA damages is the comet assay. The pea (*Pisum sativum* L.) plant is one of the few exceptions of experimental organisms on which the popular method of the comet assay has not yet been applied. The difficulties of the comet assay execution on photosynthetic cells compelled us to modify the method. In this study we present a faster and more reproducible variant of the comet assay application on photosynthetic active plant tissues.

Keywords: the comet assay, DNA damage, plant cell, singlet oxygen.

AIMS AND BACKGROUND

Single cell gel electrophoresis (SCGE) also called the comet assay has been developed as a method for detection of damages in the molecule of DNA (Ref. 1). Nowadays the method of comet assay is gaining enormous popularity due to its broad spectrum of applications. It is extensively used for genotoxicity testing of substances such as industrial chemicals, biocides, agrochemicals, food additives and pharmaceuticals[2]. The comet assay is a simple technique, faster and cheaper than the other methods for

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ORIGINAL ARTICLE

Long-term impact of sublethal atrazine perturbs the redox homeostasis in pea (*Pisum sativum* L.) plants

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Abstract Atrazine frequently contaminates soil, groundwater, rivers, and ponds. It is well know that acute doses (1–5 mM) of atrazine induce massive generation of singlet oxygen by blocking photosystem II. The sublethal concentrations of this herbicide, similar to those found in the environment, also reduce growth and disrupt photosynthesis in a long-term aspect, but exact mechanisms remain much uncertain. In this study the effects of environmentally relevant atrazine levels, ranging from 0.1 to 10 μM, on pea plants were characterized for up to 20 days. The plants exposed to continuous influence of atrazine exhibited perturbed redox homeostasis with increases of the lipid peroxides, the total and oxidized glutathione pools and elevated guaiacol peroxidase and glutathione-*S*-transferase activities. In contrast, the long-term atrazine impact did not affect superoxide dismutase activity whereas the catalase was inhibited. The perturbations of the redox status and the recruitment of the antioxidant machinery imply that the sublethal atrazine concentrations alter the poise between production and scavenging of reactive oxygen species. Taken together these results show that the long-term impact of sublethal atrazine has hallmarks of oxidative stress most probably triggered by generation of singlet oxygen.

Keywords Atrazine · Antioxidant enzymes · Singlet oxygen · Glutathione · Hydrogen peroxide · Lipid peroxidation

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Introduction

Atrazine [6-chloro-N-ethyl-(1-methylethyl)-1,3,5-triazine-2,4-diamine], and other *s*-triazines, have been widely used as herbicides in USA (according to the US Environmental Protection Agency) and probably throughout the world for the last 50 years. It is primarily used on maize, sorghum, and sugarcane. The triazines belong to the herbicides that degrade most slowly in soils and water. Under field conditions, less than 5% of the atrazine and 1% of simazine in soil is degraded for 1 month (Taylor 1995). Atrazine frequently contaminates soil, groundwater, rivers, and ponds. Concentrations were as high as 120 μg/liter in agricultural basins, 14 μg/l in urban basins, and 22 μg/l in river basins (Cox 2001). The highest measured residual amounts of simazine in agricultural surface soils reach 1,000 μg/l (Braun and Hawkins 1991). Typical environmental contaminations in agricultural lands vary in diapason 1–100 μg/l or kg soil (Van Maanen et al. 2001; Vitanov et al. 2003). Water quality monitoring data show that in England and Wales, more than 10% of the surface and groundwater samples investigated from, the amounts of *s*-triazines (including atrazine) exceeded European standard (0.1 μg/l) for quality of drinking water

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INTERACTION BETWEEN STRESSES

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"If we wish to understand life we must study death"
Levitt, J., 1980

Summary. Normally, under natural conditions, plants are subjected to the influence of at least two different stress factors. The physiological responses of plants, exposed to two subsequent stress factors differing in their intensity or duration are reviewed. In the experiments presented here, the effects of some natural (water depletion, extreme temperatures), and anthropogenic (UV-B irradiation and herbicides) stresses, applied alone and in combination were studied. As a measure of the interaction between stresses, the changes in biometric parameters, the levels of some oxidative stress markers and activity of defence enzymes were monitored in pea, wheat, or maize seedlings (grown as water culture) and in Arabidopsis plants. The relationships between the metabolic changes observed, and the degree of cross-synergism or cross-adaptation to the interacting stresses are discussed.

Key words: Stress interaction, Active oxygen species, Stress markers, Cross-synergism, Cross-adaptation

Abbreviations: MDA – malondialdehyde

Abiotic and biotic stresses cause alterations in the normal physiological processes of all plant organisms, including the economically important crops. Plant damage and decrease in their productivity take place most often due to naturally occurring unfavourable factors of the environment (natural stress factors) - extreme temperatures; water deficit or abundance; increased soil salinity; high solar irradiance; early autumn or late spring ground frosts; pathogens etc. Recently, along to these factors plant organisms are imposed to a large scale of new stressors related to human activity (anthropogenic stress factors) – toxic pollutants such as pesticides, noxious gasses (SO_2, NO, NO_2, NO_x, O_3 and photochemical smog); photooxidants; soil acidification and mineral

Short communication

Effect of Atrazine on Glutathione Levels, Glutathione S-Transferase and Glutathione Reductase Activities in Pea and Wheat Plants

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Abstract

Miteva L.P-E., Ivanov S.V., Alexieva V.S., Karanov E.N. (2004): **Effect of atrazine on glutathione levels, glutathione S-transferase and glutathione reductase activities in pea and wheat plants**. Plant Protect. Sci., **40**: 16–20.

Changes were studied in the endogenous level of glutathione (total and oxidised), and in the amount of free thiol groups as caused by the herbicide atrazine on two species of plants with different sensitivity to it.The activities of two enzymes related to glutathione metabolism (glutathione reductase and glutathione S-transferase) were also determined. The application of the herbicide on leaf increased the levels of total and oxidised glutathione in pea and wheat plants. Increased activity glutathione S-transferase in wheat plants was found.

Keywords: atrazine; pea; wheat; glutathione; glutathione S-transferase; glutathione reductase

For more than 40 years, triazines have been among the most successful selective herbicides used in agriculture; their representative most widely used is atrazine: 2-(ethylamino)-4-chloro-6-isopropylamino-1,3,5-triazine. Like all *s*-triazines, atrazine is an inhibitor of photosystem 2 (PS2). As a result of this inhibition, the excitation energy generated by PS2 cannot be dissipated through the normal electron flow beyond Q_A, and singlet oxygen is generated (Cobb 1992). The application of herbicide concentrations of triazines enhanced the amount of hydrogen peroxide and also increased the amounts of oxidative lipid peroxidation products and ion fluxes in pea plants (Pallett & Dodge 1980; Ivanov *et al*. 2003).

Glutathione (GSH) plays important and well-defined roles in the metabolism and detoxification of numerous pesticides including herbicides that can initiate oxidative stress in plants (Kömives & Gullner 2000). GSH is a component of the glutathione-ascorbate shuttle which is part of the antioxidant system in plants (Foyer *et al*. 2001). Glutathione reductase (GR, EC 1.6.4.2) plays an important role in restoring reduced form of glutathione (Foyer & Halliwell 1976; Lascano *et al*. 2001). Detoxification of different pollutants, including atrazine, by conjugation is another of the glutathione functions (Shimabukuro *et al*. 1970). The process can take place spontaneously or as a result of the activity of the enzyme glutathione S-transferase (GST, EC. 2.5.1.18) (Kömives & Gullner 2000). Beside its function in detoxification, glutathione has an additional protective role in the reduction of cytotoxic hydroperoxides (which arise as a result of oxidative stress) to the respective alcohols (Dixon

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СТРЕС И УСТОЙЧИВОСТ ПРИ РАСТЕНИЯТА

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Резюме: *Стресовата физиология на растенията изучава въздействието на неблагоприятните условия на околната среда върху растенията. В настоящата публикация се разглеждат основни положения в теорията на стреса, адаптирани към нуждите на растителните науки. Представени са възгледите на повечето от съвременните изследователи работещи в областа. Направен е опит да се дадат дефиниции на български на някои широко използвани термини. Посочени са възприетите за класификация на стресовите фактори подходи, както и основните пътища за придобиване на устойчивост от растенията. Показани са различията в механизмите на адаптация между животните и растенията. Приведени са редица примери, илюстриращи използването на различните способи за придобиване на устойчивост в земеделската практика. Дискутирани са проблемите и преспективите, произтичащи от навлизането в селското стопанство на растения с променен геном.*
Ключови думи: *стрес, устойчивост, адаптация, толерантност, избягване на стреса, трансгенни растения*

S. IVANOV, Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, 1113 Sofia. STRESS AND RESISTANCE IN PLANTS

Abstract: *Plant stress physiology deals with the effect of unfavourable conditions of environment on the plants. In this study the main topics of the theory of stress adapted to the plant science are described. The conceptions of the most of contemporary investigators working in this area are pointed out. An attempt for correct definitions of some wide used terms was made. The adopted for classification of stress factors approaches are indicated. The main ways for acquirements of plant stress resistance are noticed. The differences in mechanisms of adaptation between plants and animals are described. The various approaches for the achievement of acquired plant resistance in agricultural practice was illustrated by some examples. The problems and perspectives as a result of the entering of transgenic crops in agriculture are also discussed.*
Key words: *stress, resistance, adaptation, tolerance, stress avoidance, transgenic crops*

През своето онтогенетично развитие растенията са подложени на действието на редица неблагоприятни фактори на околната среда (наричани условно стресори). Подобни въздействия предизвикват негативни изменения в дивите растителни популации и снижават добивите от земеделските култури (табл. 1). Изучаването на последствията от действието на огромното многообразие от стресори е предмет на дейност на стресовата физиология на растенията. В настоящата публикация е направен опит да се представят някои основни положения в теорията на стреса, адаптирани към нуждите на растителните науки.

Терминът "стрес" е въведен за първи път в медицината от канадския учен Selye (1936; 1956). Като понятие обаче той произлиза от физиката (виж допълнение 1). Класическото определение на Selye за стрес е формулирано по следния начин: *Всички външни въздействия за организмите са стресиращи фактори (стресори). Те предизвикват стрес и в резултат на този стрес има специфична реакция и неспецифичен общ отговор.* През 1980 г. Jacob Levitt (1980) дава следната дефиниция за стрес (ор): *Всеки фактор от околната среда, способен да предизвика увреждания в живите организми.*

Тотален антиоксидантен капацитет на комерсиални плодови сокове

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Резюме

Превантивната роля на съединенията с антиоксидантна природа спрямо редица заболявания е отдавна известна. Тоталният Антиоксидантен Капацитет (ТАК) позволява сравняване на различни хранителни продукти по отношение на антиоксидантно съдържание и нормиране на дневния прием от антиоксиданти спрямо нуждите на организма. В работата е изследван ТАК и количеството на фенолните съединения на серия комерсиални сокове, произведени от плодове на различни растителни видове. За определяне на ТАК са използвани два широко разпространени метода (FRAP и ABTS). Резултатите показват, че независимо от метода на определяне най-висока антиоксидантна сила притежава сокът от кайсия, а най-ниска от ананас. Останалите сокове се класират на различни позиции в зависимост от използуваните аналитични тестове. Корелацията между количеството на фенолните съединения и ТАК е слаба. Антиоксидантният капацитет на изследваните сокове не е правопропорционален на ТАК на плодовете, от които те са произведени. Този резултат показва, че ТАК на плодовите сокове и нектари е силно зависим от технологията за получаването им.

Ключови думи: *ABTS анализ, FRAP анализ, плодови сокове, тотален антиоксидантен капацитет, феноли*

Total antioxidant capacity of commercial fruit juices

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Summary

The preventive role of antioxidants against numerous

Зависимост на антиоксидантния капацитет на експериментални червени вина от сортовите различия и компонентния състав

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Резюме

Редица изследвания показват, че червените вина се характеризират с високо съдържание на съединения, притежаващи антиоксидантни свойства. Настоящата работа има за цел да се изследва Тоталния Антиоксидантен Капацитет (ТАК) на серия експериментални червени вина, получени от различни сортове грозде. Допълнително е направен корелационен анализ между компонентния състав на вината и ТАК. Вината са произведени от 5 сорта червено грозде, отгледани в района на град Плевен, реколта 2004 г., преработени в подходяща технологична зрялост, в условията на микровинифициране по класическата схема за производство на червени вина. За определяне на ТАК са използвани два метода - FRAP и ABTS (TEAC). И според двата аналитични теста класацията на червените вина е следната: Памид (5,84 мкмол Fe^{2+}/мл за FRAP и 1.37 мкмол Trolox/мл) < Гъмза < Мерло < Пино ноар < Кабарне совиньон (34,81 мкмол Fe^{2+}/мл за FRAP и 8,19 мкмол Trolox/мл). Наблюдава се положителна корелация между ТАК и съдържанието на феноли (rFRAP 0,996, rABTS 0,989), антоциани (r_{FRAP} 0,834, r_{ABTS} 0,821), пролин (r_{FRAP} 0,702, r_{ABTS} 0,690), белтъци (r_{FRAP} 0,968, r_{ABTS} 0,978), титруеми к-ни, захари и алкохол. Изненадващо връзката на ТАК с количеството на реактивоспособните тиолови групи във вината е обратнопропорционална (r_{FRAP} -0,881, r_{ABTS} -0,901). Базирайки се на тези резултати ние предполагаме, че основна предпоставка за високият ТАК на червените вина е значителното съдържание на феноли, антоциани, пролин и белтъци.

Ключови думи: *ABTS (TEAC) анализ, антиоксидантен капацитет, FRAP анализ, червени вина, пролин, феноли, тиолови групи*

Antioxidant capacity of experimental red wines in relation to cultivar differences and chemical composition

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Summary

Numerous studies indicate that red wines are rich in compounds possessing antioxidant properties. The present study aims at investigating the Total Antioxidant Capacity (TAC) of a series of red wines. Additionally, a correlation coefficient between TAC and some compounds is obtained. Grape from 5 cultivars cultivated in Pleven district, vintage 2004 is used. TAC is determined according to FRAP and ABTS (TEAC) methods. Both assays rank the red wines as follows: Pamid (5.84 μmol Fe^{2+}/ml for FRAP and 1.37 μmol Trolox/ml) < Gamsa < Merlot < Pinot Noir < Cabernet Sauvignon (34.81 μmol Fe^{2+}/ml for FRAP and 8.19 μmol Trolox/ml). A positive correlation between TAC and the content of phenols (r_{FRAP} 0,996, r_{ABTS} 0,989), anthocyanins (r_{FRAP} 0,834, r_{ABTS} 0,821), proline (r_{FRAP} 0,702, r_{ABTS} 0,690), proteins (r_{FRAP} 0,968, r_{ABTS} 0,978), sugars and alcohol is observed. Surprisingly, the relationship between TAC and the amount of reactive thiol groups is reverse (r_{FRAP} -0,881, r_{ABTS} -0,901). On the basis of these results we assume that the high TAC of red wines is mainly due to the significant content of phenols, anthocyanins, proline and proteins.

Keywords: *ABTS (TEAC) assay, antioxidant capacity, FRAP assay, red wines, proline, phenols, thiol groups*

Въведение

Все по-голям брой епидемиологични изследвания показват превантивната роля на редица природни продукти спрямо разнообразни патологични състояния - от сърдечно-съдови заболявания до рак. Тези наблюдения стимулираха проучването на продукти от растителен произход, познати от векове, в търсене на терапевтични агенти. Фактът, че умерената консумация на вино е свързана с намаляването на риска от сърдечно-съдови заболявания, е известен с името "Френски парадокс". Френският парадокс (малкото случаи на сърдечно-съдови заболявания сред населението на Южна Франция на фона на богатата на наситени мастни киселини диета) бе отдаден на редовното консумиране на червено вино. (Renaud and De Lorgeril, 1992). Тъй като тези патологични състояния се свързват с увреждания, причинени от свободни радикали, би могло да се обобщи, че присъстващите в червените вина съединения проявяват антиоксидантна активност.

Вината съдържат голям брой фенолни съединения, повечето от които са с растителен произход. Почти всички от тях притежават антиоксидантни свойства. Възможността за екстрахирането им от семките и люспите на ▶

Early Detection of Changes in Leaf Reflectance of Pea Plants (Pisum sativum L.) under Herbicide Action

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Abstract - **Based on high resolution leaf spectral reflectance data a new technique was developed and applied to detect damages of agricultural plants under the action of low intensity stress factors (herbicides) which at very low concentrations could not be established by the standard biochemical and biometric techniques. Results are presented from a remote sensing study of the peculiarities of the leaf spectral reflectance of pea plants (*Pisum sativum L.*) treated with atrazine and 2.4-D (2.4 - phenoxyacetic acid) at three low concentrations (0.01 μM, 0.1 μM and 1 μM, respectively 2.15, 21.5, and 215 μg/l for atrazine and 2.59, 25.9, and 259 μg/l for 2.4-D) as compared to the field dose of these herbicides commonly used in the agricultural practice. The physiological status of the plants was assessed using biometric and biochemical parameters such as length, fresh weight, dry weight and electrolyte leakage. The high-resolution spectral data were obtained using a multichannel spectrometer in the visible and near infrared ranges of the electromagnetic spectrum in 128 channels at a spectral resolution (halfwidth) of 2.6 nm. Using the technique which employs discriminant analysis and other statistical methods we established the presence of statistically significant differences in the arising variations of the leaf spectral reflectance characteristics between control and treated plants in the green (520÷580 nm), red and near infrared (690÷800 nm) ranges of the spectrum.**

I. Introduction

Remote sensing techniques are well-established already non-intrusive tools in assessing changes in the structure and functioning of ecosystems. The remote sensing of the optical properties of different cultural plant species subjected to stress factors gives the possibility to distinguish symptoms below the subjective level of detection and timely to mitigate the risk of their action [1, 2]. In the last years there is a significant growth in the amount of published information for research efforts aimed to reveal the relation between the spectral reflectance properties of vegetation, its pigment concentration and biochemical parameters [3÷5]. The spectral characteristics of vegetation are determined mainly by scattering and absorption characteristics of the internal leaf structure and biochemical constituents. The pigments are the main factor that controls the leaf spectral response in the visible range whereas the water content is the determinant factor in the near and infrared ranges [6, 7]. Chlorophyll content, in particular, is indicative of photosynthetic capacity, productivity and stress. Its change leads to variations or is reflected by leaf spectral reflectance in the region 669÷747 nm ("red edge" and "slope") which is a reliable indicator for stress in plants [8, 9].

Atrazine and 2.4-D are the economically most important herbicides and have been widely used in crop production for the last 40 years. Atrazine [6-chloro-N-ethyl- (1-methylethyl)-1, 3, 5-triazine-2.4-diamine] is primarily used on maize, sorghum, and sugarcane. Photosynthesis is the major target of the atrazine. It blocks the photoinduced electron transport by specific binding to the Q_b site of the D1 protein in Photosystem 2 reaction center [10]. In fact, the herbicide-induced toxicity seems to require light and is thought to involve chlorophyll-mediated singlet oxygen ($^1O_2$) generation. The general view is that as the energy flow is not being used for photosynthetic electron transfer, the chances of forming triplet chlorophyll are increased, leading to $^1O_2$ formation and oxidative damage [11÷13].

2.4-D (2.4-dichlorophenoxyacetic acid) is a typical phenoxy herbicide, as it was introduced after the World War II and has shown excellent efficacy in the control of weeds. The endogenous control of auxin (plant hormone) levels is disturbed in plants treated with 2.4-D. This herbicide usually induces several abnormalities in growth, cell division, and morphology [11, 12].

It is well known that the extensive agricultural use of atrazine and 2.4-D has caused their wide accumulation in underground, surface, and tap water, soils, as well as their distribution by aerosols. The atrazine belongs to the herbicides which degradation in soils and water is occurring relatively slowly. Under field conditions less than 5 % of the atrazine and 1 % of simazine (similar herbicide) in soil is

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Fluorescence of leaves of pea plants treated with low concentrations of herbicide atrazine

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The effect of herbicide atrazine on the leaf fluorescence of pea plants (Pisum sativum; L.; cv. Scinado) is in the scope of this research. Plants were grown hydroponically in a growth chamber at standard conditions. Three day old seedlings were subjected to treatment with the sublethal amounts of herbicide. Atrazine was added to the nutrition medium in concentration 0.01, 0.1, and 1 μM. These concentrations are analogous to the environmental contaminations measured for this herbicide. The measurements were taken on the 8th and 14th days following the treatments. The fluorescence of detached leaves from 1st, 2nd and 3rd leaf nodes of control and treated plants were examined.

The leaf fluorescence was excited by 470 nm monochromatic light and registered by means of the Spectrometric System SPS-1, developed in Solar-Terrestrial Influences Laboratory, BAS.

Area and positional features (specific wavelengths) of the fluorescence spectrum were used as parameters to characterize the spectrum distribution.

Statistically significant differences were established between the mean values of indices of the fluorescence spectra of leaves of control and treated with 0.1 and 1 μM atrazine concentrations plants. The most pronounced changes in the fluorescence spectra of leaves of treated plants were observed at wavelength 684.3 nm.

Introduction

Atrazine [6-chloro-N-ethyl-(1-methylethyl)-1,3,5-triazine-2,4-diamine] belongs to the group of triazines and has been one of the most used herbicides in agriculture for the last several decades. It is mainly used for selective weed control in corn, sorghum and sugarcane. The target plants for atrazine consist of a wide variety of broadleaf and some grassy weeds.

The primary mechanism of action of atrazine is the inhibition of photosynthesis. It acts as a non-reducible analog of Qb that attaches itself to the Qb-site of the D1 protein in photosystem II reaction center and blocks electron transport in chloroplasts, thus halting CO2-fixation and production of ATP and NADPH2, all needed for plant growth. [1]. Moreover, as a result of the inhibition of photosynthetic electron flow chlorophyll molecule is not able to dissipate its excitation energy and so forms a high reactive triplet chlorophyll molecule. This triplet chlorophyll molecule reacts with oxygen resulting in the production of singlet oxygen 1O2. The singlet oxygen and triplet chlorophyll molecules cause lipid peroxidation, loss of membrane integrity and other oxidative damages [2].

The extensive agricultural use of atrazine has caused its wide accumulation in soils and waters. It is highly persistent in soils, has a relatively long half-life and significant amounts of it can be found in soil for more than a year after treatment [3]. Because atrazine does not absorb strongly to soil particles it has a high potential for groundwater pollution despite its moderate solubility in water [4]. Typical contaminations in agricultural areas are in the range of 1 – 100 μg per kg soil or liter water as maximal amounts reach up to 1 mg [5, 6]. The breakdown of atrazine is performed mainly through chemical hydrolysis followed by degradation by soil microorganisms and depends on the specific environmental conditions [7].

The influence of subherbicide concentrations analogous to the residual herbicide amounts in soils and waters on plants is poorly investigated. Jettner et al. [8] demonstrated the sensibility to atrazine of 22 plant species growing for 21–28 days on a soil-free system containing different concentrations of atrazine. The results obtained are rather noteworthy. The herbicide reduces the fresh weight of barley by 30% (ID30) up to 50% (ID50) in a concentration of 25 μg/l (sunflower–50 μg/l; wheat–100 μg/l, etc.). Moreover, De la Torre and Burkey [9] and Lambrev et al. [10] showed that sublethal concentrations of atrazine disrupted the normal photosynthetic function of plants (barley and pea, respectively) and the effect observed was amplified with time. These results are a clear indication that the long-term influence of trace amounts of atrazine probably leads to an effect similar to that observed after a single application of field doses. On the other hand it was reported that in some plants the same atrazine concentrations exhibit cytokinin-like properties [11].

Purpose of the researches

The aim of this study is to investigate the effect of the sublethal atrazine concentrations on the leaf fluorescence of young pea plants

Materials and methods

As a model system pea plants (Pisum sativum L. cv Scinado) were used. Seeds were soaked in tap water for 6-8 h and germinated on the wet filter paper in a germination chamber at 25oC in the dark for 3 days. Seedlings were grown hydroponically in Hoagland – Arnon nutrient solution in growth chamber (12/12 h day/night photoperiod; 60 – 90 μmol m-2 s-1 photon flux density; 26/22oC day/night tempera-ture; 60 – 70 % air humidity) The atrazine stock solution (0.1 mM) was prepared using Tween-80 (0.05% w/v). Three-day-old seedlings were subjected to herbicide treatment. Atrazine was added to the nutrient solution in 0.01, 0.1 and 1 μM concentration. The chosen concentrations are based upon the residual amounts of the herbicide measured in the environment. Nutrient medium was renewed twice a week. All measurements were made with the 1st, 2nd and 3rd leaves on the 8th and 14th days after the beginning of the experiment.

Effects of low concentrations of paraquat on the leaf spectral reflectance of pea plants (*Pisum sativum L.*)

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Results from a remote sensing study on arising changes in leaf spectral reflectance of pea plants in the visible and near infrared spectral ranges due to herbicide action are presented and discussed. The herbicide paraquat was applied at three low concentrations (0.01 μM, 0.1 μM and 1 μM) as compared with the herbicide field dose used in the agricultural practice. The paraquat is strongly toxic and its usage is restricted but because of the fast and typical effects forced in plants it is exceptionally appropriate for a model in studying herbicide action mechanisms. To assess the statistical significance of the differences found between leaf spectral reflectance characteristics of control and treated plants we applied an approach based on discriminant analysis and other statistical methods. High resolution spectral data were obtained using a STIL-BAS designed multichannel spectrometer. It was operated in the spectral range 480÷810 nm in 128 channels with 2.6 nm spectral resolution (halfwidth) and 2 mm² spatial resolution. The plant physiological status was assessed using biometric parameters such as fresh weight of the aboveground part of the plants and fresh weight of their root system. Statistically significant changes were established between the spectral reflectance characteristics of leaves of control and treated plants with herbicide concentrations of 0.1 μM and 1 μM.

Introduction

The spectral remote sensing in the visible and near infrared (VNIR) ranges of the electromagnetic spectrum finds more and more wide application to investigate in details the physiological responses of plants to the conditions of growth and the adaptation to environment on the basis of their optical properties. In general, the spectral reflectance of plant leaves is a function of their structure, water content and concentration of biochemical substances [1÷3]. The changes in leaf metabolism affect the basic growth processes such as photosynthesis, transpiration, mineral nutrition etc. and they are of an essential importance for the understanding of plant development and adaptation to the environmental stresses [4÷6]. In the last years there is an increasing amount of publications with results from studies on the impact of different stresses on the spectral peculiarities of plants [7÷11]. There is however a number of not yet clarified aspects regarding the stress physiology of plants and its reflection on their spectral reflectance characteristics (SRC). It is not enough clear as well the extent to which a low-intensive but with a prolonged action stress factor exerts an influence on the biochemical and spectral reflective properties of the plants.

In spite of the ever increasing requirements for protection of the environment the use of herbicides is a mandatory practice in the production of nutrition products for countries with a developed agriculture. The contemporary herbicides are the result from many years of research and selection. The yearly application of herbicides leads however not only to the acquisition of tolerance of the weed species to them but to their accumulation in the soils and the surface and underground waters. But the number of publications regarding the action of residual amounts of agrochemicals on their main targets (the plants) is very limited.

One of well studied herbicides with a contact action is the paraquat. As a chemical compound it is known since 1930 under the name of methyl viologen and it was used as an oxidizing reducing indicator but it was only just in the 50-ies to reveal its herbicide property [12]. Its advantages could be expressed most generally with the following [13, 14]: solubility in water and stability at a physiological pH, quick inactivation in soil, and an easy oxidation of its reduced form by molecular oxygen. The photo oxidation of thylakoid and other cell membrane lipids provoked by paraquat is the reason for plant death. The membranes are especially sensitive to the lipid peroxidation because they contain non-saturated fatty acids such as linolic (18:2) and linoleic (18:3). The molecular oxygen even without any herbicide effect is produced during the photolysis of water and generates active oxygen species but their amount significantly increases in the presence of paraquat and the endogenous protective systems of plants do not have enough capacity for their detoxification. It should be noted however that because of the extremely high toxicity of paraquat not only for plants but for warm-blooded as well in some countries of the world it became forbidden and in others its usage is limited. Nevertheless, because of the fast and typical effects brought in plants this herbicide is widely used as a suitable model in investigating the mechanisms of herbicide action.

During the last year our research has been focused on the influence that exert different herbicides (atrazine, glyphosate, paraquat, 2.4-D) when applied at low concentrations (0.01 μM, 0.1 μM, and 1 μM) as compared with the field doses used in agricultural practice on the reflective power of leaves of agricultural plants in the VNIR spectral range and on their biometric parameters and some stress markers [15, 16].

The present paper reports on results from a study of the particular influence of the herbicide paraquat on the spectral reflectance characteristics of leaves of pea plants and potential changes in the physiologic status of the plants which was assessed by biometric parameters.

To assess the statistical significance of the established differences between leaf spectral reflectance characteristics of untreated (control) and treated with the herbicide paraquat plants we applied the approach that we have developed

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СИНГЛЕТЕН КИСЛОРОД В РАСТЕНИЯТА – ОБРАЗУВАНЕ, ДЕТОКСИФИКАЦИЯ И ФИЗИОЛОГИЧНИ ФУНКЦИИ

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Резюме: *Публикацията представя основните аспекти от физиологичното и биохимичното действие на синглетния кислород (СК) в растенията. Описани са реакциите, водещи да образуването му, механизмите на светлинно-зависима продукция при фотосинтезата, както и свръхгенерирането на СК след въздействие с различни съединения (хербициди, багрила). Разгледани са химичните взаимодействия между СК и основните биологични макромолекули. Дискутирани са механизмите за детоксификация на СК в растителните клетки. Представени са основните хипотези за ролята му като сигнална молекула, изследванията, свързани с търсене на рецептори за СК, както и използването на мутанти и трансформанти за решаването на тези задачи. Описано е участието на СК в защитните реакции на растенията и връзките с вторичния метаболизъм. Приведени са примери, онагледяващи многообразните аспекти от действието му като активна кислородна форма.*
Ключови думи: *синглетен кислород, активни кислородни форми, растения, хербициди, антиоксиданти*

E. IVANOVA, S. IVANOV, Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Sofia. SINGLET OXYGEN IN PLANTS – GENERATION, DETOXIFICATION AND PHYSIOLOGICAL FUNCTIONS

Abstract: *The present publication features the main aspects of the physiological and biochemical action of singlet oxygen in plants. A description in made of the reactions leading to its generation, the mechanisms of light-induced production during the photosynthesis and the overproduction of singlet oxygen after treatment with various compounds, e.g. herbicides and dyes. The chemical reactions between singlet oxygen and the main biological macromolecules are presented. The mechanisms by which singlet oxygen is detoxified in plant cells are discussed. The major hypotheses for its role as a signal molecule, the search for singlet oxygen receptors and the use of mutants and transformants for achieving these objectives are reviewed. The role of singlet oxygen in plant defense response and its connections with the secondary metabolism are described. The various aspects of its function as an active oxygen species are amply illustrated.*
Key words: *singlet oxygen, reactive oxygen species, plants, herbicides, antioxidants*

Активни кислородни форми (АКФ). От времето, когато Joseph Priestley (1775) открива кислорода, проучванията върху свойствата на този изключително важен за живите организми химичен елемент не спират. Безспорната му необходимост за голяма част от биологичните процеси контрастира с негативите, съпътстващи неговото действие. Многобройни изследвания показват, че те са следствие от способността му да формира активни кислородни форми (АКФ) и спрегнатото с това развитие на радикалови реакции в биологичните обекти. Най-общо, АКФ могат да се разделят на свободни радикали и молекули (табл. 1). Свободните радикали по определение са частици, имащи несдвоени електрони. Най-съществени за живите организми са *in vivo* образуващите се супероксидни ($^{\bullet}O_2^-$) и хидроксилни ($^{\bullet}OH$) радикали, както и водородният пероксид (H_2O_2) и синглетният кислород (СК). Супероксидният радикал е първият продукт от редукцията на кислородната молекула и в този смисъл може да бъде разглеждан като предшественик на останалите АКФ **(1)**.

$$O_2 \rightarrow (H)\ ^{\bullet}O_2^- \rightarrow H_2O_2 \rightarrow {}^{\bullet}OH + H_2O \rightarrow 2H_2O \qquad \textbf{(1)}$$

Антиоксидантен капацитет на български кисели млека

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Резюме

Българското кисело мляко, получено след ферментация от млечно-киселите бактерии *Lactobacillus bulgaricus* и *Streptococcus thermophilus*, е оригинален, произвеждан от векове продукт. Редовната консумация на кисело мляко води до редица профилактични и лечебни ефекти за потребителите. В настоящата работа е изследван тоталният антиоксидантен капацитет (ТАК) на кисели млека, получени от 4 промишлени симбиотични заквaски (LBB.BY 37-18, LBB.BY 28-22, LBB.BY 144-12, LBB.BY 130-54), една стартерна култура, изолирана от растения („С") и изходното прясно краве мляко. Продуктът с крайно pH 4,7 е получен след инкубиране на 43°С. Преди определянето на ТАК пробите са разделени на нискомолекулна водоразтворима, липофилна и белтъчна фракции. За оценка на ТАК са използвани два метода - FRAP (Ferric Reducing/Antioxidant Power) и TEAC (Trolox Equivalent Antioxidant Capacity). Най-висок сумарен ТАК и според двата аналитични метода притежават млеката получени от заквaска 144-12 – 2,48 mmol Trolox/kg (TEAC) и 2,37 mmol Fe^{2+}/kg (FRAP), съответно. Най-ниски стойности са отчетени за изходното прясно мляко. Наблюдават се съществени различия между ТАК на киселите млека, получени от отделните заквaски. Основна роля за формиране на високия ТАК на киселите млека, в сравнение с прясното мляко, имат липофилните антиоксидантни съединения. Съгласно резултатите от настоящата работа ТАК на киселите млека е по-висок от този на някои зеленчуци и плодове (краставици, тиквички, банани, диня и др.).

Ключови думи: ABTS (TEAC) анализ, антиоксидантен капацитет, FRAP анализ, заквaски, кисело мляко, *Lactobacillus bulgaricus и Streptococcus thermophilus*

Antioxidant Capacity of Bulgarian Yogurts

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Summary

Bulgarian yogurt, prepared after fermentation with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, is an original product known from centuries. The regular intake of yogurt leads to various preventive and healing effects for the consumers. In the present study is investigated the total antioxidant capacity (TAC) of yogurts prepared with 4 commercial symbiotic starters (LBB.BY 37-18, LBB.BY 28-22, LBB.BY 144-12, LBB.BY 130-54), and one starter C isolated from plants. TAC of the initial bovine milk is also estimated as a standard. The product was produced at 43°C with final pH of 4.7. Prior to the determination of TAC the probes were separated on water-soluble, lipophofilic and protein fraction. Two methods for measurement of TAC were used - FRAP (Ferric Reducing/Antioxidant Power) and TEAC (Trolox Equivalent Antioxidant Capacity). Highest TAC according to the both methods was observed for the yogurt prepared with starter 144-12 – 2.48 mmol Trolox/kg (TEAC) and 2.37 mmol Fe^{2+}/kg (FRAP), respectively. The lowest values are detected in the initial fresh milk. Moreover, there are substantial differences between TAC of the yogurts prepared with distinct starters. Primary role for the higher TAC of the yogurts in comparison to the fresh milk has the lipophilic antioxidants. ⇨

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Remote sensing of the effect of the herbicide glyphosate on the leaf spectral reflectance of pea plants (*pisum sativum l.*)

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Keywords: leaf spectral reflectance, pea plant, stress, herbicide, discriminant analysis

ABSTRACT: Results from a remote sensing study on leaf spectral reflectance changes of pea plants due to herbicide glyphosate action applied at three concentrations (0.1 μM, 1 μM and 10 μM), low as compared to the herbicide field dose used in the agricultural practice are presented and discussed. The glyphosate is one of the most frequently used herbicides in Bulgaria, mainly in the common agriculture regions. Leaf spectral reflectance data were obtained by a multichannel spectrometer designed in STIL-BAS. The data were registered in the visible and near infrared spectral ranges (480 ÷ 810 nm) in 128 channels with 2.6 nm spectral resolution (halfwidth) and 2 mm^2 spatial resolution. The spectrometric measurements were performed on fresh, immediately picked off pea leaves in two leaf node on the 14th day after treating with the herbicide. To assess the statistical significance of the differences between leaf spectral reflectance characteristics of control and treated with the three glyphosate concentrations plants we applied an approach based on discriminant analysis and other statistical methods. The fresh weight of the plants was used as the biometric parameter to assess the changes in the plant physiological status. Statistically significant differences at $p < 0.05$ between the spectral reflectance characteristics of control and treated plants were established in the four most informative for plants spectral ranges: green (520 ÷ 580 nm), maximal chlorophyll absorption (630 − 680 nm) red (690 ÷ 730 nm) and near infrared (740 ÷ 810 nm).

1 INTRODUCTION

In the precision agriculture many studies have been devoted to the use of biotic information to diagnose plant responses to changing environment. To obtain the adequate information a special emphasis has been laid on the application of non-destructive and non-invasive measuring methods. In this respect remote sensing technologies provide an important tool to aid site-specific management of crops. Remote sensing has the potential to provide real-time analysis of the attributes of a growing crop. The results of such analyses form the basis for making timely management decisions that affect the outcome of the current crop. The information on plant response to growth conditions that is supplied by remote sensors such as spectrometers, radiometers etc. is used to achieve the

Изменения в антиоксидантните характеристики на пиво по време на ферментация

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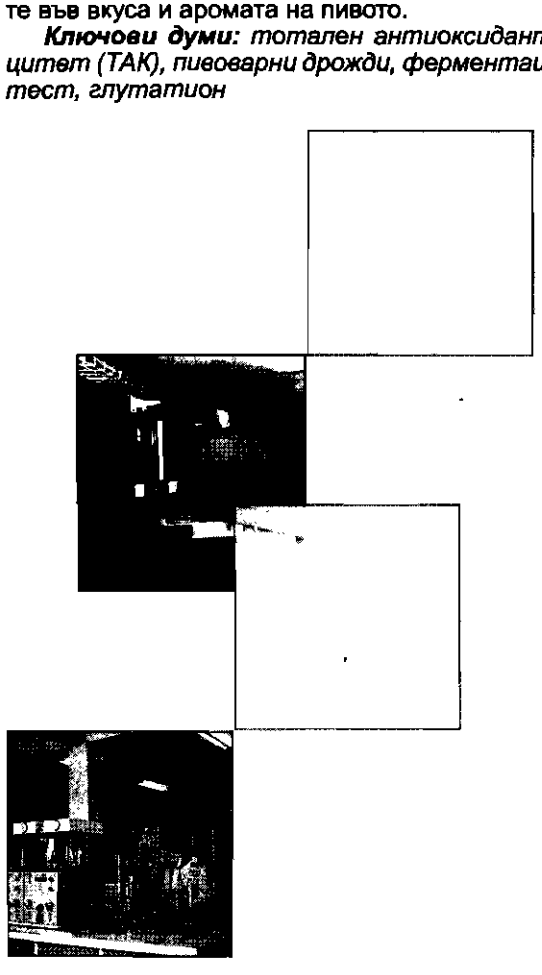
Резюме

Изследвани са промените в Тоталния Антиоксидантен Капацитет (ТАК), съдържанието на глутатион и общи феноли по време на пивоварния ферментационен процес (0 – 144 час). Установено е, че при еднакви други технологични параметри, ТАК на пивото се формира главно под действието на пивоварните дрожди. По време на ферментацията, най-високи стойности за ТАК се достигат в периода 72 – 96 час (1,102 Fe^{2+} мкмол/мл – FRAP тест), което съответства на максимумите в съдържанието на глутатион и плътността на дрождената култура. Освен като показател за здравословните ефекти от консумацията, ТАК може да се използва като критерий за подбор на подходящи щамове дрожди и маркер на процесите на стареене и промените във вкуса и аромата на пивото.

Ключови думи: *тотален антиоксидантен капацитет (ТАК), пивоварни дрожди, ферментация, FRAP тест, глутатион*



Changes In Antioxidant Characterizations During The Brewery Fermentatio

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Summary

The changes in Total Antioxidative Capacity (TA and in the amount of glutathione and total phenols dur the brewery fermentation were investigated. Under eq technological conditions, the Total Antioxidant Capacity the beer depends mainly on the type of the brewing yea During the fermentation the highest levels of TAC w reached between the 72nd and 96th hour (1,1 Fe^{2+} µm ml, FRAP assay), which corresponds to the maximu in the content of glutathione and the density of the ye culture. Apart from using TAC as an index for the he effects due to the consumption of the beverage, it can used as criteria in the selection of suitable yeast str and as a marker in the processes of aging and change the taste and flavour of the beer.

Key words: *Total antioxidant capacity (TAC), brew yeast, fermentation, FRAP assay, glutathione*

Въведение

Генерирането на активни кислородни частици (А е неизменна част от метаболизма на всички организ По своята химична природа АКЧ са както кратко щи радикали, така и стабилни молекули (Pryor 19 Високата реактивоспособност на АКЧ е причина реагират с клетъчните белтъци, липиди и нуклеин киселини. Оксидативните модификации на ключов биополимери са в основната на множество заболя ния, като атеросклероза, катаракти, някои видове р и др. Включването във всекидневната диета на хр с високо съдържание на антиоксиданти играе ва роля за поддържане на общия здравословен статус хората (Halliwell and Gutteridge 2002).

Remote Sensing Study of the Influence of Herbicides Fluridone and Acifluorfen on the Spectral Reflectance of Pea Plant Leaves (Pisum sativum L.)

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Abstract - **Results from a remote sensing study of the leave spectral reflectance of pea plants (Pisum sativum L. cultivar Scinado) treated by the photosynthetic herbicides fluridone and acifluorfen are presented. According to the mode of action, fluridone belongs to F1 (photobleaching) group of herbicides, and acifluorfen - to the group E as classified by the Herbicide Resistance Action Committee. The pea plants were grown hydroponically in a growth chamber in a nutritious medium to which the herbicides were added at two low concentrations (1 μM, 0.1 μM for fluridone, and 25 μM, 2.5 μM for acifluorfen). The high-resolution spectral data were obtained in the visible and near infrared ranges of the spectrum (450÷850 nm) using a USB2000 fiber optic spectrometer at a spectral resolution (halfwidth) of 1.5 nm. After data analysis, optimal spectral intervals for evaluation of the herbicide action were specified. The changes occurring in the spectral reflectance of the pea plants were assessed in four intervals: 520÷580 nm (region of maximal reflectivity of green vegetation), 640÷680 nm (region of maximal leave absorption), 690÷720 nm (red edge region), and 720÷770 nm (near infrared region) using the t-criterion of Student and linear discriminant analysis. Statistically significant differences were found between the spectral reflectance data of leaves of control and treated with herbicides plants at a significance level $p<0.05$ for the two fluridone concentrations and for 25 μM concentration of acifluorfen. The applied approach provides fast and reliable remote sensing of plant response to the environment.**

I. Introduction

The knowledge of spectral reflectance of crops has facilitated the development and use of various remote sensing methods for non-destructive monitoring of plants growth and development and for the detection of many environmental stresses which limit plant productivity. Coupled with rapid advances in computing and position locating technologies spectral remote sensing is now capable of providing detailed spatial information on plant response to their local environment.

In a number of studies it has been determined that spectrally similar changes in leaf spectral reflectance, transmittance, or absorptance occur in response to various biotic and abiotic stressors including pathogen inoculation, dehydration, flooding, low temperature, high salinity, tropospheric ozone, and herbicides among species that ranged from grasses to crops and trees [1÷4]. Due to the in vivo absorption properties of chlorophyll, the spectral changes are most clearly pronounced in the green-yellow and far-red spectral regions. For leaves or entire canopies, such spectral changes were shown to provide early or even pre-visible indications of plant stress [5÷7]. Increased reflectance in the red edge region (690÷720 nm) is a particularly genetic response, providing an earlier or more consistent indicator of stress. The consistency with which leaf optical properties near 700 nm change in response to stress among causes of stress and species indicates a general mechanism by which such changes occur [8, 9].

There are many attempts to achieve a better understanding of relationships between leaf spectral response to stress and changes in the content of chlorophyll and other pigments in an effort to develop improved methods of evaluating plant responses to environment [10÷15].

While spectral reflectance depends on such factors as available light, species, site, maturity, nutrient status, and leaf orientation of vegetation, one of the first general visual symptoms of physiological injury is vegetation yellowing, or chlorosis. However, depending on the nature of the stress and its effect on the plant's physiology, changes may occur in the infrared wavelengths before arising in the visible spectrum [16, 17]. In other words, chlorosis may appear after other changes appear that are not visible to the naked eye.

If a stress is manifested as a change in water concentration (turgor pressure) of a plant, typically the infrared wavelengths

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АНТИОКСИДАНТЕН КАПАЦИТЕТ НА ГРОЗДЕ И АЛКОХОЛНИ НАПИТКИ

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Резюме

Количеството и качеството на антиоксидантите са едни от най-съществените фактори, обуславящи, здравословните свойства на храните и напитките. През последните години в научната литература навлезе терминът "Тотален Антиоксидантен Капацитет – ТАК". ТАК е експериментално определян количествен показател, изразяващ способността на всеки биологичен обект да обезврежда свободни радикали. Многобройните предимства на процедурите за определяне на ТАК, наложиха бързото им усвояване в хранително-вкусовата промишленост. В настоящата работа са представени накратко методите за измерване на ТАК. Разгледани са областите на приложение на ТАК в научните и научно-приложните разработки, свързани с храните и напитките. Като примери са представени изследвания на авторите, използвали за експериментални обекти грозде, вина, пиво и др.

Ключови думи: антиоксидантен капацитет, вина, грозде, пиво, FRAP, TEAC

ANTIOXIDANT CAPACITY OF GRAPE AND ALCOHOLIC DRINKS

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Abstract

The health impact of foods and beverages presents in the human diet is due to the quality and quantity of their antioxidant content. The term "Total Antioxidant Capacity – TAC" has been widely used throughout recent years. TAC shows the ability of a sample to scavenge free radicals and is quantified by several assays. The numerous benefits of the procedures for determination of TAC, quickly established these test as useful tools in food-processing industry. Herewith, the basic methods for quantification of TAC are briefly reviewed. The areas of application of TAC related to food and beverages are discussed. As an example, the investigations of the authors regarding grape, wines and beer are described.

Key words: antioxidant capacity, wines, grape, beer, FRAP, TEAC

Количеството и качеството на антиоксидантите е един от най-съществените фактори, обуславящи здравословните свойства на храните, напитките и вината, т.к. активните кислородни частици участват в процесите на стареене на организмите, както и в повече от 100 вида болести (Ames et al. 1993; Halliwell and Gutteridge 2002). Антиоксидантите са молекули, съдържащи се в сравнително ниски концентрации във всички биологични обекти, които могат да намаляват степента на оксидативно разграждане, или напълно да предпазват останалите биомолекули от взаимодействие с активните кислородни форми. Повечето автори разделят антиоксидантите на ниско- и високомолекулни, или ензимни и неензимни (Halliwell 1990, Halliwell and Gutteridge 2002). От своя страна, нискомолекулните антиоксиданти се разделят на водоразтворими и липофилни. Фигура 1 показва най-съществените за организмите антиоксидантни съединения.

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Detection of Herbicide Contamination in Plants through Changes in Leaf Spectral Reflectance and Fluorescence

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ABSTRACT

Remote sensing techniques were applied for investigation of the leaf spectral reflectance and fluorescence of pea plants (*Pisum sativum* L.) treated by herbicides, atrazine and paraquat at three concentrations (0.01 μM, 0.1 μM and 1 μM) which are lower than the herbicides field dose used in agriculture. High-resolution spectral data for leaf reflectance in the visible and near infrared ranges (480—810 nm) of the spectrum and for fluorescence in the spectral range 650—850 nm were obtained with two multichannel spectrometers. The arising changes in spectral characteristics were estimated by a technique, which employs discriminant analysis and other statistical methods. Statistically significant differences were established between leaf reflectance of control and treated plants with herbicides concentrations 0.1 and 1 μM in the four investigated spectral ranges (green, red, red edge and near infrared). Several indices used in order to characterize the differences between fluorescence spectra of leaves of control and herbicide treated plants confirmed the presence of stress except for the lowest concentrations.

INTRODUCTION

One potential use of remote sensing is in the assessment and management of natural areas. Remote sensing in this field has focused on the spectral and spatial properties of vegetation and surrounding landscape, and the biophysical characteristics of crops (Goel *et al.* 2003). This knowledge has facilitated the development and use of various remote sensing methods for non-destructive monitoring of plant growth and helped in the detection of many environmental stresses that limit plant productivity. Coupled with rapid advances in computing and position locating technologies, remote sensing from ground, air, and space-based platforms is now capable of providing detailed spatial and temporal information on plant response to their local environment, which is needed for site-specific agricultural management approaches (Pinter *et al.* 2003, West *et al.* 2003).

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ВЛИЯНИЕ НА СОРТОВИТЕ ОСОБЕНОСТИ ВЪРХУ СЪДЪРЖАНИЕТО НА АНТИОКСИДАНТИ В ГРОЗДЕТО

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Influence of Variety Specifics on the Antioxidant Content of Grape

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Abstract

Number of investigations shows that consumation of foods and drinks with high content of antioxidants have preventive or therapeutic effect towards many illnesses. Grape, as well as wine (especially red wine) and juices prepared from grape are among the foods that have high concentration of natural antioxidants. In the present work total antioxidant capacity (TAC) of 7 different grape varieties was investigated. Three of the grape varieties are typical wine cultivars: Cabernet sauvignon, Strogosia and Gamza. The other 4 varieties Bolgar, Brestovitsa, Palieri and Hamburg misket are mainly desert varieties although they can be used for wine production as well. For TAC analysis two standard analytical tests were used: FRAP and TEAC. The highest TAC was detected in Palieri (4.4 μmol Fe^{2+}/g FW for FRAP, 2.8 μmol Trolox/g FW for TEAC) and Cabernet sauvignon. With lowest TAC was Gamza variety (2.6_{FRAP}, 1.4_{TEAC}).

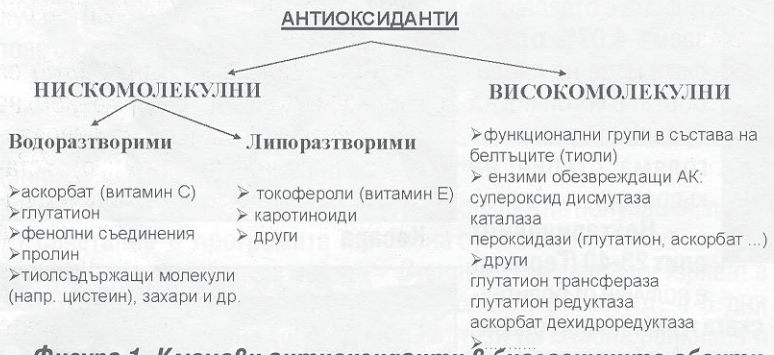
Key words: total antioxidant capacity, grape, wines, FRAP, TEAC

Редица изследвания показват, че консумацията на храни и напитки с високо съдържание на антиоксиданти оказва превантивен и/или терапевтичен ефект спрямо много широко разпространени болести. Гроздето, вината (особено червените), както и всички гроздови соковете, се причисляват към хранителните продуктите, притежаващи високи концентрации естествени антиоксиданти. В проведеното изследване е определен тоталният антиоксидантен капацитет на грозде, събирано от 7 отделни сорта лози - Каберне совиньон, Сторгозия, Гъмза, Болгар, Брестовица, Палиери и Хамбургски мискет.

АНТИОКСИДАНТЕН КАПАЦИТЕТ НА ГРОЗДЕ И АЛКОХОЛНИ НАПИТКИ

Количеството и качеството на антиоксидантите е един от най-съществените фактори, обуславящи здравословните свойства на храните, напитките и вината, тъй като активните кислородни частици участват в процесите на стареене на организмите, както и в повече от 100 вида болести (Ames et al. 1993; Halliwell and Gutteridge 2002). Антиоксидантите са молекули, съдържащи се в сравнително ниски концентрации във всички биологични обекти, които могат да намаляват степента на оксидативно разграждане, или напълно да предпазват останалите биомолекули от взаимодействие с активните кислородни форми. Повечето автори разделят антиоксидантите на ниско- и високомолекулни, или ензимни и неензимни. От своя страна, нискомолекулните антиоксиданти се разделят на водоразтворими и липофилни. Фигура 1 показва най-съществените за организмите антиоксидантни съединения.

През последните години интересът към антиоксидантите непрекъснато нараства. Вината, особено червените, се причисляват към напитките, притежаващи високи концентрации на естествени антиоксиданти. Умерената консумация на вино е свързана с намаляването на риска от сърдечно-съдови заболявания, т.нар. „френски парадокс". Френският парадокс, който представлява малкото случаи на сърдечно-съдови заболявания сред населението на южна Франция на фона на богатия на наситени мастни киселини хранителен режим, се обяснява с редовното консумиране на червено вино. Тъй като тези патологични състояния на сърдечно-съдовата система се свързват с увреждания, причинени от активните кислородни частици, би могло да се обобщи, че присъстващите в червените вина антиоксидантни съединения са ключова причина за наблюдавания профилактичен ефект. Вината съдържат голям брой фенолни съединения, повечето от които са с растителен произход. Почти всички от тях притежават способности за обезвреждане на активните кислородни частици. Разбира се, основен източник на съединения с антиоксидантни свойства във винената е суровината за тяхното производство - гроздето. Само по себе си, приемането на грозде също води до положителни здравословни ефекти за хората. Например, изследването на Stein и Keevil (1999) показва, че консумирането на гроздов сок понижава LDL окислението при болни от коронарна артерия.



Фигура 1. Ключови антиоксиданти в биологичните обекти.

За задоволяване на нарастващия интерес към антиоксидантните характеристики на храните, напитките и вината през последните години в научната литература навлезе терминът „Тотален Антиоксидантен Капацитет - ТАК". По дефиниция, ТАК е експериментално определян количествен показател, изразяващ способността на всеки биологичен обект да обезврежда свободни радикали. ТАК позволява да се отчитат синергистичните, кумулативните и антагонистичните взаимодействия между отделните антиоксиданти в пробите. **Към настоящия момент в употреба са въведени няколко десетки метода за определяне на ТАК. Най-широко използвани в практиката са 4 метода: ORAC** (Oxygen-Radical Absorbance Capacity), **TRAP** (Total Radical-Trapping Antioxidant Parameter), **TEAC** (Trolox Equivalent Antioxidant Capacity) и **FRAP** (Ferric/Reducing Antioxidant Power).

Редица предимства на процедурите за измерване на ТАК, като бързина, ниска цена, възможност за стандартизация, употреба на широко разпространена апаратура и други, наложиха бързото им усвояване в хранително-вкусовата промишленост. Областите на приложение на този вид изследвания все повече се разширяват. Засега най-разпространени са експериментите, свързани със сравнителни изследвания на антиоксидантното съдържание на храни и напитки, включително вина и пиво, от различен произход.

В следващите абзаци ще бъдат разгледани накратко някои разработки, осъществени от авторите на настоящата статия. В работата на Кърчев и съавт. (2005), се отчита ТАК на серия експериментални червени вина, получени от 5 различни сортове грозде, реколта 2004г. За определяне на ТАК са използвани два метода - FRAP и ABTS (TEAC). И според двата аналитични теста класацията на червените вина е следната: Памид < Гъмза < Мерло < Пино ноар < Каберне совиньон (Фигура 2). Допълнително е направен корелационен анализ между компонентния състав на винената и ТАК. Наблюдава се положителна корелация между ТАК и съдържанието на феноли, антоциани, аминоки-

Сравнителен анализ на антиоксидантното съдържание на пиво от търговската мрежа

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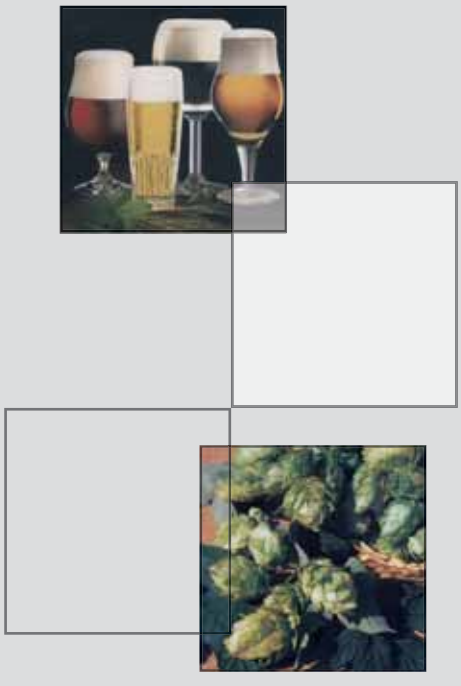
Резюме

Пивото е биотехнологичен продукт познат от древността. Понастоящем се произвеждат хиляди марки, всяка със своите уникални качества и вкус. Наред с другите компоненти, в пивото се съдържат и разнообразни антиоксиданти. В представената работа е изследвано съдържанието на антиоксиданти в 14 вида произведено в България и закупено от търговската мрежа пиво. Резултатите показват, че тоталният антиоксидантен капацитет (ТАК) на тези марки пиво е сходен с измерванията извършени от други автори. В абсолютни стойности съдържанието на антиоксиданти в пивото е аналогично на това в белите вина и по-високо от показателите отчетени за някои плодови сокове. Тъмното пиво притежава около два пъти по-висок ТАК от светлото. Между отделните брандове пиво се наблюдават големи различия в стойностите за ТАК, което най-вероятно е следствие от качеството на суровините и технологичните характеристики на производство.

Ключови думи: *тотален антиоксидантен капацитет (ТАК), пиво, TEAC тест, FRAP тест*



Comparative analysis of antioxidant content of commercially available beers

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Abstract

Beer is a biotechnological product known to the mankind since the dawn of time. Thousands of different brands are produced nowadays, each having its unique qualities and flavour. Apart from the other components, beer contains various antioxidants. We have studied the antioxidants present in 14 commercially available beer brands bought on the Bulgarian market. Our data show that the total antioxidant capacity (TAC) of those brands is similar to previously published results of other authors. TAC of beers is close to that reported for white wines and higher than some fruit juices. Dark beers are characterized with twice higher antioxidant content than the light ones. There are significant differences between the studied brands which are probably due to variability of the raw material and technological regimes.

Key words: *Total antioxidant capacity (TAC), beer, TEAC assay, FRAP assay, glutathione*

Въведение

Познато още от древността, пивото винаги е било освежителна напитка носеща със себе си характерните хмелов аромат и вкус. То се произвежда от пълноценни естествени суровини: малц от ечемик или пшеница, хмел, пивни дрожди и вода. Както и при останалите натурални храни, в пивото се съдържат различни по своята природа и значение вещества: въглехидрати, аминокиселини, витамини (особено от групата В), ан-

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AN ANTIOXIDANT CAPACITY OF SELECTED BULGARIAN WINES

ANTIOXIDAČNÁ KAPACITA VYBRANÝCH BULHARSKÝCH VÍN

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The Total Antioxidant Capacity (TAC) of 11 red and 9 white Bulgarian wines was determined using three different methods (TEAC, FRAP and DPPH). Part of the wines was cultivar defined and other part was a blend of several grape cultivars. Different vintages were used for some wines to make it possible to compare the influence of aging on the antioxidant capacity of the final product. All red wines possess more antioxidant compounds than the white ones as confirmed by the three methods. The highest TAC from the white wines showed a White wine blend, vintage 2006, and Tamyanka. The lowest TAC had the wine produced form Muskat cultivar. An interesting correlation confirmed by the three methods was observed among the wines Chardonnay vintage 2001, 2004 and 2006 – as the oldest one had the best antioxidant characteristics. No significant differences were found between the values for the first 6 red wines with highest TAC – Cabernet sauvignon, vintage 2001, 2005, 2006; Merlot, Mavrud and Melnik. The Lozishka gamza wine had the lowest antioxidant content. With the aging, the wine Cabernet sauvignon decreased its TAC measured according to the TEAC assay.

Key words: wine, antioxidant capacity, TEAC, FRAP, DPPH

Several research studies have established an inverse correlation between the consumption of food rich in antioxidants and the occurrence of diseases such as inflammation, cardiovascular disease, cancer, and ageing-related disorders (Cao et al., 1998; Lee et al., 1999; Duffy et al., 2001; Anderson et al., 2001; Roberts et al., 2002). Dietary antioxidants, including polyphenolic compounds, vitamin E and C, and carotenoids, are believed to be effective nutrients in the prevention of these oxidative stress related diseases (Halliwell and Gutteridge, 2002).

Red wines are rich in polyphenolic compounds and their moderate consumption has been recognized as beneficial to health for centuries (Waterhouse, 2002). The rare occurrence of cardiovascular diseases among the population of southern France, known for its diet rich in saturated fatty acids, was related to the regular consumption of red wine. The phenomenon is known as the "French paradox" (Renaud and De Lorgeril, 1992). The beneficial health effects of polyphenols are mainly related to their antioxidant properties (Bors and Michel, 2002). However, not all positive effects have to be related to the polyphenols. Furthermore, the synergistic and cumulative interactions between the known and unknown antioxidants and the matrix of the sample have to be taken into account when regarding the antioxidant properties of wines. The only way to detect all the substances having antioxidant properties and the exact role they play is to use different methods for determination of the Total Antioxidant Capacity (TAC). The meaning of TAC in the following investigation refers to the cumulative capacity of the antioxidants present in the sample to detoxify reactive oxygen species (Sanchez-Moreno, 2002; Kerchev and Ivanov, 2008).

Wine has been known in Bulgaria since ancient times. Today, vine-growing and wine-making plays a crucial role in the economy of the country. The wine industry contributes to the steady development of rural regions and infertile areas, maintains the ecological balance and encourages the appropriate and efficient use of the country's resources. Thus an investigation aiming at evaluating the quality and possible health effects of Bulgarian wines in terms of antioxidant properties will be useful not only as a scientific indicator for wine producers. It will also benefit the final consumer and reveal the quality of this product of importance.

The purpose of this study is to investigate the antioxidant properties of wines produced in Bulgaria. Our research was focused on the TAC of selected commercial and experimental red and white wines. Wines from different vintages and distinct grape cultivars were compared according to their antioxidant characteristics.

Materials and methods

Experimental objects and sample preparation

Twenty wine samples (9 white and 11 red) produced in different regions of Bulgaria, from different grape cultivars and vintages were used for the research. Five of them, Muskat (2004), Tamyanka (2004), Traminer (2004), Mavrud (2005) and Melnik (2005) were bought at the market. The rest of the wines was produced in the Experimental wine cellar of the Institute of Viticulture and Enology, Pleven. Two white and one red wine were blended from several cultivates. Different vintages from Chardonnay (2001, 2004, 2006), White wine blend (2005, 2006), Cabernet sauvignon (2001, 2005, 2006) and Cabernet sauvignon clone D1 (2003, 2006) were chosen in the present study.

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BIOLOGIE
Physiologie des plantes

EFFECT OF PROLONGED ACTION OF SUBHERBICIDE CONCENTRATIONS OF 2,4-D ON THE GROWTH AND SOME STRESS MARKERS OF PEA (*PISUM SATIVUM* L.) PLANTS

S. Ivanov, V. Alexieva, E. Karanov

(*Submitted on September 18, 2001*)

Abstract

The effect of prolonged action of low concentrations of herbicide 2,4-D was studied. As a model system pea plants (*Pisum sativum* L.) were used. The plants were grown as water cultures and the herbicide was added to the nutrient medium at concentrations 10^{-5}, 10^{-6} and 10^{-7} M. The chosen concentrations are 1 000 to 100 000 times lower than those used in field practice and mimic residual amounts in underground water. Fresh and dry weight ratio, chlorophyll level, relative water content and amount of some stress markers (free proline and hydrogen peroxide) were measured on the 5th, 10th, 15th and 20th day after the beginning of the stress programme. It was found that all subherbicide concentrations studied of 2,4-D inhibited growth and relative water content and increased the level of stress markers studied.

Key words: herbicide, 2,4-D, stress markers, residual amounts of herbicides

Weed control through the use of herbicides is becoming an increasing trend in modern agricultural practice. Despite of extensive attempts to save the environment, the application of pesticides is inevitable technique in crop production [1]. However, repeated use of even the most effective herbicides normally caused shifts within weed population to weed species nearly as tolerant to the herbicide as the crop themselves [2].

Unfortunately, a little information is available about the post-effect of the widely used herbicides. It is well known that even the fact that each herbicide is used for a particular crop-weed situation and is applied in a strictly determined dosage, area and period of time, usually in the following months some remaining amounts of the herbicide are spread by rains and underground water on considerably larger areas than those where they have been initially applied. Such herbicide contamination even as traces (very low concentrations) get in contact with another crop plants and ecosystems. The precise process of the herbicide dispersion is difficult to be followed, but even in low concentrations the compounds undoubtedly reach plant and animal organisms, which initially were not intended to be affected [3].

The aim of this study was to evaluate the effect of low concentrations of 2,4-D, which mimic the residual amounts of this herbicide in soils and underground water. For this purpose growth parameters, chlorophyll content and some stress markers were measured.

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BIOLOGIE
Physiologie des plantes

EFFECT OF PROLONGED ACTION OF SUBHERBICIDE CONCENTRATIONS OF 2,4-D ON THE ACTIVITIES OF SOME STRESS DEFENCE ENZYMES IN PEA (*PISUM SATIVUM* L.) PLANTS

S. Ivanov, L. Miteva, V. Alexieva, E. Karanov

(*Submitted on October 24, 2001*)

Abstract

The effect of prolonged action of low concentrations of herbicide 2,4-D was studied. As a model system pea (*Pisum sativum* L.) plants were used. The plants were grown as water cultures and the herbicide was added to the nutrient medium at concentrations 10^{-5}, 10^{-6}, and 10^{-7} M. The chosen concentrations are 1000 to 100000 times lower than those used in field practice and mimic residual amounts in underground water. The activities of some stress defence enzymes (catalase, CAT, peroxidase, POA, superoxide dismutase, SOD, glutathione-S-transferase, GST) were measured on the 5th, 10th, 15th and 20th day after the beginning of stress programme. It was found that even the lowest concentration of 2,4-D increased the activities of POA, SOD, GST, while CAT activity was strongly inhibited. The higher concentrations of herbicide caused an increase in most of the investigated enzymes activity.

Key words: herbicide, 2,4-D, stress defence enzymes, residual amounts of herbicides

Introduction. The effects and mode of action of the most widely used herbicides are known and described in details [1,2]. Moreover, for most of them the soil persistence and residual amounts are also well documented [3-5]. However, with a small exception, a little information is available about the action of their effects on the growth of crops which are not intended to be preliminary affected.

The persistence of the most used herbicides in the soil ranges from several weeks to months (and even years). Despite the fact that each herbicide is used for a particular crop-weed situation and is applied in a strictly determined dosage, area and period of time, usually in the following months some residual amounts of the herbicide are spread by rains and underground water on far larger areas than those on which they have been initially applied. These herbicide traces get in contact with other crop plants and ecosystems. The precise process of the herbicide dispersion is difficult to be followed, but even in low concentrations the compounds undoubtedly reach the plant and the animal organisms, which initially have not been the aim of this impact. Stress factors with characteristics such as low intensity, different chemicals and duration, periodically influence the plant populations, and combine with additional unfavourable environmental conditions (natural and anthropogenic).

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BIOLOGIE
Physiologie des plantes

INTERACTION BETWEEN SUB-HERBICIDE CONCENTRATION OF 2,4-D AND HIGH TEMPERATURES IN YOUNG PEA (*PISUM SATIVUM* L.) PLANTS

S. Ivanov, V. Alexieva, E. Karanov

(*Submitted on June 6, 2002*)

Abstract

The effect of sub-herbicide concentration of 2,4-D and high temperatures applied alone and in combinations was studied. As a model system young pea plants (*Pisum sativum* L.) were used. It was found that combined application of both stressors inhibited plant growth, increased the level of stress markers (leakage of electrolytes, proline, hydrogen peroxide content and amount of malondialdehyde) more significantly than those provoked by single treatment. An extra accumulation of the amount of stress markers can be considered as an oxidative burst.

Key words: herbicide, 2,4 D, stress markers, low concentrations of herbicides, high temperatures, oxidative burst

Introduction. The synthetic auxins represent one of the economically most important and widely used group of selective herbicides. A typical phenoxy herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) is a pioneer herbicide, as it was introduced after the World War II and has shown excellent efficacy in the control of weeds. 2,4-D exhibits strong hormonal auxin activity and brings about growth inhibition in broad-leaved plants. Presently, it is one of the most used herbicides of the selective weed control [1-2]. The environmental fate of 2,4-D and of its derivatives was emphasized and reviewed extensively. Accordingly, the soil persistence of 2,4-D (and some of its derivatives) varies from 59 to 390 days (depending on the soil and climatic conditions) [3]. Such contaminations even as traces come into contact with other crop plants and ecosystems. Recently, we have shown the effect of prolonged action of low 2,4-D concentrations on the growth, level of some stress markers and activities of stress defence enzymes in young pea plants [4-5]. Inhibition of plant growth and activation of defence systems were found. However, it should be clearly noted that the concentration of 2,4-D studied – 0.1 μM is almost equal to the values detected both in surface and in underground water. The low herbicide concentrations applied in our model system can be estimated as "trace amounts", and the result of their action can be considered as "post/or side effect" of 2,4-D application.

Normally, under environmental conditions plants are exposed to a complex of stress factors [6]. The changes in the seasonal or/and daily temperature is one of the most important factors affecting plant development and, in general, plant productivity. Unfortunately, little information is available about the interaction between the effect of herbicides and other environmental conditions. In this study interaction between the low concentration of herbicide 2,4-D and high temperatures on the growth and some stress markers in young pea plants was investigated. The day/night temperature

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BIOLOGIE
Physiologie des plantes

EFFECT OF INTERACTION BETWEEN SUB-HERBICIDE CONCENTRATION OF 2,4 D AND HIGH TEMPERATURES ON THE ACTIVITIES OF SOME STRESS DEFENCE ENZYMES IN PEA (*PISUM SATIVUM* L.) PLANTS

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(*Submitted on February 26, 2003*)

Abstract

The effect of sub-herbicide concentration of synthetic auxin 2,4 D and high temperatures applied alone and in combination was studied. The pea plants grown as water cultures were used as a model system. The herbicide was added to the nutrient medium at concentration 1 μM. Five days later part of the plants was subjected to 48 h high temperature stress. It was found that heat and combined treatment increased the activity of stress defence enzymes guaiacol peroxidase, superoxide dismutase, and glutathione-S-transferase but inhibited catalase activity (only at combination). The changes observed are most probably due to oxidative events induced by the stressors in the plants.

Key words: *Pisum sativum*, low concentrations of herbicides, 2,4 D, synthetic auxin, high temperatures, defence enzymes, oxidative processes, stress interaction

Abbreviations: SOD – superoxide dismutase, GST – glutathione-S-transferase, 2,4 D – 2,4-dichlorophenoxyacetic acid, IAA – indole-3-acetic acid

Introduction. For more than 50 years synthetic substances possessing auxin activity have been among the most successful herbicides used in agriculture [1]. A typical phenoxy herbicide (2,4 D) is a pioneer herbicide, as it was introduced after the World War II and has shown excellent efficacy in the control of weeds. The environmental fate of 2,4 D and its derivatives were emphasized and reviewed extensively. Accordingly, the soil persistence of 2,4 D (and some of its derivatives) varies from 59 to 390 days (depending on soil and climatic conditions) [2]. According to Environment Agency, 1999 [3], an increased level of 2,4 D was found in 5.4% of all collected underground water samples. These herbicide traces get in contact with crop plants and ecosystems. Recently we have shown the effect of prolonged action of low 2,4 D concentrations on the growth level of some stress markers and activities of stress defence enzymes in young pea plants [4,5]. Inhibition of plant growth and activation of defence systems was found.

Normally, under environmental conditions plants are exposed to a complex of stress factors. The changes in the seasonal or/and daily temperature is one of the most important factors affecting plant development and in general, plant productivity. In