РЕЗЮМЕТА

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PLANT GROWTH REGULATING ACTIVITY OF SOME PHOSPHORUS DERIVATIVES OF COUMARIN

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Summary. Plant growth regulating activity of some new synthesised phosphorus containing derivatives of coumarin was investigated. It was shown that some of the compounds inhibited the stem growth of intact pea plants, growth of wheat and cucumber seedlings as well as elongation of excised wheat coleoptile segments. Chemical structure – plant growth regulating activity relationship was also established.

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SYNTHESIS AND PLANT GROWTH REGULATING ACTIVITY OF SOME NOVEL 2-METHOXY-4-(1- OR 2-PROPENYL)-6-SUBSTITUTED PHENOLS

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Summary. The synthesis and plant growth regulating activity of some novel 2-methoxy-4-(1- or 2-propenyl)-6-substituted phenols are described. Most of the compounds possessed cytokinin like activity – they stimulated betacyanin synthesis in *A. caudatus* cotyledons, growth of excised radish cotyledons and induced retardation of chlorophyll disappearance in radish leaf discs. Some chemical structure – plant growth regulating activity relationships have been established.

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003

Cytokinin and Anticytokinin Effects on Growth and Free Polyamine Content in Etiolated and Green Radish Cotyledons

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Summary

The effects of high active cytokinins (kinetin and 4-PU-30) and their structurally related antagonists, 4-cyclopentyl-amino-2-methylthiopyrrolo[2,3-d]pyrimidine (CPP) and N-(4-pyridyl)-O-(4-chlorophenyl)-carbamate (PC), on growth and free polyamine content of green and etiolated radish (*Raphanus sativa* L.) cotyledons were studied. Both cytokinins expressed strong stimulating activity, but subsequent application of the anticytokinins partly removed kinetin and 4-PU-30 action. The effects observed were found to be stronger when structural analogy between cytokinin and antagonist existed.

Plant Growth Regulation 16: 109–112, 1995.

004

Anticytokinin effects on *in vitro* response of embryogenic and nonembryogenic genotypes of *Dactylis glomerata* L.

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Abstract

The effect of triazine and carbamate type of anticytokinins on *in vitro* response of embryogenic and nonembryogenic genotypes of orchardgrass (*Dactylis glomerata* L.) was studied. Both compounds stimulated callus production. Anticytokinins influenced both the efficiency of somatic embryogenesis and frequency of embryoid formation.

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Higher Institute of Agriculture - Plovdiv, Jubilee Scientific Session, Abstracts and papers, October 1995, volume II, book 2

005

INFLUENCE OF SOME PHYTOEFFECTORS ON THE DEVELOPMENT OF SPATHIPHYLLUM

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Abstract

Increasing the number of basal shoots of spathiphyllum can ensure better appearance and decrease the number of plants per pot which makes it significant for the commercial practice. The formation of shoots is possible to be affected by the application of substances with physiological activity.

The aim of our study was to investigate the effect of some cytokinin, cytokinin-like compounds and polyamines on the development of shoots and the number of leaves of Spathiphyllum hybridum, cv. Supreme. Foliar spray or soil drenching of 10-12 weeks old plants were provided with three different concentrations of 4-PU-30, TDZ, Glean, DETA and BA. The treatment by sprying with 4-PU-30 and DETA were found to stimulate shoot development and to increase the number of shoots and leaves. Positive response to Glean was better pronounced when applied by drenching.

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PLANT GROWTH REGULATING ACTIVITY OF SOME NOVEL HETEROCYCLIC COMPOUNDS – SUBSTITUTED HYDANTOIN, BENZOXAZOLON AND BENZOTHIAZOLE

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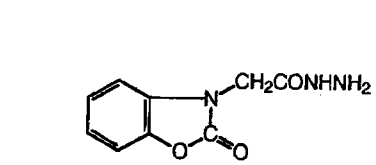
(Submitted on November 30, 1995)

Despite of the extensive attempts to safe the environment the use of herbicides and plant growth regulators is inevitable technique in crop production. 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. Resistance biotypes of normally sensitive weed species have become problems after repeated use of particular herbicides. The problem could be solved with new herbicides. However, with the increased development costs to design, synthesize, screen, characterize and document the toxicological and environmental safety of new chemicals, it is often wiser to prepare new chemicals with slight chemical modifications, which possess structurally active moieties of known herbicides or plant growth regulators.

In this paper the herbicidal and plant grow regulating activity of three novel compounds 2-(benzoxazolon-3-yl-acetyl) hydrazide (1), 6-methoxy-2-aminobenzothiazole (2) and 1-(2-hydroxyphenyl)-3-aminohydantoin are described.

Materials and methods. Synthesis of the compounds was described previously [1]. Plant growth regulating activity was determined in accordance with their influence on the growth of 2-week-old pea (*Pisum sativum* L. cv. Koray), plants grown as a water culture in a growth chamber



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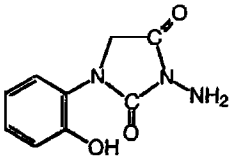

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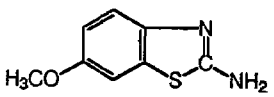

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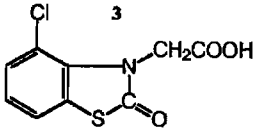

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EFFECT OF CHLORSULFURON (GLEAN-75) AND SUCROSE ON SOME POST-HARVEST PHYSIOLOGICAL EVENTS IN CUT FLOWERS

007

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Summary. Treatment of cut flowers with cytokinins is found to be beneficial in delaying senescence processes but the response to cytokinin application varies depending on cultivar, stage of flower development and type of cytokinin. The present study was conducted in view of exploring the effect of herbicide Glean-75 [chlorsulfuron; N^1-(4-methoxy-6-methyl-*sim*-triazine-2-yl)-N^2-(2-chlorophenyl-sulphonyl)urea] with cytokinin properties on the post-harvest life of cut chrysanthemum and rose flowers and of investigating the influence of this ingredient on the activity of α-amylase and invertase in petals. The experiments were conducted with open chrysanthemum flowers, cv. Westland, cold stored buds of cv. Walusa and cut at bud stage roses, cv. Sonia. The treatments were provided by holding solutions where sucrose, Glean-75 and their combination were used. Positive effect of chlorsulfuron on the elimination of leaf yellowing in chrysanthemums was established. The appearance of leaf damage was observed on the 18th day for cv. Westland which was 10 days over control flowers kept in water. Less pronounced effect was noticed on cold stored cv. Walusa where leaf injuries appeared on the 8th day after placing in the solutions. Treatment of cut roses cv. Sonia with Glean-75 + sucrose resulted in a longer vase life, successful opening and good quality of the flowers. The dynamics of α-amylase and invertase were associated with the phases of development. Decrease of the activities in controls during the phase of senescence was shown both for chrysanthemums and roses. An enhancement of enzyme activities by the compounds applied was monitored to be in a correlation with extended flower keepability.

ПРИЛОЖЕНИЕ НА СПЕКТРАЛНИ ОТРАЖАТЕЛНИ ХАРАКТЕРИСТИКИ ЗА ОТКРИВАНЕ НА РАННИ ФИЗИОЛОГИЧНИ ИЗМЕНЕНИЯ ИНДУЦИРАНИ ОТ ЕДИНИЧЕН И КОМБИНИРАН СТРЕС В МЛАДИ ЦАРЕВИЧНИ РАСТЕНИЯ

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В естествени условия растенията рядко се подлагат на единични стресови фактори. При едновременното действие на няколко стресови агенти често пъти увреждащото им действие превишава значително адитивния ефект от самостоятелното им приложение [1]. Единичен краткотрайен стрес в някои случаи обаче би могъл да повиши адаптационните възможности на растителния организъм, който впоследствие да преодолее по-лесно неблагоприятните последици от следващ стресов фактор (кръс-адаптация) [2].

Като моделна система за изследванията са използвани царевични растения хибрид "Княжа-530". При поява на трети лист растенията са експонирани 24 часа на ниска температура 5°С. След това са облъчвани за 15 минути в термостатираща ултравиолетова (UV) камера при 25°С с общо лъчение 1.37 Wm^{-2}. Експозиция от 15 минути е почти 2 пъти по-висока от естествената UV радиация за Европа [3].

Спектралните коефициенти на отражение (СКО) са получени с многоканална трасова спектрометрична система "Спектър 256", разработена в ЦЛСЗВ [4]. Високата спектрална разделителна способност на системата дава възможност отразената радиация от изследваните обекти да се измерва в 128 спектрални канала във видимата и близката ИЧ области на електромагнитния спектър (480-810 нм) през 2,6 нм. Измерванията са проведени на втори и трети лист от пет контролни царевични растения (К) и от пет растения, подложени на ниска температура (НТ), UV радиация и на комбинирано въздействие от ниска температура и UV радиация (НТ+UV). СКО са измерени на 5-тия час след преустановяване на въздействията в 20 точки от всяка проба. На фигура 1 са показани получените осреднени СКО на контролните и третираните растения.

От данните в таблица 1 се вижда, че експозицията на растенията на UV лъчение не повлиява значимо растежа им на 5-тия час, но инхибиращия ефект е ясно изразен 3 дена по-късно, като потискането на растежа е с повече от 60%. Третирането на растенията с ниска температура също не влияе на растежа им на 5-тия час, но на третия ден се установява повишаване на свежото им тегло. Предварителната обработка на растенията с ниска температура отстранява в значителна степен UV-индуцираната инхибиция на растежа, т.е. наблюдава се кръс-адаптация. Растенията са с по-голямо свежо тегло от това на самостоятелно облъчените растения, като разликите са статистически достоверни. Съдържанието на хлорофил в растенията, подложени на UV радиация намалява повече от 40% на 72-рия час. При същото измерване интерес представляват данните, които показват, че нискотемпературната експозиция запазва количеството на хлорофила в листата на облъчените растения почти на същото ниво, както е при контролните.

От получените СКО (фиг.1) се вижда, че увреждането от ниска температура, UV радиация и установената кръс-адаптация при комбинираното третиране на растенията (таблица 1) се откриват още на 5-тия час след преустановяване на въздействията, докато по направените измервания за растежа и съдържанието на хлорофил в третираните растения те се

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ВЛИЯНИЕ НА НЯКОИ ФИТОЕФЕКТОРИ ВЪРХУ СЪХРАНЕНИЕТО И РАЗВИТИЕТО НА ОТРЯЗАН ЦВЯТ ОТ ХРИЗАНТЕМА (CHRYSANTHEMUM MORIFOLIUM RAMAT), СОРТ ВЕСТЛАНД ОРИНДЖ

ЕЛЕНА ЯКИМОВА - *Институт по цветарство, Негован - София*

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Загубите на отрязан цвят от хризантема в редица случаи се дължат на неподходящо съхранение след обирането или по време на транспорт. Фазата на развитие на цветовете в момента на отрязването определя в значителна степен издръжливостта на отрязаните хризантеми. В последните години се практикува обиране на хризантемите във фаза цветна пъпка като степента на развитие на пъпката е в зависимост от сорта, маркетинга и техническото оборудване, достъпно за производителя, превозвача, търговеца или потребителя [16].

Повредите на отрязания цвят от хризантема в периода след обирането се изразяват главно в по-ранното увяхване на листата в сравнение с цветовете [15]. Установено е, че успешно съхранение на цветните пъпки от хризантеми може да се постигне само в случаите на предварително третиране с химически разтвори и съхранение при понижена температура [20] и последващо потапяне в разтвори за доразцъфтяване [17]. Според някои автори [9] третирането на цветовете за 16-20 h с разтвор на 5% захароза + физан 20 подобрява съхраняемостта и издръжливостта на хризантемата при транспортиране, като удължава и трайността на цветовете след съхранение и транспорт. Като подходящи разтвори за доразцъфтяване се препоръчват комбинации на сребърен нитрат с 8 ХХЦ и захароза, като концентрацията на захарозата варира в зависимост от сорта [10, 14]. Удължаване на живота във ваза е постигнато и при използването на натриев дихлоро-s-тразин трион (SDT) в комбинация със захароза, като е установено, че това съединение може успешно да замени сребърния нитрат [11].

Прилагането на цитокинини и цитокининподобни вещества може да бъде ефективно за удължаване на жизнения цикъл на отрязаните цветя [18]. Освен известните в практиката цитокинини от пуринов тип интерес представлява и приложението на цитокинини от групата на фенилкарбамидните, които притежават висока физиологична активност при сравнително ниски концентрации, широк спектър на физиологични ефекти и по-слаба токсичност [7]. Наблюдавано е, че производни на тиокарбамида забавят стареенето на отрязан цвят от карамфил. Забавяне стареенето на листа е установено при третиране с тидиазурон и Глийн-75 [5, 7]. Съобщава се и за цитокининова активност на хербицида Глийн-75 с проявен максимален ефект при концентрация 10^{-5}M-10^{-8}M [3, 7].

Известно е, че биологично активните вещества от групата на ретардантите удължават жизнения цикъл на някои видове отрязан цвят като е показано, че третирането с N-(диметиламино)-янтарна киселина (алар, Б-9) и 2-(хлороетил)-триметиламониев хлорид (ССС) забавя процеса на стареене [6]. Възможно е и бактерицидно действие, както това е установено при алар [13]. Ефективно е и кратковременното третиране с комбинацията алар + захароза + 8-хидроксихинолинцитрат при отрязани карамфил, роза, гербера и кученце [2, 12], както е установено и удължаване на живота във ваза на карамфил и астри под влияние на 24-часово третиране със ССС и диметил-β-брометилсулфониев бромид - БЕС [1].

Целта на нашето изследване беше да изпитаме ефекта на вещества с цитокинова и цитокининподобна активност (6БА, Глийн-75) и ретарданти (даминозид) в комбинация със захароза като компоненти на разтвори за предварително третиране и доразцъфтяване върху декоративните качества и издръжливостта на отрязани във фаза цветна пъпка краткосрочно съхранени хризантеми сорт Вестланд Ориндж.

МАТЕРИАЛ И МЕТОДИ

Изпитване на разтвори за предварително третиране

Изследванията бяха проведени с отрязан цвят от хризантема *(Chrysanthemum morifolium Ramat)*, сорт Вестланд Ориндж. Растенията са отгледани в Експерименталната база на Института по цветарство, Негован - София по приета в института стандартна технология. Цветовете бяха отрязани при диаметър на цветните пъпки 4-4,5 cm с няколко отворени венчелистчета при дължина на цветоносното стъбло 60 cm. След отстраняване на най-ниско разположените листа цветовете (включително цветоносните стъбла) бяха

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PLANT GROWTH REGULATING ACTIVITY OF SOME CYCLOALKANCARBOXYLIC ACIDS, THEIR ESTERS AND HYDRAZIDES

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(*Submitted by Corresponding Member E. Karanov on September 24, 1996*)

It is well known that some derivatives of cycloalkancarboxylic acids act as pesticides. Fluorosubstituted cyclopropanecarboxylic acids possess fumigant action and 3,4-dichloroanilide of the same acid shows herbicide activity. The most important compounds with practical uses are native derivatives of cyclopropanecarboxylic acid – pyretrines (esters of pyretrinic and chrysanthemic acids) and their synthetic structural analogues which have strong insecticide effect [1, 2]. However, plant growth regulating activity of alicyclic carboxylic acids and their derivatives are insufficiently investigated.

The aim of this paper is the study of still unexplored plant growth regulating properties of cyclopentane- and cyclohexanecarboxylic acids, and their esters and hydrazides, since these functional moieties determine in a great extent the high retardant activity of the Alar, MEIA and some other chemicals with practical importance. However, most regulators with a retardant activity, used at present, have a significant shortage: a relatively low physiological activity, owing to which their high doses are applied to obtain the effect desired.

Materials and methods. Cyclopentane acid (1), its ethyl ester (2) and hydrazide (3), cyclohexane acid (4), its ethyl ester (5) and hydrazide (6) were studied. The acids were commercial products (Fluka AG). Esters were synthesized from acid the corresponding alcohol and hydrazides – from esters and hydrazine.

The plant growth regulating activity of above mentioned compounds was determined in accordance with their influence on the growth of wheat (*Triticum aestivum* L.), cv. Sadovo-1, and cucumber (*Raphanus sativus* L.), cv. Levina, seedlings, grown in the dark for 96 h (25±1°C). The root and coleoptile (resp. hypocotyl) length was measured. The effect of the substances on the ageing of radish and barley (*Hordeum vulgare* L.), cv. Alfa leaf explants, betacyanin synthesis in *Amaranthus caudatus* cotyledons, and elongation of wheat coleoptiles was also studied [2–6]. The compounds were tested at 1 to 0.001 mM in comparison to the appropriate standard – Alar (mono-N,N-dimethylhydrazide of succinic acid), BA (benzyladenine) or IAA (3-indolylacetic acid). The data presented are averaged from 3-4 independent experiments each in 2 replication. Fisher's procedure was used for statistical calculation.

Results and discussion. The results given in Table 1 show that the standard Alar inhibited growth of cucumber roots, but it did not influence growth of wheat seedlings. With exception of

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BIOLOGIE
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EFFECT OF ULTRIA AND TRIA-85 ON THE GROWTH AND PRODUCTIVITY OF WHEAT (*TRITICUM AESTIVUM* L.), CV. SADOVO 1

G. Ts. Georgiev, V. Alexieva, E. Karanov

(Submitted on February 9, 1996)

Recently the primary alcohol triacontanol is one of the most advantageous and intensively investigated plant growth regulators. This interest originates from its high physiological activity – application in extremely low doses (0.1 to 0.5 mg/l)[1]. According to C h e n et al.[2], yields of winter wheat were increased at an average of 12% by foliar application of 0.1mg/l of this compound and this increase in yield was attributed to heavier kernels. However, the positive effects of its application depend on many factors. This could be explained by the fact that results are influenced by the purity of the substance and its low solubility, the stage of plant treatment, species and cultivar specificity, temperature and moisture of environment, etc.[1]. Some authors considered that one of the most important reasons for the lack of physiological activity of triacontanol is the presence of some alcohols with lower carbon chain (C_{16}–C_{28})[3]. The chemical synthesis of triacontanol is a complicated, time consuming and expensive procedure, that is why in most cases it is obtained by isolation from natural products.

The aim of this study was to investigate the effects of the preparation ULTRIA and its analogue obtained by us from beeswax on the wheat growth and productivity.

Materials and methods. Experiments were conducted in Experimental Station of Acad. M. Popov Institute of Plant Physiology during 1993 and 1994. A preplant application of 360kg/ha of N and 480kg/ha of P was made to the entire plot for both seasons (forerunner – earthen-up crops). The wheat, cv. Sadovo 1 was used. In the experiments the effects of the original preparation ULTRIA (Ultria, Teijin Ltd, Tokyo) was compared to those of the product obtained from natural beeswax, containing aliphatic alcohols C_{28} – C_{32}, (85% C_{30}) (Tria-85). The phenylurea cytokinin N_1-(2-chloro-4-pyridyl)-N_2-phenylurea (4-PU-30) was used as a standard. The compounds were dissolved by emulgation in Tween-80. Treatments were carried out at two stages – spindling and fully expanded flag leaf by spraying of plants (1 l/10m^2). In the greenhouse experiments the treatment was made on 14-days old plants. The chlorophyll content was measured in 80% acetone extract and the photosynthetic activity was determined radiometrically.

All the results are statistically proceeded by Fisher.

Results and discussion. The effects of the compounds on the growth of young wheat plants are presented in Table 1. All treatments did not influence significantly the length of aboveground part, but increased their fresh and dry weight, and the effect of triacontanol was better expressed

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BIOLOGIE
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PLANT GROWTH REGULATING ACTIVITY OF SOME NOVEL 3-(1-ACYLAMINOBENZYL)-2*H*-1-BENZOPYRAN-2-ONES

E. Karanov, V. Alexieva, G. Ts. Georgiev, A. Bojilova*

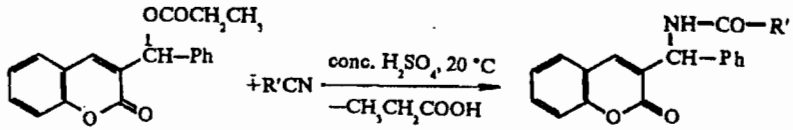
(Submitted by Corresponding Member E. Karanov on February 27, 1996)

Coumarins are widely distributed in plants. Usually native coumarins are regarded as growth inhibitors. Exogenous applied, they inhibit the auxin-induced curvature of slit pea stems and the elongation of *Triticum aestivum* and *Avena* coleoptile segments [3, 7]. Coumarins also inhibit growth of roots of intact wheat and cucumber seedlings [1, 3], *Plenum pratense* roots [4] decreasing the elasticity of cell wall [6].

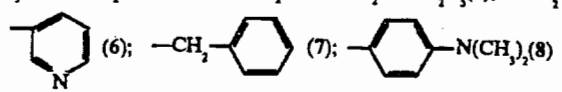
Although coumarins are usually regarded as plant growth depressors, these compounds can also promote plant growth, especially when some chemical modification in their structure is made. However, synthetic analogues of coumarin are insufficiently studied. In our previous investigations it is established that the modification in the chemical structure of some natural coumarins provoked strong alteration in the selectivity and the bias of their physiological properties – some phosphorus containing derivatives of coumarin exhibited a typical herbicide activity [1], while 2-(benzoxazolon-3-yl-acetyl-1) hydrazide simulated the growth of wheat coleoptile segments and markedly inhibited the activity of IAA-oxidase [2].

The aim of current investigation was to study the plant growth regulating properties of some novel 3-(1-acylaminobenzyl)-2*H*-1-benzopyran-2-ones.

Materials and methods. Synthesis of the compounds was performed according to the following scheme:



where R'=—CH_3(1); —CH_2Cl(2); —CH=CH_2(3); —$CH_2COOC_2H_5$(4); —$CH_2CH_2N(CH_3)_2$(5);



The stages of the chemical procedure are previously [5] described in details.

Plant growth regulating activity was determined in accordance with their influence on the growth of 2-week-old pea *(Pisum sativum* L. cv. Koray), plants grown as a water culture in a growth chamber (12 h photoperiod; 25°C±1) [9]. The influence of the compounds tested on the growth of intact seedlings was determined by measuring the hypocotyl (coleoptile respectively)

This study was supported by the National Scientific Foundation under Grant No B-317/1993.

ABSCISIC ACID AND STOMATAL ACTIVITY IN MAIZE: RELATION WITH DROUGHT CONDITION OF SEEDLINGS

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There are a lot of evidence that plant answers to the water shortage can be mediated by growth regulator substances. The central role played by abscisic acid in drought resistance with control of stomatal aperture is well recognised. Some times exogenous ABA application on leaves was used to induce water stress condition and stomatal closure. Preliminary observations gave indication that application of ABA did not wilted maize seedlings. The changes of leaves gas exchange and endogenous level of ABA was studied as consequence of ABA application or water stress inducted by PEG. Two weeks old maize seedlings, growth in hydroponical system, were treated with ABA 0.01 or 0.1 mM or transferred in 25% PEG-6000 solution. Carbon dioxide assimilation and stomatal conductance were measured daily. ABA in maize seedlings were extracted and measured by HPLC with DPA detector.

Seedlings sprayed with 0.01 mM ABA showed decrease of CO_2 assimilation and stomatal conductance (Fig 1). The 0.01 mM ABA effect was reverted after 24 h of application. Two 0.01 mM ABA application with 24h interval

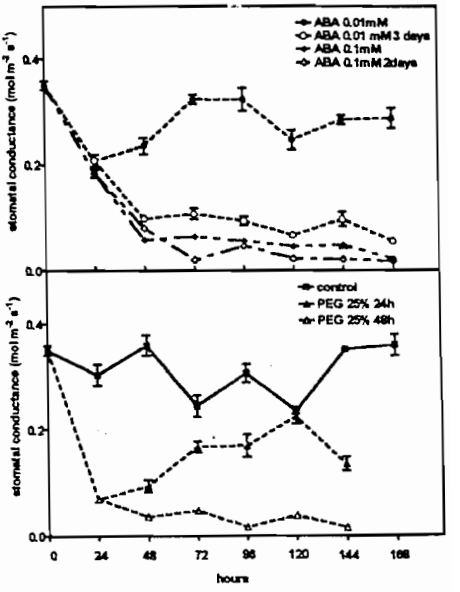

Fig.1. Change of stomatal conductance after ABA treatment or water stress induced by PEG 25%

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BIOLOGIE
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GROWTH, CHLOROPHYLL CONTENT AND GAS-EXCHANGE OF YOUNG MAIZE PLANTS TREATED WITH POLYAMINES

D. Todorov, V. Alexieva, E. Karanov

(Submitted on January 23, 1996)

Aliphatic polyamines have been described as growth promoters in cell division (10 to 100 (μ) and elongation in higher plants [7]. While the physiological role of endogenous polyamines has been intensively investigated, very little information is available on polyamine treatment of intact plants as well as on their possible application in agriculture. Spermine, spermidine and putrescine substantially increased fruit-set and yield of apples [5]. Similar effect was observed by treatment of the pears with putrescine [6]. Some natural and synthetic di- and polyamines stimulated rooting of bean [1]. That is why some authors postulated that the polyamines could be used in agricultural practice without unfavourable side-effects [2].

The aim of the present investigation was to assess the effect of spermine, spermidine and putrescine on growth, chlorophyll content and gas-exchange of young maize plants.

Materials and methods. The experiments were carried out with maize plants hybrid "Pioneer" 3737, grown in pots, containing soil with 60% humidity in a greenhouse. The plants were foliar treated with spermine (Spm), spermidine (Spd) and putrescine (Put) at concentrations 125 mg/l and 250 mg/l, when 4th leaf was fully developed. Twenty days after treatment (before 12th leaf appearance) the height of stem and the length of internodes were measured. Leaf area was calculated by the formula: leaf area = length × maximum width × 0.75. Fresh and dry weights of various organs were measured. Chlorophyll content of 4th and 7th leaves was determined on days 10 and 16 after treatment respectively by extraction with 80% acetone, followed by measurement of the optical density at 663 and 645 nm. Net photosynthetic rate (A), transpiration rate (Tr), and stomatal resistance (Rs) of 4th and 7th leaves were determined on days 9 and 15 after the treatment respectively by using a portable gas-exchange monitor LI-6000 (LI-Cor, USA) and under natural condition – leaf temperature 28-34°C, irradiance 1200-1600 $mol.m^{-2}.s^{-1}$. The results were statistically analyzed using Fisher's criteria.

Results and discussion. Even though the metabolism of polyamines in higher plants is relatively well known, the same is not true concerning the physiological effects induced for the mentioned polyamines. To clarify these effects, we studied the action of amines on young maize plants. Under the conditions mentioned, the foliar treatment with putrescine, spermine and spermidine affected the plant growth depending on the type and concentration of amine used. The

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BIOLOGIE
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PLANT GROWTH REGULATING ACTIVITY OF SOME NEW PYRIDYLOXYMETHYLENEDIMETHYLPHOSPHINE OXIDES

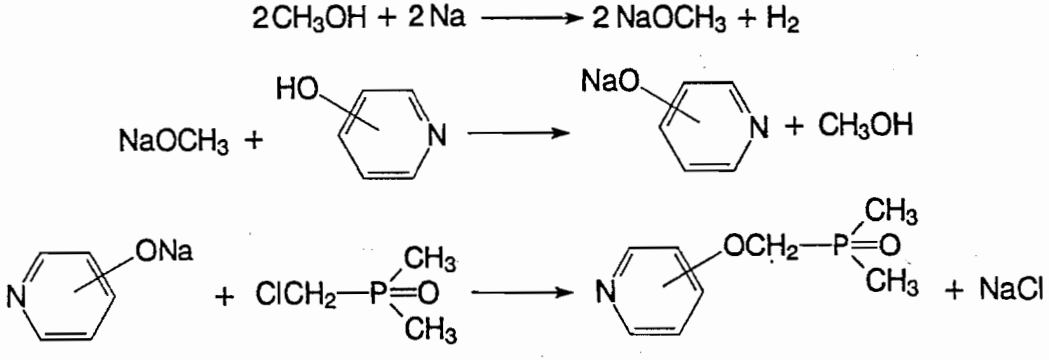
E. Karanov, Ts. Dimitrova, V. Alexieva, S. Varbanov*, E. Tashev*

(*Submitted on September 16, 1997*)

More than two hundred organophosphorus compounds have reached commercialization as herbicides, insecticides and fungicides [7]. However, only two organophosphorus compounds are marketed as plant growth regulators: glyphosate, $HOOCCH_2N(CH_2PO_3H_2)_2$ and etephon, $ClCH_2CH_2PO_3H_2$. [5,13]. Nevertheless, the interest on the search of new phosphorus containing compounds with plant growth regulating activity goes on because of their high physiological activity. HALMANN et al. [6] announced stimulating action of some novel phosphonic acid derivatives of 1,3–dioxan on the growth of beans, barley, maize, tomatoes but several herbicide effects were noted. LACHKOVA et al. [12] demonstrated retarding activity of certain new N-substituted thioamidophenylmethane phosphonates. Similarly, according to ALEXIEVA et al. [2], some 6-halogen 3-[$P(O)(OEt)_2$]-coumarins acted as retardants.

In the present work, the plant growth regulating activity of new synthesised 2-, 3-and 4-pyridyloxymethylenedimethylphosphine oxides was studied. Some chemical structure - activity relationships were discussed.

Materials and methods. The compounds with the following structure were investigated:



The details of the synthesis and chemical analysis of the compounds were shown previously [9]. The respective *ortho*, *meta* and *para* isomers of the pyridyloxymethylenedimethylphosphine oxides are numbered in the tables and figures as 1, 2 and 3. Plant growth regulating activity of the compounds was determined in accordance with their influence on the growth of 2-week-old pea (*Pisum sativum* L. cv. Koray), plants grown as a water culture in a growth chamber (12 h photoperiod, 25 °C, about 60 μmol.m^{-2}.s^{-1} photon flux density). The influence of the compounds tested on the growth of intact seedlings was determined by measuring the hypocotyl

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BIOLOGIE
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PLANT GROWTH REGULATING ACTIVITY OF SOME NOVEL N-(6-METHYLPYRID-2-YL)-α-(8-HYDROXY-QUINOLINE-7-YL)-SUBSTITUTED BENZYLAMINES

I. Sergiev, V. Alexieva, E. Karanov, I. Petkov*

(*Submitted on June 19, 1997*)

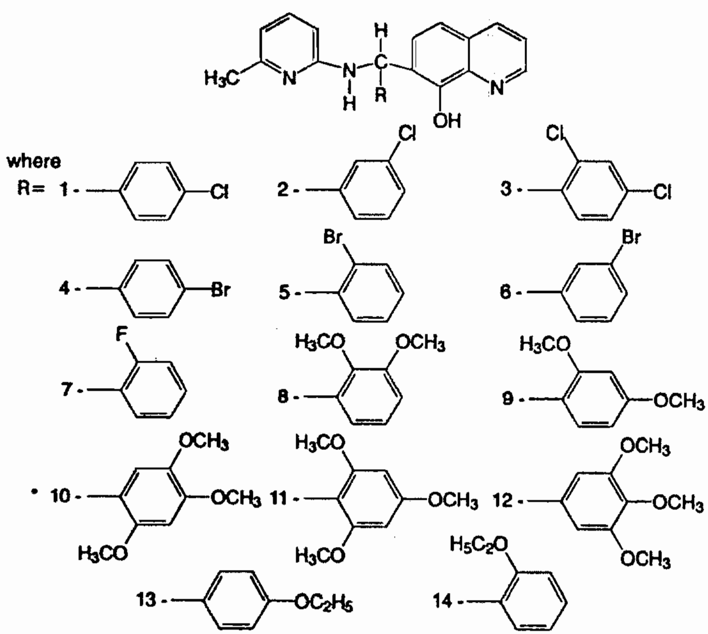
Anticytokinins are useful tools in investigation of cytokinin mode of action. In spite of the extensive empirical and QSAR studies, the problem of the structural elements, required for certain molecule(s) to possess high physiological activity remains obscure. The properties of highly active cytokinins and anticytokinins, sharing the same elements in the chemical structure have been reported [1,2]. Hitherto known cytokinin antagonists can be divided into seven chemical groups [2-4], and most of them possess structural similarities to the high active adenine or phenylurea cytokinins.

However, CHRISTOU and BARTON [5] showed that some substituted phenetylamines acted as cytokinin antagonists of cytokinin metabolism in plant tissues.

In order to elucidate to what extent the structural correlation between cytokinins and anticytokinins is an obligatory circumstance for expression of antagonism, the plant growth regulating activity of some novel derivatives of N-(6-methylpyrid-2-yl)-α-(8-hydroxy-quinoline-7-yl)-substituted benzylamines was studied.

Materials and methods. Compounds with the following chemical structure were investigated.

The influence of benzylamines on the chlorophyll degradation of detached leaves was studied using radish [6] (*Raphanus sativa* L.), cv. Red, and barley [7] (*Hordeum vulgare* L.), cv. Alpha leaf explants, incubated on test solutions in the dark for 96 (resp. 72) hours. Cytokinin-stimulated growth of excised radish cotyledons was measured according to LETHAM [8]. N^6-benzyladenine (BA) and N^1-(2-chloro-4-pyridyl)-N^2-phenylurea (4-PU-30) were used as standards. All chemicals were tested in concentration range 0.1 to 0.001 mM alone or in combination with BA or 4-PU-30 (0.01 mM). The data presented are means of three or four experiments, each in three replications. Fisher's procedure was used for statistical evaluation.



Results and discussion. The studies on the

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Effect of Putrescine, 4-PU-30, and Abscisic Acid on Maize Plants Grown under Normal, Drought, and Rewatering Conditions

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Received September 25, 1997; accepted August 10, 1998

Abstract. The experiments were carried out with maize (*Zea mays* L.) seedlings, hybrid Kneja 530, grown hydroponically in a growth chamber. Twelve-day-old plants were foliar treated with putrescine, N^1-(2-chloro-4-pyridyl)-N^2-phenylurea (4-PU-30), and abscisic acid (ABA) at concentrations of 10^{-5} M. Twenty-four hours later the plants were subjected to a water deficit program, induced by 15% polyethylene glycol (PEG; molecular weight, 6,000). Three days after drought stress half of the plants were transferred to nutrient solution for the next 3 days. The effects of the water shortage, rewatering, and plant growth regulator (PGR) treatment on the fresh and dry weights, leaf pigment content, proline level, relative water content (RWC), transpiration rate, activities of catalase and guaiacol peroxidase, hydrogen peroxide content, and level of the products of lipid peroxidation were studied. It was established that the application of PGRs alleviated to some extent the plant damage provoked by PEG stress. At the end of the water shortage program the plants treated with these PGRs possessed higher fresh weight than drought-subjected control seedlings. It was found also that putrescine increased the dry weight of plants. Under drought, the RWC and transpiration rate of seedlings declined, but PGR treatment reduced these effects. The accumulation of free proline, malondialdehyde, and hydrogen peroxide was prevented in PGR-treated plants compared with the water stress control. The results provided further information about the influence of putrescine, 4-PU-30, and ABA on maize plants grown under normal, drought, and rewatering conditions.

Plant Growth Regulation **24**: 77–89, 1998.

Polyamine concentrations in four *Poa* species, differing in their maximum relative growth rate, grown with free access to nitrate and at limiting nitrate supply

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Abstract

Polyamines are thought to play a role in the control of inherent or environmentally-induced growth rates of plants. To test this contention, we grew plants of four grass species, the inherently fast-growing *Poa annua* L. and *Poa trivialis* L. and the inherently slow-growing *Poa compressa* L. and *Poa pratensis* (L.) Schreb., at three levels of nitrate supply. Firstly, plants were compared when grown with free access to nitrate, allowing the plants to grow at their maximum relative growth rate (RGR_{max}). Secondly, we compared the plants when grown with relative nitrate addition rates of 100 and 50 mmol N (mol N)$^{-1}$ day^{-1} (RAR_{100} and RAR_{50}, respectively).

The freely-occurring polyamines, spermine, spermidine and putrescine, were separated from their conjugates; the latter were further subdivided into a TCA-soluble and a TCA-insoluble fraction. Each of the three fractions responded differently to the nitrate supply. Under nitrogen limitation, the total concentration of polyamines (free and bound ones together) decreased in both leaves and roots of all *Poa* species, whereas that in the stem remained more or less the same. These effects were to a large extent determined by the free polyamines. For the conjugates there was more differentiation, both between plant organ and among polyamine structures. A positive correlation between the RGR, LAR (leaf area per plant mass), SLA (leaf area per leaf mass), LMR (leaf mass per plant mass) and SMR (stem mass per plant mass) with the polyamine concentration was found. The RMR (root mass per plant mass) showed a negative one. No significant differences were found between the inherently fast- and slow-growing grass species.

The (putrescine)/(spermine + spermidine) ratio in the leaves increased with decreasing nitrate supply, which is associated with a decrease in leaf expansion, accounting for a decrease in LAR and SLA. For the roots, this ratio tended to decrease with decreasing nitrate supply, whereas for the stems the results were somewhat more variable.

We found no evidence for a crucial role of polyamines in the determination of inherent variation of growth in spite of a positive correlation of especially the free polyamines with growth parameters.

Plant Growth Regulation **29:** 175–180, 1999.

Subcellular aspects of the protective effect of spermine against atrazine in pea plants

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Abstract

The action of the exogenously applied tetraamine spermine in reversing the effect of atrazine stress on intact pea plants (*Pisum sativum* L., cv. Koray) was investigated at the ultrastructural level. The results indicate that atrazine increases cell senescence by lipid peroxidation and loss of unsaturated fatty acids from thylakoid membranes of pea plant chloroplasts. Spermine acts as a polyfunctional effector. It stabilises the molecular composition of the membranes by preventing lipid peroxidation and as a consequence protects the conformation of the thylakoid system and structural integrity of the chloroplasts. Spermine treatment also contributes to the process of neutralisation of the free radicals by peroxisomes.

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EXPLORATIONS COSMIQUES

EARLY DETECTION BY MEANS OF SPECTRAL REFLECTANCE COEFFICIENTS, OF PHYSIOLOGICAL CHANGES IN LEAVES OF *ZEA MAYS* L. SEEDLINGS GROWN UNDER WATER DEFICIT AND UV-B RADIATION

D. Krezhova, T. Yanev, V. Alexieva*, D. Mishev, Hr. Nikolov

(*Submitted on August 2, 1999*)

Introduction. Plant organisms are subjected during their ontogenetic development to the impact of different stress factors which cause changes in the plant physiological processes. Plant injuries are usually due to unfavourable environmental factors (extreme temperatures, water stress, high level of sun radiation, etc.). Some of the stress factors activate the cell metabolism which sometimes leads to an enhanced resistibility (cross adaptation) to other stress factors. As it is known [1,2] the action of most of the stress factors (increased UV-radiation and water deficit for example) leads to accumulation of active hydrogen species much more than is the cell capacity for their neutralization by endogenous antioxidants. Therefore, the cell membranes become damaged in result of lipid peroxidation, i.e. the injuring mechanism of different stress factors turns out to be identical or similar.

The remote sensing methods for study of natural formations and the spectral reflectance (SR) of vegetation in particular (in the visible (VIS) and near infrared (NIR) ranges of the electromagnetic spectrum) provide the possibility early and statistically significant information to be obtained about crop state on large areas. The laboratory biochemical methods in current use do not meet these requirements to the necessary degree especially with respect to the early detection of stress effects. Three SR bands in the VIS (400–680 nm) and the NIR (680–900 nm) ranges give specific information about leaf status [3]: around 550 nm (maximal chlorophyll spectral reflectance), around 660 nm (maximal chlorophyll spectral absorption) and around 700 nm (the red edge band which is most significantly influenced by chemical fertilizers, heavy metal pollution, etc.). The leaf water content affects SR in a much wider SR wavelength range (400–2500 nm): the less the water contents is, the larger the SR values are, SR departures at the different wavelengths not being equidistant from SR of leaves with normal water content.

Materials and methods. The experiments were carried out with maize (*Zea mays* L.) seedlings, hybrid Kneja 530 grown as a soil culture in a growth chamber (12×12 h photoperiod, 25 °C, 65% relative air humidity, 160 photon flux density). On the 14th day a part of the plants were subjected to a water deficit programme-drought experiment (40% soil humidity) for three days followed by UV-B treatment of drought plants a part of the control plants.

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EFFECT OF ATRAZINE AND SPERMINE ON FREE PROLINE CONTENT AND SOME ANTIOXIDANTS IN PEA (*PISUM SATIVUM* L.) PLANTS

I. Sergiev, V. Alexieva, S. Yanev*, E. Karanov

(*Submitted on March 21, 2000*)

Most of the native and anthropogenic stresses induce similar physiological responses in higher plants. These primary non-specific events include changes in oxidative enzymes activities, variations in free proline content and increase of polyamine levels.

Weeds are product of human society. Nowadays, chemical weed control has expanded to probably every crop situation in the world [1]. Despite of the extensive attempts to safe the environment, the use of herbicides is inevitable technique in crop production, and the chemical contamination is a common anthropogenic stress factor.

Selective chemical weed control has entered a new phase with the development of herbicide antidotes (safeners), which increase the physiological tolerance of crops to herbicides. Since the 1970s it is known that the addition of certain chemicals may protect cereal crops from herbicide damage, and to extend the selectivity and use of the herbicide.

In spite of the large amount of research till now there is no validated theory on how chemical safeners render their protection against herbicides. This is probably due to the huge variety of chemical herbicide structures with unestablished mode of action and practically unlimited possibilities for crop-herbicide-safener combinations [1].

Recently, ZHELEVA et al. [2] announced protective effect of exogenous polyamines against atrazine in pea plants. They concluded that polyamine spermine partially overcomes the atrazine-induced deleterious effects. Plants treated with combination of atrazine and polyamine possessed improved growth, gas exchange, chlorophyll content, and de novo protein synthesis than those treated with atrazine only.

The target site of atrazine action is established to be the reaction centre of PS II. One of the most detrimental consequences is the production of free radicals [1,3]. Currently it is established that polyamines act as free radical scavengers [4].

The aim of this investigation was to evaluate the effect of spermine as antidote for atrazine in relation to the activity of catalase, guaiacol peroxidase and free proline content in pea plants. The enzymes are assumed to be part of a system which renders protection against active oxigen species. The content of free proline was studied as a stress marker.

Some enzymes, often mixed-function oxygenases, have been implicated to take part in herbicide catabolism. Maize and sorghum contain high amounts of the enzyme glutathione-S-transferase (GST), so that atrazine is conjugated and detoxified before it reaches its thylacoid site of action [1]. In this relation we also studied the influence of atrazine and spermine on the activity of glutathione-S-transferase in pea plants.

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JOURNAL OF PLANT PHYSIOLOGY

022

Novel Physiological Properties of Two Cytokinin Antagonists

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Summary

The promotive effect of two cytokinin antagonists 2-chloro-4-cyclobutyl-amino-6-ethylamino-1,3,5-triazine and N-(4-pyridyl)-O-(4-chlorophenyl) carbamate on somatic embryos production directly or through callus in leaf explants of the grass *Dactylis glomerata* L. (orchardgrass) was established. The data for the endogenous contents of hydrogen peroxide and lipid peroxidation products during the earliest stages of somatic embryogenesis suggest that anticytokinins induce some alterations in the production of active oxygen species. Both compounds activated the defence enzymes 5 days after culture initiation. The triazine derivative enhanced catalase activity in more differentiated leaf segments while the phenylurea-type anticytokinin had the same effect on peroxidase activity. Additional activation of catalase and slight reduction of peroxidase activity were determined in 10-day-old cultures. During induction of somatic embryogenesis in *Dactylis* leaf explants the main difference in the physiological action of the cytokinin antagonists tested was related to IAA-oxidase activity. A possible role of the anticytokinin-induced changes in cell oxidative status on the explant embryogenic response is discussed.

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ALTERATIONS IN THE ACTIVITIES OF SOME HYDROGEN PEROXIDE SCAVENGING ENZYMES DURING INDUCTION OF SOMATIC EMBRYOGENESIS IN LEAF EXPLANTS FROM *DACTYLIS GLOMERATA* L.

M. Somleva, V. Alexieva, I. Sergiev, E. Karanov

(*Submitted on January 11, 1999*)

One of the unique characteristics of plant cells is totipotency in which nondividing, differentiated somatic cells, when cultured properly, start dividing and can regenerate whole plants either via organogenesis or via somatic embryogenesis. The molecular and biochemical mechanisms of the dedifferentiation process, underlying the expression of totipotency, remain largely unknown.

The embryogenic systems developed for *Dactylis glomerata* L. (orchardgrass) are unique among members of Poaceae, most of which are recalcitrant under in vitro conditions. Orchardgrass leaf explants cultured on a simple medium containing only auxin reveal a repeatable system for producing somatic embryos directly (without callus formation) and indirectly. The duration of the developmental stages – acquisition of embryogenic competence, induction of somatic embryogenesis, embryo development and maturation – is determined which makes this system suitable for biochemical studies on the earliest events during induction of somatic embryogenesis [1]. In vitro culture of isolated plant organs, tissues and cells leads to a transition from auxotrophic to heterotrophic metabolism under condititions of altered phytohormonal status which cause an oxidative stress. Some of the medium components – FeEDTA and metal cations – stimulate free radical production and lipid peroxidation. Certain metabolic changes in cultured cells inducing formation of toxic oxygen species are caused by synthetic auxins: stimulation of lipid peroxidation; increase in the alternate pathway of mitochondrial respiration and residual oxygen metabolism which is connected with activation of lipoxygenase; rise in ethylene production. It is well established that high concentrations of H_2O_2 can induce lipid peroxidation and cell injury, and enzymes related to H_2O_2 scavenging are particularly important. On the other hand, it has been supposed that hydrogen peroxide at lower, physiological concentrations may play a regulatory role in plant cells and takes part in transduction of signals induced by auxins and cytokinins [2].

The aim of our study is to evaluate the auxin-induced production of some toxic oxygen forms and the activity of H_2O_2 scavenging enzymes catalase and peroxidase, including IAA-oxidase, during the induction of indirect and direct somatic embryogenesis in leaf explants from *Dactylis glomerata* L.

Materials and methods. Developing leaves from *Dactylis glomerata* L., the highly embryogenic genotype Embryogen-P which was kindly provided by prof. B. V. Conger (University of Tennessee, USA), was used as an explant source. After surface sterilisation leaf halves from the basal parts of the innermost two leaves were cut transversely into 6 segments (3 mm long) and plated in the same order onto SH medium [3] solidified with 0.8% agar. The medium was supplemented with 30 μM 3,6-dichloro-o-anisic acid (dicamba) for induction of callus and embryo formation. Culturing was

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SYNTHESIS AND PLANT GROWTH REGULATING ACTIVITY OF NEW TRIAZOLO- AND PYRAZOLOPYRIMIDINE DERIVATIVES OF AMINOMETHYL-, AMINOALKYLOXYMETHYL DIMETHYLPHOSPHINE OXIDES AND (AMINOMETHANE)PHOSPHONIC ACID ESTERS

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New triazolo[4,5-d]pyrimidine and pyrazolo[3,4-d]pyrimidine derivatives of aminomethyl- and aminomethyloxymethyl dimethylphosphine oxides **8–14** as well as of esters of (aminomethane) phosphonic acid **18–20** were synthesized. The structure of the compounds prepared was confirmed by means of elemental analysis, IR, ^{1}H- and ^{31}P{^{1}H}-NMR spectroscopy. Tertiary phosphine oxides **8, 9** and **12** as well as phosphonate **20** showed herbicidal and plant growth regulating activity.

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BIOLOGIE
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EFFECT OF SOME PHENYL AMINES ON MAIZE PLANTS GROWN UNDER DROUGHT INDUCED BY POLYETHYLENE GLYCOL[1]

D. Todorov, V. Alexieva, E. Karanov

(*Submitted on January 11, 1999*)

Shortage of water limits plant growth and crop productivity in many regions, more than any other single environmental factor. Genetic modification of plants by breeding to allow growth and yield under unfavourable conditions is a solution to problems of environmental stress. However, this approach is time-consuming and demands sustained efforts. There is much evidence that plants' responses to unfavourable environments can be modulated by various plant growth regulators. A large number of reports refer to the capability of polyamines to ameliorate an osmotic stress [6] and a herbicide stress [12]. Although it is difficult to ascribe a single mechanism for polyamine protective action, it is assumed that due to their unique properties as biological polycations, polyamines bind to acidic sites of nucleic acids and cell membrane phospholipids, thereby stabilizing their structure and preventing the breakdown of macromolecules under stress conditions [2]. The aim of our investigation was to compare the effects of phenyl diamine, diphenyl diamine and phenyl triamine (not occurring naturally in the plants) on the growth, relative water content and transpiration rate, pigments, free proline level, malondialdehyde and hydrogen peroxide contents, and peroxidase and catalase activities of young maize plants subjected to water stress.

Materials and methods. The experiments were carried out with maize (*Zea mays* L.) seedlings, hybrid "Kneja"-530, grown hydroponically at growth chamber. Ten-day-old plants were foliar treated with phenyl diamine (PDA), diphenyl diamine (DPDA), phenyl triamine (PTA) at concentrations of 10^{-3}M, 10^{-4}M and 10^{-5}M. Twenty four hours later part of the plants were subjected to water deficit programme, induced by 10% and 20% polyethylene glycol (PEG), MW 6000 in the nutrient solution. Seven days after drought the fresh weight (FW), dry weight (DW), the relative water content (RWC) and transpiration rate (Tr) of leaves were measured. Enzymes were extracted from about 0.5 g fresh leaf material with 100mM K-phosphate buffer (pH 7.0). Extracts were centrifuged and the supernatants were used for the assays. Catalase (EC 1.11.1.6, CAT) was determined according to BRENNAN and FRENKEL [5]. Peroxidase (EC 1.11.1.7, POX) was measured by the method described by DIAS and COSTA [8]. Pigments were extracted with 80% acetone and were estimated spectrophotometrically. Proline was extracted with 3% sulfosalicylic acid and was measured according to BATES et al. [4]. Malondialdehyde (MDA) was estimated as the thiobarbituric acid reactive material using the molar extinction coefficient 155 $mM^{-1}cm^{-1}$. The endogenous level of hydrogen peroxide (H_2O_2) was measured spectrophotometrically ($\lambda = 390$ nm) after incubation of 0.1% trichloroacetic acid leaf extract with 1 M KI.

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GENOTOXICITY

P. Venkov · M. Topashka-Ancheva · M. Georgieva
V. Alexieva · E. Karanov

Genotoxic effect of substituted phenoxyacetic acids

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Abstract The potential toxic and mutagenic action of 2,4-dichlorophenoxyacetic acid has been studied in different test systems, and the obtained results range from increased chromosomal damage to no effect at all. We reexamined the effect of this herbicide by simultaneous using three tests based on yeast, transformed hematopoietic, and mouse bone marrow cells. The results obtained demonstrated that 2,4-dichlorophenoxyacetic acid has cytotoxic and mutagenic effects. The positive response of yeast and transformed hematopoietic cells was verified in kinetics and dose–response experiments. The analysis of metaphase chromosomes indicated a statistically proved induction of breaks, deletions, and exchanges after the intraperitoneal administration of 2,4-dichlorophenoxyacetic acid in mice. The study of phenoxyacetic acid and its differently chlorinated derivatives showed that cytotoxicity and mutagenicity are induced by chlorine atoms at position 2 and/or 4 in the benzene ring. The mutagenic effect was abolished by introduction of a third chlorine atom at position 5. Thus 2,4,5-trichlorophenoxyacetic acid was found to have very weak, if any mutagenic effect; however, the herbicide preserved its toxic effect.

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EXPLORATIONS COSMIQUES

SPECTRAL REFLECTANCE COEFFICIENTS TO EARLY DETECT PHYSIOLOGICAL CHANGES IN LEAVES OF *ZEA MAYS* L. SEEDLINGS TREATED WITH HERBICIDES

T. Yanev, D. Krezhova, V. Alexieva*, Hr. Nikolov

(*Submitted by Academician D. Mishev on December 15, 1999*)

Introduction. Abiotic and biotic agents exert changes in the normal physiological processes in all of the culture and wild plants. Plant damages and decrease of their productivity are most frequently due to unfavorable environmental factors (natural stress factors)—extreme temperature, water deficit, wet lands, saline soils, high Sun radiation, early autumn and late spring rimes, etc.

In recent years new impacts arising from human activity—toxic polluters decrease the plant biosynthetic activity, change the plant normal functions and cause mortal damages [1]. Such toxic polluters are pesticides, harmful gases (SO_2, NO, NO_2, N_x, O_3 and photochemical smog), photooxidants (peroxyacylnitrates), rise in acidity and mineral deficit in result of acid rainfalls, fertilization, heavy metals, increased UV-radiation, etc.

In spite of the more and more increasing requirements for environmental protection the use of herbicides continues to be an obligatory practice in food production in the countries with advanced agriculture [1]. The chemical pollution continues to be one of the most frequently met anthropogenic stresses.

The objective of our previous studies [2-6] was to examine whether damages in leaves of maize plants (in result of the stress impact of drought, low temperature, UV-B radiation and combinations of these stress factors) may be detected by means of the leaves spectral reflectance coefficients (SRC) not later than they are usually established through endogenous stress markers and important biochemical and biometric parameters.

The effect of the above mentioned stress factors was mainly estimated by:

a) The concentration of the following endogenous stress markers: proline, products of the lipid peroxidation (measured as malondialdehyde (MDA) equivalents), hydrogen peroxide content, electrolyte leakage and in some of the experiments—by parameters such as fresh weight, chlorophyll content, etc.;

b) SRC changes in the visible and near-infrared ranges of the electromagnetic spectrum (480–810 nm).

In this work the possibility to early detect through SRC the physiological changes in leaves of maize plants treated with herbicides was studied. An overview is made as well on the main results of our previous studies for early detection through SRC of physiological changes in leaves of maize plants treated with other stress factors.

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BIOLOGIE
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HERBICIDAL ACTIVITY OF SOME NOVEL PHOSPHOAMIDES

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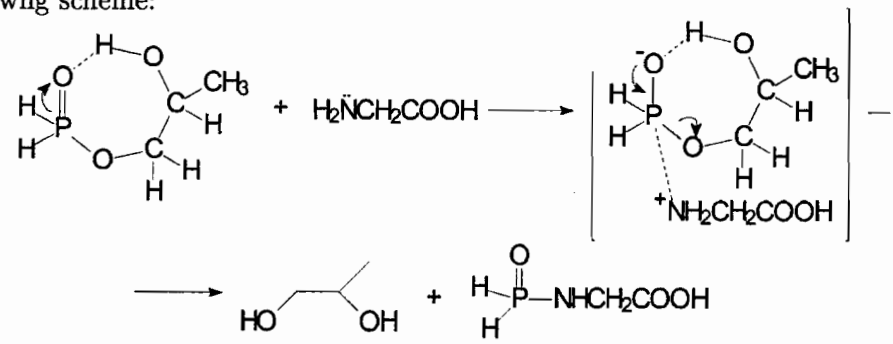
(*Submitted on July 12, 2000*)

Control of plant growth and development through the use of growth regulators and herbicides is becoming an increasingly important aspect of modern agricultural practice. Despite of the extensive attempts to safe the environment, the application of pesticides and plant growth regulators is inevitable technique in crop production. 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. The problem could be solved with new herbicides. However, during the last decade the costs of the development of new chemicals, including their design, synthesis, screening, physiological characterisation, toxicological and environmental safety registration sharply raised [1]. One of the modern, widely used and relatively cheaper approach to the development of new xenobiotics is a chemical modification of known substances (native or synthetic) with structural moieties typical of some high effective compounds. Using this approach some novel active derivatives possessing herbicidal [2–4] or plant growth regulating activity [5–7] were announced.

In this paper the herbicidal and plant growth regulating activity of five novel phosphoamides – amidodiphosphonic and amidodiphosphoric acids, amidoglycine-H-phospinate and cyclic phosphoamide esters of glycine are described.

Materials and methods. Amidoglycine-H-phospinate (I) was synthesized by the reaction of glycine with hydroxypropyl ester of hypophosphorous acid according to the followng scheme:



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The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat

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ABSTRACT

It emerged recently that there is an inter-relationship between drought and ultraviolet-B (UV-B) radiation in plant responses, in that both stresses provoke an oxidative burst. The purpose of this investigation was to compare the effects and interaction of drought and UV-B in wheat and pea. The absence of changes in relative leaf water content (RWC) after UV-B treatments indicate that changes in water content were not involved. RWC was the main factor resulting in reduced growth in response to drought. Increases in anthocyanin and phenols were detected after exposure to UV-B. The increases do not appear to be of sufficient magnitude to act as a UV-B screen. UV-B application caused greater membrane damage than drought stress, as assessed by lipid peroxidation as well as osmolyte leakage. An increase in the specific activities of antioxidant enzymes was measured after UV-B alone as well as after application to droughted plants. Proline increased primarily in drought-stressed pea or wheat. Proline may be the drought-induced factor which has a protective role in response to UV-B. The physiological and biochemical parameters measured indicate the UV-B light has stronger stress effectors than drought on the growth of seedlings of both species. The two environmental stresses acted synergistically to induce protective mechanisms in that pre-application of either stress reduced the damage caused by subsequent application of the other stress.

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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 SOME DIAMINES ON MAIZE PLANTS GROWN UNDER DROUGHT INDUCED BY POLYETHYLENE GLYCOL

D. Todorov, V. Alexieva, E. Karanov

(*Submitted on February 21, 2001*)

More than any other single environmental factor the shortage of water limits plant growth and crop productivity in many regions. There is much evidence that plant responses to unfavourable environments can be modulated by various plant growth regulators (PGRs). The protective effect of exogenous polyamines against different stresses have been intensively studied [1–3]. Although it is difficult to ascribe a single mechanism for polyamine protective action, it is assumed that because of their unique properties as biological polycations, polyamines bind to acidic sites of nucleic acids and cell membrane phospholipids, thereby stabilizing their structure and preventing the breakdown of macromolecules under stress conditions [4].

On the other hand, only a few reports about the relationship between chemical structure and plant growth regulating activity is available. ALEXIEVA [5] has compared the activity of some native and synthetic di- and polyamines in relation to their effect on aging of mono- and dicotyledonous plants' leaf explants and has found that in some cases synthetic polyamines possessed higher activity than native ones. Similar conclusions were made by YONOVA et al. [3] about some ω,ω'-disubstituted ureido- and thioureidoalkanes. However, in these experiments excised leaves were used.

Recently we have shown that some aromatic diamines alleviated to some extent the damages provoked by water shortage in young maize seedlings. However, their protective activity was lesser than those of native diamine putrescine, applied at concentration 10^{-5} M [6]. The aim of this study was to compare the effects of some aliphatic diamines on the growth, some stress markers, and stress-defence enzyme systems of young maize plants subjected to water stress, induced by polyethylene glycol, and to clarify if the

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BIOLOGIE
Physiologie des plantes

PLANT GROWTH REGULATING ACTIVITY OF SOME COUMARINS AND BISCOUMARINS

V. Alexieva, I. Sergiev, I. Manolov*, E. Karanov

(*Submitted on June 6, 2002*)

Abstract

The plant growth regulating activity of a series of mono- and biscoumarins was studied in relation to some growth parameters of pea, cucumber and wheat plants. The effects of the compounds on the elongation of excised cucumber hypocotyl and wheat coleoptile segments, as well as the activity of IAA oxidase were also investigated. It was established that most of the compounds showed a retardant activity in relation to the growth of intact pea, cucumber and wheat seedlings, and the effects of biscoumarins were better expressed. A clear selectivity of action was found in relation to the elongation of excised cucumber and wheat segments. Monocoumarins inhibited the elongation of cucumber hypocotyls and stimulated the IAA oxidase activity, while in wheat coleoptiles the opposite trend was shown.

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JOURNAL OF PLANT PHYSIOLOGY

Treatment with salicylic acid decreases the effects of paraquat on photosynthesis

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Summary

The present study investigated the possible mediatory role of salicylic acid (SA) in paraquat (Pq) toxicity on photosynthesis. Twelve-day-old barley seedlings were supplied with 500 µmol/L salicylic acid (SA) or 10 µmol/L paraquat (Pq) via the transpiration stream and kept in the dark for 24 h. Then they were exposed to 100 µmol m^{-2} s^{-1} PAR and samples were taken 1,2,3, and 6 h after the light exposure. Leaf gas exchange parameters, the activity of ribulose-1,5 bisphosphate carboxylase (RUBPC, EC 4.1.1.39) and of the photorespiratory enzymes phosphoglycolate phosphatase (PGP, EC 3.1.3.18), glycolate oxidase (GO, EC 1.1.3.1),and catalase (EC 1.11.1.6) were determined. Treatment of seedlings with SA alone resulted in decreased levels of chlorophyll, CO_2 assimilation and transpiration rates. Pq treatment led to a decrease in chlorophyll content and to a very strong inhibition of photosynthesis, the effects were manifested by 1 h of illumination. Neither SA nor Pq caused changes in RUBPC activity, but instead greatly increased the activity of the photorespiratory enzymes. Pre-treatment of seedlings with SA provided protection against subsequent Pq-induced stress. This observation was confirmed by gas exchange parameters, chlorophyll content and by changes in some important parameters associated with oxidative stress, namely H_2O_2 production, lipid peroxidation and electrolyte leakage. Pre-treatment with SA fully blocked the inhibitory effect of Pq on photosynthesis and reduced Pq-induced H_2O_2 production, lipid peroxidation, and electrolyte leakage. The data suggest that SA might protect cells against oxidative damage and prevent photosynthesis upon paraquat treatment.

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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.

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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.

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BIOLOGIE
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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.

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BIOLOGIE
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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.

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BIOLOGIE
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ROSETTE LEAF SENESCENCE IN WILD TYPE AND ETHYLENE INSENSITIVE MUTANT (*eti5*) *ARABIDOPSIS THALIANA* (L.) HEYNH DURING INFLORESCENCE AND FRUIT DEVELOPMENT: I. CHANGES IN PLASTID PIGMENTS, PRODUCTS OF LIPID AND PROTEIN OXIDATION, AND HYDROGEN PEROXIDE LEVELS

D. Todorova, I. Sergiev, V. Alexieva, E. Karanov

(*Submitted on April 24, 2002*)

Abstract

In order to characterize physiologically the senescence of rosette leaves of two *Arabidopsis thaliana* genotypes – a wild type, cv. Columbia, and an ethylene insensitive mutant *eti5* during inflorescence and fruit development, the changes in leaf pigment and protein content, products of lipid peroxidation and protein oxidation, content of hydrogen peroxide were determined. The plants were grown in plastic pots (d = 70 mm; h = 80 mm), filled with soil/perlite mixture (3:1) in a growth chamber (16/8 day/night photoperiod, 70 μmol.m^{-2}.s^{-1} photon flux density; 26/22 °C day/night temperature; 60 % air humidity). The plants were irrigated daily. All parameters were determined on the 3rd, 4th, 5th, 6th and 7th rosette leaf.

The leaves of the wild type had lower chlorophyll content in comparison with the mutant rosette leaves during the first investigated stage of plant development. Additionally, this parameter further significantly decreased in the wild type of Arabidopsis during silique maturation in comparison to *eti5* plants. The data of stress marker amounts (malondialdehyde equivalents and carbonyl groups) showed that there is a greater oxidative damage of lipids and proteins in the wild type of Arabidopsis. The levels of hydrogen peroxide measured during inflorescence were found to be higher in wild type plants than in mutant ones, but during silique maturation this difference was compensated mainly due to a reduction in H_2O_2 content in the wild type, and the slight increase in *eti5*.

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BIOLOGIE
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CHANGES IN POLYAMINE CONTENT IN WILD TYPE *ARABIDOPSIS THALIANA* PLANTS TREATED WITH ETHREL, BA AND CPPU SEPARATELY AND IN COMBINATIONS[1]

D. Todorova, V. Alexieva

(*Submitted by Corresponding Member E. Karanov on April 24, 2002*)

Abstract

Comparative studies were performed on the endogenous polyamine content in wild type of *Arabidopsis thaliana* (L.) Heynh plants after leaf treatment both separately and in combinations with two cytokinins – BA (N^6-benzyladenine), CPPU (N_1-(2-chloro-4-pyridyl)-N_2-phenylurea) and Ethrel (ethylene producer). We studied the level of free and bound putrescine (Put), spermidine (Spd) and spermine (Spm) in rosette leaves of plants, as well as their total content. It was established that when plants were treated with BA the measured levels of polyamines were higher than those measured in plants treated with CPPU. Generally, treatment with Ethrel increased polyamine levels. Despite the fact that the two groups of compounds – Ethrel and cytokinines – have usually opposite effects on most physiological processes when they were applied in combination, they caused an accumulation of polyamines. The effect was more pronounced after treatment with Ethrel and the phenylurea cytokinin CPPU.

040

Доклади на Българската академия на науките
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Tome 55, No 10, 2002

BIOLOGIE

Physiologie des plantes

EFFECT OF SOME CYTOKININS AND THEIR COMBINATION WITH ETHREL ON THE ENDOGENOUS POLYAMINE CONTENT IN ETHYLENE-INSENSITIVE MUTANT *ETI5* TYPE OF *ARABIDOPSIS THALIANA* (L.) HEYNH PLANTS[1]

D. Todorova, V. Alexieva, E. Karanov

(*Submitted on July 17, 2002*)

Abstract

The effect of two cytokinins – BA (N^6-benzyladenine) and CPPU (N^1-(2-chloro-4-pyridyl)-N^2-phenylurea) and Ethrel (ethylene producer) applied separately and in combination over the endogenous polyamine content in ethylene-insensitive mutant (*eti5*) of *Arabidopsis thaliana* (L.) Heynh plants after leaf treatment was investigated. The level of free, bound and the total content of putrescine (Put), spermidine (Spd) and spermine (Spm) in rosette leaves was determined. It was established that plants contained a higher level of polyamines after CPPU treatment. In general, in plants treated with BA the levels of PAs were reduced. The treatment with Ethrel caused increased titres of bound polyamines, but there was a decrease of free forms. Accumulation of polyamines after combined application of Ethrel and both cytokinins was observed. The effect was more pronounced after the plant treatment with Ethrel and BA.

041

Доклади на Българската академия на науките
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BIOLOGIE
Physiologie des plantes

POLYAMINE LEVELS IN *ARABIDOPSIS THALIANA* (L.) HEYNH, PLANTS DURING THEIR DEVELOPMENT[1]

D. Todorova, V. Alexieva, E. Karanov

(*Submitted on December 20, 2001*)

Abstract

Comparative studies were performed on the endogenous polyamines content in the wild type and in an ethylene insensitive mutant (*eti5*) of *Arabidopsis thaliana* (L.) Heynh. We studied the level of free and bound putrescine (Put), spermidine (Spd) and spermine (Spm) in rosette leaves of plants during different stages of plant development. It was established that mutant plants contained a higher level of endogenous polyamines comparing to the wild type. In general, the highest levels of polyamines were detected in trichloracetic acid (TCA)-insoluble fraction and the lowest – in supernatant-bound (SN-bound) polyamines (PAs) in both genotypes. The results indicated a specific correlation between the high level of total spermidine and flower formation and seed germination, too. Not so significant increase was detected in total putrescine and spermine amounts during generative phases comparing to the vegetative phase. We suggested that spermidine plays the most important role in flowering and seed maturation.

Bulg. J. Plant Physiol., Special Issue, 2003, 1–17

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.

043

Доклади на Българската академия на науките
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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

S. Ivanov, V. Alexieva, E. Karanov

(*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.

044
Доклади на Българската академия на науките
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BIOLOGIE
Physiologie des plantes

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.

045

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

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.

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

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.

BULG. J. PLANT PHYSIOL., SPECIAL ISSUE 2003, 133–152

SALICYLIC ACID - AND METHYL JASMONATE - INDUCED PROTECTION ON PHOTOSYNTHESIS TO PARAQUAT OXIDATIVE STRESS

L. Popova*, E. Ananieva, V. Hristova, K. Christov, K. Georgieva, V. Alexieva, Zh. Stoinova

Acad. M. Popov Institute of Plant Physiology, Acad. G. Bonchev Str., Bl. 21, 1113, Sofia, Bulgaria

Summary. In the present work it is demonstrated that Salicylic acid (SA) and Methyl Jasmonate (MeJA) provided protection of photosynthesis (A) against paraquat (Pq) stress and diminished the oxidative damage caused by Pq. Barley seedlings (12 d old) were supplied with 500 μM SA, 23 μM MeJA or 10 μM Pq via the transpiration stream and kept in the dark for 24 h. They were then exposed to 100 $\mu mol.m^{-2}.s^{-1}$ PAR and samples were taken 1, 2, 3, and 6 h after the light exposure. Leaf gas exchange parameters, the activity of RuBPC and of the photorespiratory enzymes PG, GO, and CAT were determined. Treatment of seedlings with SA or MeJA alone resulted in decreased levels of Chl, A and Tr. Pq treatment led to a decrease in Chl and protein content and to a very strong inhibition of A. Pq-treatment did not affect the activity of RuBPC but greatly increased the activity of the photorespiratory enzymes. Pre-treatment of seedlings with SA or MeJA fully blocked the inhibitory effect of Pq on A and provided protection against subsequent Pq-induced oxidative damage. This observation was confirmed by gas exchange parameters, Chl and protein content and by changes in lipid peroxidation, H_2O_2 level, and electrolyte leakage. The relationship between SA, MeJA and Pq toxicity and the degree of oxidative damage was examined by measuring the activities of several antioxidative enzymes such as SOD, APX, GR and POX. Treatment with 10 μM Pq reduced the activities of APX and GR. Pre-treatment with 500 μM SA for 24 h in the dark greatly improved the capacity of the antioxidative defence system and increased Pq tolerance. Pre-treatment with 23 μM MeJA only partially improved the capacity of the antioxidative enzyme system. It is suggested that the observed protection of MeJA on A against Pq-toxicity was mainly due to improvement of membrane stability and composition, Chl and protein levels.

ROSETTE LEAF SENESCENCE IN WILD TYPE AND AN ETHYLENE-INSENSITIVE MUTANT OF *ARABIDOPSIS THALIANA* DURING INFLORESCENCE AND FRUIT DEVELOPMENT

I. SERGIEV[1], D. TODOROVA[1], V. ALEXIEVA[1], E. KARANOV[1], A. SMITH[2] AND M. HALL[2]

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1. INTRODUCTION

Senescence is an important developmental process in plants, which eventually leads to whole plant, organ, tissue and cell death through highly regulated, endogenously controlled degenerative processes (Chandlee, 2001). Leaf senescence is a key developmental step in the life of an annual plant, as it is the time during which material built up by the plant during its growth phase is mobilized into the developing seed to prepare for the next generation (Smart, 1994; Smart *et al.*, 1995). Obvious visual symptoms for leaf senescence are the loss of chlorophyll pigments, desiccation and eventual abscission. Cellular and molecular events contributing to these visual symptoms include chloroplast disintegration, a decline in photosynthesis and damages of proteins and nucleic acids. Other internal symptoms of senescence are loss in the ability to accumulate protein and nucleic acids because of enhanced degradation and/or diminution of synthesis, and loss of plasma membrane and endomembrane structure with associated increases in permeability to inorganic and organic solutes, as well as an increase in the levels of active oxygen species, the later forming organic free radicals (Smart, 1994; Buchanan-Wollaston, 1997). The free radicals impose a significant impact for the deterioration of cell constituents during plant senescence. They are highly reactive, self-propagating, potentially damaging, and are also formed during the normal metabolic processes. Their effective removal is of importance for the well-being of the plant organisms (Fridovich, 1976). One of the mechanisms by which plants defend against free radical mediated damage is the

induction of the superoxide dismutase (SOD). Peroxidase and catalase then breakdown the formed hydrogen peroxide, and thus prevent the further formation of potent free radicals (Dhindsa *et al.*, 1981).

I. Machačková and G.A. Romanov (eds.), Phytohormones in Plant Biotechnology and Agriculture, 217-228

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Tome 56, No 7, 2003

BIOLOGIE

Physiologie des plantes

MODULATION OF THE PARAQUAT TOXICITY IN PEA PLANTS BY SOME PHENYLUREA DERIVATIVES

I. Sergiev, V. Alexieva, E. Karanov

(*Submitted on March 26, 2003*)

Abstract

The toxic effects of the non-selective herbicide paraquat on pea plants (*Pisum sativum*, L., cv. Citrina) were investigated by means of the dynamics of changes in the levels of free proline, hydrogen peroxide, leakage of electrolytes and malondialdehyde. It was established that the application of the phenylurea cytokinin 4PU-30 or its structural analogue MCP-3 along with the herbicide enhance the paraquat phytotoxicity.

Доклади на Българската академия на науките
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Tome 56, No 6, 2003

BIOLOGIE

Physiologie des plantes

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.

Russian Journal of Plant Physiology, Vol. 50, No. 2, 2003, pp. 163–167. From Fiziologiya Rastenii, Vol. 50, No. 2, 2003, pp. 183–187.

Influence of Root Oxygen Deficiency on Photosynthesis and Antioxidant Status in Barley Plants[1]

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Received November 2, 2001

Abstract—Roots of barley plants (*Hordeum vulgare* L., cv. Alfa) were subjected to hypoxia for 120 h. By 72 to 120 h of soil flooding, a noticeable decrease in the rate of CO_2 assimilation and transpiration was observed. A drop in the activities of Rubisco and photorespiratory enzymes was found. We examined the changes in the activities of enzymes involved in the antioxidative system and stress markers related to membrane integrity, namely, lipid peroxidation and electrolyte leakage. Catalase and peroxidase activities were increased during the experiment, whereas superoxide dismutase activity drastically decreased.

Effect of ethylene and its antagonist 1-MCP on the senescence of detached leaves of *Arabidopsis thaliana*

V.S. ALEXIEVA*[1], I.G. SERGIEV*, D.A. TODOROVA*, E.N. KARANOV*, A.R. SMITH** and M.A. HALL**

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*Institute of Biological Sciences, University of Wales, Edward Liwyd Bl., SY233DA Ceredigion, Aberystwyth, UK***

Abstract

1-Methylcyclopropene (1-MCP) applied alone did not influence significantly the chlorophyll and carotenoid content of the older leaves of *Arabidopsis thaliana* (L.) Heynh., but retarded the senescence of the younger ones (6^{th} and 7^{th} leaf nodes). However, 1-MCP effectively blocks the ethylene induced senescence of excised rosette leaves. The preliminary application of 1-MCP (3 h in advance to the treatment by *Ethrel*) almost totally eliminated the ethylene action. Similar trend was also observed after simultaneous application of *Ethrel* and 1-MCP, and the effects of both treatments on the chlorophyll and carotenoid destruction are comparable.

Bulgarian Journal of Agricultural Science, 10 (2004), 305-309
National Centre for Agrarian Sciences

053

Effect of Plant Growth Regulators Alar, MEIA and Paclobutrazole on the Phenophase of Flowering and Some Ornamental Parameters of Mini-Carnation (*D. caryophyllus f. spray* Hort.)

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[2] *Institute of Plant Physiology "Acad. M. Popov", Bulgarian Academy of Sciences, BG-1113 Sofia, Bulgaria*

Abstract

The effect of three plant growth regulators on flowering and some quality parameters of mini-carnation (*D. caryophyllus f. spray* Hort.) cut-flower was studied. Plants of Bulgarian varieties - Yanita, Naslada and Russalka were treated three times with water solutions of Alar (500 and 1000 mg/l), MEIA (500 and 1000 mg/l) and Paclobutrazole (500 mg/l). It was found that:

• PGRs Alar and MEIA stimulated the beginning of flowering and full blooming during the phenophase of flowering, while Paclo haà a retardant effect on their timing.

• All three PGRs increaseà the number of flowers per floriferous shoot but in terms of floriferous shoot length and number per plant the effect of Paclobutrazole was retardant, unlike Alar and MEIA.

SHORT COMMUNICATION

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

Lyuba P-E. MITEVA, Sergei V. IVANOV, Vera S. ALEXIEVA and Emanuil N. KARANOV

Institute of Plant Physiology Acad. M. Popov, Bulgarian Academy of Sciences, Sofia, Bulgaria

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.

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Plant Physiology and Biochemistry
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Original article

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

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Received 18 April 2003; accepted 23 October 2003

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.

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BIOLOGIE

Physiologie des plantes

SYNTHESIS AND ANTICYTOKININ PROPERTIES OF TRISUBSTITUTED UREAS AND THIOUREAS

I. Sergiev, B. Hadjieva*, V. Alexieva, V. Kalcheva*, B. Galabov*, M. Markova*, E. Karanov

(*Submitted on May 19, 2004*)

Abstract

A series of trisubstituted ureas and thioureas structural analogues to phenylurea cytokinins were synthesized and their growth regulating properties were studied on typical cytokinin-response model systems. It was found that N^1-benzyl-$N^{1'}$-methyl-N^3-phenylthiourea and N^1-benzyl-$N^{1'}$-methyl-N^3-cyclohexylthiourea expressed considerable anticytokinin activity by eliminating the cytokinin action of the standards N^6-benzyladenine and 4PU-30. Some trends in the "chemical structure – physiological activity" relationships were also outlined.

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BIOLOGIE

Physiologie des plantes

SYNTHESIS AND CYTOKININ/ANTICYTOKININ ACTIVITY OF SOME NEW DISUBSTITUTED UREIDOALKANES

I. Sergiev, B. Hadjieva*, V. Alexieva, V. Kalcheva*, E. Karanov

(*Submitted on May 19, 2004*)

Abstract

The cytokinin/anticytokinin properties of series of newly synthesized disubstituted ureidoethanes and ureidohexanes were investigated in typical cytokinin-response model systems. It was found that cyclohexyl and phenyl derivatives showed cytokinin activity by retarding the chlorophyll loss in barley leaf segments, but did not influence the growth of excised radish cotyledons. The symmetric disubstitution in the phenyl rings led to expression of anticytokinin activity. The presented compounds were less active as cytokinin antagonists as compared to the other groups of anticytokinins, and this is most probably due to the multiplication of the urea bridge.

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Доклади на Българската академия на науките
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BIOLOGIE
Physiologie des plantes

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.

Plant Growth Regulation **42:** 1–5, 2004.

059

Cytokinin oxidase/dehydrogenase in *Pisum sativum* plants during vegetative development. Influence of UV-B irradiation and high temperature on enzymatic activity

Irina Vaseva-Gemisheva[1,2,*], David Lee[1], Vera Alexieva[2] and Emanuil Karanov[2]
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Received 3 April 2003; accepted in revised form 25 July 2003

Abstract

Cytokinin oxidase/dehydrogenase (EC 1.5.99.12) specific activity was determined in leaves and roots of two *P. sativum* cultivars (cv. Scinado and cv. Manuela) during vegetative development and the effect of UV-B irradiation or elevated temperature was assessed. The measurement of CKX activity during development showed localisation of this enzyme to roots. The reduction in CKX activity in leaves after UV-B irradiation and the increased levels of the enzyme in high temperature-treated plants suggests that the enzymes from the CKX gene family have a different expression during stress responses provoked by different factors and probably are tissue specific. Differences regarding cytokinin oxidase/dehydrogenase activity stress response were observed between the two pea cultivars.

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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.

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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.

Russian Journal of Plant Physiology, Vol. 52, No. 2, 2005, pp. 213–219. From Fiziologiya Rastenii, Vol. 52, No. 2, 2005, pp. 243–249.

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.

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Доклади на Българската академия на науките
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BIOLOGIE
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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

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(*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.

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.**

Доклади на Българската академия на науките
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EXPLORATIONS COSMIQUES

METHOD FOR DETECTING STRESS INDUCED CHANGES IN LEAF SPECTRAL REFLECTANCE

D. Krezhova, T. Yanev, St. Lukov, P. Pavlova*, V. Aleksieva**, D. Hristova***, S. Ivanov**

(*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.

Доклади на Българската академия на науките
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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.

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Доклади на Българската академия на науките
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BIOLOGIE

Physiologie des plantes

INFLUENCE OF 4PU-30 AND THIDIAZURON ON HYDROGEN PEROXIDE AND SOME DEFENCE ENZYMES OF IN VITRO CULTURED APPLE AND PEACH[1]

I. Sergiev, D. Todorova, K. Kornova*, V. Alexieva, E. Karanov

(*Submitted on April 20, 2005*)

Abstract

The effect of the phenylurea cytokinins 4PU-30 and thidiazuron (TDZ) on hydrogen peroxide levels, and peroxidase, catalase and IAA-oxidase activity on in vitro cultured apple and peach was studied. Explants of apple (rootstock MM 106) and peach (rootstock GF 677) were cultured on solid Murashige-Skoog medium. Both compounds were added to the standard medium and tested at concentrations of 0.5, 0.05, 0.005 and 0.0005 mg/l. The higher concentrations increased the activity of peroxidase and catalase. The effect observed diminished with the decrease of the concentration applied. Both compounds enhanced endogenous level of hydrogen peroxide. However, both plant growth regulators had opposite effect in relation to IAA-oxidase activity, i.e. TDZ enhanced IAA-oxidase activity, while 4PU-30 induced inhibition with exception of concentration 0.5 mg/l.

Доклади на Българската академия на науките
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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.

Доклади на Българската академия на науките
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BIOLOGIE
Physiologie des plantes

EFFECT OF SOME PLANT GROWTH REGULATORS ON PHYSIOLOGICAL AND BIOCHEMICAL STATUS OF IN VITRO MICROPROPAGATED PLANTLETS FROM APPLES DURING ADAPTATION

D. Todorova, I. Sergiev, K. Kornova*, V. Alexieva, E. Karanov

(*Submitted on May 25, 2005*)

Abstract

The effect of the phenylurea cytokinin 4PU-30, gibberellic acid (GA_3) and natural (Sm) and synthetic (DETA) polyamines on some stress markers' content, enzymatic activity and free polyamine levels in two in vitro cultured apples (rootstocks MM106 and M26) was studied. All compounds were leaf applied to plants during adaptation period of rooted plantlets. The treatment with these plant growth regulators provides a promising opportunity for adaptation of apples in non-sterile medium and improves their physiological and biochemical status. The compounds applied reduce the stress-provoked damages due to the transition from in vitro to in vivo growth conditions.

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Доклади на Българската академия на науките
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BIOLOGIE

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EFFECTS OF SOME PLANT GROWTH REGULATORS ON THE ENDOGENOUS POLYAMINE CONTENT IN CULTIVATED IN VITRO *ROSA HYBRIDA* L., CV. MADELON

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(Submitted on December 22, 2004)

Abstract

The effects of the auxin indolylbutyric acid, cytokinins N^1-(2-chloro-4-pyridyl)-N^2-phenylurea (4PU-30) and N^6-benzyladenine, as well as the cytokinin antagonists 2-chloro-4-cyclobutyl-amino-6-ethylamino-1,3,5-triazine and N-(4-pyridyl)-O-(4-chlorophenyl)-carbamate on the amount of free, TCA-soluble and TCA-insoluble polyamines in in vitro cultured one-month-old plantlets of *Rosa hybrida* L., cv. Madelon were studied. Single nodes from shoot cultures were grown on standard MS medium supplemented with the tested plant growth regulators. Elevated amounts of spermine, spermidine and putrescine were detected after 4PU-30 application. In benzyladenine-treated plantlets, the levels of polyamines were reduced as compared to the control plants. An exception was observed in TCA-soluble forms of polyamines. The application of indolylbutyric acid caused a decrease of free and TCA-insoluble putrescine and spermine, but enhanced the same fractions of spermidine and all TCA-soluble forms. Accumulation of polyamines was detected after anticytokinin application. The obtained results suggest an existing physiological effect of the used anticytokinins on the endogenous polyamine levels.

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Effect of temperature stress on the endogenous cytokinin content in *Arabidopsis thaliana* (L.) Heynh plants

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Abstract

The levels of three endogenous cytokinin equivalents: zeatin (Z), *iso*-pentenyladenine (iP) and dihydrozeatin (dZ) in two *Arabidopsis thaliana* (L.) Heynh genotypes – wild type (wt) and ethylene-insensitive mutant (*eti5*), were compared using enzyme immunoassay (ELISA). Cytokinin content was measured after exposure to low (4 °C for 24 h in darkness) or high temperature (38 °C for 24 h in darkness). Measurements were performed immediately and 24, 48 and 120 h after treatments. It was found that at normal growth conditions *eti5* plants contained more endogenous cytokinins compared to the wild type. At both temperature treatments mutant plants had decreased total cytokinin levels. Wild-type plants treated with high temperature (HT) exhibited reduced total cytokinins (with the exception of rates at 48 h), while low temperature (LT) treatment resulted in elevated total amount of the studied equivalents (except at 24 h). The obtained results suggested that HT had greater effect on cytokinin levels than LT since it caused more profound changes in the total content. We assume that this was due to the natural chilling tolerance of *Arabidopsis* plants.

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Antagonistic effects of triazolo[4,5-d]pyrimidine and pyridylurea derivatives on cytokinin-induced cytokinin oxidase/dehydrogenase activity in young pea plants

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Abstract

The effect of strong and weak cytokinin antagonists, belonging to the groups of triazolo[4,5-d]pyrimidines (TP), and pyridyl-phenylurea derivatives (PU), on cytokinin oxidase/dehydrogenase activity (CKX) in the tissues of young pea plants was studied. Tested anticytokinins, with the exception of the most efficient one – PU-1, were able to promote increased CKX activity in roots, when applied alone, but they had no significant influence on the enzymatic activity in leaves. N^6-benzyladenine (BA) and 1-(2-chloropyridin-4-yl)-3-phenylurea (CPPU) provoked strong increase in CKX activity in roots, while in leaves considerable inhibition of enzymatic activity was observed. Different types of anticytokinins exhibited diverse preference towards taking off the action of purine and phenylurea cytokinins over CKX activity.

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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.

APP 074

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\Cytokinin oxidase/dehydrogenase (CKX) activity in wild and ethylene-insensitive mutant *eti5* type of *Arabidopsis thaliana* (L.) Heynh plants and the effect of cytokinin N^1-(2-chloro-4-pyridyl)-N^2-phenylurea on enzymatic activity and leaf morphology

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Abstract

The specific activity of cytokinin oxidase/dehydrogenase (EC 1.5.99.12) (CKX) was determined in leaves of wild type (*wt*) and ethylene-insensitive mutant (*eti5*) of *Arabidopsis thaliana* (L.) Heynh plants. Comparative studies showed that this mutation has lower basal CKX activity than *wt*. Application of 4PU-30 (N^1-(2-chloro-4-pyridyl)-N^2-phenylurea) resulted in decreased CKX activity in both *wt* and mutant plants. The treatment increased leaf blade thickness and the volume of chlorophyll-containing cells per unit leaf area in *wt* but these changes were not observed in the *eti5* mutant. The reduction in chlorophyll "a" and "b", as well as in carotenoids content in the treated *wt* tissues resulting from altered leaf morphology was not detected in *eti5* plants.

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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.

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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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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).

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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.

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Доклади на Българската академия на науките
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BIOLOGIE
Physiologie des plantes

HYDROGEN PEROXIDE PRETREATMENT ALLEVIATES PARAQUAT INJURIES IN PEA (*PISUM SATIVUM* L.)

Irina Moskova, Dessislava Todorova, Vera Alexieva, Iskren Sergiev

(*Submitted by Academician V. Golemansky on June 20, 2007*)

Abstract

Hydrogen peroxide is a molecule natively generated in plants during the normal physiological processes. However, under stress conditions it can be massively produced and can become toxic for the cells. Recently other authors reported that the exogenous application of hydrogen peroxide in low concentrations renders protection of plants against different stress factors. In this investigation we report the protective role of preliminary treatment with H_2O_2 against the toxic action of the herbicide paraquat in pea plants. The changes in some physiological parameters, such as plant survival, content of malondialdehyde, leaf pigments and photosynthetic rate were determined in relation to the paraquat toxicity in H_2O_2-treated plants.

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BRIEF COMMUNICATION

Influence of cytokinins and novel cytokinin antagonists on the senescence of detached leaves of *Arabidopsis thaliana*

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Abstract

Cytokinins N^6-benzyladenine (BA) and 1-(2-chloropyridin-4-yl)-3-phenylurea (4PU-30) delayed the senescence of detached leaves (3rd to 7th leaf node) of wild and ethylene insensitive *eti5* mutant of *Arabidopsis thaliana*. The novel anticytokinins, structural analogues of purine and phenylurea cytokinins also affected the senescence of detached rosette leaves of *A. thaliana*. They diminished to a significant extent the cytokinin-induced delay of chlorophyll destruction, but without a considerable difference in their action against both types of cytokinins. These results correlated with changes observed in ribonuclease (RNase) activity.

J. Amer. Soc. Hort. Sci. 132(6):770–776. 2007.

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.**

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ORIGINAL PAPER

081

Polyamine content in *Arabidopsis thaliana* (L.) Heynh during recovery after low and high temperature treatments

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Abstract Comparative studies on the effect of temperature treatment on the endogenous polyamine content in wild type and the ethylene insensitive mutant *eti5* of *Arabidopsis thaliana* (L.) Heynh were performed. The levels of free and conjugated putrescine, spermidine and spermine were measured in rosette leaves of 38-day-old plants subjected to low and high temperature for 24 h in darkness. Data for fractions measured in treated wild type plants during recovery suggest that alterations in polyamine levels may be a consequence of the conversion of the supernatant-bound into free form and *vice versa*, while in treated *eti5* plants *de novo* synthesis of spermidine and spermine could not be excluded. It was found that high temperature provoked more significant changes in polyamine levels than low temperature. The results suggest that the *eti5* mutant showed a better ability to recover after the temperature treatments than wild type partly as a consequence of changes in polyamine content.

Oxidation Communications 31, No 4, 895–903 (2008)

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.

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Changes in Endogenous Polyamines and Some Stress Markers Content Induced by Drought, 4PU-30 and Abscisic Acid in Wheat Plants

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ABSTRACT

Aerial parts of 7-day old wheat (*Triticum aestivum* L.) seedlings were sprayed with a water solution of the phenylurea cytokinin 4PU-30 or ABA. Twenty-four hours later, part of them was subjected to moderate (– 0.2MPa) or strong (–1.0MPa) water stress. It was found that both stresses induced an accumulation of free, SN-bound and pellet-bound Put in shoots and roots of wheat plants. Free Spd and Spm fractions were little affected by drought in both organ tissues. SN-bound and pellet-bound fractions of Spd and Spm were enhanced in shoots, but in roots strong water deficit provoked a decrease in these fractions. The application of ABA and 4PU-30 caused a slight rise in polyamine levels. In combination with water deficit they increased almost all polyamine levels, but the effect on the free Put in shoots was most substantial. Water shortage also provoked membrane integrity deterioration, mainly due to the lipid peroxidation. Both plant growth regulators significantly reduced the malondialdehyde levels and free proline content of drought-treated wheat seedlings. The results obtained present additional information about the physiological role of growth regulators in relation to water stress.

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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.

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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".

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ORIGINAL PAPER

086

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

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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.

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.

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088

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

Received: 14 August 2007 / Accepted: 28 September 2008 / Published online: 18 October 2008

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.

Response of chlorophyll fluorescence to salinity stress on the early growth stage of the soybean plants *(Glycine max L.)*

089

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Abstract—**The chlorophyll fluorescence in response to salinity stress of soybean plants in spectral range 650-850 nm and slow transient fluorescence kinetics were investigated using remote sensing techniques. The soybean plants were grown under controlled conditions as water cultures on nutrient solution of Helrigel. Salinity was performed at the stage of 2nd to 4th trifoliate expanded leaves by adding of NaCl in the nutrient solution at concentrations 40 mM and 80 mM. The chlorophyll fluorescence was registered by a multichannel fiber optic spectrometer USB2000 working in time-acquisition mode. As a source of actinic light, a light emitting diode with the maximum of the light output at 470 nm was used. The fluorescence spectra were registered subsequently in time at every 2 second. At least 40 spectra from each soybean leaf were obtained; the leaves being taken from 20 control plants and 20 plants treated with two NaCl concentrations. Measurements were conducted on the 14th day after treatment. Several indices such as fluorescence spectra area, halfwidth of the fluorescence spectral curve, and wavelength of spectrum maximum were used to characterize the differences between the normalized fluorescence spectra of leaves of control and treated plants. The Student t-criterion, discriminant analysis and derivative analysis were applied to estimate the statistical significance of the differences between the average values of the indices. The results revealed that the low NaCl concentration led to salinity tolerance while the high NaCl concentration caused salinity stress in the soybean plants.**

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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.

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

091

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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.

Oxidation Communications 33, No 4, 948–955 (2010)

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

092

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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.

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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.

plant biology 094

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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*.

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SPECTRAL REMOTE SENSING TECHNIQUE AS A TOOL FOR PRESERVATION OF ENVIRONMENT

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Abstract

One of the major applications of remote sensing data in agriculture is crop stress detection. In this work we demonstrate the applicability of a remote sensing technique, leaf spectral reflectance, for recognition of vegetation stress using hyperspectral data. The spectral responses of young plants from different cultural species (soybean, pea, tobacco and tomato) subjected to abiotic stresses (salinity and herbicides) and some viral infections (Tomato Mosaic Virus - ToMV, Tomato Spotted Wilt Virus - TSWV etc.) were investigated. Leaf reflectance data were collected in the visible and near infrared spectral ranges (450-850 nm) using a portable fiber-optic spectrometer. The results indicated that stress detection and discrimination by means of changes of reflectance data are realized in four specific spectral regions as follows: green, red and red-edge (520-580 nm; 640-680 nm; 690-720 nm) due to chlorophyll degradation (necrotic or chlorotic lesions); as well as near infrared (720-780 nm) due to leaf tissue changes. The statistically significant differences ($p<0.05$) were assessed by means of the Student's t-criterion.

096 Comptes rendus de l'Acade'mie bulgare des Sciences, Vol 64, No12, pp.1695-1700

Leaf Morphology and Histology Changes of Pea Plants Treated with Hydrogen Peroxide and Paraquat

Irina Moskova, Dessislava Todorova, Vera Alexieva, Iskren Sergiev

ABSTRACT

The effects of herbicide paraquat and hydrogen peroxide, applied alone and in combination, on pea leaf morphohistology were analysed. A single treatment with H_2O_2 provoked histological, but not evident morphological changes of pea leaves. Paraquat disturbed the histology organization significantly decreasing the intercellular spaces and cell sizes in all leaf tissues. Additionally paraquat caused a distinct leaf wilting. The results clearly demonstrate the leaf damages provoked by paraquat. The herbicide did not induce these effects when applied to preliminary treated with H_2O_2 leaves. The data demonstrate that hydrogen peroxide rendered some protective action against the herbicide injuries.

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(87) № и дата на PCT публикация:

(54) ХЕРБИЦИДНИ АНТИДОТИ И СИНЕРГИСТИ

(57) Антидотите и синергистите намират приложение за увеличаване на растежа и продуктивността при царевицата и соята чрез контрол върху плевелната растителност. Те са с повишена ефективност и широк спектър на действие и принадлежат към различни химични групи. Антидотите понижават токсичните ефекти на хербицидите върху културното растение, без да намаляват ефективността им по отношение на плевелите. Синергистите редуцират хербицидните дози, като запазват действието им, което допринася за опазването на околната среда. Използват се известни хербициди като ажил, флекс, фузилад, тел, титус, хлорсулфурон и техни комбинации с растежни регулатори от групите на ретардантите - монометилов естер на итаконовата киселина, фенилкарбамидните цитокинини, например N_1-(2-хлор-4-пиридил)-N_2-фенилкарбамид и 4-ПК-30 и полиамините като диетилентриамин.

1 претенция