БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ — BULGARIAN ACADEMY OF SCIENCES

ФИЗИОЛОГИЯ НА РАСТЕНИЯТА, XII, 2 — PLANT PHYSIOLOGY, XII, 2

София . 1986 . Sofia

Effect of Nitrogen Fertilizer Application and Water Stress on Bean Photosynthetic Productivity

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The Metodi Popov Institute of Plant Physiology — Sofia

(S u m m a r y)

The combined effect of nitrogen supply and water stress on bean plant photosynthetic productivity was investigated using the cultivar Dobroudžanski-2.

It was found that fertilizer application increased bean leaf photosynthetic rate about two times, retarded leaf ageing, enhanced transpiration and reduced stomatal CO_2-resistance. The biological productivity of plants provided with nitrogen fertilizer was 2-2.5 times higher than that of plants with no nitrogen fertilizer application.

Water stress inhibited the photosynthetic apparatus reaction of beans to temperature rising from 20 to 35°C. After water stress ended plants provided with nitrogen recovered their reaction and their photosynthetic rate maximum was observed at 25°C. Plants to which no nitrogen fertilizer was applied did not restore this ability.

Carboxylizing enzyme activity was also restored more fully after water stress ended in plants to which nitrogen fertilizer was applied.

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ФИЗИОЛОГИЯ НА РАСТЕНИЯТА, XII, 2 — PLANT PHYSIOLOGY, XII, 2

София . 1986 . Sofia

Changes in Photosynthetic Parameters of Different Bean Varieties Submitted to Water Stress

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(S u m m a r y)

The effect of water stress on the photosynthetic apparatus and its ability to recover after a period of drought was investigated in different bean varieties: D 81113, Garnet and Dobrudjanski-2.

Photosynthetic rate, transpiration and stomatal conductance were measured in dynamics.

It was found that under 40% full water holding capacity (FWC) soil conditions the photosynthetic rate in D 81113 and Garnet varieties decreased to 17—18% compared to the control plants cultivated under 60% FWC. The photosynthetic rate of Dobrujanski-2 variety declined to 35%. The stomatal conductance decreased to 18%, 36% and 26%, respectively. It was established also that 36 hours after recovery of the soil humidity to 60% FWC the photosynthetic rate reached approximately 65% of the control in D 81113 and Garnet varieties, and 90% in Dobrudjanski-2 variety. The stomatal conductance increased some 60% in the first two varieties and 80% in Dobrudjanski-2 variety.

It was concluded that the photosynthetic apparatus of Dobrudjanski-2 variety bean plants had greater drought resistance and better recovery ability compared to those of D 81113 and Garnet varieties. That is why the biological productivity of the former variety is less affected by the soil humidity variations.

БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ – BULGARIAN ACADEMY OF SCIENCES

ФИЗИОЛОГИЯ НА РАСТЕНИЯТА, XIII, 3 – PLANT PHYSIOLOGY, XIII, 3

София . 1987 . Sofia

Comparative Investigations of Photosynthesis in Cultivated and Wild Tomato Genotypes

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(Summary)

Differences in photosynthesis and its dependence on temperature and light were studied in some cultivated and wild tomato genotypes, namely: 1. line 6944 msms aa (homozygous for two recessive genes, plants — male sterile, stems and leaf petioles without antocyanine colour); 1a. line 6944 Msms Aa (heterozygous for two genes, plants — fertile, stems and leaf petioles with antocyanine colour); 2. line 6944 MsMs AA (homozygous for two dominant genes, plants — fertile, stems and leaf petioles with antocyanine colour); 3. line T_{25} ps c (plants functionally sterile, with potato type leaves); 4. line T_{25} ps (plants functionally sterile with normal leaves); 5. *L. pimpinellifolium* — wild species; 6. *L. cheesmanii* var. *typicum* — wild species. Seventy-day old plants, pot grown in photothermostat conditions (26°±1°C and 180 W.m^{-2}), were investigated.

It was found that the photosynthetic rate (PR) of the wild tomato forms *L. pimpinellifolium* and *L. cheesmanii* var. *typicum* was 14—30% lower than that of the cultivated ones (1, 1a, 2, 3, 4).

Light saturation of photosynthesis for all genotypes investigated was attained at 180—260 W. m^{-2}. The light curve plateau of the wild forms was considerably lower. This fact shows that the photosynthetic apparatus of cultivated tomatoes was adapted to higher light intensities. With the increase in light intensity to 260 W.m^{-2} and in temperature to 34°C stomatal resistance to CO_2 was reduced in all genotypes investigated and transpiration was enhanced.

БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ – BULGARIAN ACADEMY OF SCIENCES

ФИЗИОЛОГИЯ НА РАСТЕНИЯТА, XIV, 1 – PLANT PHYSIOLOGY, XIV, 1

София . 1988 . Sofia

Effect of Drought and Nitrogen Fertilization on Photosynthesis, Transpiration and Biological Productivity of Maize

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Summary

The effect of drought on photosynthesis, transpiration and biological productivity of maize plants at two levels of nitrogen nutrition was studied as well as the restoration ability of photosynthesis and water deficiency of leaves after normalization of soil humidity. The experiments were made with cv. 2-L-611, the plants were grown as soil culture.

It was established that the drought increased the water deficiency and stomatal resistance (r'_s) of maize leaves and decreased transpiration (Tr) and intensity of photosynthesis (IP) more strongly in fertilized plants. The rate of restoration of IP, Tr and r'_s following irrigation was not influenced by the level of nitrogen nutrition. The younger leaves had higher restoration ability. The drought decreased the activity of carboxylating enzymes (PEPC and RuBPC), but PEPC was affected more strongly. When soil humidity was restored up to 60% FWC the activity of both enzymes was restored to 140% over that of non-dried fertilized with nitrogen plants, but the activity was not restored in non-fertilized plants. The changes in water regime decreased the leaf area of maize plants almost in equal degree at the two level of nitrogen nutrition, but the dry matter formed by one plant decreased more strongly in the variant with fertilization because of the higher inhibition of photosynthesis of these plants.

Известия СО АНСССР, сер. Биологии, вып. 2, 1988

V. A. Sokolov, V. K. Shumniy.
Ts. Tsonev, V. Stanev, J. Danailov, K. Dobrinova

STOMATAL FREQUENCY, SIZE AND FUNCTIONAL CHARACTERS IN HETEROSIS HYBRIDS OF PEA

Comparative study of morphological and functional characters of stomata in heterosis hybrid and parent's forms of pea has been carried out. Parents appeared to be phenotypically different from their hybrid progeny by one mutant gene weakening chlorophyll pigmentation. Stomatal morphology was studied on both sides of a completely formed top leaf the stage of 7—8, 10—11, 13—14, 16—17 internodes.

Transpiration rate, stomatal resistance and photosynthesis under different light conditions were taken as physiological parameters for study.

It became clear that the difference in stomatal quantity on leaf area unit depends on the size of the epidermal cells, but not on the origin of the studied plants.

Heterosis hybrids did not reliably differ from normal parents in the stomatal size and frequency F_1 hybrids different from their parents functionally, they had a higher transperation rate, CO_2 conductivity, and as a consequence they had a higher photosynthesis rate.

Differences in these parametres were especially noticeable in hybrids maintained at low light efficiency.

PHOTOSYNTHETICA 28 (2): 289-295, 1993

Changes in some photosynthetic parameters in pea plants after treatment with cobalt

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Abstract

CO_2 (40, 200, 400 μM) was added to the root system of 10-d-old pea plants (*Pisum sativum* L. cv. Ran). The Co^{2+}-excess caused a reduction in the plant fresh and dry masses and water and chlorophyll contents. The rates of photosynthesis and transpiration decreased, while proline content and stomata resistance increased. The dramatic effect of Co^{2+}-toxicity was expressed both in an inhibition of ribulose 1,5-bisphosphate (RuBP) carboxylase activity and a stimulation of RuBP-oxygenase and phosphoenolpyruvate carboxylase activities on the 4th day of cultivation of plants in a solution of 400 μM Co^{2+}.

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BIOLOGIE
Physiologie des plantes

COMPARATIVE EFFECTS OF METHYL ESTER OF JASMONIC ACID, ABSCISIC ACID AND BENZYLADENINE ON CHLOROPHYLL CONTENT AND PHOTOSYNTHESIS IN EXCISED COTYLEDONS OF *CUCURBITA PEPO* L. (ZUCCHINI)

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(*Submitted by Corresponding Member E. Karanov on March 2, 1999*)

Jasmonates—jasmonic acid (JA) and related compounds represent a novel class of plant growth regulators involved in different physiological and developmental processes [9]. The role of JA and its methyl ester (MeJA) in promotion of leaf senescence has been well documented in literature [12]. Much data are available on the inhibitory effect of MeJA on chlorophyll content in senescing leaf segments [11,12]. However, very limited data exist on the effect of MeJA on chlorophyll accumulation in greening leaf tissues. Excised epigeal cotyledons represent an appropriate model system for studying jasmonate action on chlorophyll metabolism during their greening and formation of functionally active photosynthetic apparatus. Cotyledons represent reserve organs which undergo structural and functional differentiation during their transition into photosynthetic organs after emerging above the soil [1]. In addition, it is well known that the genetic programme of this transition is under the positive control of cytokinins whereas abscisic acid (ABA) acts as a cytokinin antagonist [1,5]. That is why, the aim of this work was to study the differential effects of MeJA, ABA and benzyladenine (BA) on development of photosynthetic apparatus during greening of excised marrow cotyledons based on chlorophyll accumulation and photosynthetic activity.

***Bulg. J. Plant Physiol.*, 2000, 26(1-2), 48-57**

INTERACTION BETWEEN METHYL ESTER OF JASMONIC ACID AND BENZYLADENINE DURING THE GROWTH OF EXCISED GREENING COTYLEDONS OF *Cucurbita pepo* L. (zucchini)

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Received 25 March 2000

Summary. Treatment of excised marrow cotyledons (*Cucurbita pepo* L. (zucchini) growing under photoperiod with methyl ester of jasmonic acid (MeJA) resulted in a decrease of their fresh weight. This effect did not depend on the exogenously applied MeJA concentrations. Furthermore, *in vivo* labelling experiments showed an inhibition of the total protein synthesis. On the other hand, specific quantitative changes were observed in the spectrum of soluble proteins resulting in the formation of abundant jasmonate-induced proteins (JIPs). Benzyladenine (BA) applied together with MeJA neutralized the jasmonate action on the growth of the excised cotyledons, as well as on the *in vivo* protein synthesis. Similar antagonistic interaction between BA and MeJA was observed on the formation of JIPs both when the substances were applied together and in the case of subsequent treatment of cotyledons. The antagonism observed between the two plant growth regulators during the growth and development of excised greening cotyledons can be considered as one possible mechanism in the maintenance of plant cell homeostasis under stress conditions in the earlier stages of germination.

BULG. J. PLANT PHYSIOL., 2001, 27(3–4), 76–84

EFFECT OF PHENYLMETHYLSULFONYL FLUORIDE – AN INHIBITOR OF PROTEASES, ON THE GROWTH AND POLYPEPTIDE PROFILE OF EXCISED COTYLEDONS OF *CUCURBITA PEPO* L. (ZUCCHINI) AFTER TREATMENT WITH BENZYLADENINE

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Summary. Treatment of excised marrow (*Cucurbita pepo* L., zucchini) cotyledons in darkness with 45 μM BA caused a marked stimulation of the growth accompanied by a strong decrease in cotyledons relative dry weight. PMSF – a well known inhibitor of both thiol- and serine-type proteases, present in the aqueous solutions either alone or with the cytokinin, inhibited significantly the growth of cotyledons and hindered the decrease in cotyledons dry weight in all treatments. SDS-PAGE analysis of the protein profiles showed that PMSF suppressed the gradual decline in the quantity of the 20–2 kDa polypeptide group and the low-molecular-weight polypeptide bands (below 15 kDa) both in the control and BA-treated cotyledons. On the other hand, the polypeptide bands migrating in the higher molecular weight zone of the profiles including the 97.4-kDa polypeptide and the LSU of Rubisco (55 kDa) decreased in quantity in the presence of PMSF. These results suggest that the inhibitory effect of PMSF on the breakdown of storage proteins in the excised marrow cotyledons may be due to an inhibition only of certain PMSF-sensitive proteases taking part in this process during germination.

Acta Enthomologica Bulgarica, 2001, 7(1-2), 53-59

СТИМУЛИРАЩ ЕФЕКТ НА АБАМЕКТИНА ВЪРХУ ФОТОСИНТЕЗАТА В ТРЕТИРАНИ РАСТЕНИЯ

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Резюме

Лиросект е широкоспектърен инсектициден препарат, който се произвежда от фирма „БИОВЕТ АД" - гр. Пещера. Той е емулсионен концентрат, съдържащ абамектин. Абамектинът е продукт на почвения актиномицет *Streptomyces avermitilis*, получен чрез микробиологичен синтез. Целта на настоящата работа беше да се изследва влиянието на абамектина върху основни физиологични процеси - интензивността на фотосинтезата и натрупването на хлорофил в първични диференциани листа на прорастъци от тиквичка. Според наши данни за подобен ефект на абамектина се съобщава за първи път в литературата.

Като обект на изследване бяха използвани 14-дневни прорастъци на тиквичка, отглеждани като водна култура при контролируеми условия в светлинна камера. Прорастъците бяха пръскани с разтвори на Лиросект с различна концентрация на абамектин или плацебо.

Получените резултати показват висока степен на стимулация на фотосинтезата, която тясно корелира и с увеличено съдържание на хлорофил. В ход са изследвания, които да анализират по-задълбочено възможните механизми, които лежат в основата на този стимулиращ ефект.

BULG. J. PLANT PHYSIOL., 2002, 28(1–2), 85–91

EFFECTS OF ABAMECTIN ON PROTEIN AND RNA SYNTHESIS IN PRIMARY LEAVES OF *CUCURBITA PEPO* L. (ZUCCHINI)

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Received March 22, 2002

Summary. Abamectin is the active compound of the broad spectrum insecticidal and acaricidal preparation Lirosect which is produced in Biovet JSC – Peshtera. Spraying of intact 14-day-old seedlings of *C. pepo* with solutions of Lirosect 1.5 EK, applied at different concentrations, resulted in a marked stimulation of protein and RNA syntheses measured by the incorporation of [^{3}H]-uridine and [^{14}C]-lysine in total RNA and soluble protein, respectively. The most significant increase of protein and RNA syntheses was observed at 0.1% Lirosect. This stimulation was accompanied by an increase in leaf dry weight. These results could be interpreted in view of the ability of abamectin to act as a membrane-active complexone.

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BIOLOGIE
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ELECTROPHORETIC ANALYSIS OF METHYL JASMONATE-INDUCED PROTEINS IN EXCISED COTYLEDONS OF *CUCURBITA PEPO* L. (ZUCCHINI)

M. Tchorbadjieva, K. Ananieva*, E. D. Ananiev*

(*Submitted by Corresponding Member E. Karanov on July 17, 2002*)

Abstract

Treatment of excised marrow (*Cucurbita pepo* L., zucchini) cotyledons with methyl ester of jasmonic acid (MeJA) resulted in an induction mainly of two abundant polypeptide bands with Mr 43 and 53 kD and to a lesser extent of polypeptides with Mr 60, 69 and 97.4 kD as revealed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Separation of these jasmonate-induced polypeptides (JIPs) by denaturing two dimensional electrophoresis (2-D gel electrophoresis) showed that the 53 and 43 kD polypeptides consisted of several charge-isomeric molecules of the same molecular mass. The 53 kD polypeptides had pI values between 4.4 and 4.8 whereas the 43 kD polypeptide band consisted of 5 different polypeptides with pIs between 4.8 – 5.6. In contrast to MeJA abscisic acid (ABA) did not induce the synthesis of 43 and 53 kD polypeptides. It could be supposed that the presence of differently charged isomers of JIPs in cotyledons reflects their complex role as stress-related proteins.

BIOLOGIA PLANTARUM 46 (3): 357-362, 2003

Phenylmethylsulphonyl fluoride inhibits the formation of jasmonate-induced proteins in cotyledons of *Cucurbita pepo* (zucchini)

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Abstract

Phenylmethylsulphonyl fluoride (PMSF), a well known inhibitor of both thiol- and serine-type proteases, in aqueous solutions either alone or with the plant growth regulators, methyl ester of jasmonic acid (MeJA) and N^6-benzyl-aminopurine (BAP), significantly inhibited the growth of excised *Cucurbita pepo* L. (zucchini) cotyledons. SDS-PAGE analysis of the protein profiles showed that PMSF suppressed the gradual decline of the main 20 - 25 kDa polypeptide group and the low molecular mass polypeptides (below 15 kDa) while leupeptine was not able to affect the electrophoretic pattern of cotyledon proteins. On the other hand, in the presence of PMSF, the content of the polypeptides with higher molecular mass including the 97.4 kDa polypeptide and the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (55 kDa) decreased. Besides, when applied together with MeJA, PMSF prevented the appearance of the jasmonate-induced polypeptides (JIPs; 69, 60 and 43 kDa) thus suggesting that JIPs are synthesized from aminoacids released during the breakdown of cotyledon storage proteins.

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BIOLOGIE
Physiologie des plantes

EFFECT OF METHYL ESTER OF JASMONIC ACID, ABSCISIC ACID AND BENZYLADENINE ON ENDOGENOUS NUCLEAR RNA POLYMERASE ACTIVITY IN EXCISED COTYLEDONS OF *CUCURBITA PEPO* L. (ZUCCHINI)

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(*Submitted by Academician E. Golovinsky on July 23, 2003*)

Abstract

"Run-on" transcription experiments with isolated nuclei showed that treatment of excised cotyledons of *Cucurbita pepo* L. (zucchini) with methyl ester of jasmonic acid (MeJA) or abscisic acid (ABA) resulted in inhibition of RNA polymerase I and RNA polymerase II activities. The inhibitory effect of MeJA on RNA polymerase II was more pronounced than on RNA polymerase I. ABA affected both RNA polymerases to the same extent but its effect was stronger than MeJA. No additivity in the action of MeJA and ABA on RNA polymerase system was found when they were applied in a mixture of equimolar concentrations. ABA neutralized the specific stimulation of RNA polymerase I by cytokinin 6-benzyladenine (BA) when cotyledons were treated with the mixture of their equimolar concentrations, whereas MeJA was not able to eliminate the cytokinin effect in the first hours of treatment.

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Changes in endogenous cytokinin levels in cotyledons of *Cucurbita pepo* (zucchini) during natural and dark-induced senescence

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Cytokinin (CK) levels in cotyledons of *Cucurbita pepo* L. (zucchini) were investigated through the processes of post-germination, greening, natural senescence and subsequent rejuvenation. The concentrations of the physiologically active CK bases, ribosides and nucleotides, as well as the *cis*-isomers of zeatin derivatives, decreased between the first and fifth weeks of cultivation under controlled light conditions. At the same time, the levels of storage CK *O*-glucosides and physiologically inactive CK 7- and 9-glucosides increased with senescence. With plant decapitation and subsequent cotyledon rejuvenation, not only the chlorophyll content but also the levels of physiologically active CKs, nucleotides and *cis*-zeatin derivatives increased. The levels of *O*-glucosides, however, decreased. When 1-week-old seedlings were transferred to the dark, there was a progressive reduction in cotyledon chlorophyll content, deterioration of chloroplast ultrastructure and a decrease in physiologically active CKs and their nucleotides. In contrast with natural senescence, the storage CK *O*-glucosides decreased under dark conditions, suggesting different metabolic regulation of endogenous CK levels during natural and dark-induced senescence of zucchini cotyledons. The chlorophyll loss of dark-treated cotyledons could be partially reversed, even after 5 days, with return to light conditions. During this recovery, physiologically active CKs and their nucleotides again increased, whereas the storage CK *O*-glucosides and *cis*-zeatins decreased. The present results suggest that dark-induced destruction and subsequent restoration of chloroplasts during light shifts are controlled by changes in the levels of physiologically active CKs and their nucleotides.

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Methyl jasmonate down-regulates endogenous cytokinin levels in cotyledons of *Cucurbita pepo* (zucchini) seedlings

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Endogenous cytokinins (CK) were measured in *Cucurbita pepo* L. (zucchini) cotyledons after spraying 7-day-old seedlings with methyl jasmonate (MeJA) to examine whether the senescence-promoting action of MeJA is associated with changes in metabolic regulation of CK levels. MeJA promoted senescence as estimated by the loss of chlorophyll and injury to chloroplast ultrastructure. This was already detected one day after treatment. The contents of physiologically active CKs determined by high-performance liquid chromatography-mass spectrometry (CK bases: *trans*-zeatin, dihydrozeatin and isopentenyladenine and their ribosides), especially *trans*-zeatin and its riboside declined considerably. CK nucleotides and physiologically inactive CK 7- and 9-glucosides were also markedly decreased whereas the content of storage CK *O*-glucosides as well as *cis*-isomers of zeatin declined only slightly. Chlorophyll content and the normal chloroplast structure recovered 5 days after the MeJa treatment. The process of photosynthetic apparatus recovery correlated with an increased concentration of physiologically active CKs, especially Z. In contrast, the content of CK nucleotides and storage CK *O*-glucosides decreased further. We suggest that the ability of MeJA to promote some aspects of senescence in intact zucchini cotyledons is at least partially due to down-regulation of endogenous CK levels and their interconversion between active and inactive forms.

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BIOLOGII
Biochemie

TWO-DIMENSIONAL GEL ELECTROPHORETIC ANALYSIS OI PROTEINS IN EXCISED COTYLEDONS OF *CUCURBITA PEP* L. (ZUCCHINI) AFTER HORMONE TREATMENT

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(*Submitted by Academician E. Karanov on July 28, 2004*)

Abstract

Two dimensional electrophoretic analysis (2-D PAGE) of soluble proteins from excised cotyledons of *C. pepo* L. (zucchini) revealed approximately 70 polypeptides within a wide range of molecular masses (10–120 kDa) and pIs (3.5–9.5). About half of them represented neutral and slightly acidic polypeptides (pIs 5.5–7.5) with molecular masses higher than 25 kDa. The other polypeptides corresponded to reserve proteins and could be divided into three groups according to their charge and molecular masses. The first set of polypeptides consisted of approximately 12 polypeptides with low molecular masses and acidic nature (10–13 kDa and pIs 3.5–5.2). These polypeptides were mostly accumulated in control and abscisic acid (ABA)-treated cotyledons and less presented in cotyledons either treated with 6-benzylaminopurine (BAP) alone or with the mixture of BAP+MeJA (methyl ester of jasmonic acid). The second group comprised about 15 polypeptides migrating between 19–25 kDa and situated in the alkaline zone of the gel (pI 7.6–8.5). These polypeptide spots represented the main products of proteolytic degradation of cotyledon reserve proteins (globulins). They were accumulated in ABA-treated cotyledons and their content diminished mostly in BAP-treated cotyledons. The large subunit of ribulose-1,5-biphosphate carboxylase (Rubisco) was heavily reduced after cytokinin treatment together with other polypeptides of higher molecular mass. The third polypeptide group consisted of several polypeptides (8–10) with lower molecular mass (10–12 kDa) and basic nature (pIs 7–8.3).

EFFECT OF METHYL ESTER OF JASMONIC ACID, ABSCISIC ACID AND BENZYLADENINE ON CHLOROPHYLL SYNTHESIS IN EXCISED COTYLEDONS OF *CUCURBITA PEPO* (ZUCCHINI)

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Summary. Except for the first stages of illumination, exogenous methyl ester of jasmonic acid (MeJA) inhibited much stronger than abscisic acid (ABA) the accumulation of chlorophyll in excised cotyledons of *C. pepo* L. (zucchini) exposed to controlled light conditions. Benzyladenine (BA) applied in a combination with MeJA or ABA at equimolar concentrations completely neutralized their inhibitory effect. In contrast to ABA, preincubation of cotyledons with MeJA in the dark increased its inhibitory effect on chlorophyll synthesis while the effect of ABA remained almost unchanged. Determination of δ-aminolevulinic acid (δ-ALA) in greening cotyledons revealed a close correlation between the effect of phytohormones on chlorophyll content and δ-ALA accumulation. MeJA inhibited to a greater extent than ABA the synthesis of δ-ALA. Our results suggest different mechanisms of inhibition of chlorophyll synthesis by both inhibitors. MeJA preferentially inhibitied chlorophyll accumulation at the level of chlorophyll precursors in the dark while ABA exerted its effect on the light-dependent reactions of chlorophyll synthesis.

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Short Communication

Some biochemical properties of cytokinin oxidase/dehydrogenase in *Cucurbita pepo* L. (zucchini) leaves

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Cytokinin oxidase/dehydrogenase (CKX) was extracted and partially purified from intact leaves of *Cucurbita pepo* (zucchini) and its activity determined by an *in vitro* assay based on the measurement of the rate of conversion of [2-^{3}H]iP to [2-^{3}H] adenine. The optimum incubation time of the enzyme reaction under standard assay conditions was 3h. Under these conditions the conversion of the substrate was almost linear and did not exceed 20%. The addition of copper-imidazole complex to the assay mixture resulted in a significant increase in the enzyme activity compared with the standard assay, especially during the first 6h of incubation. The partially-purified enzyme extract exhibited a pH optimum at pH7.0–7.5 (Tris-HCl). The biochemical properties of zucchini enzyme preparations are discussed in view of the existing evidence for biochemical diversity of CKX.

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BIOLOGIE
Physiologie des plantes

SPECIFIC EFFECTS OF DARKNESS AND MeJA TREATMENT ON SENESCENCE RELATED PHOTOSYNTHETIC PARAMETERS IN INTACT *CUCURBITA PEPO* (ZUCCHINI) COTYLEDONS

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(*Submitted by Corresponding Member O. Poljakova on September 28, 2005*)

Abstract

Cotyledons of 7-day-old *Cucurbita pepo* L. (zucchini) seedlings were induced to senescence either by transfer of the seedlings to darkness or spraying with methyl jasmonate (MeJA). Measurements were done 24 h after treatment. Senescence was quantified by the changes in functional activity of PSII (estimated by the F_v/F_m ratio and the actual quantum yield of PSII electron transport in the light-adapted state, ΦPSII) as well as photosynthetic rate. While 24-h dark treatment did not affect both senescence related parameters studied, MeJA caused a significant reduction in photosynthetic rate without affecting the functional activity of PSII. These results indicate that MeJA is a more potent inducer of senescence compared to darkness. The lack of effect of both stress factors used to promote senescence in zucchini cotyledons on the photochemical efficiency of PSII could imply the existence of mechanisms protecting the functional activity of PSII under definite stress conditions, thus making this parameter more stable compared to others used to quantify senescence.

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Methyl jasmonate is a more effective senescence-promoting factor in *Cucurbita pepo* (zucchini) cotyledons when compared with darkness at the early stage of senescence

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KEYWORDS
Cotyledons;
Cytokinins;
Dark treatment;
Methyl jasmonate;
Senescence

Summary

The effects of short-term darkening and methyl jasmonate (MeJA) on cotyledon senescence were studied 24 h after transfer of intact 7-day-old *Cucurbita pepo* (zucchini) seedlings to darkness or spraying with 100 μM MeJA. The jasmonate inhibitory effect on chlorophyll content and chloroplast transcriptional activity was stronger compared with darkness. Further, MeJA reduced the photosynthetic rate whereas darkness did not affect photosynthesis. Neither stress factor affected the photochemical quantum efficiency of photosystem II (PSII) estimated by the variable fluorescence (F_v)/maximal fluorescence (F_m) ratio, suggesting the existence of mechanisms protecting the functional activity of PSII at earlier stages of senescence, thus making this parameter more stable compared to others used to quantify senescence. Both stress factors caused a decrease in the content of physiologically active cytokinins, especially *trans*-zeatin (Z), with the jasmonate effect being much more pronounced when compared to darkness. Our results indicate that MeJA is a

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ORIGINAL PAPER

Changes in photosynthetic capacity and polypeptide patterns during natural senescence and rejuvenation of *Cucurbita pepo* L. (zucchini) cotyledons

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Abstract Natural senescence of *Cucurbita pepo* (zucchini) cotyledons was accompanied by a gradual degradation of reserve proteins (globulins) and an intensive decrease in the content of both large subunit (LSU) and small subunit (SSU) of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). The net photosynthetic rate, the primary photochemical activity of PSII, estimated by the variable fluorescence (F_v)/maximal fluorescence (F_m) ratio (F_v/F_m) and the actual quantum yield of PSII electron transport in the light-adapted state (ΦPSII) also progressively decreased during natural senescence. In contrast, the fraction of the absorbed light energy, which is not used for photochemistry (LNU) increased with progression of senescence. The decline in the photosynthetic rate started earlier in ontogenesis compared with the down-regulation of the functional activity of PSII, thus suggesting the existence of protective mechanisms which maintain higher efficiency of the photochemical electron transport reactions of photosynthesis compared with the dark reactions of the Calvin cycle during earlier stages of natural senescence. Decapitation of the epicotyl above the senescing cotyledons resulted in full recovery of the polypeptide profile in the rejuvenated cotyledons. In addition, the photosynthetic rate increased reaching values that exceeded those measured in juvenile cotyledons. The photochemical efficiency of PSII also gradually recovered, although it did not reach the maximum values measured in the presenescent cotyledons.

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Senescence progression in a single darkened cotyledon depends on the light status of the other cotyledon in *Cucurbita pepo* (zucchini) seedlings: potential involvement of cytokinins and cytokinin oxidase/dehydrogenase activity

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Darkness mediates different senescence-related responses depending on the targeting of dark treatment (whole plants or individual leaves) and on the organs that perceive the signal (leaves or cotyledons). As no data are available on the potential role of darkness to promote senescence when applied to individual cotyledons, we have investigated how darkness affects the progression of senescence in either a single or both individually darkened cotyledons of young 10-day-old *Cucurbita pepo* (zucchini) seedlings. Strong acceleration of senescence was observed when both cotyledons were darkened as judged by the damage in their anatomical structure, deterioration of chloroplast ultrastructure in parallel with decreased photosynthetic rate and photochemical quantum efficiency of PSII. In addition, the endogenous levels of cytokinins (CKs) and IAA were strongly reduced. In a single individually darkened cotyledon, the structure and function of the photosynthetic apparatus as well as the contents of endogenous CKs and IAA were much less affected by darkness, thus suggesting inhibitory effect of the illuminated cotyledon on the senescence of the darkened one. Apparently, the effect of darkness to accelerate/delay senescence in a single darkened cotyledon depends on the light status of the other cotyledon from the pair. The close positive correlation between CK content and the activity of CK oxidase/dehydrogenase (CKX; EC 1.4.3.18/1.5.99.12) suggested that CKX was essentially involved in the mechanisms of downregulation of endogenous CK levels. Our results indicated that CKX-regulated CK signaling could be a possible regulatory mechanism controlling senescence in individually darkened cotyledons.

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ORIGINAL PAPER

Different effects of dark treatment on pigment composition and photosystem I and II activities in intact cotyledons and primary leaves of *Cucurbita pepo* (zucchini)

Kiril Mishev · Detelin Stefanov · Kalina Ananieva · Chavdar Slavov · Evguéni D. Ananiev

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Abstract Darkness mediates different senescence-related responses depending on the targeting of dark treatment (whole plants or individual leaves) and the organs that perceive the signal (leaves or cotyledons). In this study, we presented data on the differential effects of 2-day dark treatment on progression of senescence in cotyledons and primary leaves of 14-day-old plants of *Cucurbita pepo* L. (zucchini). The lack of changes in chlorophyll *a*, chlorophyll *b* and carotenoid contents as well as in the PSI activity measured by the far-red induced alterations in the P_{700} oxidation levels and the quantum yield of electron transport from Q_A^- to PSI end acceptors (φ_{Ro}) indicated higher resistance of cotyledons to the applied dark stress compared to the primary leaves. In contrast to cotyledons, PSI activity in the primary leaves was significantly inhibited. Concerning the activity of PSII analyzed by the changes in the JIP-test parameters (the maximal efficiency of PSII photochemistry, φ_{P_0}; the performance index, PI_{ABS}; the efficiency of Q_A^- reoxidation, ψ_0 and the effective dissipation per reaction center, DI_0/RC), no differences were observed between cotyledons and primary leaves, thus suggesting that PSI activity in the true leaves was more susceptible to the applied dark stress. The transfer of the darkened plants to normal light regime resulted in delayed senescence in cotyledons which was in contrast to results on *Arabidopsis*, thus implying the existence of specific mechanisms of cotyledon senescence in different monocarpic plants.

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ORIGINAL PAPER

Local induction of senescence by darkness in *Cucurbita pepo* (zucchini) cotyledons or the primary leaf induces opposite effects in the adjacent illuminated organ

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Abstract Local darkening of zucchini cotyledons or the primary leaf affected in an organ-specific manner the adjacent ones which remained under the initial light regime. Individual darkening of either the pair of cotyledons or the primary leaf led to acceleration of senescence expressed by lowering of chlorophyll content and net photosynthetic rate. Darkening of the pair of cotyledons induced a reduction in total cytokinin (CK) levels and increased CK oxidase/dehydrogenase (CKX) activity in the adjacent illuminated primary leaf. In addition, abscisic acid (ABA) content was increased which correlated with reduced stomatal aperture leading to decreased stomatal conductance and transpiration rate. In contrast, darkening of the adjacent primary leaf led to increased metabolic activity in the illuminated cotyledons including increased total CK levels in parallel with decreased CKX activity, decreased ABA content in correlation with increased stomatal aperture, stomatal conductance and transpiration rate. On the other hand, the functional activity of the photosynthetic apparatus as well as the transcript levels of the three photosynthesis-related genes *psbA*, *psaB* and *rbcL* remained almost unaffected in both illuminated organs. Thus, compared with the primary leaves, cotyledons appeared to be much more resistant to the dark stress applied either directly or to the adjacent primary leaf. Our results indicated the involvement of CKs and ABA signalling in the control of the communication mechanisms between cotyledons and the primary leaf that could operate in response to changing environmental factors like shading during earlier stages of plant development.

Keywords Abscisic acid · Cotyledons · Cytokinins · Cytokinin oxidase/dehydrogenase · Dark treatment · Primary leaves

Abbreviations

ABA	Abscisic acid
CK	Cytokinin
*cis*Z	*cis*-Zeatin
*cis*ZR	*cis*-Zeatin riboside

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Review

Decapitation as an approach to study control mechanisms of leaf senescence

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Abstract. The removal of the top stem bud independently or in combination with the leaves, known as decapitation, can lead to changes in the physiological state of plants at the same age. The decapitation procedure reverses the symptoms of senescence which makes it an effective physiological approach to study the control mechanisms of delayed leaf senescence and rejuvenation. The process of rejuvenation that develops in the decapitated plants is characterized by increased pigment content and photosynthetic rate. In addition, photosynthetic electron transport, Rubisco activity and changes in photorespiratory metabolism are recovered to the maximal values observed in mature leaves. Thus, through decapitation leaf life span can be prolonged. Decapitation leads to substantial changes in the biosynthesis, metabolism and transport of plant hormones, especially auxin and cytokinins (CKs). Based on the model of the mutual interactions of auxin and CKs in regulating correlative dominance, the effects of rejuvenation observed after decapitation of plants could be explained by the increased levels of endogenous CKs due to the elimination of both the auxin-inhibiting effect on CK supply from the roots and the competition from the epicotyl as a sink for CKs. Thus, decapitation leads to elimination of the dominant phloem sink and causes marked changes in sink-source relationships. Decapitation can be applied also as an approach for investigation of defoliation, which is often induced by herbivores, insects, hail, etc., as well as in

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COMPARATIVE STUDY OF THE EFFECTS OF METHYL JASMONATE AND ABSCISIC ACID ON RNA AND PROTEIN SYNTHESIS IN EXCISED COTYLEDONS OF *CUCURBITA PEPO* L. (*ZUCCHINI*)

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Summary. The effects of methyl jasmonate and abscisic acid on the polypeptide pattern of soluble proteins as well as *in vivo* protein and RNA synthesis and endogenous nuclear RNA polymerase activity were compared in excised marrow cotyledons grown in the light. Treatment of cotyledons with MeJA resulted in the induction of two abundant polypeptide bands with Mr 43 and 60 kDa and a significant accumulation of a polypeptide band with Mr 97.4 kDa as analysed by SDS–PAGE. In contrast to MeJA, ABA did not cause the formation of these specific polypeptide bands. Furthermore, the inhibitory effect of ABA on protein and RNA biosynthesis as well as the activity of total endogenous RNA polymerases was much stronger than the effect of MeJA. It is suggested that in excised marrow cotyledons MeJA and ABA act by different mechanisms in the regulation of cotyledon growth and development during the earlier stages of germination.

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BIOLOGIE
Biologie moléculaire

METHYL JASMONATE-INDUCED POLYPEPTIDES IN EXCISED COTYLEDONS OF *CUCURBITA PEPO* L. (ZUCCHINI) AND COUNTERACTION BY CYTOKININS

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(*Submitted by Corresponding Member E. Karanov on May 3, 1997*)

Methyl jasmonate(MeJa) and its related compounds are widespread in the plant kingdom and regarded as putative plant growth regulators [1, 2]. Two major hypotheses have been put forward explaining the mode of action of jasmonates and their multiple physiological effects in plants. The first one is based on the observation that MeJa is involved in senescence promotion in excised leaf segments of a large number of mono- and dicotyledonous plants [3-5]. The second hypothesis concerns their involvement in major genetic and metabolic processes as putative effector molecules in the signal transduction chain in some stress responses, especially desiccation, osmotic and wounding stresses. It is supported by many experimental data that methyl jasmonate-induced polypeptides (JIPs) accumulate upon jasmonate treatment in all plant species tested so far [4-9]. JIPs-gene expression and protein accumulation can be considered as a general response of the plant cell to certain stresses.

In the present work we studied the effect of exogenously applied MeJa in the model system of excised zucchini cotyledons and its interaction with cytokinins which are the major phytohormones regulating transition of cotyledons from reserve organs to photosynthesizing leaves which occurs in the early stages of seed germination.

BIOLOGIA PLANTARUM 42 (4): 549-557, 1999

Effect of methyl ester of jasmonic acid and benzylaminopurine on growth and protein profile of excised cotyledons of *Cucurbita pepo* (zucchini)

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Abstract

Treatment of excised marrow (*Cucurbita pepo* L., zucchini) cotyledons with methyl ester of jasmonic acid (MeJA) had no effect on their growth in darkness. On the other hand, MeJA induced the synthesis of three polypeptides (69, 60 and 43 kDa) and stimulated the accumulation of other polypeptides (97.4 and 53 kDa). These changes in the polypeptide profile were accompanied by a suppression of total protein and RNA synthesis as well as the activity of nuclear RNA polymerases. In contrast to MeJA, N^6-benzylaminopurine (BAP) significantly enhanced cotyledon growth and stimulated protein and RNA synthesis. Furthermore, BAP, when applied together with MeJA, was able to counteract some effects of MeJA including the appearance of specific MeJA-induced polypeptide bands.