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Influence of Succinate on Zinc Toxicity in *Zea mays*

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INTRODUCTION

High concentrations of heavy metals are toxic in most plant species. The overall toxic reaction to heavy metals is the depressed growth due to the depressed photosynthesis, to the direct inhibition of growth processes and to the decreased nutrient uptake and transport (Foy *et al.*, 1978; Greger *et al.*, 1991). There are many data about the mechanisms of trace metals tolerance in plants (Steffens, 1990; Verkleij and Schaat, 1990). The postulated tolerance mechanisms are: biochemical detoxi- fication after the toxic metal pass through the plasma membrane, compartmentalization of metals within the cell by synthesis of metal- binding proteins or chelation by organic ligands, and limited uptake of the toxic ion (Meharg, 1994).

It appears that mechanisms of trace metals tolerance exist in plant no matter whether they are tolerant to the metal or not (Steffens, 1990). Metal tolerance of plants is specific for each metal. For example, copper is partially bound to metallothioneins in the cytoplasm or to similar low-molecular-weight thiol containing proteins (Lolkema *et al.*, 1984). Zn is accumulated in the vacuoles mediated by Zn malate shuttle (Mathys, 1980; Verkleij and Shaat, 1990). Mathys (1980) suggests that Zn might be chelated by malate in the cytosol and transported to the vacuole

where after dissociation from malate, forms a stable complex with oxalate.

Since Mathys model was published, many researchers have demonstrated positive correlations between the organic acid content of plant tissues and the tolerance to metals. In *Triticum aestivum* the observed increase in the organic acid content (aconitate, α-ketoglutarate, and succinate) reflects responses to Mn toxicity (Macfie *et al.*, 1994). The concentrations of citrate and malate increased upon Al stress in Al-tolerant cultivars of *Sorghum bicolor* (Cambraia *et al.*, 1983) and *Zea mays* (Suhayda and Haug, 1986).

The aim of the present work was to understand whether the exogenous high concentrations of succinate could reduce the toxicity effect of Zn in maize plants thus, increasing the resistance to the metal.

with different zinc concentrations and after 18 day-Zn treatment (after appearance of symptoms of zinc toxicity) the plants were exposed to 1 μM Na- succinate by foliar treatment.

Plant growth parameters

After 18 day-treatment, plants were harvested, divided into roots, stems and leaves and their fresh weight was determined. Thereafter, the roots, the stems, and the leaves were oven-dried at 60°C to a constant weight.

Atomic absorption determination of zinc content

Dry plant material (1 g) from roots, stems and leaves was separately ashed at 550°C. The dried residue was brought to standard

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BIOLOGIE
Physiologie des plantes

APPLICATION OF HYDROXIDE SALTS OF COPPER AND ZINC AS MICROELEMENT SOURCES FOR GERMINATING OF LETTUCE SEEDS (*LACTUCA SATIVA* L.)

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(*Submitted by Corresponding Member E. Karanov on May 7, 1998*)

The heavy metals Cu and Zn are important microelements in biological systems, and they take part in many physiological systems as cofactors or prosthetic groups of many enzymes [1,2]. Increased concentrations of copper and zinc are toxic for all living organisms [1-6]. As their sources generally salts of H_2SO_4 and HCl and rarely Zn—EDTA are used because they are water soluble and easily penetrate into the subsoil water [7,8]. To maintain the soil nutrition content with easily available Cu and Zn without environmental pollution, it is necessary to find out new chemical compounds with lower solubility than sulphates and chlorides, but higher than that of respective hydroxides. It is well known that the hydroxide-salts of Cu and Zn meet these requirements and it could be suggested that these compounds are proper sources of the microelements Cu and Zn.

In our previous investigations we found that newly synthesized mixed hydroxide carbonates and hydroxide sulphates of Cu and Zn could be used for lettuce growth. We also found out that in optimal concentration they stimulate the lettuce root system growth which is essential to obtain seedlings of good quality [9,10].

The lettuce is a small grained plant with a very limited amount of nutrient element stored in the seed. Data exist [11] that small-seeds plants with initial period of germination about 14 days are very sensitive to nutrient regimen and have increased requirements to the concentration of nutrient elements in the zone around the roots.

ARRAMBARRI et al. [12] when growing lettuce as sand-culture also conclude that correctly balanced concentrations of hydroxide-chlorides of Cu and Zn are proper for normal growth and development of the plants.

The aim of the present study was to choose the most suitable combination of type of hydroxide salts, concentration and proper carrier, and to investigate the influence of mixture obtained on the germination of lettuce seeds.

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BIOLOGIE
Physiologie des plantes

UPTAKE AND TRANSPORT OF ZINC IN ZINC-SENSITIVE *PISUM SATIVUM* IN THE PRESENCE OF SUCCINATE

Z. Stoyanova, S. Doncheva

(*Submitted by Corresponding Member E. Karanov on October 19, 1999*)

Zinc is an essential micronutrient for all living organisms, but like other essential nutrient ions [1] it can be toxic at high levels [10]. It has been well documented that many species exhibit ability to resist Zn toxicity [4,8]. Considerable effort has been made to understand the mechanisms involved in the ability of some plants to grow in the presence of Zn levels that are toxic to most plants, the phenomenon known as tolerance [12]. There are some strategies that plant can use to deal with Zn toxicity: binding of Zn to cell wall polymers, other forms of complexation in cell walls [9], complexation with heavy metal-binding peptides, i.e. phytochelatins and vacuolar sequestration with organic acids [11]. ERNST [2] was the first to postulate vacuolar accumulation of Zn-organic acid complexes as a mechanism for Zn tolerance in naturally tolerant ecotypes. MATHYS [8] investigated this proposal further and developed an original model for its operation in *Silene cucubalus*. He suggested that Zn might be chelated by malate in the cytosol and served to deliver Zn to the vacuole where after dissociation from malate it formed a stable complex with oxalate. Since Mathys' model was published, many researchers have demonstrated a positive correlation between the organic acid content of plant tissues and tolerance to zinc [5]. WANG et al. [11] suggested that vacuolar sequestration of Zn by high levels of vacuolar citrate might be considered as a major mechanism of Zn accumulation in plants exposed to high levels of the metal. On the other hand, data are available showing that organic acids are not included in the Zn specific tolerance mechanisms.

The ability to tolerate high Zn concentrations was related predominantly to high malate concentrations. The possible implication of other organic acids in Zn tolerance is less clear.

The present study was undertaken to examine the possible involvement of succinate in Zn accumulation in pea plants exposed to high levels of zinc in order to establish a potential role of succinate in detoxication of zinc in plants.

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БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ

ИНСТИТУТ ПО ФИЗИОЛОГИЯ НА РАСТЕНИЯТА "АКАД. М. ПОПОВ"

ПОСТИЖЕНИЯ И ПЕРСПЕКТИВИ НА ВОДНИЯ РЕЖИМ И МИНЕРАЛНОТО ХРАНЕНЕ НА РАСТЕНИЯТА В БЪЛГАРИЯ

ТОМ 2

СОФИЯ - 2001 г.

СТРУКТУРНИ И УЛТРАСТРУКТУРНИ ПРОМЕНИ В ЦАРЕВИЧНИ РАСТЕНИЯ, ТРЕТИРАНИ С МЕДНИ ЙОНИ

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Тежките метали присъстват във всички екосистеми. В еволюцията на растителните видове са се развили специфични изисквания към определени химични елементи, включително и към тежки метали. В метаболизма на висшите растения централна роля играят желязото, мангана, медта, цинка и молибдена, както и никела при бобовите и кобалта в симбиотичната система бобови-ризобиум. За поддържане на оптималния растеж и развитие на растенията са необходими определени количества от тежки метали. Обратно, концентрации от тези метали, надвишаващи многократно оптималното ниво имат токсичен ефект (Foy et al., 1978).

Екотоксичността на тежките метали не може да бъде определена чрез общото количество на тези елементи в почвите, а само чрез количеството, което е погълнато от корените. На този процес оказват влияние pH на почвата, редокс потенциала на мембраните и биологичната активност на корените и организмите, обитаващи ризосферата (Egnı, 1998). Излишъкът от тежките метали оказва влияние върху всички нива на организация на растителните организми.

Видимите симптоми на стрес от тежки метали в растенията са израз на метал-индуцираните изменения на структурно и ултраструктурно ниво. Тези изменения на клетъчно, тъканно и организмово ниво са резултат от директното взаимодействие на токсичния метал със структурните компоненти на различните нива на организация на растителните организми или са индиректен резултат от промените в метаболизма на клетките ни. Електронната микроскопия, комбинирана с аналитични техники като енергетичнодисперсионен X-ray микроанализ, микролазерен анализ (LAMMA), вторична йон-мас спектрометрия (SIMS), спектроскопия за изследване енергетичната загуба на електроните (EELS, Lichteaberger and Neuman, 1997) и цитохимични методи, е ценен метод, който спомага за изучаване на първичните механизми на металната токсичност и толерантност на молекулно ниво.

Растежът на растението или на отделните му органи са често използвани параметри за оценка на ефекта на металната токсичност. В настоящото проучване е изследван ефекта на медната токсичност върху структурното и функционално състояние на коренови меристемни клетки от царевични растения, третирани с концентрации от медни йони в диапазона от 0.41μmol/L до 78μmol/L.

Коренът е основния орган, който акумулира медни йони (Jarvis, 1980; Jensen and Adalsteinsson, 1989; Doncheva et al., 1996). Съдържанието на медта в корените е по-високо от това в стъблата и листата и се повишава с увеличаване на концентрацията на медта в хранителния разтвор (Doncheva et al., 1996). Задържането на медта в корените може да се приеме като важен ограничаващ фактор на транспорта на медта към надземните части на растенията. Кореновият растеж е в пряка зависимост от концентрацията на тежките метали в хранителния разтвор. Основните морфологични и структурни изменения на корените, причинени от медната токсичност, могат да се обобщят по следния начин: 1) потискане на кореновата елонгация; 2) увреждане на кореновия връх; 3) намаляване на броя на кореновите власинки; 4) намаляване на кореновата биомаса и 5) потискане на формирането на латералните корени. С повишаване на медната концентрация от 1.5 до 78 μmol/L се намалява дължината и свежото и сухото тегло на корените. При третиране на царевичните растения с най-високата изследвана медна концентрация (78 μmol/L Cu), дължината на корените им се намалява с 31%, а свежата и сухата им биомаса с 31 и 28%, съответно в сравнение с тези на контролните растения. Инхибирането на кореновото удължаване е първия видим ефект от металната токсичност. Кореновата елонгация може да бъде намалена чрез инхибиране на клетъчното делене или чрез намаляване на клетъчното разширяване /expansion/ в зоната на удължаване. Клетъчното делене на кореновите апикални меристемни клетки е най-чувствително към токсични концентрации на тежките метали и се използва като тест за оценка на риска на околната среда.

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INFLUENCE OF SUCCINATE ON ZINC TOXICITY OF PEA PLANTS

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ABSTRACT

The response of pea plants (*Pisum sativum*, cv. Citrine) to various zinc (Zn) concentrations (0.67 to 1000 μM Zn) in the presence and absence of succinate (200 μM Na-succinate) were investigated. Treatment of pea plants alone with excess of Zn reduced plant growth, chlorophyll content and induced alterations in the structure of the chloroplast, resulting mainly in decreased granal thylakoids. The photochemical activity of photosystem II estimated by the ratios F_v/F_m and F_v/F_o was less affected by Zn treatment. The presence of succinate lead to an increase in plant growth and chlorophyll content, improved chloroplast structure of and recovered photosystem II activity in Zn-treated plants. This stimulation was accompanied by an increased zinc root concentration and a decreased zinc shoot concentration. The higher root zinc concentration and decreased zinc translocation from root to shoot by succinate treatment suggest that succinate facilitates the formation of metal-succinate complexes in the roots and may play a role in zinc accumulation. These results provide indirect evidence for a possible role of succinate in Zn-resistance of plants.

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Research Note

Influence of nitrogen deficiency on photosynthesis and chloroplast ultrastructure of pepper plants

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Pepper plants (*Capsicum annuum* L. cv. Zlaten Medal) were grown on nutrient solution without nitrogen, and photosynthetic response of plants was examined by determination of leaf CO_2 fixation and chlorophyll and carotenoid contents. The absence of nitrogen in the medium resulted in a decrease of the leaf area and of plant biomass accumulation, and in an increase of the root-shoot dry weight ratio. The photosynthetic activity and chlorophyll and carotenoid contents decreased significantly under nitrogen deprivation. Examination of nitrogen deficient leaves by transmission electron microscopy showed dramatic changes in chloroplast ultrastructure. The proportion of starch granules and plastoglobules in the stroma matrix was increased and internal membrane system was greatly reduced. It seems that nitrogen plays an important role in the formation of chloroplast structure and hence to the photosynthetic intensity and productivity of pepper plants.

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RESEARCH ARTICLE

The effect of zinc supply and succinate treatment on plant growth and mineral uptake in pea plant

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The influence of succinate treatment on Zn toxicity was investigated using plant growth and mineral uptake as stress indicators. Pea plants (*Pisum sativum* L., cv. Citrine) were treated with various Zn concentrations (0.67 to 700 µM Zn) in the presence and absence of 0.2 mM Na-succinate. Plants pre-treated with succinate and then exposed to Zn exhibited higher dry root, stem and leaf weight than the plants treated with Zn alone. An increase in Zn supply resulted in a decrease in the concentrations of Ca, Mg, P in the roots and an increase of Ca and N levels in the stems and leaves. The amount of Zn in the roots, stems and leaves increased with greater Zn rates. The succinate treatment increased P in the roots but did not affect the Ca, N and Mg contents in Zn-treated plants. Most of the Zn taken up was retained in the roots after succinate treatment. The ameliorative effect of succinate on plant growth could be due to a lower Zn translocation in the leaves and stems and increased Zn accumulation in the roots. Lower Zn translocation in aboveground parts seemed to result from Zn complexing by organic anion in the roots. This probably caused less Zn transport to the stems and leaves and suggested that succinate has potential for complexing with Zn and may play a role in tolerance to high Zn levels.

Microtubule organization but not *α Tub 1* and *α Tub 3* expression are primary targets of aluminum toxicity in maize root tips

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Introduction

Aluminium-induced inhibtion of root elongation in sensitive plants can be observed upon approximately 30 minutes of exposure (Llugany et al., 1995; Barceló and Poschenrieder, 2002). Root tips are considered the most Al sensitive plant part (Ryan et al. ,1993; Sivaguru et al. 1998). In Al sensitive maize root cell elongation, but also root tip cell division, are inhibited after short-term exposure to Al (Gunsé et al., 1997; Poschenrieder et al., unpublished). The primary mechanisms of these inhibitory effects are still unclear. Changes in growth rates induced by environmental factors are reflected by the structure of the cytoskeleton. Reorientation of microtubuli from a transversal to longitudinally orientated position has frequently been observed under growth inhibiting conditions. Studies on oat coleoptiles have found a parallelism between a tubulin mRNA levels and growth inhibition induced by continuous light treatment (Moser et al., 2000). *Tubα3* but not *Tubα1* are activated during mycorrhizal infection of maize roots and *Tubα 1* is involved in nuclear divisions (Bonfante et al., 1996; Montoliu et al., 1990). The reorganization of microtubules also seems to be involved in early toxicity responses to Al (Blancaflor et al., 1998; Sivaguru et al., 1999). However, up -to-date no time-dependent investigation on the expression of a-tubulin genes in relation to Al-induced root growth inhibition has been performed.This study investigated the short-term (5 minutes to 3 h) effects of Al (50 µM total concentration; 17 µM Al^{3+} activity) on the organization of microtubules in the transition and elongation zones of root tips of two maize varieties different in Al sensitivity. The influence of the three-hour Al treatment on the expression of the genes codifying for a-tubulin synthesis in root tips of maize (*Tubα1 and Tubα3*) was investigated using RT-PCR.

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INFLUENCE OF SILICON PRETREATMENT ON ASCORBIC ACID CONTENT AND REDOX STATUS IN Mn-TREATED MAIZE PLANTS

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(*Submitted by Academician E. Karanov on June 22, 2005*)

Abstract

The influence of silicon (Si) pretreatment on the total ascorbate content (ASC) and histological localization of ASC in leaves of two *Zea mays* L. varieties differing in Mn-resistance was investigated. After 5 day treatment with 200 μM Mn, Mn-tolerant plants exhibited a greater capacity to maintain ASC content than sensitive ones. Both Mn-tolerant and Mn-sensitive plants pretreated with 1 mM Si and then exposed to Mn exhibited higher ASC content than the plants which were not pretreated with Si. The received results for histochemical ASC localization indicate that Mn treatment induced an increase in the number of detectable silver granules in the Mn-tolerant plants. Si-pretreated leaf cells contained more detectable granules than Mn-treated alone. However, the silver granules were regularly distributed in the mesophyll cells of both tolerant and sensitive plants.

Ascorbate redox status was expressed as the ratio AA/ASC. In Mn-sensitive maize plants, AA/ASC ratio was approximately 0.25 for control leaf tissue and decreased in Mn-treated plants. The Mn-tolerant exhibited greater ratio (0.40) than the sensitive ones. Si pretreatment improved Mn tolerance without an increase in the AA/ASC ratio suggesting that Si cannot increase the tolerance to Mn by increasing the leaf ascorbate redox status.

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Effects of Succinate on Manganese Toxicity in Pea Plants

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ABSTRACT

Pea (*Pisum sativum* cv. Citrine) plants were grown in nutrient solution containing various manganese (Mn) concentrations in the presence or absence of succinate to evaluate the potential role of succinate in the plant tolerance to Mn excess. Supplying pea plants with excess Mn led to a reduction in the relative growth rate (RGR), chlorophyll *a* and *b* content, photosynthetic O_2 evolution activity, and photosystem II (PSII) activity, as measured in the light-adapted state (ϕPSII) in comparison to the control. The primary photochemical efficiency of PSII, estimated by the F_v/F_m ratio, was less affected by increasing Mn concentration. Chloroplasts from Mn-treated leaves exhibited significant changes in their ultrastructure, depending on the strength of Mn toxicity. The concentration of Mn in roots, stem, and leaves increased with the increase of Mn in the nutrient solution. Addition of succinate before and after Mn treatment did not reduce the inhibitory effect of Mn on the plant growth, chlorophyll fluorescence parameters, photosynthetic O_2 evolution activity, and chloroplast structure of the pea plants. It was found that supply of exogenous succinate at a high Mn concentration (over 1500 μM) in the nutrient solution led to an increase of Mn uptake in the roots accompanied by a decrease in a Mn translocation to the leaves and stems compared to Mn-treated pea-plants. However, differences in the toxicity effect of Mn in both Mn and Mn/Succinate-treated pea plants were not detected. Thus, such changes in Mn distribution within the Mn/succinate-treated plant did not confer tolerance of Mn excess to pea plants. These results suggest that succinate probably has an affinity for Mn and may function as a "terminal acceptor" of large amounts of Mn, decreasing Mn transport to the stem and leaves, but does not contribute to Mn tolerance.

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RESEARCH PAPER

Root cell patterning: a primary target for aluminium toxicity in maize

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Abstract

The short-term influence (5–180 min) of 50 μM Al on cell division was investigated in root tips of two *Zea mays* L. varieties differing in Al-resistance. The incorporation of bromodeoxyuridine into S-phase nuclei was visualized by immunofluorescence staining using confocal laser fluorescence microscopy. In Al-sensitive plants 5 min Al exposure was enough to inhibit cell division in the proximal meristem (250–800 μm from the tip). After 10 or 30 min with Al only, a few S-phase nuclei were found in the cortical initials. By contrast, cell division was stimulated in the distal elongation zone (2.5–3.1 mm). After 180 min the protrusion of an incipient lateral root was observed in this zone. These observations suggest a fast change in cell patterning rather than a general cariotoxic effect after exposure to Al for a short time. No such changes were found in Al-resistant maize. This is the first report showing such fast Al-induced alterations in the number and the position of dividing cells in root tips. The observation that similar changes were induced by a local supply of naphthylphthalamic acid to the distal transition zone suggests that inhibition of auxin transport plays a role in the Al-induced alteration of root cell patterning.

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Abbreviations: BrdU, bromodeoxyuridine; DEZ, distal elongation zone; DTZ, distal transition zone; NPA, naphthylphthalamic acid.

Physiological Response of Pea Plants to Different Substrate Moisture Levels in a Ground Experiment in SVET-2 Space Greenhouse

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Abstract

Maintenance of adequate moisture in the plant growth medium (substrate) to ensure appropriate amounts of water, nutrients and oxygen to the roots is still a problem in microgravity. The aim of this study was to examine the effect of different substrate moisture levels on plant growth and photosynthesis in pea plants (Pisum sativum L. cv. Ran-1). Pea plants were grown in the laboratory prototype of the SVET-2 Space Greenhouse (SG) during May - June 2005 in 1-1,5 mm particle sized substrate Balkanine, at 18-20°C temperature, 400 μmol.m^{-2}.s^{-1} photosynthetic photon flux, 0,2 m/s wind speed and 55 % initial substrate moisture. On the 16th day of plant development the substrate moisture was increased to 60 % for 24 hours. The plant growth expressed by shoot height and dry weight was not changed with the increase of substrate moisture. The photosynthetic rate was reduced and in contrast the dark respiration rate was increased. The content of chlorophyll a, b and carotenoids at 60 % substrate moisture were similar to that at 55 %. The obtained results showed that pea plants grown in SVET-2 SG at the above substrate moisture levels were with reduced photosynthesis and with no changes in the growth parameters.

SG during small rise of the substrate

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Exogenous succinate increases resistance of maize plants to copper stress

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Summary

The effect of copper (Cu) excess (1.5, 4.7, 31, 78, 156 μM) and exogenously supplied succinate on plant growth, chlorophyll content, chlorophyll fluorescence, and isoenzym profiles of some antioxidant enzymes in maize plants was studied. Excessive Cu supply led to a reduction in the relative growth rate (RGR), tolerance index (TI), chlorophyll *a* and chlorophyll *b* contents, and the quantum yield of PSII electron transport in the light-adapted state (ΦPSII). Copper treatment induced several changes in the anionic and cationic peroxidases (PODs), as well as superoxide dismutase (SOD) isoenzyme profiles. After 8 d of 78 μM–Cu treatment, two new anionic and two new cationic peroxidase isoenzymes in the roots were registered. Copper applied at concentrations above 31 μM resulted in higher levels of manganese superoxide dismutase (Mn-SOD) in the roots and Cu,Zn-superoxide dismutase (Cu,Zn-SOD) in the leaves. However, the addition of Na-succinate (200 μM) to the root medium prior to Cu treatment increased the capacity of the plants to partially overcome Cu toxicity.

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Second Scientific Conference with International Participation
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EXPERIMENT INVESTIGATING THE INFLUENCE OF OXYGEN DEFICIENCY ON PLANTS GROWN IN MICROGRAVITY

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Abstract

Plant growth experiments in the Bulgarian developed SVET Space Greenhouse onboard the MIR Orbital Station proved that precise monitoring and control of the substrate moisture levels is not enough to provide adequate moisture for plant roots because the water distribution in microgravity differs significantly from the one on Earth. Microgravity changes the behavior of fluids and gases, disturbs the water-air balance in the substrate medium and causes difficulties in providing even substrate moisture in the whole volume. As a consequence excessive irrigation may occur and the high water content may cause oxygen deficiency in the plant root area. Some ground-based biotechnology experiments were carried out to improve the scientific knowledge of water distribution in the root substrate media and its impact on plants. A space experiment with Arabidopsis thaliana plants grown in the ISS European Modular Cultivation System, with the objective to investigate plant response to oxygen deficiency occurring in microgravity in the process of substrate moisture control, is planned. Plant growth and development, the ultrastructure of cell organelles in plant roots and leaves, the activity of specific marker enzymes and cytosolic Ca^{2+} concentration in plants grown under both microgravity and synchronous ground - based 1-g conditions will be investigated.

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GROUND BASED VERIFICATION TESTS AND EQUIPMENT FOR SELECTION OF ROOT-ZONE MEDIA FOR HIGHER PLANT CULTIVATION IN SPACE

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ABSTRACT

The study proposed gives an approach for early evaluation of the substrate properties. This includes a course of measurements for preliminary evaluation of a part of substrate physical, hydro-dynamical, and agro-chemical characteristics. In case some parameter exceeds the bounds of admissibility the substrate is not subjected to accurate study in specialized laboratories. Three kinds of substrates of different trade-marks and similar particle size range (1-2 mm) were tested in a laboratory. A number of measurements were carried out using standard methods to make analysis of some agro-physical properties. Seed germination and plant growth-testing apparatus was developed to evaluate substrate agro-properties at different constant moisture, light intensity and temperature levels. Photosynthetic and transpiration rates as well as stomatal conductivity of the grown experimental samples were measured by the infrared gas-analyzer LICOR 6400. The experimental results are reported.

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MODEL OF WATER AND NUTRIENTS SUPPLY TO PLANTS IN A SPACE GREENHOUSE

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Key words: space greenhouse, substrate media, water and nutrient supply

Abstract

Particulate media are currently used as artificial soils (substrates) in the root growth modules of space greenhouses. The water necessary for plant vegetation is supplied into the substrate and, flowing through it, reaches the roots. One of the methods of supplying nutrients to plants is their being stored in advance in the pores of the particles. Wetting the substrate, the water penetrates the particle pores and the mineral nutrients dissolve. Diffusing inside the pores, the nutrients reach the solution outside the particles and become accessible to the plant roots.

In the proposed model consisting of two sub-models, the root module is considered as a system of lumped parameters. The first sub-model is based on the material balance of the water in the substrate and takes into account the effects of irrigation, transpiration and evaporation. The dependence of the water losses at transpiration and evaporation on the "meteorological" conditions in the space greenhouse and on the grown plants parameters is described by a system of algebraic equations. The second sub-model, based on the first one, describes, in terms of the concentration of the respective nutrient component, its extraction from the substrate grains and consumption by the plant roots.

The model gives a possibility to simulate various conditions of irrigation and nutrition during the plant vegetation.

Impact of Different Substrate Moisture Levels on Lettuce Plants during Ground Based Experiment in SVET-2 Space Greenhouse

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Abstract - **Plant experiments carried out in space has proved that microgravity alters conditions in the plant growth facilities especially in the root modules and thus affects plant growth and development. Microgravity changes behavior of fluids and gases in the porous media used as plant growth substrates which causes problems with the control of water supply systems and this often leads to excess water input (overmoistening) and oxygen deficiency.**

The pattern of fluid and gas distribution in substrate medium in microgravity could not be repeated on Earth but some processes could be imitated. Overmoistening of the substrate medium and the subsequent oxygen deficiency could be replaced with the waterlogging on Earth.

Ground experiment was carried out in the laboratory prototype of SVET-2 Space Greenhouse (SVET-2 SG) to study the effect of different root-zone moisture conditions and waterlogging on growth, photosynthesis and chlorophyll content of lettuce plants (*Lactuca sativa* L. cv. Lolo Rossa).

The increase in height and biomass was suppressed while leaf dry matter increased during the waterlogging treatment suggesting assimilates accumulation in the leaves and slow translocation to the roots.

Waterlogging caused a rapid decline in net photosynthetic rate (Pn). The reduction of Pn could not be attributed only to diffusion limitation resulting from stomatal closure but also to metabolic inhibition due to accumulation of assimilates in the leaves.

The chlorophyll content decreased during the waterlogging and slowly recovered after termination of the waterlogging treatment.

Lettuce plants showed decline in Pn and overall growth during waterlogging and demonstrated fast recovery after waterlogging removal. The results suggested that lettuce plants were waterlogging-resistant to a certain degree but yield was greatly affected.

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.

Abbreviations – CK, cytokinin; *cis*-Z, *cis*-zeatin; *cis*-ZR, *cis*-zeatin riboside; CKX, cytokinin oxidase/dehydrogenase; DHZ, dihydrozeatin; FW, fresh weight; iP, N^6-(2-isopentenyl)adenine; iPR, N^6-(2-isopentenyl)adenine 9-riboside; Z, *trans*-zeatin; ZR, *trans*-zeatin 9-riboside; ZOG, *trans*-zeatin O-glucoside; ZROG, *trans*-zeatin 9-riboside O-glucoside; ZRMP, *trans*-zeatin 9-riboside-5′-monophosphate; (abbreviations for cytokinins according to Kamínek et al. 2000).

EFFECT OF OXYGEN DEFICIENCY ON GROWTH COMPONENTS OF LETTUCE DURING GROUND BASED EXPERIMENT IN SVET-2 SPACE GREENHOUSE

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ВЛИЯНИЕ НА НЕДОСТИГА НА КИСЛОРОД ВЪРХУ РАСТЕЖА НА САЛАТЕНИ РАСТЕНИЯ ПРИ НАЗЕМЕН ЕКСПЕРИМЕНТ В КОСМИЧЕСКА ОРАНЖЕРИЯ СВЕТ-2

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Ключови думи: *Космическа оранжерия СВЕТ-2, преовлажняване на субстратната среда, салатени растения, растежни показатели, фотосинтеза*

Резюме: *Салатени растения (Lactuca sativa L. var Lolo Rosa) бяха отгледани в лабораторния образец на Космическа оранжерия СВЕТ-2 върху субстрат Балканин при контролируеми условия. На 21-ден от тяхното развитие растенията бяха подложени на 10-дневно преовлажняване на субстратната среда, последвано от 10-дневен възстановителен период. Чрез преовлажняването на субстрата бяха създадени условия, с които се имитира специфичното прекъсване на въздухоносните пори в субстратната среда в микрогравитация, възникващо вследствие на променената физика на флуидите и проблемите при управлението на влажността в субстратната среда в космически условия. По време на преовлажнителния и възстановителния периоди бяха взети проби за определяне на растежните параметри на растенията, съдържанието на хлорофил и интензивността на фотосинтезата.*

По време на преовлажняването нарастването на растенията на височина и натрупването на свежа биомаса бяха значително инхибирани. През възстановителния период растенията увеличиха височината си и натрупаната биомаса в сравнение със същите, измерени през периода на преовлажняване, но в края на възстановителния период натрупаната от тях биомаса беше с 4 пъти по-малка от тази на непреовлажнените растения. Интензивността на фотосинтезата се понижи с 75% при преовлажнените растения в сравнение с непреовлажнените, а съдържанието на хлорофил в листата на растенията намаля с 29% в хода на преовлажняването. В процеса на възстановяване тези показатели бяха намалени съответно с 30% и 15% .

Получените от нас резултати показаха, че преовлажняването забавя развитието на салатените растенията и оказва влияние върху добива им. Установените от нас изменения обаче са обратими и след прекратяване на преовлажняването, растенията възстановиха фотосинтетичната си активност, биосинтезата на хлорофил и растежа си.

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THE EFFECT OF SILICON ON THE SYMPTOMS OF MANGANESE TOXICITY IN MAIZE PLANTS

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The effect of exogenously applied silicon (Si) on plant growth, lipid peroxidation, total phenolic compounds and non-protein thiols was studied in two maize varieties (*Zea mays* L.vars. *Kneja 605, 434*) differing in sensitivity to excess manganese (Mn). Based on the density of brown spots per leaf area and relative shoot weight (RSW) used to define Mn tolerance var. *Kneja 434* was found to be more Mn-tolerant than *Kneja 605*. The lipid peroxidation level and total phenolic compounds were enhanced with increasing Mn concentration in the nutrient solution. In addition, the Mn-sensitive var. *Kneja 605* with markedly expressed first visible Mn toxicity symptoms had higher levels of total phenolic acids than var. *Kneja 434* thus supporting the hypothesis that a stimulating effect of Mn on phenol content reflected rather a stress response to Mn excess than a tolerance mechanism. In contrast, non-protein SH content increased to a higher extent in the Mn-tolerant var. *Kneja 434*. The increased amount of non-protein SH compounds was accompanied by a much stronger oxidative stress in the Mn-sensitive plants when compared with the Mn-tolerant variety, thus suggesting that non-protein SH compounds may play a role in Mn tolerance in maize. The addition of silicon (Si) reduced the density of brown spots per leaf area as well as lipid peroxidation level and improved plant growth in Mn-treated plants.

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Silicon amelioration of manganese toxicity in Mn-sensitive and Mn-tolerant maize varieties

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ABSTRACT

Differences in tolerance to Mn excess and amelioration by Si were evaluated in two maize varieties. Dry weight, callose accumulation, chloroplast ultrastructure, and photosynthesis parameters were used as stress indicators. Variety Kneja 605 was much more Mn-sensitive than variety Kneja 434. In Kneja 605 excess Mn caused severe chloroplast damage and enhanced carotenoid production, symptoms similar to those triggered by photoinhibiton. In Mn-tolerant Kneja 434, in contrast, a Mn-induced decrease of the carotenoid concentrations, and only slight alterations in the chloroplasts were observed. These effects were similar to light Fe-deficiency symptoms. The threshold tissue concentration for Mn-induced callose accumulation was much lower in Kneja 605 than in Kneja 434. Therefore tolerance to excess Mn in Kneja 434 was not due to more efficient exclusion but to more efficient detoxification and compartmentation of Mn. The constitutively thicker epidermal layers in Kneja 434 and the observation that Si-induced amelioration of Mn toxicity in Kneja 605 substantially increased the thickness of the epidermal layers suggest that Mn storage in non-photosynthetic tissue could be a Mn tolerance mechanism in maize.

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Root Behavior in Response to Aluminum Toxicity

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Abstract Roots have an extraordinary capacity for adaptive growth which allows them to avoid toxic soil patches or layers and grow into fertile sites. The response of roots to aluminum toxicity, a widespread problem in acid soils, is an excellent model system for investigating the mechanisms that govern this root behavior. In this review, after a short introduction to root growth movement in response to chemical factors in the soil, we explore the basic mechanisms of Al-induced inhibition of root growth. The actinomyosin network and endocytic vesicle trafficking are highlighted as common targets for Al toxicity in cell types with quite different origins: root tip transition zone cells, tip-growing cells like root hairs or pollen tubes, and astrocytes of the animal or human brain. In the roots of sensitive plants, the perception of toxic Al leads to a change in root tip cell patterning. The disturbance of polar auxin transport by Al seems to be a major factor in these developmental changes. In contrast, Al activates organic acid efflux and the binding of Al in a nontoxic form in Al-resistant genotypes.

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SPECIAL ISSUE (PART I) – PROCEEDINGS OF THE XI NATIONAL CONFERENCE ON PLANT PHYSIOLOGY
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PHYSIOLOGICAL CHARACTERISTICS OF *IN VITRO* AND FIELD CULTIVATED *LEUCOJUM AESTIVUM* L. PLANTS

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Summary. A living collection of *Leucojum aestivum* L. (Amaryllidaceae) was established at the Institute of Botany, Sofia, in 2001. *In vitro* cultures originating from a single mother bulb have been maintained since 2003 on MS-based agar-solidified medium supplemented with 30 g L^{-1} sucrose, 2 mg L^{-1} BAP and 0.15 mg L^{-1} NAA under equal temperature and light conditions in plastic containers. Part of the regenerated plantlets were successfully adapted *ex vitro* and planted in open-air trenches in 2007. Uniform shoot clumps were selected and grown for seven weeks during the vegetation peirod in spring 2009 on solid and in a liquid medium with the same compostion. Main physiological characteristics of the field cultivated plants and plantlets from long-term *in vitro* cultures were compared. Leaves were taken from field grown and field adapted plants, and in vitro obtained plantlets on solid and in liquid media, all genetically identical. The photosynthetic rate of field grown plants was twice higher than that of in vitro plantlets. Moreover, the results for the plants adapted to open-air conditions were similar to those initially planted on the field, whereas the photosynthesis and the biomass accumulation were more expressed by the in vitro plantlets cultured in a liquid medium compared to those grown on agar-solidified one. The liquid shoot-clump culture of *L. aestivum* was determined as a suitable system for *in vitro* alkaloid production.

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ORIGINAL ARTICLE

Characterization of the tolerance to excess manganese in four maize varieties

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Abstract

Manganese (Mn) is an essential micronutrient in all organisms, but may become toxic when present in excess. Four maize (*Zea mays* L.) varieties, Kneja 605, Kneja 434, Kneja 509 and Kneja 537, were studied with respect to their responses to excess Mn in hydroponic solution. In the varieties Kneja 605, Kneja 509 and Kneja 537, increasing Mn concentrations in the nutrient solution negatively affected biomass accumulation, photosynthetic rate, transpiration, stomatal conductance and chlorophyll content. In addition, these varieties showed increased electrolyte leakage and lipid peroxidation (malondialdehyde [MDA] content). Increased Mn leaf concentrations, higher contents of chlorophyll *a* and chlorophyll *b*, higher photosynthetic rate and transpiration, lower concentrations of MDA and insignificant changes in the electrolyte leakage in the leaves were found in var. Kneja 434 compared with the other maize varieties studied. This variety appeared to possess a stronger ability to cope with Mn phytotoxicity, suggesting high potential for Mn detoxification and var. Kneja 434 could be a good candidate for improving maize productivity on acid soils under non-tropical conditions.

Key words: electrolyte leakage, lipid peroxidation, maize production, manganese tolerance, manganese toxicity.

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Early responses of roots to Aluminium: alteration of vesicle transport as a target

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Introduction

Aluminium toxicity is among the most important abiotic constraints for plant growth on acid mineral soils (Kochian et al., 2004; Ma, 2007; Poschenrieder et al., 2008). Aluminium not only causes a fast inhibition of root cell division and elongation, but alters the entire root architecture. The brittle, stunted root system of plants affected by Al toxicity has poor capacity to supply the shoot with water and nutrients. As a consequence multiple secondary stresses occur.

Recognition of the primary targets of Al toxicity is essential for understanding the mechanisms of Al-induced alterations of root growth. Moreover, the early root responses to Al represent a valuable model system for studying the basic events that control root growth and development. Here we present a summary of our recent research activities in this field making special reference to the role of Al-induced alteration of vesicle transport in the Al toxicity syndrome.

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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.

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Lead toxicity in sunflower plants as affected by EDTA

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Abstract

Objective The present work was aimed to study lead (Pb) biosorption by sunflower plants cultivated in liquid media and the role of exogenously applied EDTA in ameliorating Pb toxicity.

Methods The effects of Pb (ionic) and the EDTA-Pb complex on plant growth of cultivated sunflower *H. annuus* cv. 1114 and the interspesific line *H. annuus* x *H. argophyllus* grown in Hoagland solution were investigated. Phytotoxicity was assessed using several well-established stress markers: plant growth, leaf anatomy, electrolyte leakage, total antioxidant activity and free radical scavenging capacity.

Results Pb treatment induced a decrease in the relative growth rate (RGR), disturbance of plasma membrane integrity and changes in the morphological characteristics of the leaf tissues, the number of trichomes and stomatal aperture index. In addition, the antioxidant capacity was also affected. The addition of EDTA together with Pb into the culture solution induced amelioration of Pb toxicity effects on all parameters tested in both the cultivated sunflower *H. annuus* cv. 1114 and the interspesific line. Our results showed that in the presence of EDTA the content of Pb in the roots, stems and leaves was reduced compared with Pb treatment alone suggesting that the uptake of Pb was limited. However, EDTA enhanced the translocation of Pb from roots to stems and leaves as evidenced by the increased translocation factor (TF). The interspecific line demonstrated higher tolerance to excessive Pb levels when compared with the cultivated sunflower. This was due mainly to an increased photosynthetically active area, maintenance of plasma membrane integrity, permanently high total antioxidant activity and free radical scavenging capacity as well as total flavonoids content.

Conclusions The comparative analysis of the response to Pb application showed that the deleterious effects were more pronounced in the cultivated sunflower *H. annuus* cv. 1114. The toxic effects of Pb on RGR, leaf morphometric parameters, electrolyte leakage, SOD izoenzyme spectra, total antioxidant activity and free radical scavenging capacity were alleviated in the presence of EDTA in both *H. annuus* cv. 1114 and the interspesific line. The interspesific line demonstrated higher tolerance to excessive Pb levels as well as higher capacity for Pb accumulation in the roots which makes it a good candidate for phytoremediation in contaminated soils.

Response of sun- and shade-adapted plants of *Haberlea rhodopensis* to desiccation

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Abstract

The variabilities in some morphological and physiological characteristics of sun- and shade-adapted *Haberlea rhodopensis* plants were compared. Changes in the photosynthetic activity, electrolyte leakage from leaf tissues, malondialdehyde content and leaf anatomy were studied at different degrees of desiccation as well as after rehydration of plants. The malondialdehyde (MDA) content in well-watered sun *Haberlea* plants was higher compared to shade plants suggesting higher lipid peroxidation, which is commonly regarded as an indicator of oxidative stress, but desiccation of plants at high light did not cause additional oxidative damage as judged by the unaffected MDA content. The electrolyte leakage from dried leaves (8% RWC) from both shade and sun plants increased 4-fold indicating similar membrane damage. However, the recovery after rehydration showed that this damage was reversible. Well-watered sun plants had higher photosynthetic activity probably due to the higher thickness of the mesophyll layer. On the other hand, desiccation at high light reduced strongly CO_2 assimilation which was in accordance with the stronger reduction of stomatal conductance. Stomata were visible only on the abaxial side of sun leaves having also higher abundance of non-glandular trichomes. Increased trichomes density and epicuticular waxes and filaments upon desiccation could help plants to increase reflection, reduce net radiation income, slow down the rate of water loss and survive adverse conditions.

Effect of light on the photosynthetic activity during desiccation of the resurrection plant *Haberlea rhodopensis*

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Abstract: The effect of light during desiccation of the resurrection plant *Haberlea rhodopensis* on the photosynthetic activity and some morphological parameters was evaluated using plants growing at low or high irradiance in natural habitat. Chlorophyll content was not only lower in sun plants compared to shade plants, but it declined to a higher extent when desiccation was carried out at high light irradiance. Regardless of lower chlorophyll content in sun plants their photosynthetic activity (P_N) was about 30% higher compared to shade plants. However, during dehydration P_N declined more rapidly in sun plants. The mean leaf thickness of fully hydrated leaves from sun plants was larger when compared with shade plants, which was due to higher thickness of the mesophyll. Following rehydration plants rapidly recovered and P_N was higher by about 70% in sun than in shade plants. The results showed that the sun-exposed *Haberlea* plants exhibited good adaptation to desiccation under high irradiance.

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BRIEF COMMUNICATION

Fatty acid content during reconstitution of the photosynthetic apparatus in the air-dried leaves of *Xerophyta scabrida* after rehydration

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Abstract

Desiccation of *Xerophyta scabrida* caused considerable damage of chloroplast ultrastructure together with a complete loss of chlorophyll. Upon rehydration, the relative water content of the pale-green leaves almost reached that of the dark-green ones, however, the Chl content and photosynthetic activity remained lower. The process of reconstitution of the photosynthetic apparatus in the re-greening leaves was accompanied by changes in fatty acid (FA) content. The amount of the FA methyl esters was more than 2-fold higher in the green leaves as compared to the dry ones and slightly increased after rehydration in the pale-green leaves. Among the three main fatty acids in the leaves, oleic, palmitic and linoleic acid, the latter increased more than 3-fold during rehydration. This acid is concentrated mainly in the glycolipids and this was an indirect indication for the restoration of the photosynthetic apparatus. Our results showed that rehydration of *X. scabrida* led to a decrease of the saturated FA in parallel with an increase of the unsaturated FA, thus indicating increased membrane permeability. The observed changes in the lipid content can be considered as a characteristic feature of *X. scabrida* and most probably of other poikilochlorophyllous species.

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Abbreviations: Chl - chlorophyll; FA - fatty acids; FAME - fatty acids methyl esters; FID - flame ionization detector; F_0 - initial yield of chlorophyll fluorescence in the dark adapted leaves; F_m - maximum chlorophyll fluorescence of dark adapted leaves; F_m' - maximum fluorescence in light-adapted leaves; F_s - steady state fluorescence of light-adapted leaves; F_v - variable fluorescence of dark adapted leaves; F_v/F_m - maximum photochemical quantum efficiency of PS 2; F_v'/F_m' - excitation capture efficiency of PS 2; $1 - F_v'/F_m'$ - proportion of the energy dissipated as heat in the PS 2 antenna; FR - far-red; HDT - homoiochlorophyllous; PDT - poikilochlorophyllous; PPFD - photosynthetic photon flux density; PS 1 - photosystem 1; PS 2 - photosystem 2; P_{700} - reaction center of PS 1; P_{680} - reaction center of PS 2; RWC - relative water content; Rfd - chlorophyll fluorescence decrease ratio; Φ_{PS2} - quantum yield of Photosystem 2 photochemistry in the light-adapted state.
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