Centre for Agricultural Research

HUN-REN

3rd Plant Polyamine Research Workshop

October 12-13, 2023

Budapest and Martonvásár, Hungary

PROGRAM AND ABSTRACTS

Ágnes Szepesi and Tibor Janda

Dear Colleagues and Friends,

On behalf of the Scientific Committee of **3rd Plant Polyamine Research Workshop** and the local organizing committee it is a great pleasure to welcome you in Budapest and Martonvásár for the Plant Polyamine Workshop!

Plant Polyamine Research Workshops were established in 2021 at Martonvásár with the aim to provide a platform for scientific discussion and exchange for research on polyamines in plants. This event was very well received by the scientific community interested in polyamines, so in 2022 at Szeged a hybrid event was organized with collecting more researchers and PhD students to celebrate the importance of polyamines in plants. Now, in 2023 there is an opportunity to make this event broader with an exciting scientific program in two days.

The main aims of our international meeting are to establish a strong community of researchers working in plant polyamine field, and highlight the latest results in this topic. Following two successful meetings in the last two years, we would like to continue our started discussion, making valuable cooperation with different institutes and universities and bring together all researchers in plant polyamine research field.

Thank everybody who has contributed to this workshop and all of you who participate in Plant Polyamine Workshop in Hungary.

We wish you a great and successful scientific meeting and pleasant days in Budapest and Martonvásár!

Tibor Janda Ágnes Szepesi

3rd Plant Polyamine Research Workshop

Local Organizing Committee

Tibor Janda	Centre for Agricultural Research	Hungary
Ágnes Szepesi	University of Szeged	Hungary
Gabriella Szalai	Centre for Agricultural Research	Hungary

Scientific Committee

Tibor Janda	Centre for Agricultural Research	Hungary
Ágnes Szepesi	University of Szeged	Hungary

3rd Plant Polyamine Research Workshop is sponsored by

General Information

Organizers

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Conference Venue

Day 1: ATK INSTITUTE FOR SOIL SCIENCES (TAKI), 1022 Budapest, Herman Ottó út 15.

Day 2: ATK Agricultural Institute (MGI), 2462 Martonvásár Brunszvik. u. 2.

Conference Language

The language of the Workshop is English.

Online access

Topic: Poliamin Workshop

Time: Oct 12, 2023 Budapest

Join Zoom Meeting

https://us06web.zoom.us/j/83430105868?pwd=i7cc0EzD3duoIEvWHXrw2C2Za9v6yy.1

Meeting ID: 834 3010 5868

Passcode: 474519

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Workshop Program

Day 1 **12th Oct 2023**

Location: ATK INSTITUTE FOR SOIL SCIENCES (TAKI), 1022 Budapest, Herman Ottó út 15.

9:00-9:55 Registration
10:00-10:15 Opening (Tibor Janda and Ágnes Szepesi)

Session 1: Role of polyamines in growth and development

Chairs: Magda Pál, Jolán Csiszár

10:20-11:00 Plenary Lecture
CHANGES IN POLYAMINE METABOLISM DURING COLD ACCLIMATION IN MAIZE
Tibor Janda

11:00-13:30 Oral Presentations (25 min+5 min discussion)

11:00-11:30 Daily changes in polyamine metabolism under light and dark condition after polyamine treatments
Magda Pál

11:30-12:00 Involvement of polyamine metabolism in the cytokinin induced shoot meristem formation from lateral root primordia of Arabidopsis
Katalin Gémes

12:00-12:30 The role of polyamine metabolism in epilepsy
László Héja

12:30-13:30 ***Lunch***

Section 2: Polyamine metabolism and stress-related signal pathways in plants

Chairs: Tibor Janda, Katalin Gémes

13:30-14:50 Oral Presentations (25 min+5 min discussion)

13:30-14:00 The role of polyamines in metal-induced phytotoxicity – where do we stand at the moment?
Cristiano Soares

14:00-14:30 Blue and white light-induced differential adaptation to cadmium stress in wheat, influenced by putrescine pre-treatment
Altafur Rahman

14:30-14:50 Effect of osmotic stress on antioxidants and polyamines in cereals
Kristóf Jobbágy

14:50-15:20 ***Coffee break***

15:20-17:40 Oral Presentations (25 min+5 min discussion)

15:20-15:50 Polyamine catabolism and salt stress in plants
Ágnes Szepesi

15:50-16:20 Bio-Polymer Nanocomposites Improve Salinity Stress Resilience in Maize: Insights into Polyamine and Transcription Factor Genes
Fatemeh Gholizadeh

16:20:17:00 Plenary Lecture by invited speaker (online)
NOVEL METHODOLOGY FOR DELIVERING PUTRESCINE INTO THE APPLE EXPLANTS USING HORMONE ANCHORED NANOTUBE
Gholamreza Gohari

17:00-17:40 Plenary Lecture by invited speaker (online)
ORNITHINE DECARBOXYLASE GENE EXPRESSION MEDIATES POLLEN REJECTION IN SOLANUM
Roger T. Chetelat (UC Davis, USA; online lecture)

Day 2 13th Oct 2023

Location: ATK Agricultural Institute (MGI), 2462 Martonvásár Brunszvik. u. 2.

Section 3: The significance of polyamines in plant stress responses

Chairs: Ágnes Szepesi, Cristiano Soares

9:00-9:40 Plenary Lecture by invited speaker
POLYAMINES IN ARABIDOPSIS GLUTATHIONE PEROXIDASE MUTANTS: FUNCTION AS ANTIOXIDANTS OR GROWTH REGULATORS?
Jolán Csiszár

9:40-12:30 Oral Presentations (25 min+5 min discussion)

9:40-10:10 Changes in polyamine content of maize seedlings as a response to Fusarium infection
Anett Klaudia Kovács

10:10-10:40 The effect of light quality on physiology, photosynthesis and polyamines in Eruca sativa
Dilyana Doneva

10:40-11:00 ***Coffee break***

11:00-11:30 The effects of ionic and osmotic stresses on polyamine-dependent hypusination in the early developmental stage of tomato plants
Péter Pálfi

11:30-12:00 Can microalgae biomass's utilization significantly impact both the qualitative and quantitative yield?
Lamnganbi Mutum

12:00-12:30 Comparative study of LED and non-LED illumination of silica nanoparticle treated broccoli microgreens
Henrietta Kovács

12:30-13:20 ***Lunch***

13:20:13:50 Redox control of polyamine metabolism in maize
Kalpita Singh

13:50:14:20 Antioxidant and polyamine metabolism: the role of ethylene signalling in regulating the salt stress response in mature tomato fruit
Zalán Czékus

14:20:14:50 New potential biostimulants: Effects of effusol and juncusol on polyamine metabolism
László Bakacsy

15:00-16:00 Visiting the Agroverzum and Beethoven Museum

16:00-17:00 Round table discussion **"Present and future of polyamine research"**, future plans and announcement of Best Lecture Competition

17:00-20:00 Gala Dinner

Abstracts

Session 1:

Role of polyamines in growth and development

Role of Polyamines in Growth and Development

Changes in polyamine metabolism during cold acclimation in maize

Tibor Janda*, Magda Pál, Fatemeh Gholizadeh, Gabriella Szalai

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Low temperature is one of the most important factors limiting the spread and production of plants worldwide. This is especially true for the chilling-sensitive field crops of tropical or subtropical origin. In the case of maize plants, temperatures in the 10–15°C range decrease the capacity for biomass production, and exposure of plants to still lower temperatures for a prolonged period may lead to irreversible damage and even the death of the plants. It has been known for a long time that light is a critical factor for the development of frost hardiness in cold tolerant plants (Janda et al., 2014). Cold acclimated state, although to a lesser extent than in the case of winter cereals, can also be reached in the chilling sensitive plants. We have also shown that in spite of its photoinhibitory effects, light may also have a similar role in the cold acclimation processes in maize plants as it was found in wheat (Szalai et al., 2018). In this previous work we have shown that although exposure to photoinhibitory light during the cold acclimation period caused various stress symptoms, it also enhanced the effectiveness of acclimation processes to a subsequent severe cold stress. Greater accumulations of soluble sugars were also detected during hardening at relatively high light intensity. Furthermore, it has also been shown that certain stress responses were light-dependent not only in the leaves, but also in the roots. In the present work the effects of light during the cold acclimation period were further studied in maize, focusing on the stress responses not only in the leaves, but also in the roots. Before exposure to chilling temperature at 5°C, plants were cold acclimated at non-lethal temperature (15°C) under two different light conditions: normal growth light and under reduced light conditions. Polyamines such as putrescine, spermidine, and spermine are small aliphatic amines that are ubiquitous in all plant cells, and they also play important role in the responses to low temperatures in various plant species, including the chilling sensitive ones (Song et al., 2015). In the present work, the effects of light during the cold acclimation period on polyamine metabolism will be demonstrated in different organs of maize plants.

This work was supported by the National Research, Development, and Innovation Office (grant TKP2021- NKTA-06).

References

Janda, T., Majláth, I., and Szalai, G. (2014) Interaction of temperature and light in the development of freezing tolerance in plants. J. Plant Growth Regul. 33, 460–469.

Szalai, G., Majláth, I., Pál, M., Gondor, O.K., Rudnóy, S., Oláh, C., Vanková, R., Kalapos, B., Janda, T. (2018) Janus-faced nature of light in the cold acclimation processes of maize. Front. Plant Sci. 9:850.

Song, Y., Diao, Q., Qi, H. (2015) Polyamine metabolism and biosynthetic genes expression in tomato (*Lycopersicon esculentum* Mill.) seedlings during cold acclimation. Plant Growth Regul. 75, 21–32.

Role of Polyamines in Growth and Development

Daily changes in polyamine metabolism under light and dark condition after polyamine treatments

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The biosynthesis of polyamines (PAs) is controlled by light (Ioannidis and Kotzabasis, 2007). Earlier we demonstrated that longer lighting hour conditions or higher light conditions increased the amounts of PAs, in addition decreased their catabolism in the leaves of wheat (Gondor et al., 2021). In tomato, the accumulation of PAs also showed an increasing tendency after the beginning of light conditions, but their concentrations fluctuated more frequently under dark conditions (Takács et al., 2016). PA diurnal rhythm was also described in the leaves of tobacco plants (Gemperlová et al. 2006). In the present work, we focused on circadian rhythm-related changes in PA metabolism under light and dark conditions in wheat plants. Leaf and root samples were collected before and 1, 3, 5, 7, 9 and 24 hours after the beginning of the illumination. Besides the determination of the levels of PAs, namely putrescine (PUT), spermidine (SPD) and spermine (SPM), the catabolite product of the latter two, 1,3-diaminopropane (DAP) was also measured; furthermore, the gene expression levels of certain PA metabolism-related gene were analysed, too. PA treatments (PUT and SPD) were also applied in order to reveal whether the excess of PAs modify the daily fluctuation of the PA pool.

References:

Gemperlová L., Nováková M., Vaňková R., Eder J., Cvikrová M., (2006) J Exp Bot, 57: 1413-1421.

Gondor O.K., Tajti J., Hamow K.Á., Majláth I., Szalai G., Janda T., Pál M., (2021) Int J Mol Sci, 22: 11717.

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Funding: This work was funded by the National Research Development and Innovation Office, Hungary (NKFIH K134395).

Role of Polyamines in Growth and Development

Involvement of polyamine metabolism in the cytokinin induced shoot meristem formation from lateral root primordia of Arabidopsis

Katalin Gémes[1,2,*] , Péter Benkő[1,2], Ágnes Szepesi[1], Jolán Csiszár[1], Attila Fehér[1,2], Nikolett Kaszler[1,2]

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Both in nature and in vitro, plant organogenesis is one of the alternative pathways in plant tissue culture techniques for propagation. During these process plants can be regenerated from different explants via de novo shoot meristem (SM) formation. The success of in vitro regeneration depends on several factors, such as the presence of a suitable explant, environmental conditions and different hormones, mainly auxin and cytokinin. Besides plant hormones, the role of polyamines has been implicated in this process. We have found that the level of spermidine (spd) and expression of genes related to spd synthesis (ADC1,2; SPDS1,2) and back-conversion of thermospermine (t-spm) to spd (PAO5) enhanced during the direct conversion of lateral root primordia to shoot meristem. Gene expression analyses supported the view that the pao5-2 mutation as well as exogenous T-Spm downregulate the expression of the class 3 haemoglobin coding genes AtGLB1 and AtGLB2. AtGLB1 and 2 have been reported to augment cytokinin sensitivity, indirectly inhibiting the expression of type-A ARABIDOPSIS RESPONSE REGULATORs (ARRs). In agreement, the same ARR-coding genes were found to be upregulated in the pao5-2 mutant. Although GLB proteins might also control cytokinin-induced nitric oxide (NO) accumulation, we could not find experimental evidence for it. Rather, the negative effect of NO-donor treatment on AtPAO5 gene expression and SM formation was seen. Nevertheless, a hypothetical pathway is set up explaining how AtPAO5 may affect direct shoot meristem formation, controlling cytokinin sensitivity through T-Spm and GLBs.

Funding: This work was supported by the grant from the National Research, Development and Innovation Office of Hungary—NKFIH (NKFIH FK 128997).

Role of Polyamines in Growth and Development

The role of polyamine metabolism in epilepsy

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In the brain, polyamines can be regarded as gliotransmitters since they are accumulated and stored in astrocytes and can be released by various mechanisms. The polyamine putrescine (PUT) is utilized to synthesize GABA which can also be released from astrocytes and provide tonic inhibition on neurons. The polyamine spermine (SPM), synthesized from PUT through spermidine (SPD) is known to unblock astrocytic Cx43 gap junction channels and therefore facilitate astrocytic synchronization. In addition, SPM released from astrocytes may also modulate neuronal NMDA, AMPA and kainate receptors. As a consequence, astrocytic polyamines possess the capability to significantly modulate epileptiform activity.

We explored different steps in polyamine metabolism and coupled GABA release to assess their potential to control seizure generation and maintenance in two different epilepsy models: the low-[Mg^{2+}] model of temporal lobe epilepsy in vitro and in the WAG/Rij rat model of absence epilepsy in vivo. Importantly, we found that inhibition of SPD synthesis completely prevented seizure generation in WAG/Rij rats. We hypothesize that this anti-epileptic effect is attributed to the subsequent enhancement of PUT to GABA conversion in astrocytes, leading to GABA release through GAT-2/3 transporters. This interpretation is supported by the observation that intraperitoneally applied PUT and GABA also significantly shortens in vivo seizures. In addition, we show that SPM is a gliotransmitter that is released from astrocytes and significantly contributes to network excitation. Our findings conclusively suggest that the major pathway through which astrocytic polyamines contribute to epileptiform activity is the production of GABA. Modulation of astrocytic polyamine levels, therefore, may serve for a more effective anti-epileptic drug development in the future.

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Session 2:

Polyamine metabolism and stress-related signal pathways in plants

Polyamine metabolism and stress-related signal pathways in plants

The role of polyamines in metal-induced phytotoxicity – where do we stand at the moment?

Cristiano Soares[1]*, Sofia Spormann[1,2,3], Ágnes Szepesi[4], Fernanda Fidalgo[1]

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Polyamine (PA) metabolism and biological roles have started to raise attention from plant scientists in recent decades, with a growing number of publications exploring the features and functions of these polycations. Up to now, plant PAs have been described to act as efficient membrane- and protein-stabilizers, thus ensuring cellular homeostasis under stress, while also serving as direct and indirect signalling agents, interacting with phytohormones and reactive oxygen species (ROS). Moreover, PAs have also been found to regulate stress priming, acting as epigenetic regulators of gene expression, and to enhance the efficiency of the plant antioxidant (AOX) system. Although the stress-ameliorating effects of PAs have been widely studied for several abiotic stresses, such as drought and salinity, there is still an evident gap concerning their effects on metal-induced stress. Still, studies usually found an increased accumulation of PAs in response to metal exposure, while being also reported that PAs can regulate ion homeostasis and transport by interacting with ion channels, promoting the formation of metal conjugates and/or inhibiting metal accumulation. In the last years, a growing number of publications have also explored the potential of PA exogenous application in plants grown under metal toxicity. Still, further insights on PA-mediated signalling and physiological adjustments are needed to draw a clearer picture. Recently, studies from our research group have highlighted that Ni-exposed tomato plants (*Solanum lycopersicum* L. cv. Gold Nugget and cv. Purple Calabash) modulate PA metabolism in a cultivar-dependent manner, with differences in antioxidant potential. Interestingly, there was no accumulation of spermidine (Spd) nor spermine (Spm) in response to Ni-stress in any cultivar, but plants of the Gold Nugget variety showed higher levels of H2O2, and a higher putrescine (Put) content, coupled with an increased activity of polyamine oxidase (PAO) and diamine oxidase (DAO). This batch of results point to the stimulation of the back-conversion catabolism, especially in the Gold Nugget variety, which leads to the subsequent formation of important stress-responsive signalling molecules such as H2O2, Put, and γ-aminobutyric acid (GABA).

This work was supported through FCT/MCTES within the scope of UIDB/05748/2020 and UIDP/05748/2020 (GreenUPorto).

Polyamine metabolism and stress-related signal pathways in plants

Blue and white light-induced differential adaptation to cadmium stress in wheat, influenced by putrescine pre-treatment

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Blue light exerts a profound influence on various plant functions, including plant morphology, photosynthesis, and primary and secondary metabolism. While earlier research has suggested that blue light may have advantageous impacts under certain stress conditions, the underlying mechanisms are still largely unknown. At the same time, growing evidence emphasizes how important polyamines are for improving plant stress tolerance. In this study, we investigated the notion that, in contrast to white light, blue light modulates the potential protective effects of exogenous putrescine in wheat plants, triggering unique adaptation processes in response to cadmium (Cd) stress. It has been discovered that compared to white light conditions, less pronounced Cd stress was seen under blue light conditions. Blue light exerted its unique influence on metabolite and gene expression levels, resulting in reduced Cd uptake, accompanied by lower phytochelatin levels, but increased accumulation of conjugated polyamines. Notably, pre-treatment with putrescine protected in both light environments, with a stronger benefit observed in the white light condition. In addition, differences between blue and white light conditions during Cd stress were revealed by putrescine pre-treatment, specifically in phytochelatin production, polyamine metabolism, and the accumulation of phenolic compounds and plant hormones. Our data unequivocally established that blue light-regulated Cd tolerance in wheat and reshaped defense strategies in the presence of excess putrescine.

Acknowledgment: This work was financed by a grant from the National Research Development and Innovation Office, Hungary (NKFIH K134395).

Polyamine metabolism and stress-related signal pathways in plants

Bio-Polymer Nanocomposites Improve Salinity Stress Resilience in Maize: Insights into Polyamine Genes

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Polyamines (PAs), are low-molecular weight aliphatic compounds found in all living organisms. They play a role in many physiological phenomena of plants such as growth, development and response to various stresses. Salinity is one of the most important stress factors that can affect growth, yield and crop quality in plants. In this study, treatments with chitosan (CTS), proline-coated chitosan nanoparticles (CTS-Pro NPs), glycine-coated chitosan nanoparticles (CTS-Gly NPs), proline (Pro), glycine (Gly) and distilled water (DW) to reduce the effects of salinity were sprayed for 5 days of maize seedlings at 25 °C in the greenhouse. Then we applied salt stress with 3 levels (0, 100 and 400 mM NaCl) for 5 days. Seven days after salinity stress sampling was done for gene expression analysis. In this study, we examined nine ZMPAO (ZMPAO1- ZMPAO9) genes in different treatments. The application of CTS, CTS-Gly NPs and CTS-Pro NPs respectively increased the expression level of ZMPAO1, ZMPAO3 and ZMPAO6 genes under salinity, while the expression level of ZMPAO4 gene decreased more than other treatments at 400 mM salinity. Moreover, Gly and CTS-Gly NPs under salinity increased the expression level of ZMPAO7 and ZMPAO8 genes. Also, the expression level of ZMPAO9 increased in all treatments under salinity except DW and Pro treatments. The level of ZMBHLH95 was highly expressed under salinity and CTS treatment. We also found that some genes including ZMZIP1, ZMPIF1 and ZMDREB80 are extremely high expressed under salinity and Gly and CTS-Gly treatments respectively, while the expression level of ZMPMP3-5 increased under CTS treatment. In general, the expression analysis showed that some PAO family members and transcription factors are involved in stress tolerance in maize with the application of nanoparticles. These results confirmed the benefit of NPs that could be applied as an innovative protective agent to mitigate the effects of salinity in maize.

This work was supported by the National Research, Development, and Innovation Office (grant TKP2021- NKTA-06).

Polyamine metabolism and stress-related signal pathways in plants

Polyamine catabolism and salt stress in plants

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Polyamine (PA) catabolism is a crucial mechanism to regulate and fine tune the proper PA content in plants, not only in growth and development but also in stress responses. Salt stress as one of the most threatening stress types worldwide, causes significant yield loss reducing food safety and supply. Our research is focusing to study the degradation processes of polyamines during salt stress in different plant species, cultivars, developmental ages to provide evidence for using these processes as a target for future breeding efforts. Diamine oxidase and polyamine oxidase are the main PA degrading enzymes producing ammonia, aldehydes and also hydrogen peroxide as a by-product. Using inhibitor compounds, we showed that the proper PA degradation by these enzymes are crucial to fine tune not only the PA levels but also the ROS and RNS balance during salt stress. As the free PAs, especially spermidine is essential for hypusination, which is a metabolite-dependent posttranslational modification of eukaryotic translation factor 5A, we investigated the role of hypusination in salt stress-induced responses in *Arabidopsis thaliana* and *Solanum lycopersicum*. Based on our results, PA catabolism has a central role in salt stress responses but further studies are needed to demonstrate the potential regulation of PA catabolism by hypusination or the PA catabolism-mediated responses of hypusination.

Reference:

Szepesi Á, Bakacsy L, Kovács H, Szilágyi Á, Köhler ZM. Inhibiting Copper Amine Oxidase Using L-Aminoguanidine Induces Cultivar and Age-Dependent Alterations of Polyamine Catabolism in Tomato Seedlings. *Agriculture*. 2022; 12(2):274. https://doi.org/10.3390/agriculture12020274

Szepesi Á, Bakacsy L, Fehér A, Kovács H, Pálfi P, Poór P, Szőllősi R, Gondor OK, Janda T, Szalai G, et al. L-Aminoguanidine Induces Imbalance of ROS/RNS Homeostasis and Polyamine Catabolism of Tomato Roots after Short-Term Salt Exposure. Antioxidants. 2023; 12(8):1614. https://doi.org/10.3390/antiox12081614

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Polyamine metabolism and stress-related signal pathways in plants

Effect of osmotic stress on antioxidants and polyamines in cereals

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In our experiments, we compared the polyamine contents, thiol levels and specific, stress-related gene expressions of two *Aegilops biuncialis* (Ae. b.) Vis. genotypes (382 - drought tolerant, 642 - drought sensitive) and three *Triticum aestivum* L. genotypes (Cappelle-Desprez (C.D.) - drought sensitive, Plainsman V and Mv9 kr1 - drought tolerant), following osmotic stress. A 7-day treatment with 15% polyethylene glycol (PEG) reduced shoot and root growth in all genotypes. Total cysteine (glutathione (GSH) precursor) content remained unchanged after the treatment, but levels of the oxidized form were significantly increased in several genotypes in both root and shoot. GSH levels were not significantly altered by PEG, however the amount of oxidized (GSSG) glutathione was most definitely affected in several genotypes. The GSH/GSSG ratio increased in the root of Ae. b. 382 and Plainsman, while it decreased in the other genotypes. In the shoots, the GSH/GSSG ratio remained unchanged for most genotypes. While the spermidine content did not show any alteration during the treatment (with the exception of Mv9kr1), the spermine content – especially in roots – exhibited a significant increase in Ae. b. 382, Mv9kr1 and Plainsman as well. The 1,3-diaminopropane content did show a general increase in the shoots, but not in the roots, while the putrescine content was highly variable. The investigated genes changed significantly in most genotypes during PEG treatment. In summary, PEG treatment affected GSH metabolism, thiol levels, polyamine content and stress related gene expressions in the investigated genotypes, which changes may have an important role in the response to osmotic stress.

The experiments were supported by the National Research, Development, and Innovation Office (grant TKP2021- NKTA-06).

Polyamine metabolism and stress-related signal pathways in plants

Novel methodology for delivering putrescine into the apple explants using hormone anchored nanotube

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Multi wall carbon nanotubes (MWCNTs) have been successfully exploited as growth regulator considering their potentials in manipulation of plant developmental patterns. Beside the biochemical characteristics of nanoparticles, they are gradually involved in target delivery systems as the carrier of nutrients and hormones. On the other hand, polyamines (PAs) and their derivations play crucial roles in plant growth and development. Take the mentioned subjects into consideration, putrescine anchored carbon nanotube which had been labelled with fluorescein (MWCNT-Put-Fl) was synthetized in this study. A set of physiological, biochemical and morphological parameters were assessed in an attempt to examine the usage potential of de novo synthetized MWCNT-Put in terms of plant in-vitro culture. For this purpose, the nanotube was treated on to the in-vitro plantlets of red-leafed apple (*Malus niedzwetzkyana*) in three concentrations (0, 50 and 100 mg/l). Localization of the nanotube in the plantlets was successfully accomplished using fluorescence microscopy. Bio-imaging of tissues indicated the existence of nanotube in nearly all studied organs. Application of MWCNT-Put at both concentrations (50 and 100 mg/ l) could enhance the growth of plantlets by increasing the rate of leaf formation and speeding up the plastochron. Also, lateral outgrowth and proliferation of the plantlets were enhanced using MWCNT-Put. The levels of the photosynthetic pigments, including chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Car), increased following application of the nanotube. Glutathione peroxidase (GPX) activity was significantly affected by the nanotube. However, polyphenol oxidase (PPO) and peroxidase (POX) were not influenced by MWCNT-Put. Stomatal density and form were considerably impacted by the nanotube. The higher the concentration of MWCNT-Put, the higher the stomatal density was achieved. Representing geometrical transformation of shape as a thin plate spline revealed that the nanotube effectively increased longitudinally of stomata and changes their aspect ratio.

Keywords: Polyamine, Nanotechnology, Smart delivery, Stomata, Bio-imaging

Polyamine metabolism and stress-related signal pathways in plants

Ornithine decarboxylase gene expression mediates pollen rejection in Solanum

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Crosses between cultivated tomato (*Solanum lycopersicum*) and its green-fruited wild relatives succeed only when tomato is used as the pistillate parent; the reciprocal crosses (i.e. wild x cultivated) are blocked by unilateral incompatibility (UI) in which *S. lycopersicum* pollen tubes are arrested in the style. Multiple pistil barrier factors and corresponding pollen resistance loci are involved in recognizing heterospecific pollen. One form of UI occurs in pistils of self-incompatible (SI) species, which contain S-RNases that are cytotoxic to pollen tubes from self-compatible (SC) species lacking either functional S-locus F-box (SLF) or Cullin1 proteins. Pollen of cultivated tomato and other red fruited species lack functional Cullin1, and as a result are incompatible on pistils of SI species and accessions (i.e. the 'SI x SC rule'). Another form of UI is independent of the S-RNase rejection mechanism and is based on expression of ornithine decarboxylase (ODC2) in pistils; the corresponding resistance factor is a farnesyl pyrophosphate synthase (FPS2) expressed in pollen. Pollen of *S. lycopersicum* contain low levels of *FPS2* mRNA, and are rejected by UI on pistils of *S. pennellii* LA0716 (SC), which contain no *S-RNase* but high levels of *ODC2*. Pollen of *fps2* knockout mutants in LA0716 are rejected on pistils of the introgression line IL 3-3, which contains four *ODC2* paralogs from *S. pennellii* and expresses high levels of *ODC2* in pistils. Introgression line IL 12-3 expresses the pistil barrier factors HT-A and HT-B, which have been shown to function in SI as well as UI. Combining IL 3-3 (ODC2) with IL 12-3 (HT-A, HT-B) increases the strength of pollen rejection in the pistil: *S. lycopersicum* pollen are compatible on pistils of either IL 3-3 or IL 12-3 individually but are rejected on IL 3-3 + IL 12-3 double ILs. Thus HT-A and -B contribute to both the S-RNase- and ODC2-dependent forms of UI. ODC2/FPS2-based pollen rejection was observed in all tested *S. pennellii* accessions (SC or SI), and in some *S. habrochaites* populations (mostly SI), but not in the other wild tomato species. While the biochemical/physiological mechanism of this type of UI is unknown, ODC is a key enzyme in the synthesis of putrescine and other polyamines, suggesting that PAs may be involved in heterospecific pollen rejection in *Solanum*.

Session 3:

The significance of polyamines in plant stress responses

The significance of polyamines in plant stress responses

Polyamines in Arabidopsis glutathione peroxidase mutants: Function as antioxidants or growth regulators?

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Plants contain glutathione peroxidase-like (GPXL) proteins, which catalyse the reduction of H_2O_2 or hydroperoxides to water or alcohols using glutathione or thioredoxin as electron donor. They can fine-tune ROS level and redox homeostasis, but their relationships with ethylene metabolism and signalling were also described. *Arabidopsis thaliana* possesses 8 *AtGPXL* genes. We aimed functional characterization of the membrane localized AtGPXL4 and -5 proteins using *Atgpxl4* and *-5* mutants, *Atgpxl4*5* double mutant and plants overexpressing *AtGPXL4* or *-5* (OX-AtGPXL4 and -5). While the mutant plants had generally smaller rosette diameters and roots than that of the Col-0 wild type, overexpression of OX-AtGPXL4 or -5 had increased rosette size. To investigate the involvement of these genes in redox processes under control conditions and after salt stress, 6-week-old plants were treated with 100 mM NaCl for 24 h. Besides detecting several oxidative stress-related parameters and antioxidants, the polyamines (PAs) and the expression levels of some genes determining their biosynthesis were analysed. While the H_2O_2 and PA contents were at control levels in both OX-AtGPXL lines, their amounts were elevated especially in the leaves of the single and double mutants. Some genes involved in PA biosynthesis were upregulated either in the mutants and overexpressing lines (e.g., *ADC2*, *ADC1* and *SAMDC*3), but less differences were found in the expression of selected genes involved in the conversion of PAs (*SPDS*, *SPMS*) under control conditions or after applying salt stress. The changes in the PA levels indicated that they might have important roles in compensation the effects of the AtGPXL isoenzyme deficiencies and in maintenance of the redox homeostasis.

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The significance of polyamines in plant stress responses

Changes in polyamine content of maize seedlings as a response to Fusarium infection

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Maize (*Zea mays* L.) is the most produced field crop all over the world, thanks to its excellent adaptability and high yield potential. One of its most important diseases that results qualitative and quantitative crop losses is ear rot caused by various *Fusarium* species. Several *Fusarium* species are known to infect maize, and among them, *F. graminearum* and *F. verticillioides* are the two most important pathogens. Previous studies have shown that polyamines (PAs) play a crucial role in biotic stress responses. In our work, we examined the changes in PA contents induced in the seedlings of two maize genotypes of different susceptibility by isolates of *F. verticillioides* and *F. graminearum*. In addition, we studied how infection rates and changes in PA contents are modified by putrescine pre-treatment (PUT) applied as seed soaking compared to distilled water priming or without any treatment.

Our data showed that initial- and stress-induced changes in PA contents either in maize coleoptile or radicle are not directly related to tolerance. However, the two *Fusarium* species induced specific changes in PA concentrations, which variations were in relation with the lifestyles of the pathogens (in maize, *F. verticillioides* is an endophytic pathogen in most cases, while *F. graminearum* behaves as a necrotrophic microorganism during its life cycle). The effect of PUT pre-treatments depended on both the pathogen and the plant resistance, namely against *F. verticillioides*, PUT seed treatment had beneficial effects, whereas in the case of *F. graminearum* infection, seed soaking in distilled water already alone positively affected biomass parameters in the tolerant genotype. The accumulation of PUT in parallel with the decrease in spermidine/spermine content and accompanied with the reduced catabolism of the latter ones could decrease the infection of the necrotrophic pathogen, *F. graminearum*. At the same time, the accumulation and metabolism of PAs leads to an increase in H_2O_2 content, which prevents the spread of the endophytic pathogen *F. verticillioides* in plants. Our results suggest that PAs are important compounds during *Fusarium* infections, but further information and researches are needed to clarify the PA-related resistance and changes in PA metabolism.

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The significance of polyamines in plant stress responses

The effect of light quality on physiology, photosynthesis and polyamines in Eruca sativa

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The extensive development in light emitting diodes (LEDs) industry in recent years provides the opportunity to adjust wavelength selection for plant growth. On the other hand, year-round growing of quality greens for the market became an area of increasing interest. By precisely establishing an appropriate spectrum production yields can be increased, as well as the nutritional qualities of vegetable crop can be improved.
In this study, rocket salad (*Eruca sativa*) was grown from seeds under three light regimes – white light (WL) provided by fluorescent lamps, red-blue (RB) and red-green-blue (RGB) provided by LEDs. The effect of the different light spectra on the biomass accumulation, photosynthetic performance, nitrate, antioxidant and polyamine content was investigated. WL and RB produced grater biomass, but RB was characterized with highest level of polyamines necessary for growth and development No photosynthetic stress was observed under the three lighting regimes, but leaves grown under RB and RGB light displayed higher levels of maximum quantum yield of PSII and photochemical quantum efficiency and better non-photochemical quenching. The thermoluminescence reveals that better developed plants (WL and RB) possess a larger AG band, indicating their potential role as a source leaves for younger sink leaves. Notable differences in antioxidants and nitrates content under the three lighting regimes were detected with higher concentrations of antioxidant compounds and less nitrates levels under the RB light.

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The significance of polyamines in plant stress responses

The effects of ionic and osmotic stresses on polyamine-dependent hypusination in the early developmental stage of tomato plants

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Salt stress is one of the most threatening factors in agricultural production, causing both osmotic and ionic stresses to plants. In the tolerance of salt stress hypusination may also play an important role [1]. Hypusination is an essential spermidine-dependent post-translational modification of eukaryotic translation factor eIF5A. The specific lysine side chain of the translation factor eIF5A is converted into a hypusine side chain by the enzymes deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH), thus activating the protein to be able to assist the translation process (reviewed by [2]). In order to investigate the specific effect of salt stress components on hypusination we germinated tomato seeds (*Solanum lycopersicum* cv. Rio Fuego) in the presence of treatments NaCl, LiCl (for ionic stress) or sorbitol (for osmotic stress). We compared the development of the seedlings, measured the activities of the antioxidant enzyme, the enzymes involved is polyamine catabolism, and examined the enzymes of hypusination, DHS and DOHH by Western blot. The treatments had negative effects on the development of the seedlings compared to the control, the changes of enzyme activities were non-significant and the Western blot showed an increase of DHS protein level in the NaCl samples and an increase of DOHH protein level in the LiCl samples compared to the control.
Our results suggest that the ionic component has a more important effect on hypusination than the osmotic component, suggesting the significant role of hypusination in salt stress.

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The significance of polyamines in plant stress responses

Can microalgae biomass's utilization significantly impact both the qualitative and quantitative yield?

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The beneficial effects of microalgae extract on the growth and development of higher plants have been demonstrated earlier through some studies. The present work aimed to test three microalgae strains (MACC-612, MACC-430, MACC-922 from the Mosonmagyaróvár Algae Culture Collection, MACC) on winter wheat varieties (*Triticum aestivum*) in field condition to study the biochemical changes and eventually the quantitative and qualitative impact on yield. The metabolic contents of the selected strains were quantified and metabolomic analyses of the treated leaves were conducted. The metabolomic analysis shows the presence of secondary metabolites in the selected species and indole-3-acetic-acid was in the detectable range only in strain MACC-612 (*Nostoc sp*.). The present result suggested changes in salicylic acid, abscisic acid, jasmonic acid-leucine/isoleucine conjugate composition in the plant after the application of microalgae biomass. Furthermore, in the two successive field trial, 2020-21 and 2021-22, three microalgae strains MACC-612, MACC-430 and MACC-922 were added without or with adjuvants, Trend 90 as separate treatments taking BAP-6 as standard check. The application was done at the critical flowering stage and positively impacted the yield attributes and protein quality that reflects on the flour quality. In conclusion, the type of microalgae strain applied created no huge difference among the treatments, however, MACC-922, Chlorella vulgaris was slightly superior results than the other two strains, MACC-612, *Nostoc linckia* and MACC-922, *Chlamydopodium fusiforme*. Since the effect of polyamines on stress tolerance has already been confirmed by numerous experiments, we plan to focus in the future on: 1. the relationships between the polyamine content and effects of microalgae; 2. to combine algae treatments with polyamine treatments.

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Keywords: Microalgae biomass, Biochemical, secondary metabolites, metabolomic analysis

The significance of polyamines in plant stress responses

Comparative study of LED and non-LED illumination of silica nanoparticle treated broccoli microgreens

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Microgreens are tiny, "micro-scale" crops that are becoming increasingly popular and more widely grown due to their impact on human health, their ability to be easily grown and their cheap availability. Containing more nutrients than their mature counterparts is making them an increasingly important part of a healthy diet and in the fight against hidden hunger [1]. The application of nanoparticles (NPs) and the light source could be possible methods to increase the nutrient content of microgreens and their suitability for biofortification. The small size, long lifetime and high photoelectric conversion efficiency of LEDs are properties that are making them an increasingly popular light source in crop production. Silicon is considered a "quasi-essential" element for plants and regulates many physiological processes, including germination, vegetative growth, photosynthesis and stress tolerance [2]. Our aim was to compare the developmental parameters, pigment content and some biochemical and antioxidant parameters, as well as the process of polyamine-dependent hypusination under controlled conditions in a hydroponic system under LED and non-LED light conditions [3]. Future studies will aim to investigate the simultaneous effects of different nanoparticles and illumination on microgreen plants.

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The significance of polyamines in plant stress responses

Redox control of polyamine metabolism in maize

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Maize is one of the most consumed cereals after rice and wheat as well as an important feed and industrial crop. Plant growth and development are tightly regulated by the balance of ROS and antioxidant system. The critical balance of oxidants and reductants influences gene expression, protein activity, and subsequent metabolism. Therefore, the aim of the study is to study the effect of an oxidant (5 mM H2O2) and two reductants (5 mM ascorbate and 1 mM NaHS) on the redox-dependent regulation of polyamine metabolism in maize. Interestingly, it was observed that the external application of NaHS after 7 days showed a significant positive effect on shoot length and fresh weight in comparison to the other two compounds along with a peak in endogenous H2O2 content. The activity of H2O2 degrading enzyme- ascorbate peroxidase (APX) was lower after 3 days of NaHS treatment but elevated after 7 days. The application of H2O2 resulted in a higher activity of APX than Asc application after both 3 and 7 days. The modulating effect of the redox environment on the polyamine metabolism, the endogenous contents of putrescine (PUT), spermine (SPM), spermidine (SPD), and 1, 3-diamino propane (DAP) was evaluated. Notably, Asc and H2O2 led to a peak in PUT content after 3 days of the treatment but a lower level of SPD and SPM. These results were further confirmed by the gene expression of the enzymes spermidine synthase and spermine synthase. Furthermore, the gene expression of the enzyme S-adenosylmethionine decarboxylase was lowered after 7 days of Asc treatment and so was the endogenous content of SPD and SPM after 7 days of Asc application. Moreover, the endogenous content of DAP showed a significantly lower level in response to Asc after 3 days but a peak in the case of H2O2 treatment. Therefore, our results indicate the redox control of polyamine content at metabolite and gene expression levels.

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The significance of polyamines in plant stress responses

Antioxidant and polyamine metabolism: the role of ethylene signalling in regulating the salt stress response in mature tomato fruit

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The phytohormone ethylene (ET) plays a crucial role in plant defence responses, which can be dependent on plant organs. In this work, the effects of salt stress were analysed in fruits of wild-type (WT) and Never ripe (Nr) - ET receptor mutant – tomatoes to understand the role of ET in this process. Treatments with 75 mM NaCl reduced fruit weight and size in both genotypes, which was more pronounced in Nr tomato. In addition, significantly higher oxidative stress was detected in Nr compared to the untreated control in WT, based on changes in H2O2 and malondialdehyde levels after NaCl treatment. ET affected the major antioxidant enzymes, especially ascorbate peroxidase (APX) in WT, but in Nr fruits the activity of APX did not change, and superoxide dismutase and catalase activities were downregulated compared to untreated controls upon salinity stress, contributing to a higher degree of oxidative stress in Nr fruits. The dependence of polyamine metabolism on active ET signalling was also analysed under salt stress. Salt stress increased the accumulation of spermine in WT fruits, which was not detected in Nr, but the levels of putrescine and spermidine were increased by salt stress in these tissues. In addition, PA catabolism was much higher under salt stress in Nr fruits, contributing to higher oxidative stress, which was only partially alleviated by the increased total and reduced ascorbate and glutathione pools. It can be concluded that ET-mediated signalling plays a crucial role in the regulation of salt-induced oxidative stress and PA levels in tomato fruits at the mature stage.

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The significance of polyamines in plant stress responses

New potential biostimulants: effects of effusol and juncusol on polyamine metabolism

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Juncus species have been valuable sources of phenanthrene compounds used in traditional Chinese medicine for millennia. Among these compounds, effusol and juncusol are the most extensively studied, known for their antimicrobial and anticancer properties; however, their effects on higher plants have not been investigated. Our aim was to examine the effects of effusol and juncusol on Arabidopsis thaliana model plant, and understand their impact on polyamine metabolism and enzymes, along with their consequences on the growth of the model plant. To achieve this, we treated the plants with different concentrations of phenanthrene (Phe), effusol, and juncusol. We measured biomass, photosynthetic pigments, protein levels, and hydrogen peroxide content. The activities of antioxidant enzymes (SOD, CAT, POD) were also analyzed. We measured polyamine (PA) levels and the activities of PA degradation enzymes (DAO and PAO). Our results showed that Phe had a negative influence on plant growth, photosynthesis, and reduced protein levels. In contrast, effusol and juncusol increased biomass, maintained the antioxidant defense system, and did not alter polyamine levels. However, significant differences were observed in the activities of the PA degradation enzymes (DAO and PAO) among the treated groups. Effusol effectively reduced PA degradation, while juncusol increased DAO enzyme activity at some concentrations. These findings suggest that effusol and juncusol may enhance plant biomass and sustain an optimal antioxidant defense system, potentially resulting in reduced polyamine degradation. Effusol and juncusol hold promising potential for the development of new biopesticides or biostimulants for regulating plant growth.

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