

**Department of Plant Biology**

University of Szeged

## **2nd Hungarian Polyamine Research Workshop**

**December 2, 2022**

**Szeged, Hungary**



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Hungarian Society for Plant Biology



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## 2<sup>nd</sup> Hungarian Polyamine Research Workshop

### Local Organizing Committee

Ágnes Szepesi	University of Szeged	Hungary
Péter Pálfi	University of Szeged	Hungary
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## 2<sup>nd</sup> Hungarian Polyamine Research Workshop is sponsored by



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# General Information

## Organizer

Department of Plant Biology, Institute of Biology, Faculty of Science and Informatics,  
University of Szeged

## Conference Venue

Conference Room

Institute of Biology

Szeged, Középfasor 52.

H-6726

## Conference Language

English

## Coffee and Lunch Break

Snacks, lunch and coffee are serviced.

## Link by Zoom

<https://us02web.zoom.us/j/88603873552?pwd=aTQ1eG04U2F1cTdyU3BrU3BMa3lCUT09>



# Programme

9:45-10:00	Registration/Online access
10:00-10:10	Opening/Welcome <b>Szepesi, Ágnes</b>
10:10-12:00	<b>Session I: <i>Polyamines in Growth and Development</i></b> Chair: <b>Ágnes Szepesi and Magda Pál</b>
10:10-10:25	Polyamines in plants: facts and questions <b>Janda, Tibor</b>
10:30-10:45	Analytical challenges in the determination of conjugated polyamines <b>Nagy, Katalin</b>
10:50-11:05	Fine-tuning of polyamine metabolism and its light-related modulation <b>Pál, Magda</b>
11:10-11:25	L-aminoguanidine induced responses of tomato plants: when the specificity matters <b>Szepesi, Ágnes</b>
11:30-11:45	Involvement of polyamines and their metabolism in the direct conversion of lateral root primordia to shoot meristem of <i>Arabidopsis</i> <b>Gémes, Katalin</b>
11:50-12:00	Discussion
12:00-13:00	<b>Lunch Break</b>
13:00-16:00	<b>Session II: <i>Polyamines in Stress Responses</i></b> Chair: <b>Tibor Janda</b>
13:10-13:25	Unraveling the genetics of polyamine metabolism in barley for senescence-related crop improvement <b>Sobieszczuk-Nowicka, Ewa</b>
13:30-13:45	Genome-wide exploration of the genetics of biogenic polyamines in barley <b>Tanwar, Umesh Kumar</b>
13:50-14:05	Effects of putrescine treatment on wheat genotypes subjected to drought <b>Peeva, Violeta</b>
14:10-14:25	Influence of phyA mutation on polyamine metabolism in <i>Arabidopsis</i> in the presence of spermine excess under different light conditions <b>Rahman, Altafur</b>
14:30-14:45	Identification of polyamine oxidase gene family in wheat ( <i>Triticum aestivum</i> ) and evaluation of their expression in response to exogenous polyamines and cold treatment <b>Gholizadeh, Fatemeh</b>
14:50-15:05	Application of putrescine conjugated with engineered nano-carriers to lessen cadmium stress in grapevine ( <i>Vitis vinifera</i> cv. 'Sultana') <b>Gohari, Gholamreza</b>
15:10-15:30	Poster presentations
15:35-16:00	Closing and future plans



## Lectures

- [L1] Tibor Janda, Magda Pál, Mihály Dernovics, Fatemeh Gholizadeh, Gabriella Szalai  
**Polyamines in plants: facts and questions**
- [L2] Katalin Nagy, Mihály Dernovics  
**Analytical challenges in the determination of conjugated polyamines**
- [L3] Magda Pál and Tibor Janda  
**Fine-tuning of polyamine metabolism and its light-related modulation**
- [L4] Katalin Gémes, Nikolett Kaszler, Péter Benkő, Árpád Molnár, Abigél Zámori, Attila Fehér  
**Involvement of polyamines and their metabolism in the direct conversion of lateral root primordia to shoot meristem of *Arabidopsis***
- [L5] Ágnes Szepesi, Péter Pálfi, Henrietta Kovács, Márton Zoltán Köhler, Laura Zsigmond, László Bakacsy  
**L-aminoguanidine induced responses of tomato plants: when the specificity matters**
- [L6] Ewa Sobieszczuk-Nowicka, Umesh Kumar Tanwar, Ewelina Stolarska, Ewelina Paluch-Lubawa, Autar K. Mattoo, Magdalena Arasimowicz-Jelonek  
**Unraveling the genetics of polyamine metabolism in barley for senescence-related crop improvement**
- [L7] Umesh Kumar Tanwar, Ewelina Stolarska, Ewelina Paluch-Lubawa, Autar K. Mattoo, Magdalena Arasimowicz-Jelonek, Ewa Sobieszczuk-Nowicka  
**Genome-wide exploration of the genetics of biogenic polyamines in barley**
- [L8] Dilyana Doneva, Magda Pál, Liliana Brankova, Gabriella Szalai, Tibor Janda and Violeta Peeva  
**Effects of putrescine treatment on wheat genotypes subjected to drought**
- [L9] Altafur Rahman, Judit Tajti, Magda Pál  
**Influence of *phyA* mutation on polyamine metabolism in *Arabidopsis* in the presence of spermine excess under different light conditions**
- [L10] Fatemeh Gholizadeh  
**Identification of polyamine oxidase gene family in wheat (*Triticum aestivum*) and evaluation of their expression in response to exogenous polyamines and cold treatment**
- [L11] Sima Panahirad, Mohammadreza Dadpour, Rubén Alcázar, Vasileios Fotopoulos and Gholamreza Gohari  
**Application of putrescine conjugated with engineered nano-carriers to lessen cadmium stress in grapevine (*Vitis vinifera* cv. 'Sultana')**



## Poster presentations

- [P1] Péter Pálfi, Lilla Sípos, Márk Lackó, Ágnes Szepesi  
**Effect of inhibition of deoxyhypusine synthase with ciclopirox treatment on tomato plants at different developmental stages**
- [P2] Szegő, A.; Miramazloun, I.; Gyöngyik, M.; Mednyánszky, Zs. and Papp, I.  
**Gene expression and activity of polyamine- and diamine oxidases under nitrate stress**



## **Abstracts**

## **Talks**

### **Session I: *Polyamines in Growth and Development***



## Polyamines in Growth and Development

### Polyamines in plants: facts and questions

Tibor Janda\*, Magda Pál, Mihály Dernovics, Fatemeh Gholizadeh, Gabriella Szalai

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Polyamines (PAs) are a group of aliphatic amines that are found in all living cells, and that appear to be essential for life. The major PAs in plants are putrescine, spermidine, spermine and cadaverine. They occur in free, conjugated (associated with small molecules like phenolic acids) and bound forms (associated with various macromolecules). The main role of PAs was originally assumed to be as direct protective compounds, since this interaction could be important for stabilizing the structure of these macromolecules, especially under stress conditions. Recent results strongly suggest that they also play a regulatory role in various fundamental cellular processes. Besides their direct protective role, they also serve as signalling molecules (Pál et al., 2015; 2021). They are able to regulate the oxidative defence systems of cells partly as direct scavengers of reactive oxygen species and also by activating the expression of genes encoding certain antioxidant enzymes. Besides their direct or indirect role in the regulation of the redox balance, they are able to potentiate  $\text{Ca}^{2+}$  efflux, a basic component of the general stress response of cells. However, there are still a large number of open questions, which have not yet been satisfactorily answered. First of all, if PAs are fundamental for life, which of their functions are really essential, and which can be replaced by other mechanisms? Secondly, what is the specific role of the individual polyamines? What are the roles of the conjugated forms? How can the PA metabolism contribute to the whole acclimation processes? These and many other similar questions still need to be answered.

### References:

- Pál, M., Szalai, G., Janda, T. (2015) Speculation: Polyamines are important in abiotic stress signaling. *PLANT SCIENCE* 237: 16-23.
- Pál, M., Szalai, G., Gondor, O.K., Janda, T. (2021) Unfinished story of polyamines: Role of conjugation, transport and light-related regulation in the polyamine metabolism in plants. *PLANT SCIENCE* 308: 110923.

**This research was supported by the TKP2021-NKTA-06 project.**



## Polyamines in Growth and Development

### Analytical challenges in the determination of conjugated polyamines

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Polyamines (PAs) are low-molecular-weight, mostly positively charged biogenic amines and they are present in all plant cells. They can be conjugated to plant metabolites, such as hydroxycinnamic acids (caffeic, coumaric, ferulic acids, etc.) and sugars. Mapping of the polyamine cycle together with the conjugated forms has been an ongoing project with the important aim to obtain a more detailed picture of the structure and amount of conjugated polyamines through qualitative and quantitative analysis. Indeed, the list of conjugated species that are theoretically present in plant tissues might contain up to 100 molecules. To address them, UPLC-QQQ-MS/MS and UPLC-qTOF-MS techniques should be applied, as the majority of conjugated polyamines are not commercially available as analytical standards, therefore their identification is possible only through their chromatographic properties, m/z values and fragmentation patterns.

The majority of conjugated polyamines can be detected in positive ion mode, but the determination of metabolites involved in the conjugation (e.g., hydroxycinnamic acids) in negative ion mode can provide additional information. Since these latter ones render the molecules UV-detection amenable, the recorded UV chromatograms can also provide additional help for identification. Spotting of unprecedented or out-of-the-focus forms might be also facilitated by neutral loss search and mass defect based visual seeking. In our presentation, examples of all sorts of detecting methods will be presented.

This research was supported by the TKP2021-NKTA-06 project.



## Polyamines in Growth and Development

### Fine-tuning of polyamine metabolism and its light-related modulation

Magda Pál\* and Tibor Janda

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The level of polyamines (PAs) changes extremely dynamically due to the PA-cycle. In order to highlight and understand the key role of PAs in plant development and stress responses, not only the strictly speaking actual PA pool should be investigated, but other processes, which influence and regulate it need to be in focus. The relationship between PAs and photosynthesis has been demonstrated by several facts at different levels as PAs are able to influence photosynthesis and vice versa, PA biosynthesis is controlled by light (Pál et al., 2021). However, limited information is available, especially in monocot plants, on how characteristics of light influence the PA metabolism.

Our hypothesis was that different light conditions (light periods, light intensities and spectral compositions) influence the PA metabolism in different ways and modify the effect of exogenous PAs in wheat plants. Based on our results, it can be concluded that light from the beginning of the illumination, the longer lighting hour condition and the higher light intensity induced the accumulation of PAs in parallel with a decrease of their catabolism. Light quantity has higher influence on PA levels than the light quality. However, different spectral conditions resulted in characteristic gene expression pattern of the synthesis and catabolism-related genes. The fine-tuning of synthesis, back-conversion and terminal catabolism may be responsible for the observed different PA metabolism modulating strategies that result in successful adaptation to different light conditions.

### References:

- Pál M, Szalai G, Gondor OK, Janda T. (2021) *Unfinished story of polyamines: role of conjugation, transport and light-related regulation in the polyamine metabolism in plants*. Plant Sci., 308: 110923.
- Gondor OK, Tajti J, Hamow KÁ, Majláth I, Szalai G, Janda T, Pál M. (2021) *Polyamine metabolism under different light regimes in wheat*. Int J Mol Sci. 22, 11717.
- Pál, M, Hamow, KÁ, Rahman, A, Majláth, I, Tajti, J, Gondor, OK, Ahres, M, Gholizadeh, F, Szalai, G, Janda, T. (2022) *Light spectral composition modifies polyamine metabolism in young wheat plants*. Int. J. Mol. Sci. 23, 8394.

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



## Polyamines in Growth and Development

### Involvement of polyamines and their metabolism in the direct conversion of lateral root primordia to shoot meristem of Arabidopsis

Katalin Gémes<sup>1,3\*</sup>, Nikolett Kaszler<sup>1,2,3</sup>, Péter Benkő<sup>1,3</sup>, Árpád Molnár<sup>3</sup>, Abigél Zámori<sup>3</sup>, Attila Fehér<sup>1,3</sup>

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Vegetative plant propagation can be achieved by several pathways. One of them is de novo shoot meristem formation. Lateral root primordia can be directly converted to shoot meristem (SM) by the application of exogenous cytokinin. Besides cytokinin, role of polyamines (PAs) and nitric oxide (NO) have been implicated in this process. We have found that among PAs endogenous level of spermidine (Spd) enhanced during the direct formation of shoot meristem which could be due to the increased synthesis of PA synthesis genes (AtADC1, AtADC2, AtSAMDC2, AtSAMDC4, AtSPDS1 and AtSPDS2). Moreover, application of exogenous Spd improved direct regeneration efficiency. PA catabolism may also affect shoot meristem formation. We have found that expression level of Arabidopsis POLYAMINE OXIDASE 5 (AtPAO5) was higher during all phases of direct shoot meristem formation. Furthermore, in *pao5-2* mutants, which contain two fold higher thermospermine (t-spm), both AtGLB1 and AtGLB2 were downregulated, but interestingly NO level was shown to be same in mutant and wild type (WT) plants. Cytokinin sensitivity could be modulated by PAO5 through the regulation of type B cytokinin response regulators (B-ARR). The potential way how PAO5 may affect direct shoot meristem formation through GLBs mediated cytokinin sensitivity are discussed.

This work was supported by grants from National Research, Development and Innovation Fund (Grant no. FK 128997). Katalin Gémes was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (Grant no. 00580/19/8) and UNKP-21-5-SZTE-587 new national excellence program of the ministry for innovation and technology.



## Polyamines in Growth and Development

### **L-aminoguanidine induced responses of tomato plants: when the specificity matters**

Ágnes Szepesi<sup>1\*</sup>, Péter Pálfi<sup>1</sup>, Henrietta Kovács<sup>1</sup>, Márton Zoltán Köhler<sup>1</sup>, Laura Zsigmond<sup>2</sup>,  
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In order to investigate the participation of PA catabolism in stress conditions, pharmacological inhibitor application is a widely used strategy. However, sometimes the specificity of these inhibitors are questionable and their application requires more attention. L-aminoguanidine (AG) is known as a specific DAO inhibitor, but the related literature is contradictory. AG is evidenced that it could be act as potential iNOS inhibitor, or DAO inhibitor or in some cases as antioxidant. For DAO inhibition, the similar structure to putrescine could help AG to inhibit this enzyme. We studied the effect of AG in tomato plants (*Solanum lycopersicum* cv. Rio Fuego, Tigerella, and Romus) in greenhouse conditions at different developmental stages. Based on our results, we provided evidence about that inhibiting DAO enzyme by AG could induces cultivar- and age-dependent alterations of PA catabolism in tomato seedlings (Szepesi et al., 2022). Careful application of AG is needed to decipher the precise role of DAO in tomato plant development and also more investigations are needed to be done to analyze the effect of AG during stress conditions.

### **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>

### **Acknowledgements:**

The funding for our research was provided by the NKFIH grant FK129061.



## Polyamines in Growth and Development

### Unraveling the genetics of polyamine metabolism in barley for senescence-related crop improvement

Ewa Sobieszczuk-Nowicka<sup>1\*</sup>, Umesh Kumar Tanwar<sup>1</sup>, Ewelina Stolarska<sup>1</sup>, Ewelina Paluch-Lubawa<sup>1</sup>, Autar K. Mattoo<sup>2</sup>, Magdalena Arasimowicz-Jelonek<sup>3</sup>

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Leaf senescence is a highly-controlled sequence of events comprising the final stage of development. Understanding its molecular mechanism is important for the improvement of crop yield and postharvest storage. Robust polyamine (PAs) catabolism can impact the rate of senescence progression in plants (Sobieszczuk-Nowicka, 2017 and references therein). The senescence-dependent PA-mediated multidirectional metabolic crosstalks are important to understand regulation and involvement of PAs in plant death and re-mobilization of nutrients during senescence. Dark-induced leaf senescence model (DILS) has been utilized to study early and late events in barley leaf senescence (Sobieszczuk-Nowicka et al. 2018).

We identified and analyzed the expression of PAs metabolic pathway genes (HvPMG) in barley to better understand their role(s) in metabolic and genetic reprogramming during senescence in Gramineae. 21 members of the polyamine metabolic pathway gene families (PMGs) were identified and characterized. The DILS model was applied by keeping the barley plants in dark (for 3, 7 and 10 days) and gene expression pattern was analyzed for the identified *HvPMG* genes. An early transient response was observed in the expression of *HvSPDS1*, *HvSPMS1* and *HvSPMS2* genes at day-3 in dark, which further decreased as the senescence progressed. The expression of *HvCuAO3* and *HvCuAO7* was upregulated during senescence till day-7, with a decline thereafter. On the other hand, the *HvPAO7* and *HvPAO8* level was upregulated during senescence progression, being highest at day 10. We did not detect *HvPAO2* expression during dark. *HvSPDS1* and *HvSPMS1* were significantly upregulated at day-3 and day-7, comparing control versus dark at each timepoint.

Our results extend novel findings and provide valuable information about Gramineae crop senescence and its physiology vs PAs, and future prospects for genetic improvement programs associated with PAs.

**References:** Sobieszczuk-Nowicka E. **2017**. Amino Acids.49:49-56, doi.org/10.1007/s00726-016-2377-y; Sobieszczuk- Nowicka E, Wrzesiński T, Bagniewska- Zadworna A et al. **2018**. Plant Physiol. 178, 2, 654-671, doi.org/10.1104/pp.18.00516; Tanwar UK, Stolarska E, Paluch-Lubawa E et al. **2022**. Inter. J. Biol. Macromolecules, doi.org/10.21203/rs.3.rs-1665637/v1.

**Funding and acknowledgements:** This work was supported by the National Science Centre, Poland (project numbers 2018/29/B/NZ9/00734)



## Talks

### **Session II: *Polyamines in Stress Responses***



## Polyamines in Stress Responses

**Genome-wide exploration of the genetics of biogenic polyamines in barley**

Umesh Kumar Tanwar<sup>1</sup>, Ewelina Stolarska<sup>1</sup>, Ewelina Paluch-Lubawa<sup>1</sup>, Autar K. Mattoo<sup>2</sup>, Magdalena Arasimowicz-Jelonek<sup>3</sup>, Ewa Sobieszczuk-Nowicka<sup>1\*</sup>

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We explored the polyamine (PAs) metabolic pathway genes at genome-level in barley to understand their role(s) in plant development and stress adaptation in Gramineae crops. Barley (*Hordeum vulgare*), a member of the grass family (Gramineae species), is unique among crop plants with importance to agriculture and science. The bioinformatics and functional genomics tools were utilized for genome-wide identification, comprehensive gene features, comparative assessment, evolution and, developmental and stress-related expression analysis of the PA metabolic pathway gene (*HvPMG*) families. We identified three S-adenosylmethionine decarboxylases (*HvSAMDCs*), two ornithine decarboxylase (*HvODCs*), one arginine decarboxylase (*HvADC*), one spermidine synthase (*HvSPDS*), two spermine synthases (*HvSPMSs*), five copper amine oxidases (*HvCuAO*) and seven polyamine oxidases (*HvPAOs*) members of *HvPMG* family in barley. All the identified genes were distributed on all seven chromosomes of barley except for *HvCuAO3* with ChrUn. Gene structure analysis revealed that four genes *HvODC1-2*, *HvPAO5*, *HvSPDS1* and *HvSAMDC2* were intron-less. Gene duplication analysis showed two tandemly and six segmentally duplicated genes with estimated average divergence time of 82.490 MYA and 51.48 MYA, respectively. Phylogenetic analysis with other plant species revealed that PA metabolic pathway is highly conserved in plants. The analysis for potential regulatory mechanisms controlling the gene expression resulted in the prediction of nine *H. vulgare* miRNAs target sites in the coding sequences, and 961 putative *cis*-acting elements (CREs) in the promoter region of *HvPMGs*. Furthermore, *HvPMG* genes significantly respond to various stress conditions including heat, cold, salt, osmotic and drought conditions. This novel study is the first to systematically and comprehensively analyze the PAs metabolic pathway gene families in barley.

This work was supported by the National Science Centre, Poland (project number 2018/29/B/NZ9/00734).



## Polyamines in Stress Responses

### Effects of putrescine treatment on wheat genotypes subjected to drought

Dilyana Doneva<sup>1</sup>, Magda Pál<sup>2</sup>, Liliana Brankova<sup>1</sup>, Gabriella Szalai<sup>2</sup>, Tibor Janda<sup>2</sup> and Violeta Peeva<sup>1\*</sup>

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A broader understanding of the role of the polyamine pool fine regulation and the alterations of polyamine-related physiological processes could be obtained by comparing the stress effects in different genotypes. In this study, the impact of pretreatment with putrescine in response to drought stress was investigated in the highly adaptable to unfavorable growth conditions Katya and more sensitive Zora wheat (*Triticum aestivum*) varieties. Photosynthetic performance, *in vivo* thermoluminescence emission from leaves, polyamine and salicylic acid levels, contents of osmoprotectants, and activities of antioxidant enzymes in the leaves were investigated not only to reveal differences in the physiological processes associated to drought tolerance, but to highlight the modulating strategies of polyamine metabolism between wheat genotypes with different drought tolerance. Results showed that the resistance of Katya under osmotic stress conditions was characterized by higher photosynthetic ability, stable charge separation reactions in photosystem II, higher proline accumulation and antioxidant activity. Katya variety exhibited higher constitutive levels of the signaling molecules putrescine and salicylic acid compared to the sensitive Zora. However, responses to exogenous putrescine were more advantageous for the sensitive variety under PEG treatment, which may be in relation with the decreased catabolism of polyamines, suggesting the increased need for polyamine under stress conditions.

**Acknowledgements:** This work was supported by Bulgarian-Hungarian bilateral program.



## Polyamines in Stress Responses

### **Influence of phyA mutation on polyamine metabolism in *Arabidopsis* in the presence of spermine excess under different light conditions**

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The unique photoreceptor PhyA coordinates the high-irradiance response of seedlings to far-red light through a complex network of metabolite and gene expression fine-tuning mechanisms. Although there are a few findings on the alterations in polyamine (PA) metabolism in *phyA* mutant *Arabidopsis* plants, the focus of these studies was on the modifiable effect of the far-red ratio on specific components of PA metabolism. The most comprehensive study on the link between *phyA* mutation and PA metabolism demonstrated the participation of *phyA* in putrescine biosynthesis and its role in the control of *S-adenosylmethionine decarboxylases* 2 and 4 in response to far-red light. (Jumtee et al., 2008). In order to determine the effect of the *phyA* mutation in PA synthesis and catabolism, the present study aims to compare the PA metabolism of *phyA* mutant *Arabidopsis* plants to those of the wild type, Col-0, under different light spectral compositions and with or without exogenous spermine (SPM) application. As several data revealed that phys have a role in particular blue-light-mediated responses (Chun et al., 2001), not only was the effect of far-red dominating spectra compared to white light, but also the effect of supplemental blue light was explored. Our findings indicate that the *phyA* mutation has some effect on PA metabolism, particularly in the context of an excess of SPM, which may be modestly changed by spectral compositions. However, the maintenance of a stable PA pool is essential for normal plant development. To attain this, gene expression level fine-tuning of the synthesis and catabolism processes is required.

## References

- Jumtee K, Bamba T, Okazawa A, Fukusaki E, Kobayashi A, (2008) *Integrated metabolite and gene expression profiling revealing phytochrome A regulation of polyamine biosynthesis of Arabidopsis thaliana*, J. Exp. Bot. 59 1187–1200.
- Chun L, Kawakami A, Christopher DA. (2001) *Phytochrome A mediates blue light and UV-A-dependent chloroplast gene transcription in green leaves*. Plant Physiol. 125: 1957-66.

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



## Polyamines in Stress Responses

### Identification of polyamine oxidase gene family in wheat (*Triticum aestivum*) and evaluation of their expression in response to exogenous polyamines and cold treatment

Fatemeh Gholizadeh

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Polyamines (PAs), are low-molecular weight compounds found in all living organisms. They are involved in plant in response to abiotic stresses including cold stress (1). Low temperature is one of the most common abiotic stresses experienced by plants and negatively affects plant growth, development, and survival (1). In this study, we identified 30 *PAO* genes in bread wheat (*Triticum aestivum*), and their protein characteristics, evolutionary relationships, gene structure and expression profiles of the wheat *PAO* genes (*TaPAO*) were investigated using bioinformatics tools. Domain analysis of proteins showed that most *TaPAO* members contained an amino oxidase catalytic domain, and there were six conserved motifs in wheat *TaPAO* proteins. Based on the phylogenetic study, *TaPAO* genes were classified into six groups, in general, these genes did not show the same distribution in subgenomes A, B and D. Expression analysis using publicly available RNASeq data showed that a large percentage of *TaPAOs* respond significantly to abiotic stresses, especially temperature (i.e. heat and cold stress). Also, we investigated proline content, antioxidant enzymes (catalase and ascorbate peroxidase) activity and gene expression response of PA-treated wheat (*Triticum aestivum* L.) varieties Mihan (cold tolerant winter variety) and Rakhshan (cold sensitive spring variety) under cold conditions. Seedlings were foliarly sprayed with 10 mg L<sup>-1</sup> spermidine (Spd), 10 mg L<sup>-1</sup> putrescine (Put) and distilled water as a control followed by the exposure of the plants to seven days of low temperature at 4 °C. Under cold conditions, exogenous PA application increased free proline content and antioxidant enzymes activity in wheat, especially in the cold-tolerant cultivar Mihan. The results of this study showed, cold stress increased not only the expression levels of *TaADC* but also *TaSAMDC*, *TaSPDS*, *TaPAO11-7B* and *TaPAO11-7D* compared to control. Overall, *TaPAOs* may have various functions in stress tolerances responses, and play vital roles in different tissues and developmental stages. Our results provided a reference for further functional investigation of *TaPAO* proteins.

### Reference

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## Polyamines in Stress Responses

### Application of putrescine conjugated with engineered nano-carriers to lessen cadmium stress in grapevine (*Vitis vinifera* cv. 'Sultana')

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Abiotic environmental stress factors like heavy metal toxicity impact plant performance and yield but also the environment and human health. Putrescine (Put) is a metal chelator with important functions in combating heavy metal toxicity [1]. Nowadays, nanotechnology provide a smart delivery system for phytohormones by using different nano-carriers such as chitosan (CTS) and carbon quantum dots (CQDs) nanoparticles (NPs) [2,3]. The potential protective roles of CTS-Put and CQD-Put NPs against Cd stress were investigated in *Vitis vinifera* cv. Sultana by assaying an array of agronomic, physiological and biochemical traits. As a first experiment, CTS-Put NPs application significantly enhanced photosynthetic pigments, chlorophyll fluorescence parameters, proline, phenols, anthocyanins antioxidant enzymatic activities and decreased leaf and root Cd content, EL, MDA and H<sub>2</sub>O<sub>2</sub> under Cd-stress condition. Furthermore, in second experiment the affirmative effects of CQD-Put NPs were manifested as increases in leaf FW and DW, photosynthetic pigments,  $F_v/F_m$ , Y (II), phenols, proline, antioxidant enzymatic activities and PA content, while decreasing Cd content in leaf and root, Y (NO), malondialdehyde (MDA), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and electrolyte leakage (EL). Overall, current findings suggest that CTS-Put and CQDs-Put NPs might be considered as a remarkable and an innovative 'green' approach with stress protecting properties in plant production, focusing on the alleviation of climate change-related abiotic stress conditions.

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# Poster Abstracts

## Polyamines in Growth and Development

### **Effect of inhibition of deoxyhypusine synthase with ciclopirox treatment on tomato plants at different developmental stages**

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The translation factor eIF5A is essential in eukaryotes, can only perform its function after activation by posttranslational modification, so called hypusination. Hypusination, which according to our current knowledge occurs only on the specific lysine side chain of eIF5A isoforms, is a two-step enzymatic biosynthesis of the amino acid hypusine. In the first step, deoxyhypusine synthase (DHS; EC 2.5.1.46) converts the specific lysine side chain to deoxyhypusine using polyamine spermidine (Spd), and in the second step, deoxyhypusine hydroxylase (DOHH; EC 1.14.99.29) forms hypusine by hydroxylation of deoxyhypusine (reviewed in Pálfi et al. 2021, Plants).

In order to investigate the significance of hypusination it is possible to inhibit the process of hypusine formation by treatment with pharmacological inhibitors of enzymes that are involved in its biosynthesis. DOHH can be inhibited by chelating the non-heme iron which is necessary its function. For this purpose, ciclopirox (CPX) is often used, but in plants this study is the first to investigate its effect.

CPX was applied to tomato plants (*Solanum lycopersicum* cv. Rio Fuego) at early state of development and mature plants in different concentrations. In a concentration-dependent manner, CPX had a negative effect on the development of seedlings, and toxic effects were observed in mature plants after the second day of treatment.

Our results suggest that DOHH is necessary for the normal development of tomato plants. Further research is needed to investigate the effects of DOHH, and through it hypusination on plants.

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Pálfi P, Bakacsy L, Kovács H, Szepesi Á. Hypusination, a Metabolic Posttranslational Modification of eIF5A in Plants during Development and Environmental Stress Responses. *Plants*. 2021; 10(7):1261. <https://doi.org/10.3390/plants10071261>

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## Polyamines in Stress Responses

### Gene expression and activity of polyamine- and diamine oxidases under nitrate stress

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Polyamines (PAs) play an important role in biotic and abiotic plant stress responses. The most common polyamines in plants are putrescine, spermidine and spermine. The PA level in cells is regulated by biosynthesis, degradation, and transport. Diamine- (DAOs) and polyamine oxidases (PAOs) are involved in the degradation of PA. In the work presented here we set out to investigate whether amounts of spermine and spermidine as well as regulation of amine oxidases may display any characteristic pattern corresponding to enhanced nitrate supplementation (4 mM, 20 mM and 50 mM) in ‘Joker’ and ‘Oitol’ cucumber hybrids. In the experiments the  $\text{NO}_3^-$  level and the NR activity of the cucumber leaves were increased when nutrient solutions with higher nitrate content were applied. We assumed that cucumber plants supplied with more  $\text{NO}_3^-$  as a source of N would produce more NO via nitrite:NO reductase activity of nitrate reductases. Our result show that in cucumber plants supplied with increasing concentration of nitrate spermine level of the leaves is increased, which is associated with a decreasing spermidine amount. Nitric oxide (NO) is a signalling molecule with a connection to PA metabolism. Enhanced intracellular spermine accumulation was observed in sunflower seedling under salt stress, in response to NO donor application. NO positively regulates intracellular PA homeostasis by increasing the abundance of PA biosynthetic enzymes like arginine decarboxylase (Tailor et al, 2019). In parallel to the increasing nitrate nutrition descending arginine level was observed in ‘Oitol’ leaves. Moreover, total PAO and DAO activities of ‘Joker’ leaves declined under increasing nitrate content of fertigation solution, although gene expression of many PAO genes was upregulated. It can be assumed that the higher nitric oxide level formed due to the increasing NR activity may have had an effect on the activity of PAO and DAO enzymes in negative correlation.

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Tailor, A., Tandon, R., & Bhatla, S. C. (2019). Nitric oxide modulates polyamine homeostasis in sunflower seedling cotyledons under salt stress. *Plant signaling & behavior*, 14(11), 1667730.



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