Role of polyamines in connection with their relation with plant hormones
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Investigations on compounds capable of reducing the stress sensitivity of plants are of great importance in the ever changing environment. Polyamines (PAs) could be promising compounds for the reduction of abiotic stress sensitivity in plants. However, as PA metabolism and signalling are involved in direct interactions with other metabolic routes and hormonal cross-talks, the precise mechanism how polyamines control plant responses are largely unknown. The main aims of the present proposal to reveal what relationships exist between PA metabolism and plant hormones, such as abscisic acid, salicylic acid and gibberellins and/or other protective compounds under control and/or stress condition. In order to achieve our goals salicylic acid (SA) induction-deficient *Arabidopsis* mutants and gibberellin acid (GA)-insensitive dwarf wheat genotypes has been also used in the experiments.

The plant hormone abscisic acid (ABA) regulates several physiological processes and may also induce tolerance to various abiotic stresses The involvement of ABA in drought stress tolerance has been studied extensively at physiological and molecular levels, and a sharp increase in its endogenous level was also detected during water deficit conditions ABA plays a central role for improvement of plant drought resistance not only by its effect on stomatal closure but also by inducing activities or gene expression of antioxidant enzymes and the synthesis of other protective compounds, such as proline. ABA also increases the gene expression level of the PA biosynthesis genes. On the other hand, overexpression of the genes of PA synthesis enzymes resulted in increased ABA biosynthesis due to the higher expression level of 9-cis-epoxycarotenoid dioxygenase (NCED). These results suggest that there is a positive feedback loop between ABA and PAs. It should be also taken into consideration, that there is connection between PA and proline metabolisms, as their synthesis shares a common precursor, glutamate; furthermore, the putrescine (PUT) degradation has also been implicated in proline accumulation, but the precise role of PA catabolism in proline production remains elusive.

According to the relationships described above, understanding the regulation of PA metabolism in plants is of major interest. However, the exact relationship between PA, ABA and proline metabolism is still poorly understood. The main aim of the present experiment was to find answers to the following questions: 1. How does ABA treatment influence the PA metabolism, and vice versa: how do PAs influence the ABA level in wheat? 2. Do specific steps in the PA metabolism respond differently under control or mild osmotic stress conditions? 3. What relationship exists between PAs and proline content and synthesis? In order to achieve our goals in the first part of the experiments the effects of PUT and ABA treatments either alone or in combination with polyethylene glycol-induced osmotic stress were investigated in young wheat plants. It was observed that ABA plays a role in the coordinated regulation of the proline and PA biosynthetic pathways, which compounds are related to each other through a common precursor. ABA pre-treatment induced similar alteration in the pattern of PA contents as it was found after osmotic stress, namely increased the PUT, but decreased the spermidine (SPD) contents in the leaves. These changes were mainly related to the PA cycle, as both the synthesis and peroxisomal oxidation of PAs have been induced at gene expression level. Although ABA and osmotic stress influenced the proline metabolism differently, the highest proline accumulation was observed in the case of ABA treatments. It was also revealed that proline metabolism partly regulated independently and not in an antagonistic manner from PA synthesis. Results suggest that the connection, which exists between PA metabolism and ABA signalling leads to the controlled regulation and maintenance of PA and proline levels under osmotic stress conditions in wheat seedlings (results have been published: Pál et al., (2018)

Interaction of polyamines, abscisic acid and proline under osmotic stress in the leaves of wheat plants. *Scientific Reports*, volume 8, Article number: 12839, **IF: 4.011**).

Reduced height (*Rht*) genes in wheat are often used in breeding programs to increase harvest index. Some of these genes are encoding DELLA proteins playing role in gibberellic acid (GA) signalling. The aim of the next experiment was to reveal the interaction between the mode of action of PAs and GAs, in other words how the mutations in *Rht* gene modify the PA-regulated processes in wheat. The main question in focus, how PA treatments influence 1. plant growth, physiological status and endogenous PA metabolism; 2. the level of selected plant hormones in GA-insensitive wheat lines compared to the wild type. Wild type and two Rht mutant genotypes (Rht 1: semi-dwarf; Rht 3: dwarf mutants) were treated with PAs, PUT, SPD and spermine (SPM). PA treatments differently influenced the PA metabolism, the plant growth parameters and certain hormone levels (SA and ABA) in these genotypes. The observed distinct metabolism of Rht 3 may more likely reflect more intensive PA exodus from PUT to SPD and SPM, and the catabolism of the higher PAs. The lower root to shoot translocation of PUT can contribute to the regulation of PA pool, which in turn may be responsible for the observed lack of growth inhibition in Rht 3 after SPD and SPM treatments. Lower accumulation of SA and ABA, plant hormones usually linked with growth inhibition, in leaves may also be responsible for the diminished negative effect of higher PAs on the shoot growth parameters observed in Rht 3. These results provide an insight into the role of PAs in plant growth regulation based on the investigation of GA-insensitive Rht mutants (results have been published: Pál et al., (2019) **Role of polyamines in plant growth regulation of Rht wheat mutants.** *Plant Physiology and Biochemistry* 137: 189-202. **IF: 3.404**).

After investigations on the effect of exogenous PAs treatments, in the GA-insensitive dwarf (Rht3) compared to the tall, wild type (WT), we focused on the role of PA metabolism in these genotypes under heavy metal and PEG-induced osmotic stress.

Wheat lines carrying *Rht* genes are important in plant breeding due to their both higher yield capacity and better tolerance to certain environmental stresses. However, the effects of dwarf-inducing genes on stress acclimation mechanisms are still poorly understood. When Cd enters the cytosol, synthesis of a number of metal-binding compounds, e.g. phytochelatins (PCs), proline and polyamines (PA) is activated in plants. Our results characterized the response of WT and Rht 3 plants to 7-day Cd treatment (50 μM). The data of lipid peroxidation indicated that Rht 3 line with repressed GA signalling could better tolerate high Cd level. In order to further reveal the background of the different levels of Cd tolerance of the wild-type and mutant line at molecular level, analysis of PA metabolism was also performed. As the synthesis of PAs is related not only to proline (glutamate is the common precursor of PUT as well as proline), but in the case of higher PAs (SPD and SPM) also with phytochelatins (PCs) (cysteine is precursor for the synthesis of glutathione and also for the synthesis of S-adenosyl methionine, which is necessary for the formation of SPD and SPM from PUT) (PC synthesis has been demonstrated as a major metal detoxification mechanism in plants), there seems to be an important metabolic relationship between these protective compounds. Thus, the changes in the proline, PA, PC, ABA and SA synthesis were evaluated in order to highlight the differences between the WT and the more Cd tolerant dwarf genotypes under Cd stress conditions. Despite the numerous studies demonstrating the protective role of SA against Cd stress, indicating that ABA is required in Cd signal transduction only a few experiments have been focused on their effect on PC synthesis.

Our results revealed that the altered expression level of Δ^1-pyrroline-5-carboxylate synthetase (P5CS) and not that of the ornithine aminotransferase (OAT) was responsible for the

Cd-induced differences in the root proline content between the wild and mutant genotypes. However, changes in the proline contents could not explain the better tolerance of Rht 3 to Cd. Cd also induced formation of PCs in the roots of the tested genotypes, with the highest accumulation of PC4, which was much higher in the mutant. However, differences in the *in vitro* PCS activity or PCS gene expression could not be detected. The terminal catabolism of higher PAs was induced in the WT, while the interconversion of SPM to SPD and decreased terminal catabolism were responsible for the increment of leaf SPD content in the Rht 3. In roots, the SPD content increased both in the wild-type and mutant plants, in accordance with the increased PUT level, which indicated that PA synthesis was induced after Cd stress. However, the fact that the initial leaf SPD content in the WT was double than that in the Rht 3 plants may be in relation with the lower inducibility of the PC synthesis in the roots under Cd stress, indicating antagonistic relationship between PCs and higher PAs.

Our results also showed that, after Cd treatment the ABA synthesis increased in the leaves of the wild type, which in turn might induce the proline and PA metabolisms in the roots. However, in the mutant line the slight increment in the leaf ABA content companied with the relatively high SA accumulation was not efficient to induce so high proline and PUT accumulation. Although changes in proline and PA, especially PUT, showed similar pattern, the accumulation of these compounds showed antagonistic relationship with the capacity of PCs synthesis in the roots of the wild type after cadmium stress. In dwarf genotype, a favourable metabolic shift from the synthesis of PA and proline to PC production was responsible for the observed higher cadmium tolerance. (results have been published: Szalai et al., (2020) **Molecular background of cadmium tolerance in Rht dwarf wheat mutant is related to a metabolic shift from proline and polyamine to phytochelatin synthesis.** *Environmental Science and Pollution Research,* Paper: 08661, 13 p. **IF: 2,914**)

Impact of putrescine pre-treatment on osmotic stress-induced responses in Rht lines of wheat has been also investigated. Although no differences were observed in the net photosynthesis, lower leaf MDA level was detected after PEG treatment in Rht 3 than in the wild type, supporting its higher drought tolerance compared to the wild type. PUT pre-treatment alone did not induce changes in the photosynthesis parameters, the lipid peroxidation (MDA content) and proline contents or the antioxidant enzyme activities after 5 days in either genotypes. However, despite of the lower root MDA content, and higher leaf proline accumulation, results also showed that the PUT pre-treatment under osmotic stress conditions could not mitigate the negative effects of PEG on the photosynthetic process. Moreover, pre-treatment with PUT had some negative effect in case of the WT, based on net photosynthesis parameter compared to the PEG-treated one. Nevertheless, despite the similar net photosynthesis rate in the two lines, higher stomatal conductance and intracellular CO_2 concentration parameters could be detected in the PUT+PEG-treated dwarf line. This maybe resulted from the induced metabolic shift, in order to facilitate metabolic reprogramming to induce stress tolerance in Rht 3. Interestingly, the level of proline accumulation was different in the leaves of both lines, but within each genotypes it was the highest where the highest leaf PUT accumulation was observed, viz. in the case of PUT+PEG treatment. However, the pattern of the proline accumulation in the leaves showed reversed pattern with the root PUT levels in PEG- or PUT+PEG-treated plants The lack of an increase in PUT level together with a decrease in SPD content, but increase in SPM amount in the leaves may be resulted from, that PUT was used for the synthesis of SPM during osmotic stress, and the catabolism of the higher PAs may be resulted in the 1,3-diaminopropane accumulation. Nevertheless, excess of PUT may also induce oxidative stress in plants, due to the fact that both the catabolism or back-conversion of polyamines produce H_2O_2 in the apoplast or peroxisomes. The changes in PA pool may be responsible for the partly negative effect of pre-treatment with PUT in the wild type under osmotic stress conditions, which was confirmed by the more intensive gas stomatal closure. The remarkable difference in the PA pool - as in the roots the lowest PUT content was found in the roots of the dwarf mutant - resulted in the lowest PUT/(SPD+SPM) ratio of the Rht 3 compared to WT after PEG treatment either alone or in combination with PUT. Nevertheless, plant responses to excess of PAs can be more advantageous for dwarf line, as the fine balance in the PA pool may responsible for a shuttle between the beneficial and deleterious effects of PAs, which may be responsible for the differences induced in the Rht lines (manuscript has been submitted to *Növénytermelés*: Pál et al., **Putreszcin által indukált válaszreakciók különbségei különböző Rht búzavonalakban ozmotikusstressz-körülmények között**. under review).

The impact of pre-treatment with PUT in response to osmotic stress was also investigated in the drought tolerant Katya and drought-sensitive Zora wheat cultivars. Photosynthetic performance, *in vivo* thermoluminescence (TL) emission from leaves, leaf temperature, PA and SA 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 PA metabolism between a drought-tolerant and a drought-sensitive wheat genotype. Results showed that the tolerance of Katya under osmotic stress conditions was characterized by higher photosynthetic ability, stable charge separation across the thylakoid membrane in photosystem II, higher proline accumulation and antioxidant activity. TL also revealed differences between the two varieties − a downshift of the B band and an increase of the afterglow band under osmotic stress in Zora, providing original complementary information to leaf photosynthesis. Katya variety exhibited higher constitutive levels of the signalling molecules PUT and SA compared to the sensitive Zora. However, responses to exogenous PUT were more advantageous for the sensitive variety under PEG treatment, which may be in relation with the decreased catabolism of PAs, suggesting the increased need for PA under stress conditions. Although PSII is generally considered as a “drought-tolerant” complex, TL measurements revealed different stress-induced responses in dependence of drought tolerance level to water deficit and to treatment with PUT in the two genotypes. Until now this is the first study where the potential effect of PUT pre-treatment on leaf photosynthesis has been studied by *in vivo* leaf chlorophyll TL technique (results have been accepted for publication: Dilyana Doneva, **Magda Pál**, Liliana Brankova, Gabriella Szalai, Judit Tajti, Radwan Khalil, Beti Ivanovska, Violeta Velikova, Svetlana Misheva, Tibor Janda , and Violeta Peeva (2020) **The effects of putrescine pre-treatment on osmotic stress responses in drought-tolerant and drought-sensitive wheat seedlings.** *Physiologia Plantarum* IN PRESS, IF: 3,0).

PA treatments have been also performed on *Arabidopsis* mutants (*sid2*: plastid-localised SA-synthesis *Arabidopsis* mutant; *eds5*: chloroplasts SA-transport deficient *Arabidopsis* mutant) compared to the wild type under normal plant growth conditions.

The main aim of this experiment was to reveal how exogenous PAs influence the SA synthesis, with a special emphasis on the effect of the SA deficiency on the PA metabolism and PA-induced changes in other plant hormone contents. Our hypothesis was that the individual PAs induced different changes in the PA and SA metabolism of the wild type and SA-deficient *Arabidopsis* mutants, which in turn influenced other hormones. To our knowledge, such a side-by-side comparison of the influence of *eds5-1* and *sid2-2* mutations on PAs has not been reported yet. To achieve our goals, wild and mutant genotypes were tested after PUT, SPD or SPM treatments. PA and plant hormone metabolism was investigated at metabolite and gene expression levels. Individual PAs induced different changes in the *Arabidopsis* plants, and the responses were also genotype-dependent. Exogenous PAs upregulated the PA synthesis and catabolism, and remarkable changes in hormone synthesis were found especially after SPD or SPM treatments. The *sid2-2* mutant showed pronounced differences compared to Col-0. Interactions between plant hormones may also be responsible for the observed differences (these results have been published: Tajti et al., (2019) **Polyamine-induced hormonal changes in eds5 and sid2 mutant Arabidopsis plants.** *International Journal of Molecular Sciences* 20(22). pii: E5746 **IF: 4,183**).

In summary, exogenously applied PAs treatments were used under normal growth and stress conditions. It has been found that PAs are generally considered to be a family of similar molecules, but that different PAs may have different or even opposite effects, and their action mechanism depends on the plant species/genotypes used. The potential benefit also varies depending on the type of treatment and the stress factor. In our research, we have demonstrated the key role of the PA-cycle in PA metabolism, which is dynamic, but the intensity of each step of the cycle varies depending on plant species, organs, and stress conditions. We have shown that the protective effect of PAs can be realized at the molecular level on the one hand, but in a complex way due to their relationship with the synthesis of other compounds (e.g. proline and phytochelatins). On the other hand, the role of PAs in signal transduction in combination with plant hormones (such SA and ABA), and their effect at the gene expression level is also becoming more and more clear. Fine-tuning of the PA pool through the PA cycle may be the key to balancing the pros and cons and may be responsible for the differences in different plant species/genotypes.

As a side branch of the present research, we investigated the Cd-induced SA signalling in wheat. The results have been published, the NKFIH KH124472 has been indicated in the acknowledgements. Tajti et al., (2019): **Pattern of changes in salicylic acid-induced protein kinase (*SIPK*) gene expression and salicylic acid accumulation in wheat under cadmium exposure**, *Plant Biology*, 21 (2019) 1176–1180 **IF: 2,156**).

Briefly, SIPK (salicylic acid-induced protein kinase) is known as a “master switch” for stress responses in plants. It can be induced by SA and several stress factors. The main aim of the present study was to reveal relationship between salicylic acid accumulation and the gene expression level of *SIPK* during 50 and 250 µM Cd stress in wheat plants. Quantitative real-time PCR reaction was used for the determination of the gene expression level of *SIPK*. SA content measurement was performed by HPLC system equipped with fluorescence detector. Cd treatment increased the endogenous SA level and the expression level of *SIPK* in a concentration dependent manner. Induction of *SIPK* expression preceded the accumulation of endogenous salicylic acid. Although SA treatment induced dramatic endogenous SA accumulation, its SIPK-inducing effect was moderate. In the roots, higher induction of SIPK was observed than in the leaves. The same tendency of the *SIPK* expression was observed in both Cd- or salicylic acid-treated plants, as decisively the highest transcript level was detected after 30 minutes of the treatments, but afterwards, the expression decreased rapidly to the control levels or even below. The induction of *SIPK* was transient in all cases, and even a very high SA level in either the leaves or roots was not able to maintain the elevated expression level of this gene. The results suggested that SIPK has a role in the initiating of cadmium stress and exogenous SA-induced signalling process.

Cumulative IF of the two and half year-project: 22.141