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Gabriella Szalai, Katalin Janda, Éva Darkó, Tibor Janda, Violeta Peeva, Magda Pál

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Comparative analysis of polyamine metabolism in wheat and maize plants

Gabriella Szalai[1], Katalin Janda[1], Éva Darkó[1], Tibor Janda[1], Violeta Peeva[2] and Magda Pál[1]

[1]Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Hungary 2462 Martonvásár, POB 19.

[2] Department of Photosynthesis, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Bulgaria 1113 Sofia, Acad. G. Bonchev Street, Bldg. 21

Corresponding author: Magda PÁL

pal.magda@agrar.mta.hu

Tel: +36-22-569-502

Fax: +36-22-569-576

Abstract

In the present work changes in polyamine contents were investigated after various hydroponic polyamine treatments (putrescine, spermidine and spermine at 0.1, 0.3 and 0.5 mM concentrations) in two different crop species, wheat and maize. In contrast to putrescine, higher polyamines (spermidine and spermine) induced concentration-dependent oxidative damage in both crops, resulting in decreased biomass. The unfavourable effects of polyamines were more pronounced in the roots, and maize was more sensitive than wheat. The adverse effects of polyamine treatment were proportional to the accumulation of polyamine and the plant hormone salicylic acid in the leaves and roots of both plant species. Changes in polyamine content and catabolism during osmotic stress conditions were also studied after beneficial pre-treatment with putrescine. The greater positive effect of putrescine in wheat than in maize can be explained by differences in the polyamine metabolism under normal and osmotic stress conditions, and by relationship between polyamines and salicylic acid. The results demonstrated that changes in the polyamine pool are important for fine tuning of polyamine signalling, which influences the hormonal balance required if putrescine is to exert a protective effect under stress conditions.

Keywords: osmotic stress; maize; polyamines; salicylic acid; wheat

Abbreviations: Ci: intracellular CO_2 concentration; DAO: diamine oxidase; DAP: 1,3-diaminopropane; $\Delta F/F_m$': effective quantum efficiency of photosystem II; Fv/Fm: optimal quantum efficiency of photosystem II; E: transpiration; g_s: stomatal conductance; PAL: phenylalanine ammonia lyase; PAO polyamine oxidase; PA: polyamine; P_n: CO_2 assimilation rate ; PUT: putrescine; SA: salicylic acid; SPD: spermidine; SPM: spermine.

1. Introduction

Polyamines (PAs) occur in free, conjugated or bound forms. Putrescine (PUT) is synthesized by the decarboxylation of ornithine or indirectly by the decarboxylation of arginine, while higher polyamines (spermidine: SPD and spermine: SPM) are produced by the sequential addition of aminopropyl moieties to the PUT skeleton through enzymatic reactions. PAs are catabolized by diamine oxidase (DAO) and polyamine oxidases (PAOs). Besides the apoplastic PAOs, which catalyse the terminal catabolism of SPD or SPM, other peroxisomal PAO enzymes are involved in the partial and/or full back-conversion of SPM to SPD and of SPD to PUT. The polyamine pool is thus dynamic, changing over time, and PAs undergo rapid interconversion in the "polyamine cycle" (Pál et al., 2015). The activation of PA biosynthesis and their direct protective role under stress conditions have been extensively reported in several plant species (recently reviewed by Minocha et al., 2014). Many reports have indicated that the stress tolerance of plants is correlated with their capacity to enhance the synthesis of polyamines upon exposure to stress, and several elegant studies have shown that the overexpression of PA biosynthetic genes is an effective strategy to elevate the endogenous PA pool and to modify stress tolerance (Liu et al., 2015). However, the accumulation of PUT under stress conditions, leading to a high PUT/(SPD+SPM) ratio may even result in plant injury under stress conditions (Shu et al., 2012). PAs have double-edged roles: as free radical scavenger in the nucleus, and as sources of free radicals in the apoplast (Takahashi and Kakehi, 2010). Besides their direct protective role as antioxidants, they also regulate various fundamental cellular processes as signalling molecules (Pál et al., 2015). There is accumulating evidence that the PA pool undergoes extensive changes in response to a range of abiotic stresses (Do et al., 2013; Liu et al., 2015; Shu et al., 2015), but these studies have mainly shown the positive effects of PAs on stress resistance, without discussing the

important influence of exogenous PAs on the PA pool or the fact that a proper balance in PA pool may be responsible for the positive effect observed.

Salicylic acid (SA) has also been shown to participate in the signalling of various stresses in plants (Pál et al., 2013b; Janda et al., 2014). It has been suggested that SA treatment influences the polyamine content (Németh et al., 2002; El-Khawas, 2012) and catabolism (Szepesi et al., 2011), and it is evident that different concentrations of SA had different effects on the PA metabolism (Wang et al., 2012). Similarly, pre-treating seed with SPD or SPM was reported to enhance the SA content in wheat under salt stress (Iqbal et al., 2006). In a few studies, parallel changes in endogenous SA and PA contents were also described under stress conditions (Iqbal and Ashraf, 2006; Majláth et al., 2011; Pál et al., 2013a). Based on these results a relationship is thought to exist between endogenous SA and PA contents.

The general aim of the present study was to help in understanding the importance of the PA cycle in stress acclimation processes, particularly regarding the different effects and roles of individual exogenous and endogenous PAs. To achieve this goal, treatments with different PA compounds at various concentrations were compared in wheat and maize plants without stress conditions, after which beneficial pre-treatment with PUT was applied before osmotic stress to highlight differences between the two plant species under stress conditions. Furthermore, since the relationship between the PA and SA signalling pathways is poorly understood, the effects of PA treatments and changes in PA contents on SA levels were also studied.

2. Materials and methods

2.1. Plant material and growth conditions

Wheat (*Triticum aestivum* L. TC33) (Thatcher-based near-isogenic line, TC33: Thatcher*6/P.I.58548) and maize (*Zea mays* L. Mv255) plants were used in the experiments. After 3 days of germination between moistened filter papers at 20°C for wheat and 22°C for maize in the dark, seedlings were grown in modified Hoagland solution (Pál et al., 2005) at 20/18°C for wheat and 22/20°C for maize with 16/8-h light/dark periodicity and 75% relative humidity in a Conviron GB-48 plant growth chamber (Controlled Environments Ltd, Winnipeg, Canada). Plastic containers were planted with 12 wheat seedlings or 6 maize seedlings and placed in the growth chamber in a fully randomized manner. The photosynthetic photon flux density (PPFD) was 250 μmol m^{-2} s^{-1}. The plant growth solution was changed every two days.

In the first experiment, after 7 days of growth in Hoagland solution the wheat and maize plants were treated hydroponically with 0.1, 0.3 or 0.5 mM of putrescine (PUT-treated), spermidine (SPD-treated) or spermine (SPM-treated), and these treatments were repeated every 2nd day using fresh hydroponic solution containing the same concentration of the relevant polyamine. The growth solution of the control plants (without PA treatment) was also changed every 2nd day. After 7 days of treatment the roots and youngest fully developed leaves were sampled for analysis (Suppl. Fig. 1A).

Based on the results of the first experiment, a concentration of 0.5 mM PUT was chosen as the pre-treatment in further experiments. After 7 days of growth in Hoagland solution, the wheat and maize seedlings were either grown further under control growth conditions in hydroponic solution or treated with 0.5 mM PUT hydroponically for 7 days. After this, the roots were washed in distilled water, then half of each group was grown further under control conditions; in the case of PUT-pretreated plants this functioned as a recovery period. The other half of each group was treated with 15% PEG-6000, for 5 days in the case

of wheat and for 2 days in the case of maize (control+PEG or PUT-pretreated+PEG). The duration of the PEG treatment was established based on previous results (Németh et al., 2002; Kovács et al., 2014). Samples were collected as described above at the end of the PEG treatment for both stressed and non-stressed plants (Suppl. Fig. 1B).

2.2. Chlorophyll-a fluorescence induction measurements

Chlorophyll-*a* fluorescence was measured using a pulse amplitude modulated fluorometer (Imaging-PAM M-Series fluorometer; Walz, Effeltrich, Germany). The maximum quantum yield of PSII photochemistry, F_v/F_m, as $F_v/F_m = (F_m-F_0)/F_m$, where F_m is the maximal fluorescence induced by a saturating flash (8000 μmol m^{-2} s^{-1} PPFD for 0.8 s) in leaves dark-adapted for 20 min, and F_0 is the minimum chlorophyll fluorescence yield in the dark (PPFD < 1 μmol m^{-2} s^{-1}). (As no significant changes were observed in the F_v/F_m parameter, these data are not shown.) The effective PSII quantum yield (ΔF/Fm') which represents the proportion of absorbed light energy consumed in photochemistry, and was measured at a light intensity of 250 μmol m^{-2} s^{-1} calculated as (Fm'-F)/Fm', where Fm' is the maximal fluorescence level induced by a saturating light pulse in the steady state, and F is the steady state chlorophyll fluorescence immediately prior to the flash. Measurements were performed on the last fully expanded leaves.

2.3. Gas exchange measurements

Gas exchange analyses were performed 5 or 2 days after osmotic stress treatment, on the intact, last fully expanded leaves using a Li-6400 instrument (Li-Cor, Lincoln, USA) for wheat and a Ciras 2 Portable Photosynthesis System (Amesbury, USA) for maize. The reference level of CO_2 was 380 μL L^{-1} and the light intensity was 250 μmol m^{-2} s^{-1}. The gas exchange analysis was performed at room temperature, and the air humidity was 50±3% in

both cases. The parameters CO_2 assimilation rate (Pn), stomatal conductance (g_s), intracellular CO_2 concentration (C_i) and transpiration (E) were determined at the steady-state level of photosynthesis.

2.4. SA extraction and analytical procedure

SA extraction and analysis were performed according to Pál et al. (2005). After separation on a reverse phase column (ABZ+, 150x4.5 mm, 5 μm, Supelco, Bellefonte, USA) SA (excitation: 305 nm; emission: 407 nm) was quantified fluorimetrically (W474 fluorescence detector, Waters, USA).

2.5. Measurement of phenylalanine ammonia lyase (PAL) activity

PAL activity was measured according to Gao et al. (2008) using 1 g leaves or roots and expressed as enzyme units per gram fresh weight (U g^{-1} FW).

2.6. Polyamine and 1,3-diaminopropane (DAP) analysis

The analysis was carried out as described by Németh et al. (2002), by 200 mg of leaves homogenizing with 1 ml 0.2 M ice-cold perchloric acid and leaving them to stand for 20 min on ice. The extract was centrifuged at 10000g for 20 min and the supernatant was used. The polyamines, namely PUT, SPD and SPM, together with DAP, the product of SPD and SPM terminal catabolism, were analysed as dansylated derivatives via HPLC using a W2690 separation module and a W474 scanning fluorescence detector with excitation at 340 nm and emission at 515 nm (Waters, Milford, MA, USA). Conjugated forms of PAs were measured after 1 hour of acid hydrolysation at 96°C.

2.7. Diamine oxidase and polyamine oxidase enzyme activities

The activity of the diamine oxidase (DAO, EC 1.4.3.6.) and polyamine oxidase (PAO, EC 1.5.3.3.) enzymes was estimated by the method of Takács et al. (2016). Enzyme activity was expressed in nmol Δ^1-pyrroline min^{-1} g^{-1} FW using an extinction coefficient of 1.86 x 10^3 mol^{-1} cm^{-1}.

2.8. Statistical analysis

Three independent repetitions were performed for each experiment and representative data are presented. The results were the means of at least ten replicates for each treatment for chlorophyll induction, and of 5 replicates for enzyme activity and HPLC analysis. The data were statistically evaluated using the standard deviation and *t-test* methods. The SPSS 17.0 statistical program (Statistical Package for the Social Sciences) was used to examine correlations between the parameters.

3. Results

3.1. Polyamine treatment under control conditions

3.1.1. Concentration dependence of polyamine treatment on biomass parameters and lipid peroxidation

Treatment with 0.1, 0.3 or 0.5 mM PUT for 7 days did not induce pronounced changes in the shoot and root length or shoot and root FW of wheat and maize plants. However, exogenous SPD or SPM induced a concentration-dependent decrease in all the biomass parameters of both plant species (Fig. 1A-B). The parameters investigated showed that the roots are more sensitive to polyamine treatment than the shoots in both species and that the shoots of maize are inhibited to a greater extent those of the wheat. Like the biomass parameters, the results of lipid peroxidation measurements also showed that the roots of maize

plants were more sensitive than the shoots after different polyamine treatments, and that greater oxidative stress was induced by higher polyamines in both plant species (Fig. 2A-B).

3.1.2. Polyamine contents in PUT-, SPD- and SPM-treated plants

Since the PAs were added to the hydroponic solution, it was not surprising that the concentration-dependent effects of PA treatment were generally more obvious in the roots than in the leaves in both plant species. PA treatments only had a slight effect at a concentration of 0.1 mM, while substantial PA accumulation was found after treatment with 0.3 or 0.5 mM PUT, SPD or SPM (Fig. 3-4).

In wheat PUT treatment resulted in a dramatic increase in PUT content, especially in the roots, and this was also accompanied by an increased amount of 1,3-diaminopropane (DAP), the product of the oxidation of SPD and SPM by PAOs. PUT only caused pronounced SPD accumulation in both the leaves and roots at 0.5 mM concentration (Fig. 3A-B). Exogenous SPD was taken up and at 0.3 and 0.5 mM concentrations translocated to the wheat leaves, increasing the content of endogenous SPD and PUT in the leaves, and of PUT and SPM in the roots (Fig.3 A-B). SPM treatment increased the content of all the PAs and of DAP in both the leaves and roots, especially at higher concentrations (Fig. 3A-B).

Slight, concentration dependent increasing trends were noted in the root PUT and leaf SPD contents of maize after PUT treatments, which were the most pronounced and statistically significant at 0.5 mM concentration of PUT (Fig. 4A-B). Exogenous SPD or SPM induced the accumulations of PUT, SPD and SPM, especially in the roots at higher concentrations, with the highest amount of PUT in the leaves and roots of plants treated with 0.3 or 0.5 mM SPM (Fig. 4A-B).

3.1.3. Salicylic acid content in PUT-, SPD- and SPM-treated plants

In order to reveal the relationship between PA and SA, the effects of different PAs (PUT, SPD and SPM) on the SA content in wheat and maize was investigated at a concentration of 0.5 mM. In wheat, SPD and SPM increased the free and bound SA content of the leaves. Interestingly, the free SA content was decreased by PUT, and did not influenced by SPD, while both forms were increased by SPM in the roots (Table 1). Under these conditions, only SPD and SPM influenced the endogenous SA content of maize plants. The 0.5 mM SPD and SPM treatment increased the amounts of free and bound SA in the leaves and induced an extraordinary accumulation of free SA in the roots of maize plants (Table 1). Correlation analysis on plants treated with 0.5 mM PUT, SPD or SPM revealed that the endogenous PA contents showed a close, positive relationship with the amount of SA, especially in maize. Close positive relationships were detected in most cases between the free and conjugated fractions of the individual PAs, and also in the different tissues (leaves or roots) of wheat and maize plants (Suppl. Table 1, 2). Correlation analysis does not reveal cause and effect relationships and can only indicate linear correlations. It may, however, provide useful information on the direction and closeness of the correlations. In the present work, the significant, positive correlations between endogenous SA and PA contents suggested that higher PA content is accompanied by SA accumulation.

3.2. Putrescine pre-treatment and PEG-induced osmotic stress

In the first set of experiments the adverse effects of SPD and SPM treatment were demonstrated by the biomass parameters and lipid peroxidation measurements, and these stress symptoms were accompanied by SA accumulation, which was in positive correlation with the increase in PA content. PUT application had no significant negative effect, but induced a significant accumulation of PA in both plant species. In order to obtain further

information on changes in the PA pool and how these are related with SA under stress conditions, pre-treatment with 0.5 mM PUT was applied, followed by 15% PEG treatment.

3.2.1. Changes in biomass, chlorophyll-a fluorescence induction, gas exchange parameters and proline content after PUT pre-treatment, PEG treatment or their combination (PUT pretreated+PEG)

The positive effect of 7 days of 0.5 mM PUT pre-treatment was observed when PUT pre-treated wheat plants were returned to control nutrition solution for 5-day recovery period. This was manifested as increased shoot FW and DW, and increased Pn compared to the same day control (Table 2 and 3). Although PUT pre-treatment was unable to prevent the PEG-induced decrease in root FW and DW in the combined treatment (PUT pre-treated+PEG) (Table 2), the photosynthetic activity (Pn) was nevertheless higher than in plants treated with PEGwithout PUT pre-treatment (Table 3).

In maize, however, not only did PUT pre-treatment cause a decrease in root FW and Pn, which remained even during the recovery period, but no positive effect on the biomass parameters was observed under osmotic stress (Table 2). The lowest shoot FW and DW were observed in PUT pre-treated+PEG maize plants. However, the ΔF/Fm' and Pn parameters demonstrated higher photosynthetic activity in the combined treatment compared to PEG treatment, without PUT pre-treatment (Table 3).

PUT pre-treatment resulted in slight stomatal opening in wheat, while in maize the slight decrease observed in Pn was due to stomatal closure (as a typical stress syndrome). However, this slight difference in Pn was not manifested in ΔF/Fm', which is related to the photochemical energy conversion in PS II. The higher value of stomatal conductance in PUT pre-treated+PEG-treated plants also resulted in a significantly higher assimilation rate compared to maize and wheat plants subjected to osmotic stress without PUT pre-treatment

(Table 3). These results suggested that PUT pre-treatment may alleviate the negative effect of PEG on the photosynthetic process to a certain extent.

Similar changes were found in maize and wheat plants for the proline content, which did not change in the leaves after PUT pre-treatment, but increased in PEG-treated or PUT pre-treated+PEG plants. In the same way, the combination of PUT pre-treatment and PEG induced proline accumulation in the roots of maize plants, while in wheat roots PEG treatment alone caused a significant increase (Table 3).

3.2.2. Polyamine contents after PUT pre-treatment, PEG treatment or their combination (PUT pretreated+PEG)

When 0.5 mM PUT pre-treatment was followed by 5 days of recovery, the exogenously applied PUT did not influence endogenous PA contents of wheat plants compared to the control. In the leaves PEG treatment alone increased the content of both free and conjugated forms of PUT and decreased the SPD and SPM contents in the free fraction (Fig. 5A-B), while it reduced the free and conjugated fractions of SPD in the roots (Fig. 5C-D). When osmotic stress was induced after PUT pre-treatment the increase in free PUT and decrease in free SDP remained, but no decrease in free SPM content was detected in wheat leaves (Fig. 5A-B). In the roots decreases in free PUT, free SPD and conjugated SPM were observed in the combined treatment (PUT pre-treatment+PEG) (Fig. 5C-D). The quantity of the bound forms of PAs was an order of magnitude lower, and no significant changes were induced (data not shown).

In maize plants neither 0.5 mM PUT pre-treatment nor PEG-induced osmotic stress induced changes alone, but when the application of exogenous PUT was followed by PEG treatment, it increased the endogenous PUT content in both the free and conjugated forms in

the leaves (Fig. 6A-B). In contrast, when applied alone as a pre-treatment or followed by osmotic stress PUT caused a significant increase in the free and conjugated PUT content in the roots (Fig. 6C-D). The contents of the bound forms of PAs was an order of magnitude lower, and showed a pattern similar to that described in the case of the free forms (data not shown).

3.2.3. Polyamine catabolism after PUT pre-treatment, PEG treatment or their combination (PUT pretreated+PEG)

The main PA catabolic process is exerted through DAO and PAO, the former showing a strong preference for diamines (PUT and cadaverin) and being mainly active in dicotyledons, while the latter only oxidizes higher PAs (SPD and SPM) and is dominant in monocotyledons. Apoplastic PAOs, which are responsible for this terminal catabolism of PAs, oxidize SPD and SPM to DAP.

Although PEG treatment alone or after PUT pre-treatment greatly increased the amount of DAP in wheat roots, the PAO activity only showed a significant increase in the roots of PEG-treated plants (Table 4). Parallel with this, the DAO activity in the leaves decreased in plant pre-treated with PUT whether this was followed by PEG treatment or not, while in the roots it only increased in the case of PEG-induced osmotic stress.

In maize neither of the treatments induced any change in the DAP content or in PAO activity (Table 4). In contrast, combined PUT pre-treatment+PEG induced a significant increase in DAO activity in the leaves and roots of maize plants (Table 4).

3.2.4. Salicylic acid content after PUT pre-treatment, PEG treatment or their combination (PUT pretreated+PEG)

Changes in the contents of plant hormones in wheat plants differed from those in maize. PEG treatment either alone or after PUT treatment increased the free SA content in wheat leaves, while in the roots PUT slightly decreased the free SA, while PEG-induced osmotic stress increased the amount of bound SA (Table 5).

PUT pre-treatment or PUT pre-treatment+PEG decreased the amount of endogenous free SA significantly, but not substantially form in the leaves of maize plants. In contrast, PUT pre-treatment increased the free SA in the roots, and was able alleviate the negative effect of subsequent PEG application (Table 5). The bound SA content increased significantly in the leaves of PUT pretreated+PEG-treated maize plants, while changes in the roots were similar to those described in the case of free SA (Table 5).

3.2.5. PAL activity

Although PUT treatment decreased the PAL activity in the leaves of wheat plants, the other treatments caused no significant changes. In the roots, PEG treatment alone or after PUT treatment increased the PAL activity (Table 5). In the leaves of maize plants the PAL activity increased after PUT treatment, but was decreased by PEG treatment either alone or after PUT treatment. In the roots only the combined treatment led to a decrease (Table 5).

4. Discussion

Several authors demonstrated the ameliorative role of PAs against various stress factors through the regulation of cation concentration, antioxidants, citrate secretion, phytohormones, etc. (Minocha et al., 2014; Li et al., 2015a,b). However, this protective effect may vary as a function of plant species, the type or concentration of PAs. So can it be said that the more PAs the better? Up to now only a few studies have dealt with the adverse effect of PAs. In order to clarify this question, the present work investigated the background of the

potential effects of PA treatments in relation with changes in the PA pool by applying exogenous PA to two different crop species. A possible relationship with SA was also studied.

In the first experiment PUT, SPD and SPM were applied in concentrations (Suppl. Fig. 1A) previously reported to be effective against stress factors in various plants (Lakra et al., 2006; Cuevas et al., 2008; Pandolfi et al., 2010; Gupta et al., 2012; Mandal et al., 2013; Kotakis et al., 2014). While PUT induced no negative changes in either wheat or maize plants, the results also revealed that maize plants were more sensitive to higher PAs (SPD and SPM). The concentration-dependent negative effects of SPD, and especially of SPM, were manifested as growth inhibition and oxidative stress. SPD treatment has been reported to lead to root growth inhibition and changes in plant morphology in Arabidopsis (Tassoni et al., 2000) and in maize, where it also induced programmed cell death (PCD) (Tisi et al., 2011). Indeed, cytotoxic products of the PA metabolism have been reported to be involved in the PCD cascade (Moschou and Roubelakis-Angelakis, 2014). However, it has also been reported that transgenic wheat plants expressing an oat arginine decarboxylase cDNA exhibited higher PA level. The PUT content, especially PUT content increased to approximately 300-400 nmol g^{-1} FW, but the plants were phenotypically normal and fertile (Bassie et al., 2008). These PA contents and the rate of accumulation were similar to those found in wheat and maize after PA treatment in the present experiments.

In the present study PA treatment led to PA accumulation in both wheat and maize plants, but these species may use several different mechanisms to control endogenous PA levels, including translocation from the roots to the shoots, conjugation to small molecules, further synthesis to higher PAs or the interconversion of higher PAs to PUT in the polyamine cycle (Kubiś et al., 2014; Pál et al., 2015). Although all the PA treatments caused pronounced PUT accumulation in both wheat and maize plants, especially in the roots, indicating the existence of interconversion, the application of PUT or SPD also induced the accumulation of

higher PAs (SPD or SPM), suggesting that PUT or SPD uptake resulted in further synthesis to SPD or SPM. However, some differences were found between the plant species. The PUT taken up by wheat was translocated to the leaves and further metabolised, while in maize it remained in the roots and only slight SPD accumulation was found. In the case of SPD treatment, the endogenous SPD level increased in the roots of wheat, resulting in its translocation to the leaves, interconversion to PUT, further synthesis to SPM, and also - although less intensively than in maize - oxidation to DAP (Fig. 7). The SPD taken up by maize was interconverted to PUT or formed SPM even at low (0.1 mM) concentration in the roots, but only PUT and DAP increased in the leaves (Fig. 7). The most interesting difference between the two plant species was found after SPM treatment. Wheat roots took up the SPM and translocated it to the leaves, but this was also accompanied by a high accumulation of PUT and SPD, mainly in the roots, presenting proof of interconversion, while SPM was also oxidised, as a large quantity of DAP was detected. The SPM taken up by maize did not result in a high endogenous SPM content, but induced a steep accumulation of PUT in both leaves and roots, which may be responsible for the greater negative effect compared to that found in wheat (Fig. 7).

As the application of 0.5 mM PUT had no significant negative effect, but induced significant PA accumulation in both plant species, a study was made of how exogenous PUT influences endogenous PA contents under stress conditions, and how this is related to the effects observed. After testing the protective effect of 0.5 mM PUT as pre-treatment, a second experiment investigated how PUT-treated plants responded to under PEG-induced osmotic stress (Suppl. Fig. 1B). In wheat, PEG treatment induced stomatal closure and a decrease in Pn, as also observed in maize, but without a decrease in $\Delta F/Fm'$ or an increase in Ci, suggesting that the effect of PEG treatment was less serious in wheat than in maize. This was supported by the growth parameters. Nonetheless, PUT pre-treatment provided protection to

both plants under osmotic stress conditions, as demonstrated by the significantly higher effective quantum efficiency and CO_2 assimilation rate compared to those given no PUT pre-treatment. The highest proline accumulation in the roots was also found after the PUT pre-treated+PEG treatment in both species, indicating that proline may also contribute to PUT-induced osmotic tolerance.

The differences observed between wheat and maize after PUT pre-treatment in the first set of experiment suggest that the PA metabolism may differ in these species. PUT pre-treatment alone had no influence on the endogenous PA levels in either the leaves or roots of wheat after a 5-day recovery period, indicating that some other mechanism (translocation, catabolism and/or further synthesis) effectively reduced the PA content to the control level. Although PEG treatment increased the level of free PUT and decreased that of free SPD and SPM in the leaves, neither the DAP level nor the DAO and PAO activities changed, suggesting that higher PAs may be interconverted to PUT. PEG treatment was also reported to increase PUT and reduce SPD and SPM content in wheat leaves by other authors (Marcińska et al., 2013; Kovács et al., 2014). In the roots osmotic stress decreased the level of SPD in the free and conjugated fractions, accompanied by increased PAO activity, resulting in a substantial accumulation of DAP. Increased DAO activity in wheat roots may be responsible for the unchanged PUT content. The results of the combined treatment were similar in part to those obtained for PEG alone: in the leaves free PUT increased, free SPD decreased, but there was no decrease in SPM, while in the roots free PUT and SPD also decreased. These results are in accordance with earlier findings, where PUT to SPM canalization was revealed in the desiccation-tolerant plant *Craterostigma plantagineum* (Alcázar et al., 2011). In the present study, PAO activity did not change, but the level of DAP increased in both leaves and roots. The less pronounced increase or even decrease in PUT

content in PUT pre-treated wheat plants during osmotic stress may indicate that PUT was used for synthesis of SPM in the leaves, which therefore showed no decline.

In maize leaves only the combined treatment (PUT pre-treated+PEG) increased the PUT level, which was accompanied with higher DAO activity. In contrast, in the roots PUT pre-treatment, alone after a 2-day recovery period or followed by PEG treatment, induced pronounced PUT accumulation parallel with an increase in DAO activity. This suggests that, as in the first experiment, root to shoot PA translocation in maize is not as pronounced as in wheat, but PUT oxidation is higher. Interestingly, PEG treatment induced PAO activity in maize roots, but there was no change in the amount of the degradation product, DAP. The fact that the PUT content remained high during the recovery period may be responsible for the lower level of stress tolerance.

The PA metabolism is linked with other hormones or signalling molecules. SA, a phenolic compound that regulates several physiological processes, has also been demonstrated to play role in acclimation mechanisms during abiotic stress (Pál et al., 2006; Janda et al., 2014). Parallel changes in SA and PA contents under biotic (Pál et al., 2013) or abiotic stress conditions have only been described in a few studies (Kovács et al., 2014), but other results have suggested that SA treatment influences PA synthesis and/or catabolism (Németh et al., 2002; Szepesi et al., 2011; Wang and Zhang, 2012; Hassannejad et al., 2012). There appear to be few reposts on the effect of exogenous PA application on SA content under optimum conditions. In one such paper 0.5 mM SPD treatment increased leaf SA in wheat, but at the same concentration PUT and SPD treatments decreased root SA levels (Rahdari and Hoseini, 2013), while SPD had no influence on endogenous SA contents in cucumber (Radhakrishnan and Lee, 2013). The accumulation of SA may be harmful to plants. A negative relationship was observed between SA and growth in the cpr1 Arabidopsis mutant, which has elevated levels of free and glucosyl SA and showed increased oxidative damage

under chilling conditions (Scott et al., 2004). In the present study 0.5 mM PUT did not influence the endogenous SA content in either wheat or maize, while at the same concentration SPD and SPM induced the accumulation of free and bound SA in the leaves of wheat and maize and that of free SA in the roots of maize, indicating an interaction between PA and SA signalling pathways, and confirming that maize is more sensitive to higher PAs.

Differences have also been observed in the way the SA content in wheat and maize plants changes in responses to osmotic stress. PUT pre-treatment also induced different changes in SA levels in wheat and maize plants. The positive effect of UV-B radiation on SA content during PEG-induced osmotic stress has been reported in wheat (Kovács et al., 2014). An increase in the endogenous SA level promotes stomatal closure, probably due to the generation of reactive oxygen species induced by SA (Melotto et al., 2006). In the present experiments PUT pre-treatment induced a slight decrease in free SA in wheat, which reflected changes in PAL activity, a crucial enzyme in the synthesis of lignins, flavonoids, anthocyanins and simple phenolic acids. It was previously reported that the SA content was reduced by PUT, increased by SPD in wheat leaves during salt stress, while in the roots was decreased it by PUT and SPD (Rahdari and Hoseini, 2013). Similarly, SPD treatment decreased SA levels in cucumber under salt stress conditions (Radhakrishnan and Lee, 2013). In these cases the SA-decreasing effect of SPD was beneficial. In the present experiment PEG treatment caused a considerable accumulation of free SA in wheat leaves and an increase in bound SA in the roots, possibly due to the increased activity of PAL in the roots. However, only free SA was found to accumulate in wheat leaves in the combined treatment, to a similar extent to that recorded in plants exposed to PEG without PUT pre-treatment. In contrast, although a slight decrease in free SA was observed in the leaves of maize plants pre-treated with PUT, a high level of free SA accumulation was observed in the roots, accompanied by an increase in bound SA content in both leaves and roots. PEG treatment alone decreased the

free SA levels in maize leaves and roots, which was accompanied by decreased PAL activity in the leaves, while the SA accumulation induced by PUT prevented this decrease in the roots in the combined treatment; furthermore the bound SA content in the leaves was the highest in this treatment. Interestingly, SA accumulation was accompanied by higher PUT content in maize and by a relatively higher PUT content compared to SPD and SPM in wheat plants. On the other hand, PUT accumulation itself also induced oxidative stress in maize, as both the catabolism and back-conversion of polyamines by DAO and PAOs result in the production of H_2O_2 in the apoplast and peroxisomes (Moschou et al., 2008; Pál et al., 2015). The changes in PA contents and their interrelationships may be responsible for the effect of PUT pre-treatment in maize under control conditions, which was manifested in the gas exchange parameters.

5. Conclusions

Although PAs are usually considered as a family of similar molecules, different PAs may have different or even opposite effects. The PA cycle functions intensively in both the leaves and roots in both wheat and maize plants (Fig. 7). However, differences may exist in the intensity of the individual steps in the cycle. In the roots of wheat the dominant direction is from SPM or SPD to PUT and DAP, while there is also pronounced level of SPD accumulation in the leaves, which may be due to the less significant catabolism and interconversion. In contrast, in maize roots interconversion is dominant, resulting in high PUT accumulation, while the product of terminal oxidation, DAP, is dominant in the leaves. Plants try to maintain an optimum PA ratio, especially attempting to decrease the excessive amounts of SPM; however, the resulting high PUT level may also have negative effects, as shown by the decrease in biomass parameters. The fine balance in the PA pool via the PA cycle may serve as a shuttle between the beneficial and deleterious effects of PAs, which may increase

or decrease the fitness of the plant, and may be responsible for the differences induced in different plant species. The positive relations observed here between PAs and SA may also lead to a deeper understanding of the roles and mechanisms of PAs during stress.

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Table 1. Effects of 7 days of 0.5 mM putrescine (PUT), spermidine (SPD) or spermine (SPM) treatment on the free and bound salicylic acid (SA) content in 14-day-old wheat and maize plants. Data represent mean values ±SD, n=5. Different letters indicate significant differences between the treatments at $P< 0.05$.

		Free SA ng g^{-1} FW		Bound SA ng g^{-1} FW	
	Treatment	**Leaf**	**Root**	**Leaf**	**Root**
Wheat	*Control*	58.5±1.78 **a**	45.09±10.78 **b**	126.36±54.78 **a**	31.11±8.28 **a**
	PUT-treated	66.21±7.83 **a**	19.07±8.3 **a**	183.39±54.92 **a**	41.24±11.85 **a**
	SPD-treated	109.42±16.45 **b**	36.28±1.16 **b**	225.92±46.66 **b**	31.22±16.47 **a**
	SPM-treated	100.64±21.8 **b**	92.6±9.63 **c**	280.37±55.06 **b**	62.85±7.79 **b**
Maize	*Control*	106.05±32.76 **a**	79±14.14 **a**	54.16±12.88 **a**	22.19±6.35 **a**
	PUT-treated	120.54±23.2 **a**	111±46 **a**	59.1±7.26 a	34.59±11.02 **ab**
	SPD-treated	224.87±43.7 **b**	384.88±51.2 **b**	118.22±18.84 **b**	31.97±0.68 **b**
	SPM-treated	267.41±12.25 **b**	1987±258.4 **c**	169.97±31.58 **c**	31.55±2.15 **ab**

Table 2. Effects of 15% PEG with or without 7 days of 0.5 mM putrescine pre-treatment on the shoot and root fresh weight or dry weight in wheat and maize plants after 5 and 2 days, respectively. Data represent mean values ±SD, n=10. Different letters indicate significant differences between the treatments at $P< 0.05$.

		Fresh weight (g plant $^{-1}$)		Dry weight (g plant $^{-1}$)	
	Treatment	**Shoot**	**Root**	**Shoot**	**Root**
Wheat	*Control*	0.89±0.06 **a**	0.81±0.05 **b**	0.1049±0.009 **a**	0.0503±0.009 **b**
	PUT pretreated	1.12±0.07 **b**	0.89±0.05 **b**	0.124±0.001 **b**	0.0589±0.007 **b**
	PEG	0.85±0.11 **a**	0.63±0.07 **a**	0.1167±0.001 **a**	0.0377±0.006 **a**
	PUT pretreated+ PEG	0.93±0.08 **a**	0.61±0.07 **a**	0.114±0.004 **a**	0.0484±0.005 **ab**
Maize	*Control*	2.61±0.36 **a**	1.61±0.25 **b**	0.45±0.06 **b**	0.12±0.02 **a**
	PUT pretreated	2.1±0.27 **a**	1.05±0.16 **a**	0.31±0.04 **a**	0.09±0.01 **a**
	PEG	2.04±0.38 **a**	0.98±0.2 **a**	0.35±0.06 **ab**	0.11±0.02 **a**
	PUT pretreated+ PEG	1.89±0.4 **a**	0.95±0.22 **a**	0.29±0.06 **a**	0.1±0.02 **a**

Table 3. Effects of 15% PEG with or without 7 days of 0.5 mM putrescine pre-treatment on gas exchange parameters (Pn: CO_2 assimilation rate; Ci: intracellular CO_2 concentration; gs: stomatal conductance and E: transpiration), the quantum efficiency of PSII (ΔF/Fm') and proline content were determined at the steady-state level of photosynthesis after 5 days in wheat plants and 2 days in maize. Data represent mean values ±SD, n=10. Different letters indicate significant differences between the treatments at P< 0.05.

		Pn	Ci	gs	E		Proline µg g^{-1} FW	
	Treatment	**(µmol CO_2 m^{-2} s^{-1})**	**(µmol mol^{-1})**	**(mmol H_2O m^{-2} s^{-1})**	**(mmol H_2O m^{-2} s^{-1})**	**ΔF/Fm'**	**Leaf**	**Root**
Wheat	*Control*	9.38±0.28 **c**	255.17±12.56 **b**	164.83±21.53 **c**	2.27±0.3 **b**	0.414±0.022 a**b**	7.87±0.65 **a**	6.38±0.18 **a**
	PUT pretreated	10.3±0.19 **d**	273.33±7.47 **b**	188.78±17.5 **c**	2.59±0.12 **b**	0.397± 0.015 a	8.62±0.32 **a**	7.45±2.23 **ab**
	PEG	5.23±0.31 **a**	180.44±46.4 **a**	49.88±3.52 **a**	0.81±0.13 **a**	0.369±0.052 **a**	26.06±2.21 **b**	12.62±1.93 **bc**
	PUT pretreated+ PEG	6.69±0.23 **b**	196.44±8.43 **a**	66.67±4.53 **b**	0.96±0.25 **a**	0.449±0.024 **b**	43.3±3.97 **c**	15.89±0.31 **c**
Maize	*Control*	12.07±0.37 **d**	226.17±6.03 **a**	308±14.98 **d**	2.32±0.22 **c**	0.326±0.037 **b**	11.41±2.37 **a**	9.83±2.8 **a**
	PUT pretreated	10.88±0.56 **c**	241.33±10.16 **a**	249.83±4.06 **c**	1.88±0.16 **b**	0.37±0.028 **b**	11.75±0.6 **a**	9.92±1.8 **a**
	PEG	4.55±0.66 **a**	324.58±13.93 **c**	122.92±4.32 **a**	1.38±0.11 **a**	0.17±0.06 **a**	23.28±3.5 **b**	12.26±1.87 **a**
	PUT pretreated+ PEG	7.4±0.71 **b**	296.67±4.64 **b**	140.1±7.61 **b**	1.63±0.09 **b**	0.329±0.05 **b**	21.7±4.7 **b**	22.5±1.87 **b**

Table 4. Effects of 15% PEG with or without 7 days of 0.5 mM putrescine pre-treatment on the 1,3-diaminopropane (DAP) content and the diamino oxidase (DAO) and polyamine oxidase (PAO) enzyme activities after 5 days in wheat plants and 2 days in maize. Data represent mean values ±SD, n=5. Different letters indicate significant differences between the treatments at P< 0.05.

		DAP nmol g^{-1} FW		DAO (Δ^1-pyrroline min^{-1} g^{-1} FW)		PAO (Δ^1-pyrroline min^{-1} g^{-1} FW)	
	Treatment	**Leaf**	**Root**	**Leaf**	**Root**	**Leaf**	**Root**
Wheat	*Control*	22.56±12.72 **ab**	41.18±34.95 **a**	7.42±0.33 **b**	3.6±1.48 **a**	11.32±2.85 **a**	11.5±1.28 **a**
	PUT pretreated	21.88±7.96 **ab**	85.2±15.84 **a**	5.1±0.69 **a**	3.63± 0.78 **a**	11±2.94 **a**	9.68± 1.29 **a**
	PEG	17.07±2.66 **a**	181.62±41.5 **b**	8.42±0.64 **b**	9.28±1.83 **b**	12.01±1.17 **a**	16.06±1.17 **b**
	PUT pretreated+ PEG	32.39±3.22 **b**	182.31±40.83 **b**	5.52±0.29 **a**	3.07±0.58 **a**	7.04±2.66 **a**	11.3±3 **a**
Maize	*Control*	18.18±3.37 **a**	10.88±3.57 **a**	42.32±6.09 **a**	40±1.77 **a**	76.12±15.13 **a**	25.67±2.59 **a**
	PUT pretreated	22.7±2.74 **a**	11.26±1,26 **a**	33.5±11.74 **a**	46.69±2.26 **b**	66.36± 13.79 **a**	21±6.11 **a**
	PEG	11.76±9.2 **a**	9.2±1.32 **a**	37.82±10.47 **a**	44.31±10.46 **abc**	77.48±5.71 **a**	36.33±5.5 **b**
	PUT pretreated+ PEG	14.43±2.98 **a**	11.04±3.78 **a**	78.93±10.1 **b**	56.67±7.5 **c**	77.96± 4.9 **a**	24.22±8.64 **ab**

Table 5. Effects of 15% PEG with or without 7 days of 0.5 mM putrescine pre-treatment on salicylic acid (SA) and phenylalanine ammonia lyase (PAL) activity after 5 days in wheat plants and 2 days in maize. Data represent mean values ±SD, n=5. Different letters indicate significant differences between the treatments at $P< 0.05$.

		Free SA (ng g^{-1} FW)		Bound SA (ng g^{-1} FW)		PAL (U g^{-1} FW)	
	Treatment	**Leaf**	**Root**	**Leaf**	**Root**	**Leaf**	**Root**
Wheat	*Control*	72±28.28 **a**	20.7±0.7 **b**	384±90.92 **a**	97.5±18.54 **a**	36.6±8.78 **b**	69.87±8.6 **a**
	PUT pretreated	57.3±19.23 **a**	14.05±0.49 **a**	273.5±115.82 **a**	83±6.79 **a**	19.8±5.65 **a**	65.75±16.75 **a**
	PEG	189.89±44.53 **b**	20.85±0.78 **b**	373.4±87.68 **a**	138.63±11.72 **b**	28.36±15.9 **ab**	125.76±1.65 **b**
	PUT pretreated+ PEG	179.4±37.33 **b**	20.65±7 **ab**	373.3±16.54 **a**	87.53±10.56 **a**	38,06±8.28 **b**	132.16±6.29 **b**
Maize	*Control*	85.13±16.39 **b**	39.44±5.67 **b**	311.11±46.45 **a**	30.71±12.33 **a**	74.8±16.37 **b**	157.4±14 **b**
	PUT pretreated	51.11±6.68 **a**	201.74±25.64 **d**	392.44±22.2 **b**	201.52±13.9 **c**	105.25±12.65 **c**	140.8±10.46 **b**
	PEG	57.2±14.1 **ab**	16.12±5.2 **a**	376.48±51.4 **ab**	25.06±9.9 **a**	40.37±9.3 **a**	114.43±10.09 **b**
	PUT pretreated+ PEG	44.88±12.25 **a**	71.19±13.41 **c**	515.08±81.66 **c**	76.29±6.21 **b**	35.13±15.55 **a**	105.3±11.08 **a**

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Legends:

Figure 1. Effects of 7-day 0.1, 0.3 and 0.5 mM putrecine (PUT), spermidine (SPD) or spermine (SPM) treatments on biomass parameters (shoot length, root length, shoot fresh weight and root fresh weight) of wheat (A) and maize plants (B) expressed as a percentage of the control values (100 % values are 28.57, 24.44, 0.49 and 0.38, respectively, for wheat and 32.4, 38.5, 1.78 and 0.88 for maize).

Figure 2. Effects of 7-day 0.1, 0.3 and 0.5 mM putrecine (PUT), spermidine (SPD) or spermine (SPM) treatments on the lipid peroxidation in the leaves and roots of wheat (A) and maize plants (B). Data represent mean values ±SD, n=5. *, ** and ***: significant differences between the control and PA-treated plants of each polyamine at the $P<0.05$, 0.01 and 0.001 level, respectively.

Figure 3. Effects of 7-day 0.1, 0.3 and 0.5 mM putrecine (PUT), spermidine (SPD) or spermine (SPM) treatments on the free polyamine contents in the leaves (A) and roots (B) of wheat plants. Grey and black gridded bars: 1,3-diaminopropane (DAP); white bars: putrescine (PUT); grey bars: spermidine (SPD); black bars: spermine (SPM). Data represent mean values ±SD, n=5. *, ** and ***: significant differences between the control and PA-treated plants of each polyamine at the $P<0.05$, 0.01 and 0.001 level, respectively.

Figure 4. Effects of 7-day 0.1, 0.3 and 0.5 mM putrecine (PUT), spermidine (SPD) or spermine (SPM) treatments on the free polyamine contents in the leaves (A) and (B) of maize plants. Grey and black gridded bars: 1,3-diaminopropane (DAP); white bars: putrescine (PUT); grey bars: spermidine (SPD); black bars: spermine (SPM). Data represent mean values ±SD, n=5. *, ** and ***: significant differences between the control and PA-treated plants of each polyamine at the $P<0.05$, 0.01 and 0.001 level, respectively.

Figure 5. Effects of 15% PEG treatment after 5 days with or without 7-day 0.5 mM putrescine pre-treatment, on the free (A, C) and conjugated (B, D) polyamine contents in the

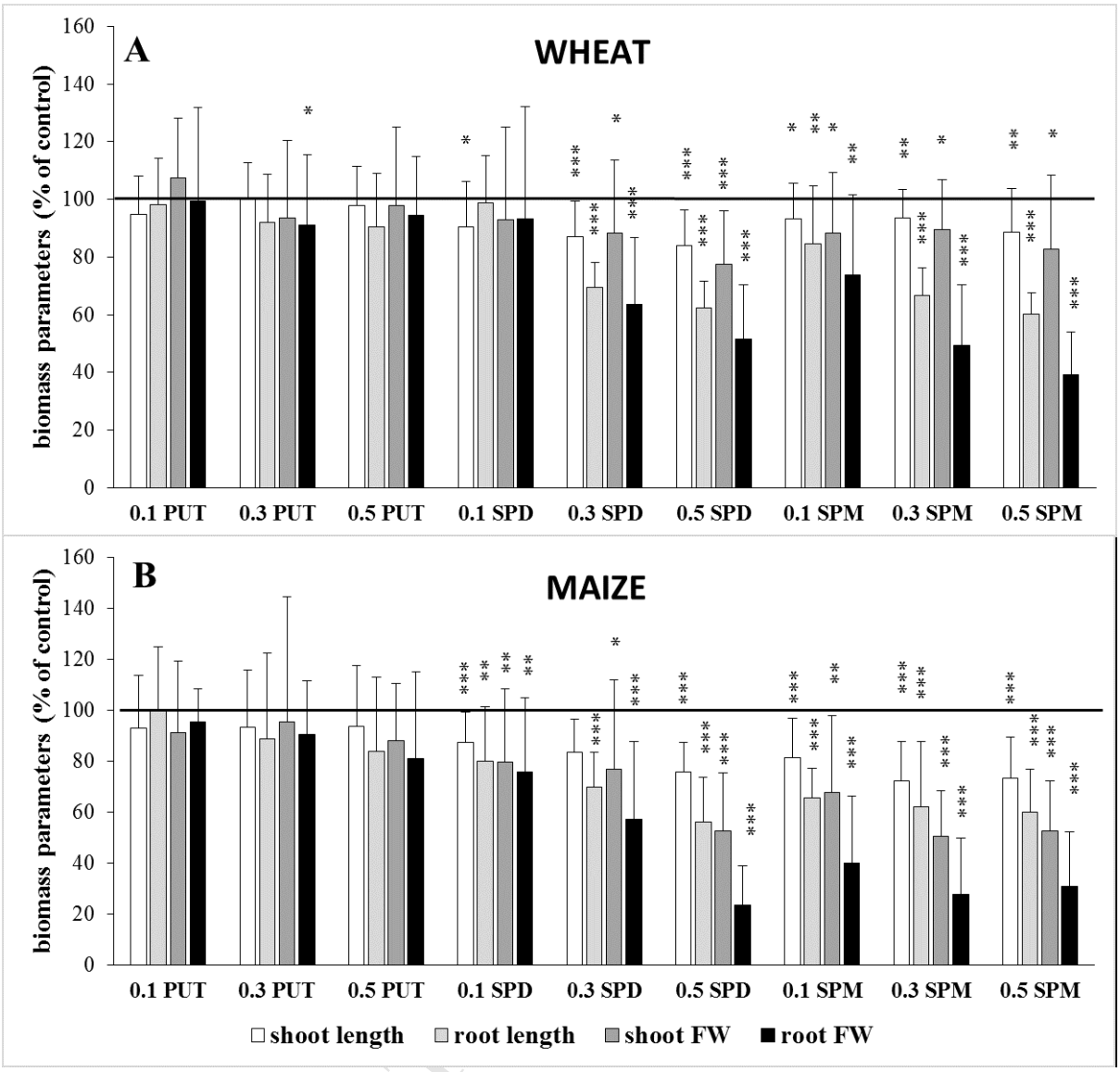
leaves (A, B) and roots (C, D) of wheat plants. White bars: putrescine (PUT); grey bars: spermidine (SPD); black bars: spermine (SPM). Data represent mean values ±SD, n=5. ** and ***: significant differences between the same polyamines at the P<0.01 and 0.001 level, respectively.

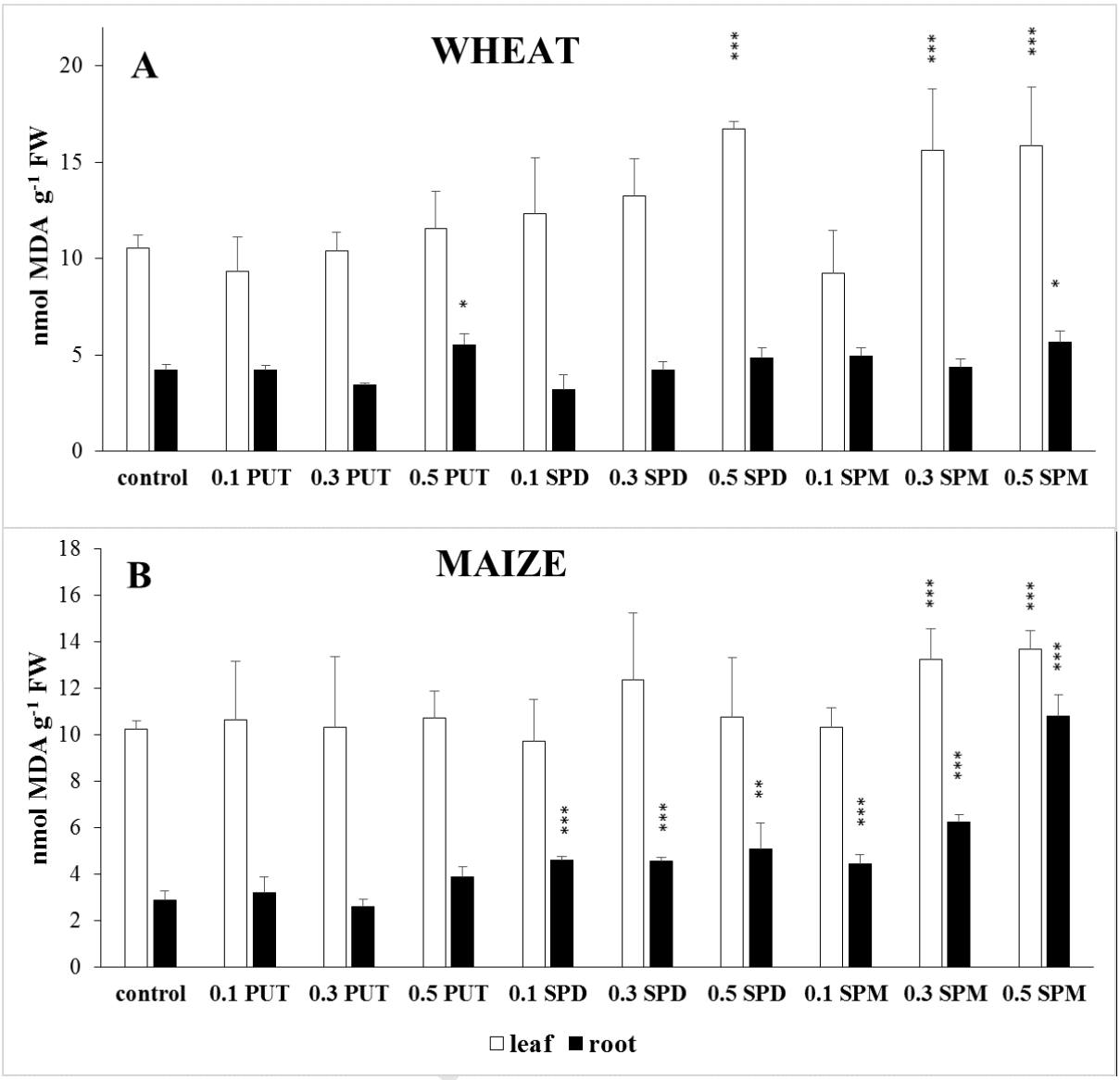
Figure 6. Effects of 15% PEG treatment after 2 days, with or without 7-day 0.5 mM putrescine pre-treatment, on the free (A, C) and conjugated (B, D) polyamine contents in the leaves (A, B) and roots (C, D) of maize plants. White bars: putrescine (PUT); grey bars: spermidine (SPD); black bars: spermine (SPM). Data represent mean values ±SD, n=5. *, ** and ***: significant differences between the same polyamines at the P<0.05, 0.01 and 0.001 level, respectively.

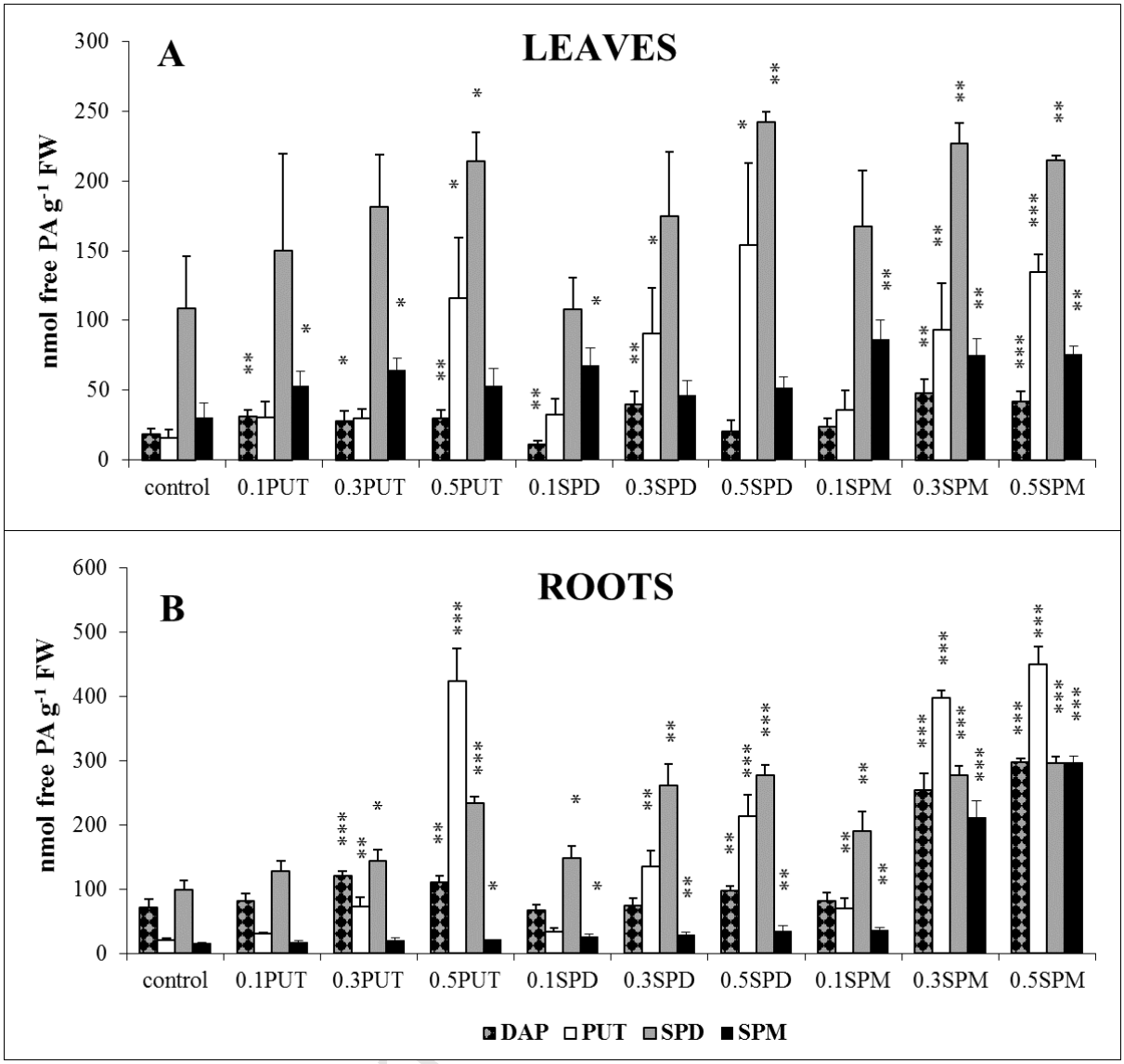
Figure 7. ***Proposed mechanism for the polyamine metabolism in the leaves and roots of polyamine-treated wheat and maize plants.*** Polyamine treatment led to the accumulation of the applied polyamine in the roots of both maize and wheat plants. The interconversion of higher polyamines to putrescine or spermidine in the polyamine cycle may occur in both plant species. Accordingly all the polyamine treatments caused marked putrescine accumulation, especially in the roots. Besides translocation from roots to shoots, the given increase in endogenous polyamine levels may induce further synthesis to higher polyamines, or terminal catabolism may occur. The thickness of the arrows indicates differences in the intensity of the individual step in the PA cycle in wheat and maize leaves or roots (for details see text).

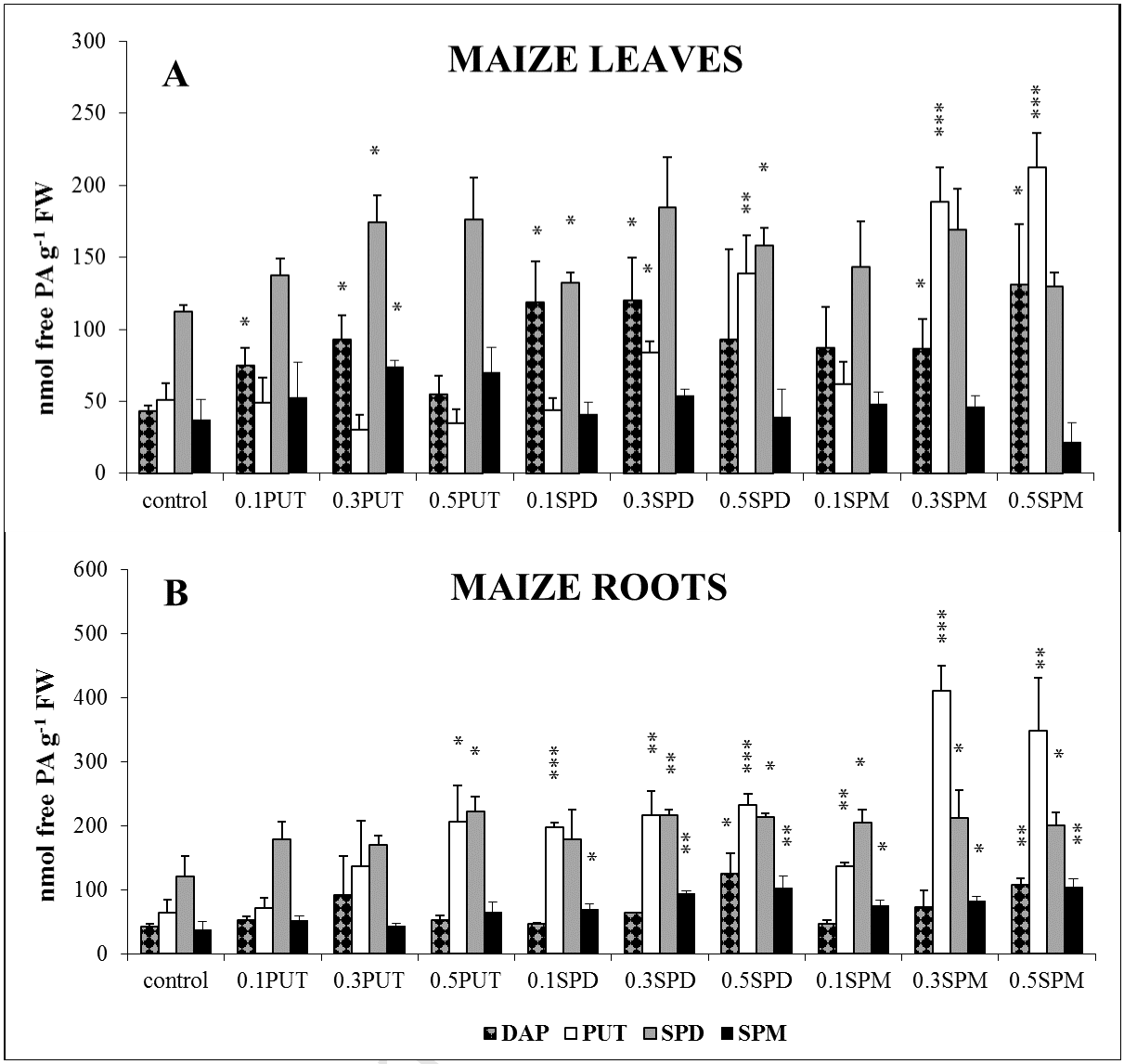
Supplementary Figure 1. Experimental design for plant growth conditions. A: First experiment, investigation on the effect of 7-day 0.1, 0.3 and 0.5 mM putrescine (PUT), spermidine (SPD) and spermine (SPM) treatments in wheat and maize plants grown in a hydroponic system. **B**: Second experiment, investigation on the effect of 7-day 0.5 mM

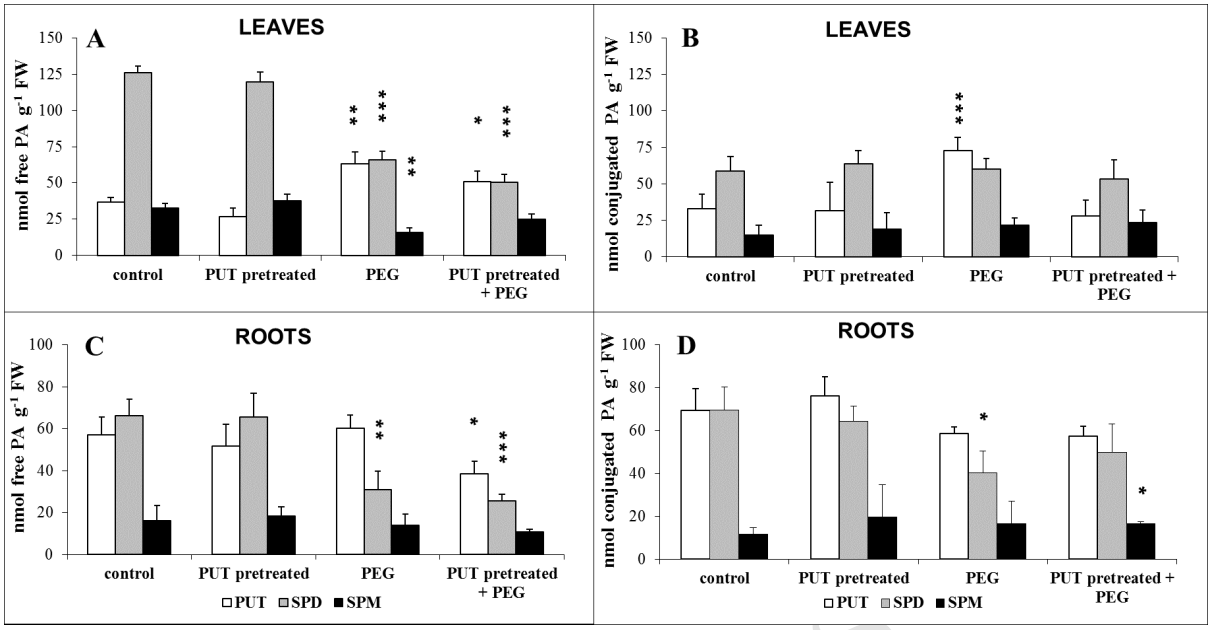
putrescine (PUT) pre-treatment followed by polyethylene glycol (PEG)-induced osmotic stress for 5 days in wheat or 2 days in maize plants in a hydroponic system.

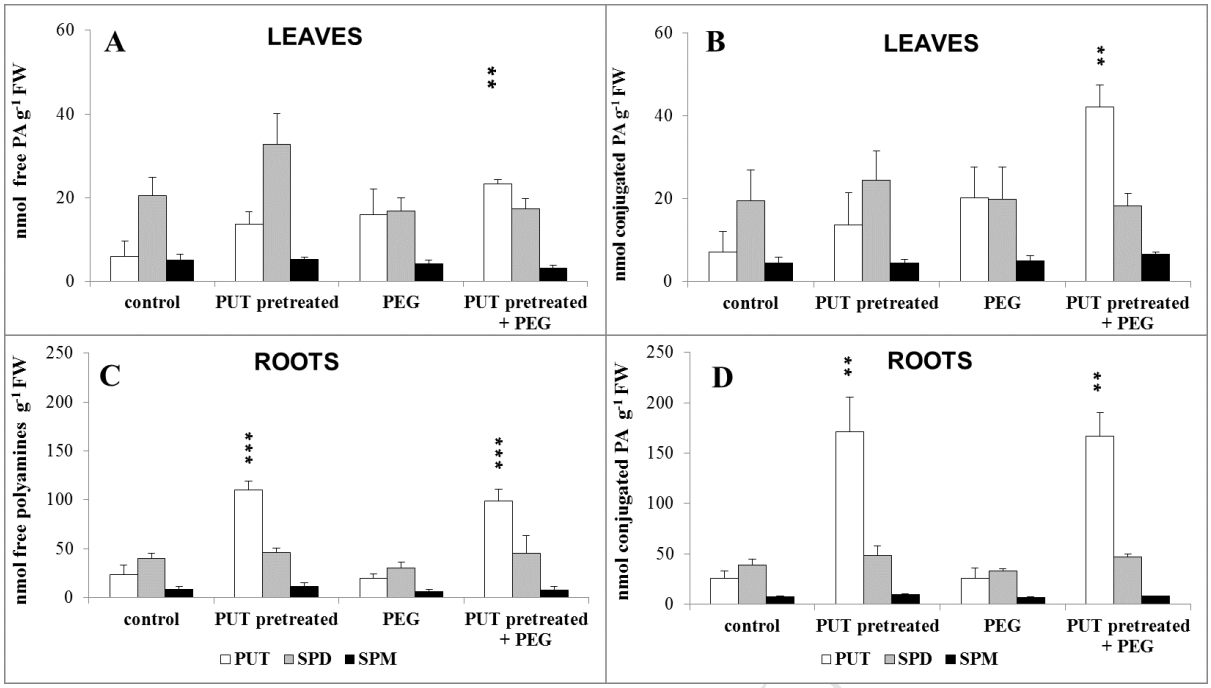


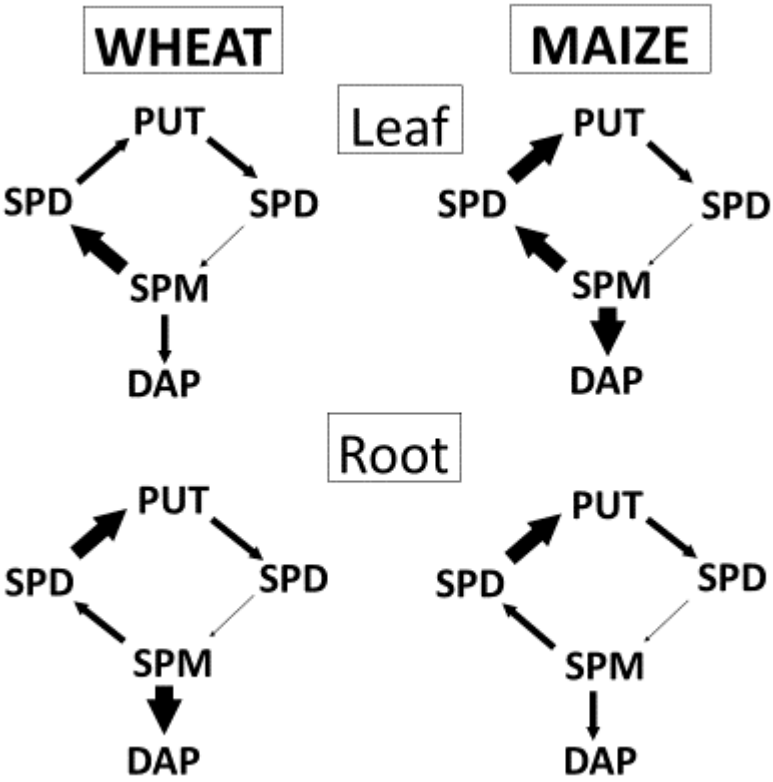












1. Spermidine and spermine, but not putrescine had negative effects at the same concentration
2. Effect of higher polyamines was paralleled with salicylic acid accumulation
3. Putrescine had protective effect in both wheat and maize under osmotic stress conditions
4. Greater beneficial effect of putrescine in wheat may be related to the polyamine cycle
5. Putrescine pre-treatment differentially affected the SA-contents in wheat and maize

ATable 1. Correlation analysis of the polyamine and salicylic acid contents in the leaves and roots of 0.5 mM PUT, SPD or SPM-treated wheat plants. Parameters were related to each other. Significant correlations at 0.05 and 0.01 levels were marked with * and **. respectively. Abbreviations: b: bound fraction, c: conjugated fraction, f: free fraction, PUT: putrescine, SA:salicylic acid, SPD: spermidine, SPM: spermine.

	fSA leaf	***fSA root***	***bSA leaf***	***bSA root***	***fPUT leaf***	***cPUT leaf***	***fPUT root***	***cPUT root***	***fSPD leaf***	***cSPD leaf***	***fSPD root***	***cSPD root***	***fSPM leaf***	***cSPM leaf***	***fSPM root***	***cSPM root***
fSA leaf	1															
fSA root	-0.040	1														
bSA leaf	**0.706***	0.024	1													
bSA root	0.086	0.378	0.450	1												
fPUT leaf	**0.689***	0.478	0.523	0.046	1											
cPUT leaf	0.539	**0.620***	0.506	0.167	**0.961****	1										
fPUT root	-0.219	-0.025	0.049	-0.104	0.215	0.263	1									
cPUT root	-0.378	0.004	-0.064	-0.169	0.128	0.185	**0.982****	1								
fSPD leaf	0.544	0.266	0.307	-0.369	**0.885****	**0.776****	0.227	0.190	1							
cSPD leaf	0.146	0.111	0.066	-0.243	0.560	0.499	**0.614***	**0.615***	**0.620***	1						
fSPD root	0.355	**0.606***	0.550	**0.693***	0.549	**0.623***	-0.120	-0.177	0.247	-0.022	1					
cSPD root	0.439	0.375	**0.599***	**0.618***	0.513	0.535	-0.137	-0.203	0.264	0.007	**0.957****	1				
fSPM leaf	0.563	0.473	**0.666***	**0.648***	**0.707***	**0.738****	0.107	0.007	0.392	0.215	**0.932****	**0.920****	1			
cSPM leaf	0.459	0.546	**0.634***	**0.687***	**0.668***	**0.729****	0.109	0.024	0.345	0.188	**0.956****	**0.923****	**0.992****	1		
fSPM root	**0.622****	-0.007	0.569	0.386	0.387	0.312	-0.435	-0.520	0.236	-0.139	**0.717****	**0.859****	**0.695****	**0.654****	1	
cSPM root	0.328	**0.765****	0.453	0.362	**0.781****	**0.857****	-0.017	-0.042	**0.590***	0.233	**0.863****	**0.770****	**0.808****	**0.839****	0.5	1

ATable 2. Correlation analysis of the polyamine and salicylic acid contents in the leaves and roots of 0.5 mM PUT, SPD or SPM-treated maize plants. Parameters were related to each other. Significant correlations at 0.05 and 0.01 levels were marked with * and **. respectively. Abbreviations: b: bound fraction, c: conjugated fraction, f: free fraction, PUT: putrescine, SA:salicylic acid, SPD: spermidine, SPM: spermine.

	fSA leaf	*fSA root*	*bSA leaf*	*bSA root*	*fPUT leaf*	*cPUT leaf*	*fPUT root*	*cPUT root*	*fSPD leaf*	*cSPD leaf*	*fSPD root*	*cSPD root*	*fSPM leaf*	*cSPM leaf*	*fSPM root*	*cSPM root*
fSA leaf	1															
fSA root	**0.885****	1														
bSA leaf	**0.861****	**0.875****	1													
bSA root	0.335	0.152	0.262	1												
fPUT leaf	**0.922****	**0.823***	**0.934****	0.594	1											
cPUT leaf	**0.941****	**0.857****	**0.937****	0.247	**0.993****	1										
fPUT root	0.365	0.253	0.322	**0.790****	**0.754***	0.296	1									
cPUT root	**0.699***	**0.806****	**0.761****	0.559	**0.747***	**0.743****	**0.963****	1								
fSPD leaf	0.437	0.466	0.498	0.388	0.589	0.489	**0.652***	**0.807****	1							
cSPD leaf	-0.291	-0.298	-0.412	-0.261	-0.438	-0.338	-0.132	-0.086	0.271	1						
fSPD root	**0.632***	**0.725***	**0.693***	0.596	**0.828****	**0.654***	**0.785****	**0.929****	**0.710****	-0.1	1					
cSPD root	0.449	**0.674***	0.537	0.502	0.622	0.437	**0.701***	**0.841****	**0.632***	0.097	**0.929****	1				
fSPM leaf	0.043	0.137	0.047	0.579	0.232	-0.017	**0.817****	**0.720***	**0.591***	0.231	**0.665***	**0.731****	1			
cSPM leaf	-0.206	-0.177	-0.237	0.188	-0.129	-0.188	0.337	0.037	0.058	0.31	0.309	0.397	**0.658***	1		
fSPM root	**0.672***	0.272	**0.603***	0.436	**0.853****	**0.714****	0.406	0.504	0.353	-0.497	0.357	0.039	-0.06	-0.25	1	
cSPM root	**0.706***	0.328	**0.609***	0.445	**0.891****	**0.711****	0.39	0.515	0.414	-0.438	0.32	0.02	-0.07	-0.372	**0.974****	1

Gabriella Szalai was responsible for HPLC analyses of salicylic acid and abscisic acid contents.

Katalin Janda was responsible for spectrophotometric measurements of diamine and polyamine oxidase activities.

Éva Darkó was responsible for the measurements of gas exchange parameters.

Tibor Janda was responsible for discussion of the results and preparing MS.

Violeta Peeva was responsible for chlorophyll-*a* fluorescence induction measurements

Magda Pál was responsible for the experimental design, plant growth and treatments, HPLC analyses of polyamine contents, spectrophotometric measurements of phenyl alanine ammonia lyase activity and proline contents and preparing MS.