Cereal Research Communications 44(4), pp. 537–548 (2016)
DOI: 10.1556/0806.44.2016.038

Priming Seed with Salicylic Acid Increases Grain Yield and Modifies Polyamine Levels in Maize

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(Received 5 March 2016; Accepted 8 June 2016;
Communicated by A. Pécsváradi)

The application of naturally occurring biologically active compounds could be an effective method to improve crop productivity under changing environmental conditions. In the present work the effects of priming maize seed with salicylic acid were tested on the grain yield under field conditions, and on the salicylic acid and polyamine metabolism under controlled environmental conditions. The field data suggested that the beneficial effects of pretreating maize seed with salicylic acid were mainly detectable in the yield in the case of early sowing dates. When young maize seedlings were exposed to low temperature stress, priming the seed with salicylic acid only modified the salicylic acid levels in the seed but not in the roots or leaves. The data suggested that salicylic acid was taken up by the seed and was then converted to bound forms. In contrast to salicylic acid, 5 days after sowing there was a substantial increase in the free form of *ortho*-hydroxy cinnamic acid in the seed, roots and leaves. Priming with salicylic acid also led to an increase in the putrescine content and a slight decrease in spermidine in the seed. The levels of putrescine, spermidine and spermine also increased in the roots of plants treated with salicylic acid under normal growth conditions. The results suggest that polyamines may also contribute to the stress tolerance of plants primed with salicylic acid.

Keywords: cold, polyamines, priming, salicylic acid, *Zea mays* L.

Introduction

Improvement of stress tolerance of cultivated plants and thus the increase of crop yields and nutritional values in environmentally friendly ways is a crucial problem in agriculture. Beside breeding of tolerant genotype or using transgenic plants, application of naturally occurring biologically active compounds can be an alternative approach to improve crop productivity under changing environmental conditions.

Several studies suggest that salicylic acid (SA) may positively affect the germination or plant growth in various plant species or may improve stress tolerance of crop plants (Krantev et al. 2008; Hayat et al. 2010; Poór and Tari 2012; Saruhan et al. 2012; Janda et al. 2014) by inducing a wide range of stress acclimation mechanisms. From practical

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*Corresponding author; E-mail: janda.tibor@agrar.mta.hu point of view not all types of application methods can be used under field conditions, especially for cereals growing on large areas. Reason for existence of hydroponic cultures may only be in greenhouses. Spraying of canopies may also have undesirable environmental impacts, and it may also be rather expensive. Priming of seed seems a potential way to improve crop productivity in various plant species (Jafar et al. 2012; Mahboob et al. 2015); and has it several advantages. It needs lower amount of chemicals than spraying, so it is relatively cheap; and the dose can also be better controlled. Previous comparative study also suggested that priming of seed was more effective than foliar application in improvement the productivity of maize hybrid (Ahmad et al. 2014).

In the present work, using field and phytotron experiments, the following hypotheses were tested: i) May the soaking of maize seed in SA solution improve its grain yield under field conditions in a region with continental climate? ii) How can the sowing date influence the effect of SA pre-treatment? iii) Polyamines are found in all living cells, and they appear to be essential for life (Bitrián et al. 2012). Their main role originally assumed to be as direct protective compounds but recent results suggest they also serve as signalling compounds (Pál et al. 2015). So the next question was whether pre-treating seed with SA may modify the polyamine levels in early growth stage under different temperature conditions?

Materials and Methods

Field experiments

Field experiments were carried out in the area of experimental fields of the Institute of Agriculture, MTA ATK close to Martonvásár, Hungary in 2013 and 2014 (N47°19’, S18°47 and N47°18’, S18°48’, respectively). Maize seeds (*Zea mays* L., hybrid Mv 255) were soaked in 0, 0.3 or 0.5 mM SA solutions (dissolved in distilled water; DW) for 1 day. After soaking seeds were air dried, then they were sown in 2 time points (early and late) in each year (April, 07th and 13th May in 2013 and on 19th April and 3rd May in 2014) in 4 different parcels. Control plants were not treated before sowing, while the effect of regular seed coating with fludioxonil procedure was also investigated. Fludioxonil is a contact fungicide which penetrates the seed surface and concentrates around the seed, thus providing a long-lasting protection zone around the young seedling against targeted fungi, such as seedborne and soilborne Fusarium. Harvest dates were 30th October and 29th November in 2013 and 2014, respectively. Temperature data for the vegetation periods are given in Fig. 1.

Phytotron experiments

Growth conditions

For experiments under controlled environmental conditions maize seeds (hybrid Norma) were soaked in 0 or 0.5 mM SA solution for 1 day, then were sown in the 3:1:1 (v:v:v) mixture of loamy soil : humus containing additive, Vegasca : sand. Plants were grown for

7 days in Conviron PGR-15 plant growth chamber (Controlled Environment Ltd. Winnipeg, Canada) at 22/20 °C day/night temperatures with 16/8 h light/dark period at 200 mmol m^{-2} s^{-1} Photosynthetic Photon Flux Density and 75% relative humidity. After 1 week some of the plants were exposed to low temperature at 5 °C for 5 days in the same growth chamber. Leaf, root and seed samples for biochemical analyses were collected before and after the cold stress and were stored at –80 °C.

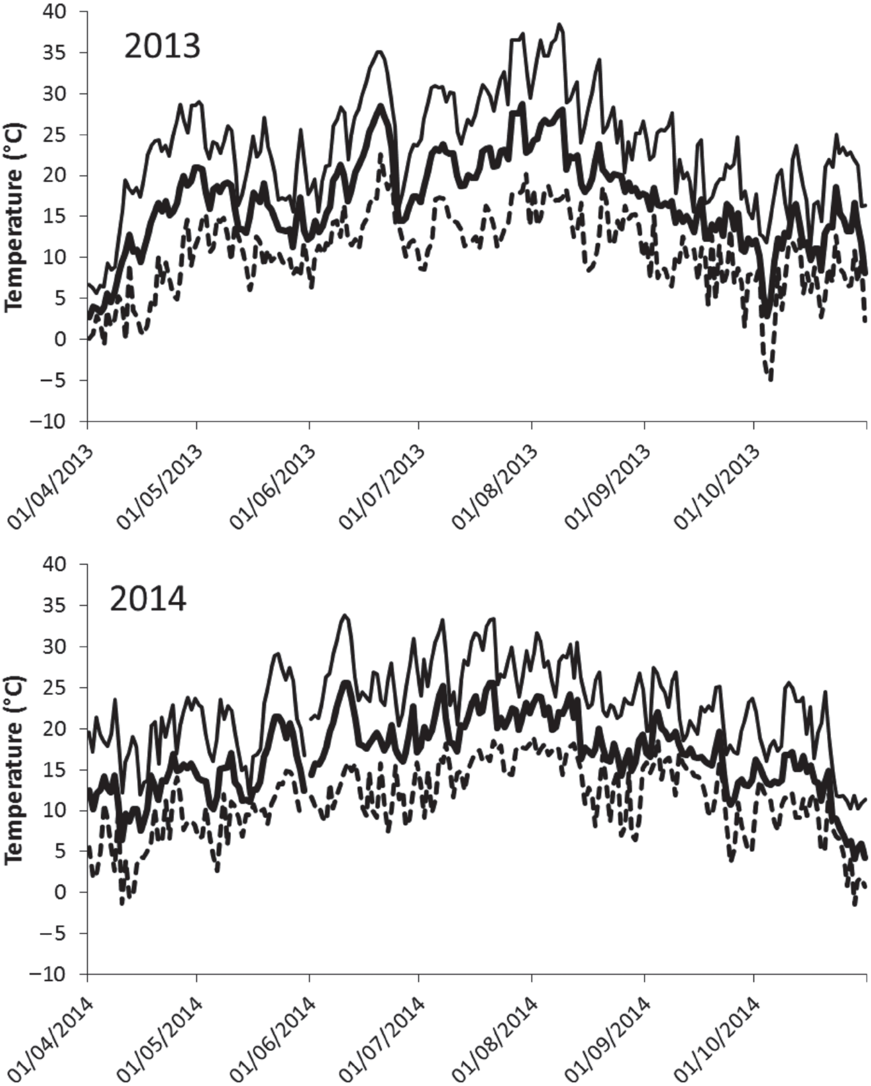

Figure 1. Temperature values during the experimental periods in 2013 and 2014 in Martonvásár, Hungary. Thin lines: maximum; thick lines: average; dotted lines: minimum daily temperatures

Measurements of SA and oHCA

A 1.5 g plant material was extracted for determination of SA and *ortho*-hydroxy cinnamic acid (oHCA). The sample preparation and HPLC analysis was carried out as described by Meuwly and Métraux (1993).

Polyamine determinations

For analysis of polyamines 200 mg of plant material was used. The extraction, sample derivatization and HPLC measurement was made according to Smith and Davies (1985).

Statistical analyses

Data from field experiments were collected in 2 years (2013 and 2014). Average values represent percentage data from 4 parcels. The results of the biochemical analyses are the means of at least 5 measurements and were statistically evaluated using the standard deviation and T-test methods.

Results

Effects of seed pre-treatment on grain yield of maize under field conditions

In order to get know the effects of exogenous SA on yield under field conditions, maize seeds were soaked in 0, 0.3 or 0.5 mM SA solution for 1 day, then seeds were dried in air before sowing using 4 different parcels in each year with 2 sowing times in a 2-year ex-

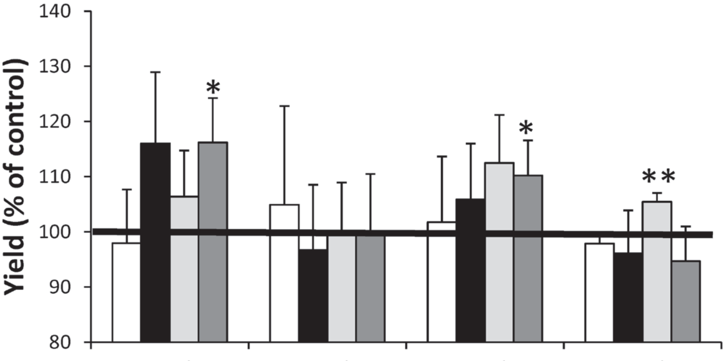

Figure 2. Crop yield data as the percentage of non-treated control values (100%; represented by horizontal line). Seeds were treated with distilled water (DW; white bars); normal seed coating with fludioxonil (dressed, black bars), soaked in 0.3 or 0.5 mM SA for 1 day (light or dark grey bars, respectively). A: early; B: late sowing. The absolute control values (100%) are: 6.3; 6.7; 7.0 and 7.6 t/ha for 2013/A, 2013/B, 2014A and 2014/B, respectively. * and ** represent significant difference from the control at $p<0.05$ and 0.01 levels, respectively

periment. Relative grain yield data of each year are shown in Fig. 2. Since in some cases the absolute yield values were very variable between the different parcels data are given as a percent of control plants (without any treatment or soaking: non-treated control) in the same parcel. Yield data show that while coating of seed with fludioxonil had no significant effect on the grain yield in any treatment, pre-soaking of seed in SA solution significantly increased the yield in more cases. Treatment with 0.5 mM SA was efficient in early sowing in both cases, while treatment with 0.3 mM SA also increased the yield in 2014 at late sowing.

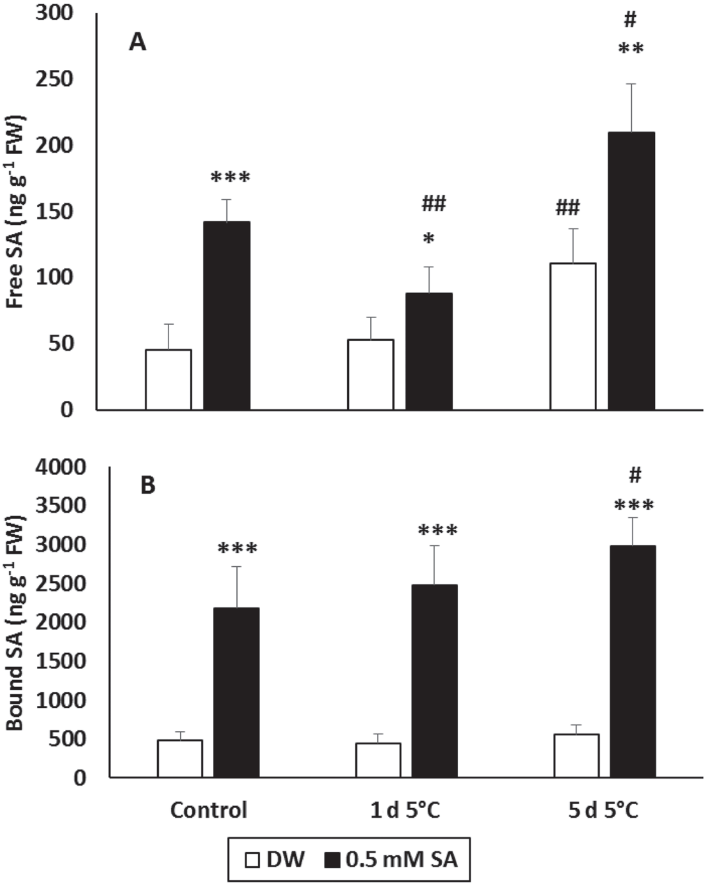

Figure 3. Effects of soaking of maize seed with SA on the free (A) and bound SA (B) levels before and after 1 and 5 days of cold treatment at 5 °C in the seed of young maize plants (hybrid Norma). ** and *** represent significant differences between treatments with SA and distilled water at $p < 0.01$ and 0.001 levels, respectively. # and ## represent significant differences between the control and cold treated plants at $p < 0.05$ and 0.01 levels, respectively

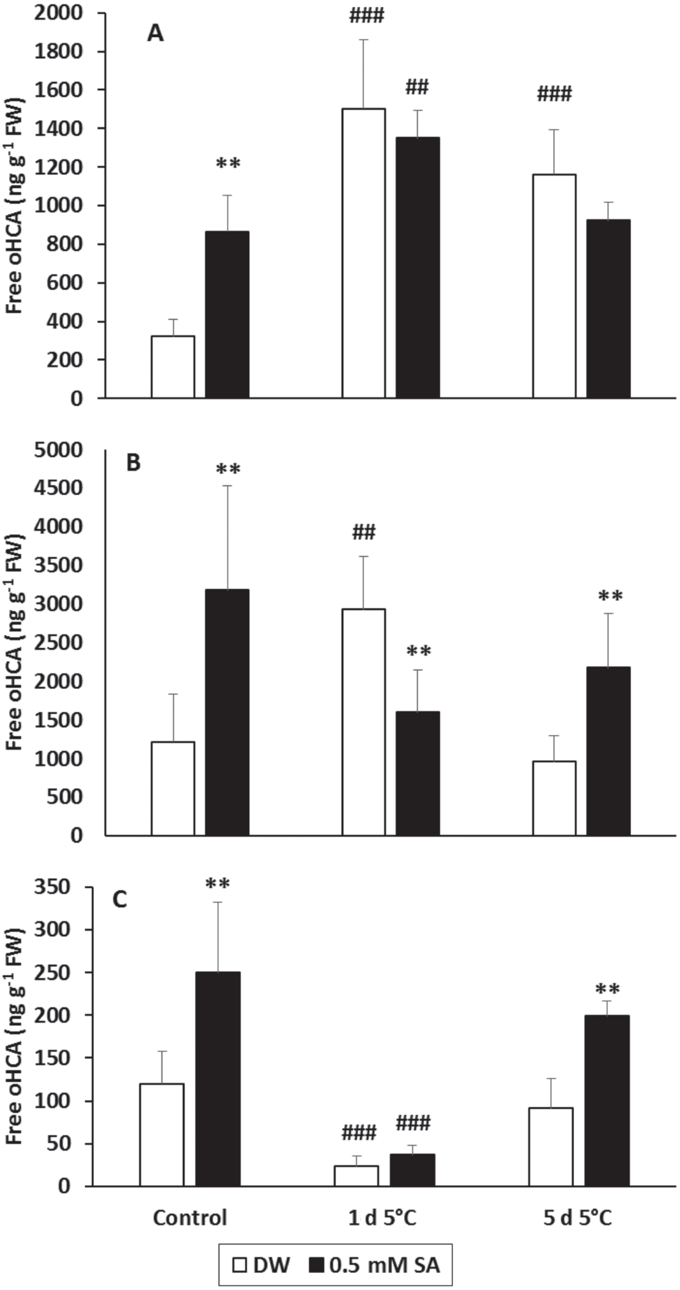

Figure 4. Effects of soaking of maize seed with SA on the free oHCA contents in the leaves (A), seed (B) and roots (C) of young maize plants (hybrid Norma) before and after 1 and 5 days of cold treatment at 5 °C. ** represents significant differences between treatments with SA and distilled water at $p<0.01$ level, ## and ### represent significant differences between the control and cold treated plants at $p<0.01$ and 0.001 levels, respectively

Phytotron experiments

The field data suggested that the beneficial effects of pre-treatments with SA on crop yield were mainly detectable at early sowing. Early sowing increases the chance of the occurrences of chilling injury in young maize seedling. Therefore a phytotron experiment was designed under controlled environmental conditions, where young maize plants (Norma hybrid) were exposed to low temperature stress for 5 d at 5 °C; and the effects pre-treatment of seed with 0.5 mM SA on the changes in the concentration of endogenous SA and its related compound oHCA, as well as the changes in polyamines playing general role in various stress acclimation processes were determined.

Effects of soaking of maize seed in SA solution on the SA and oHCA contents

Concentrations of SA and its putative precursor, oHCA were determined before and after the cold treatment at 5 °C in seed, roots and leaves of young maize seedlings. Interestingly, significant changes in SA contents, either in free or bound forms, could only be detected in the seed of the plants. Neither soaking of seed nor low temperature treatment caused substantial changes in the SA content in the leaves or in the roots (data not shown). In the seed soaking in SA solution significantly increased its level, especially in the bound form; and its elevated level was also detectable after 5 days of cold treatment (Fig. 3). Chilling itself increased the free SA content after 5 days in plants treated either with DW or SA, while this increase in the bound form was only significant in the seeds pre-treated with SA.

Interestingly, not only the levels of SA were modified in seed by SA priming, but also the oHCA content. In contrast to the SA level, free oHCA content substantially increased in all the plant organs in plants primed with SA (Fig. 4). Low temperature also increased the free oHCA levels in the leaves and seed after 1 day, while it was reduced in the roots (Fig. 4). Bound oHCA also substantially increased in the SA-treated seed, it was ca. 4 times higher than in the controls, and this difference was also detected in the cold stressed plants (data not shown). In the leaves and roots bound oHCA did not show significant changes.

Effects of soaking of maize seed in SA solution on polyamine contents

Changes in the PA contents were also followed in control and SA-treated maize plants after 1 and 5 d cold treatments at 5 °C. Soaking of seed in 0.5 mM SA led to an increase in the putrescine (Put) content in the leaves, while the level of spermidine (Spd) decreased in the SA-treated plants (Fig. 5). There were no significant differences in the spermine (Spm) and cadaverine contents between the leaves of the control and SA-treated plants under normal growth temperature (22 °C). Cold treatment for 5 d led to a decrease in the contents of Put and Spd; however, the cadaverine level substantially increased in leaves exposed to low temperature (Fig. 5).

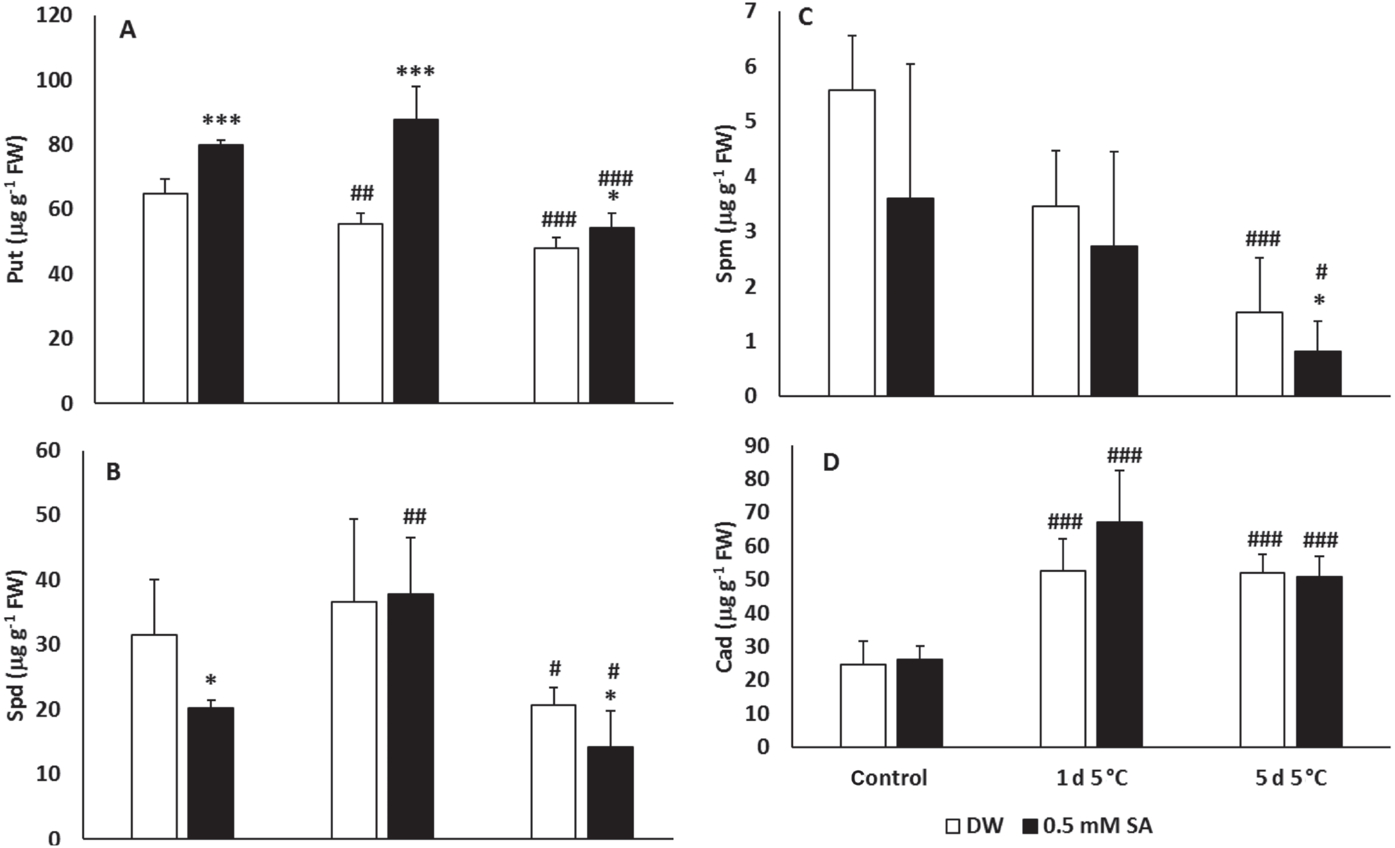

Figure 5. Effects of soaking of maize seed with SA on the free Put (A), Spd (B), Spm (C) and cadaverine (Cad; D) contenst before and after 1 and 5 days of cold treatment at 5 °C in leaves of young maize plants (hybrid Norma). * and *** represent significant differences between treatments with SA and distilled water at $p<0.05$ and 0.001 levels, respectively. #, ## and ### represent significant differences between the control and cold treated plants at $p<0.05$, 0.01 and 0.001 levels, respectively

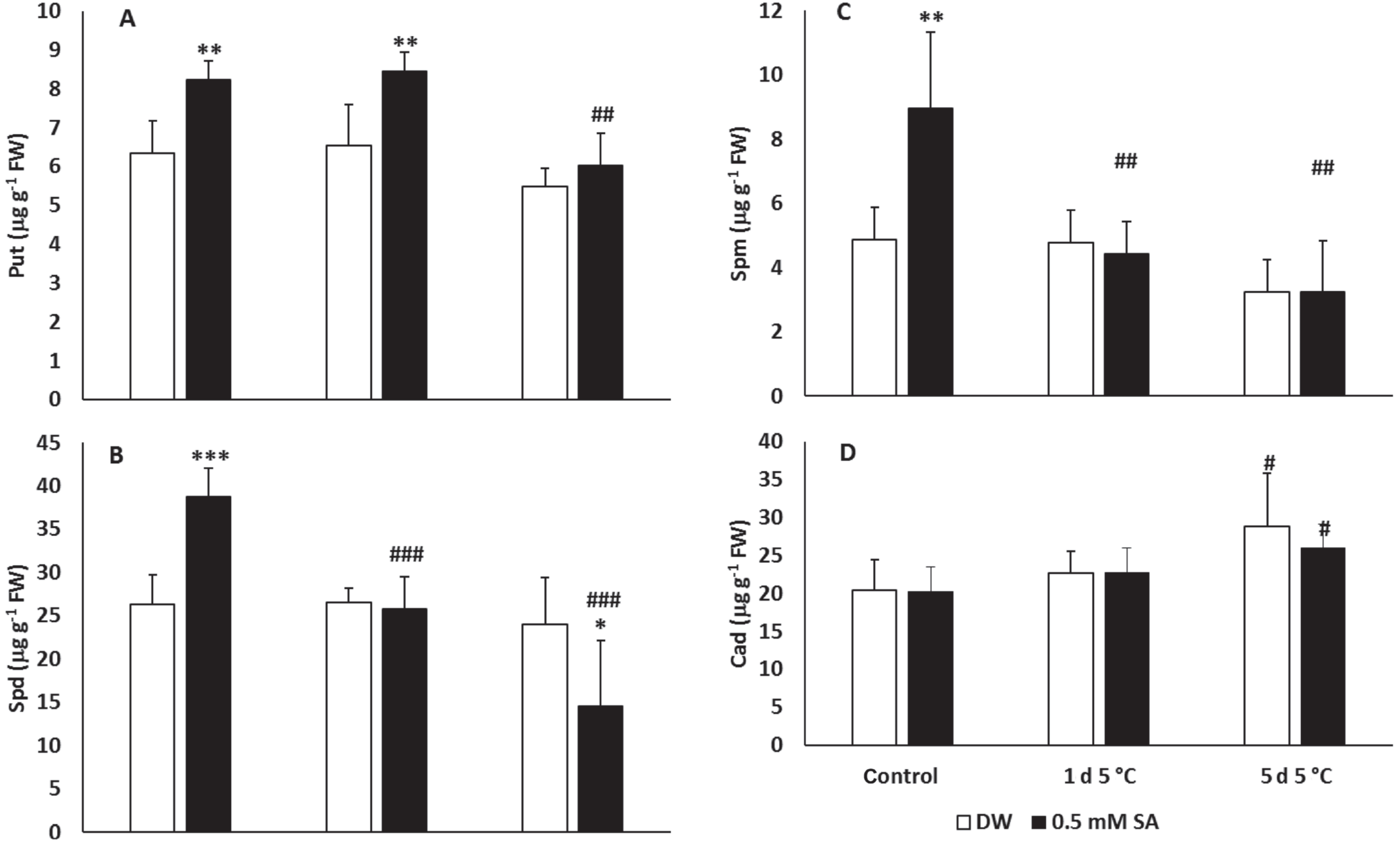

Figure 6. Effects of soaking of maize seed with SA on the free Put (A), Spd (B), Spm (C) and cadaverine (Cad; D) contents before and after 1 and 5 days of cold treatment at 5 °C in roots of young maize plants (hybrid Norma). *,** and *** represent significant differences between treatments with SA and distilled water at $p<0.05$ and 0.001 levels, respectively. #, ## and ### represent significant differences between the control and cold treated plants at $p<0.05$, 0.01 and 0.001 levels, respectively

The contents of Put, Spd and Spm were also higher in the roots of the SA-treated plants under normal growth conditions (Fig. 6). However, although Put level was still higher after 1 day of cold treatment in the SA-primed plants, this increase could not be detected after cold treatment at 5°C for 5 d. In contrast to the leaves, pre-soaking in SA did not significantly modify the cadaverine content in the roots, but severe cold stress slightly increased its level (Fig. 6).

Discussion

Recent results showed that priming of seed with SA effectively improved performance of cereals such as rice (Ruan et al. 2002) or maize plants. In the case of maize it significantly increased the number of grains per cob, the grain yield and harvest index parameters both at optimum and late sowing dates (Rehman et al. 2015). Early sowing in spring season at continental climate may dramatically increase the probability of exposure of plants to low temperatures. Since the beneficial effect of priming with SA was more obvious at early sowing dates, it might also be related to cold acclimation mechanisms induced by SA. It has been shown that SA may provide protection against chilling injury in maize plants when it was added to hydroponic growth solution before exposure chilling stress conditions (Janda et al. 1999). Furthermore, not only SA, but certain related compounds, such as acetyl salicylic acid, oHCA or benzoic acid had similar beneficial effects under low temperature conditions (Senaratna et al. 2000; Janda et al. 2014). However, since the way of application was different, these results cannot be generalised. Under the present experimental conditions, when young maize seedling where exposed to low temperature stress at 5°C under controlled environmental conditions, priming of seed in SA did not significantly modify the SA levels in the roots or in the leaves. The data suggested that SA was taken up by the seed and it was converted to bound forms. These results are in accordance with our earlier findings where it was shown that soaking of pea seed in radioactively labelled SA solution led an increased level of labelled bound SA in the seed, while in the epicotyl the increased level of SA was mainly due to *de novo* synthesis of SA (Szalai et al. 2011).

In contrast to SA, there was a substantial increase in the free form of a putative SA precursor, oHCA in all the organs (roots, seed, and leaves) in plants previously primed with SA. Since low temperature also modified the level of oHCA, the effects of soaking in SA in the cold-stressed plants were not so obvious. Since oHCA has been shown to have an antioxidant property (Foley et al. 1999; Gallardo et al. 2006); and its level substantially increased in cold-hardened wheat plants (Janda et al. 2007), it can be assumed that it may also contribute to the cold acclimation in plants either as an antioxidant molecule and/or as an intermediate regulator of the synthesis of signal molecule salicylic acid. However, exact mechanism and the significance of the contribution of oHCA in acclimation processes have not been made clear yet.

In the present work the changes in polyamine contents were also detected. Polyamines can be found in all living cells, and are generally assumed to act as protective compounds playing role in stress acclimation processes. They directly or indirectly are able to influence several physiological processes, including photosynthesis, the antioxidant system and ion channels (Tiburcio et al. 2014; Pál et al. 2015). Priming of seed with SA increased the level of Put both under control or cold conditions. Low temperature also increased the level of another polyamine, cadaverine, which has been also reported to accumulate in winter wheat during cold hardening (Pál et al. 2015), and has been reported to act as free radical scavenger (Kuznetsov et al. 2007). The major polyamines, Put, Spd and Spm can be converted into each other in the polyamine cycle (Pál et al. 2015). It means that not only their synthesis, but also their degradation catalyzed by polyamine oxidases may determine their role in stress signalling processes. Furthermore, chilling tolerance of maize can be improved by soaking of seed in Put underlining the specific role of polyamines in stress responses (Cao et al. 2008).

In conclusion, it was demonstrated in the present paper that treatment of maize seed with SA before sowing is not only beneficial during the early growth stage of the plants, but it may also significantly increase the grain yield under natural field conditions. At continental climate this advantage was mainly detectable at early sowing, when the probability of chilling injury is higher. The results also suggest that the polyamine metabolism and the synthesis of certain phenolic compounds, such as oHCA may also contribute to the beneficial effects of priming with SA.

Acknowledgements

This work was supported by grants from National Scientific Research Fund (OTKA K101367). Thanks are due to the Department of Maize Breeding at MTA ATK in Martonvásár for providing maize seeds for the experiments.

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