EXPERIMENTAL ANALYSIS FOR AFFECTING THE EXOGENOUS SALICYLIC ACID ON DROUGHT TOLERANCE IN MAIZE IN IRAN

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Abstract

In a previous work, it was shown that 5.5 mM salicylic acid (SA) added to corn hydroponic solution increased its tolerance to low temperature stress. The effect of SA and cold treatments on the polyamine content in leaves was investigated using HPLC method. Both 0.5 mm SA and cold treatment significantly increased putrescine content. Spermidine increased only when it increased in addition to temperature stress. Sperm content decreased after both SA and low temperature treatments. The parallel use of 0.5 mM SA and 15 mM PEG significantly increased electrolyte leakage and decreased the specific photosynthetic parameters in maize and wheat. It is concluded that 0.5 mm SA pretreatment, which enhances cold tolerance, increases susceptibility to drought. The experiments were conducted at the Azad University of Firuzabad.

1 INTRODUCTION

The role of salicylic acid (SA) in defense mechanisms against pathogen attack has been known for several years [2,3]. In recent years its role has been widely investigated in both biotic and abiotic stresses. It was also reported that SA accumulated during exposure to ozone or UV light [4,5]. SA ameliorated the damaging effects of heavy metals on membranes in rice [6] and improved the heat-shock tolerance of mustard (Sinapisalba L.) [7,8] and tobacco plants [9]. Acetyl SA (aspirin) induced heat tolerance in potato microplants [10] and decreased the inhibitory effect of drought [11] and salt stress [12] in wheat. It has already been shown that salicylic acid may alter the synthesis of the plant hormone ethylene in maize [13]. The addition of salicylic acid [1] or certain related compounds [14] to the hydroponic solution of maize plants (Zea mays L.) reduces the effect of low temperature stress. Polyamines are essential components of living organisms and their role in the physiological and biochemical processes has long been studied [15]. It was shown in several cases that stress tolerance is associated with changes in the polyamine metabolism [16]. An increase in the endogenous polyamine

content was reported during low temperature stress [17], especially when chilling was accompanied by light stress [18]. The role of polyamines in osmotic and salinity stresses [19,20] and in frost hardening [21] is also well documented. Besides low temperature, drought is one of the most important limiting factors of crop production all over the world. The aims of the present work were: (1) to investigate the effect of SA pre-treatment on the polyamine content in maize; (2) to discover whether SA was able to induce drought tolerance in maize and wheat plants under the same conditions where it induces chilling tolerance.

2 MATERIALS AND METHODS

Sterilized seeds of maize (Z. mays L., hybrid Norma) and wheat (Triticumaestivum L., Mv Emma) were germinated for 4 days at room temperature, then grown in Hoagland's solution [22] at 22/20 8C with a 16-h light:8-h dark period in a Conviron PGR-15 plant growth chamber in a phytotron. The irradiance at leaf level was a constant 250 mmol m2 s 1, provided by metal halide lamps, and the relative humidity was75%. Before the cold treatment (5 8C for 3 days) and drought stress (15% PEG-6000 for 3 days at 22/20 8C) the plants were treated with 0.5 mM salicylic acid for one day. The polyamine content was analyzed after a 3-day chilling period. Photosynthetic parameters and electrolyte leakage were measured after the 1st, 2nd or 3rd day of drought stress.The youngest fully developed leaves were chosen for net photosynthesis measurements, which were made on attached leaves using a LI-6400 infrared gas analyzer (LICOR, Lincoln, NE, USA) operated with a 6400-02 LED light source (LICOR) providing 250 mmol m2 s 1 PPFD. The measurements were carried out at 22 8C. Gas exchange parameters were calculated according to von Caemmerer and Farquhar [23]. The results are the means of at least four measurements. Electrolyte leakage was measured according to Szalai et al. [24] with slight modifications. Leaf disks cut to a size of 5 mm in diameter from maize or of approximately 1 cm from wheat plants were placed three to a vial in 1.5 ml ultrapure water. After 1 h, the conductivity was measured and the samples were frozen at /70 8C for 1 day to achieve 100% electrolyte leakage. The results are the mean of ten measurements.Two hundred milligrams of leaves were homogenized with 1 ml 0.2 M ice-cold perchloric acid and were allowed to stand for 20 min on ice. The extract was centrifuged at 10 000/g for 20 min and the supernatant was used. Polyamines were analyzed as dansylated derivatives via HPLC using a W2690 separation module and a W474 scanning fluorescence detector (Waters, Milford, MA, USA) as described by Smith and Davies [25]. The results are the mean of at least four measurements.

3 RESULT

Effect of SA pre-treatment on polyamine content in maize.
Young maize plants grown in hydroponic solution were treated with 0.5 mM SA at growth temperature (22/20 8C) for 1 day followed by a 3-day low temperature stress at 5 8C. The amount of polyamines was determined before and after the cold treatment in the control (no addition) and the SA pre-treated plants. As the result of 1 day of salicylic acid treatment the putrescine level almost tripled at 22/20 8C and remained at this level even after 3 days of chilling treatment at 5 8C. In plants not treated with

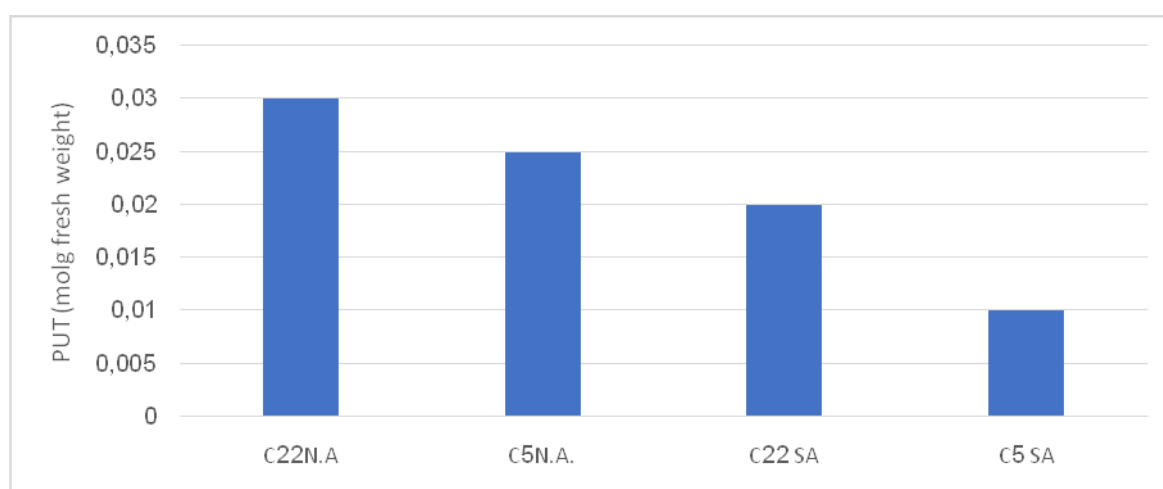salicylic acid the putrescine level was approximately doubled after cold treatment (Fig. 1).

Figure 1: Changes in polyamine content (putrescine) in young maize (Norma hybrid) plants after a 3-day low temperature stress (5 8C) with or without a 1-day 0.5 mM SA pretreatment at 22/20 8C. (N.A., No addition. **, ***, significant at the P 50.01 and 0.001 levels, respectively, compared with N.A., unchilled plants).

Neither low temperature treatment (3 days, 5 8C), nor 1 day SA treatment at the growth temperature caused a significant change in the spermidine content in young maize plants. However, the spermidine content showed an approximately 50% increase in plants which were chilled after a 1 day SA pre-treatment (Fig. 2).
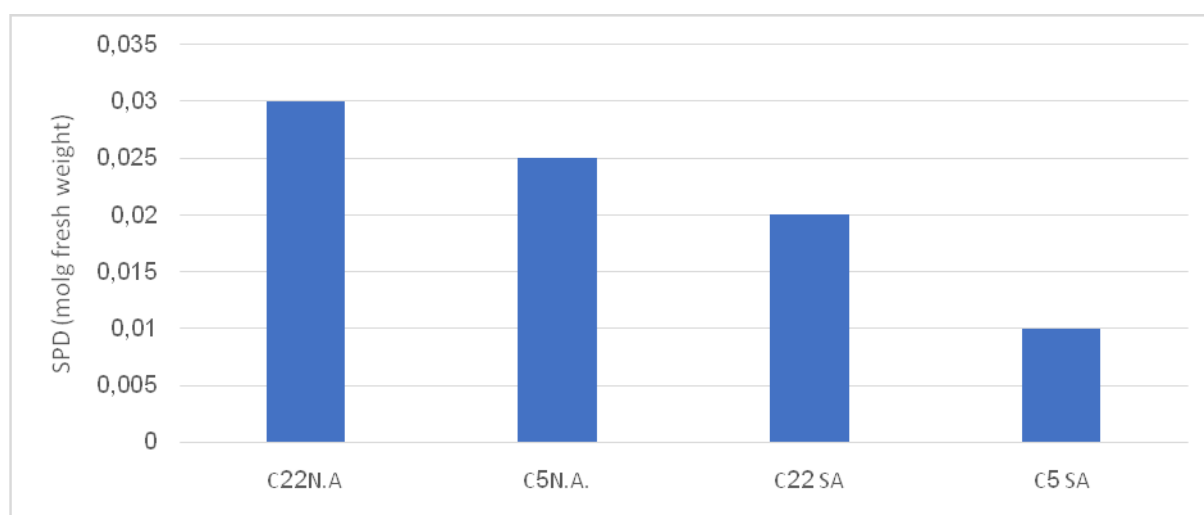

Figure 2: Changes in polyamine content (spermidine) in young maize (Norma hybrid) plants after a 3-day low temperature stress (5 8C) with or without a 1-day 0.5 mM SA pretreatment at 22/20 8C. (N.A., No addition. **, ***, significant at the P 50.01 and 0.001 levels, respectively, compared with N.A., unchilled plants).

The spermine content in the plants is much lower than that of putrescine or spermidine. Both chilling stress and SA pre-treatment caused only a slight, statistically nonsignificant decrease in spermine content. However, similarly to the change in the

spermidine content, the spermine level changed by more than 50% in plants where the chilling treatment followed SA pre-treatment (Fig. 3).
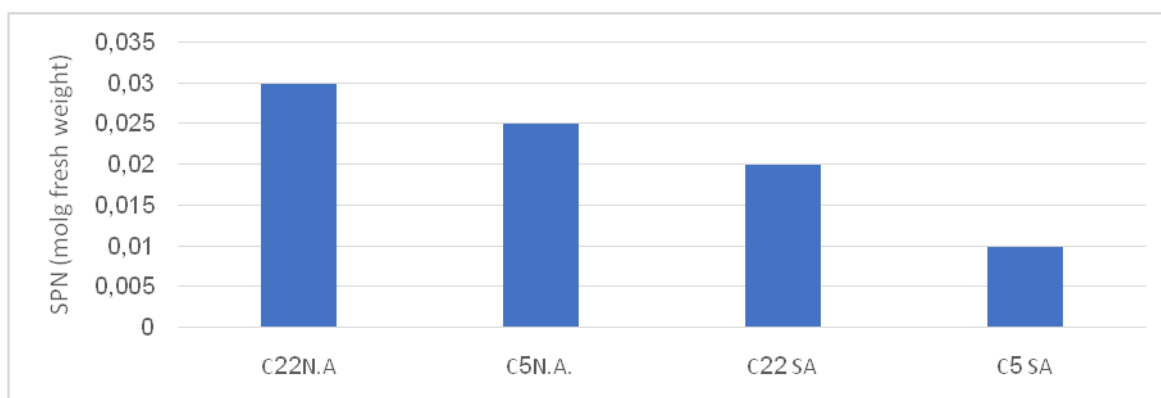

Figure 3: Changes in polyamine content (spermine) in young maize (Norma hybrid) plants after a 3-day low temperature stress (5 8C) with or without a 1-day 0.5 mM SA pretreatment at 22/20 8C. (N.A., No addition. **, ***, significant at the P 50.01 and 0.001 levels, respectively, compared with N. A., unchilled plants).

Effect of SA treatment on drought tolerance in maize.

In order to test the drought tolerance of young maize plants grown in nutrient solution the plants were treated with 15% PEG for 3 days. Some of the plants received pre-treatment with 0.5 mM SA prior to the PEG treatment. As the result of the 0.5 mM salicylic acid treatment the maize plants exhibited an approximately 25% reduction in net photosynthesis (averaged over four measurement times). The 15% PEG treatment initially caused a similarly small reduction, but by the 3rd day this decrease became substantial (around 75%; Fig. 4). When the two compounds were used in combination this drastic decline was already noticed on the 1st day (Fig. 4). The changes were correlated to a reduction in the conductivity of the stomata (Fig. 5).
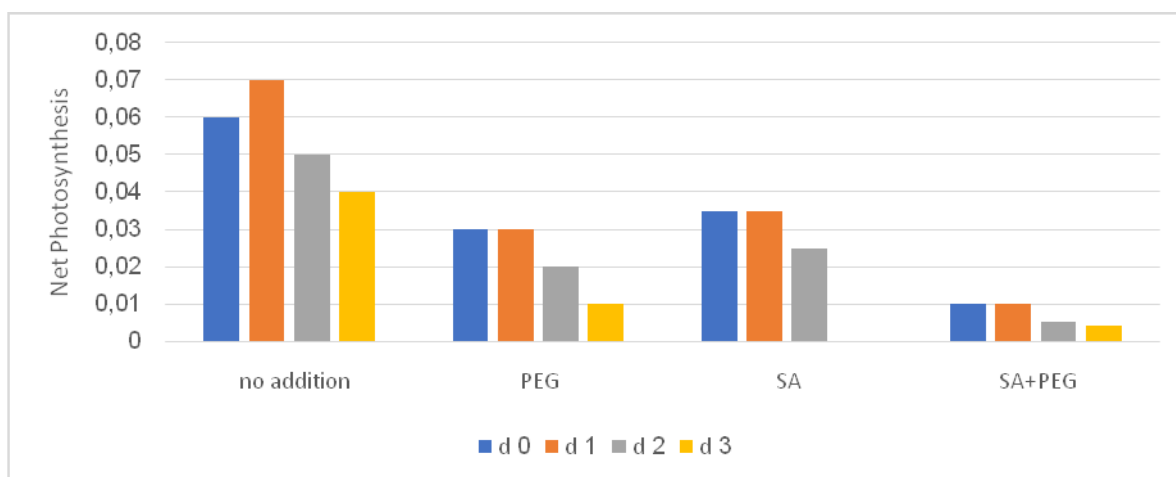

Figure 4: Effects of treatment for 3 days with 15% PEG-6000 (PEG), for 1 day with 0.5 mM hydroponic salicylic acid (SA) or for 1 day with SA followed by 3 days with PEG (SA+PEG) at 22/20 8C on the net photosynthesis in young maize (Norma hybrid) plants. The measurements were carried out under light-saturated (PPFD+1500 mmol m2 s 1) conditions at 22 8C. (n5; vertical bar represents the least significant difference at the 5% level).

Electrolyte leakage was also determined after the 3rd day of the PEG treatment. The slight increase in the electrolyte leakage from plants treated with either 0.5 mM SA or 15% PEG was not statistically significant (Fig. 4A), but a significant deviation could be observed in plants treated with both salicylic acid and PEG, the greatest electrolyte leakage being registered for maize plants.
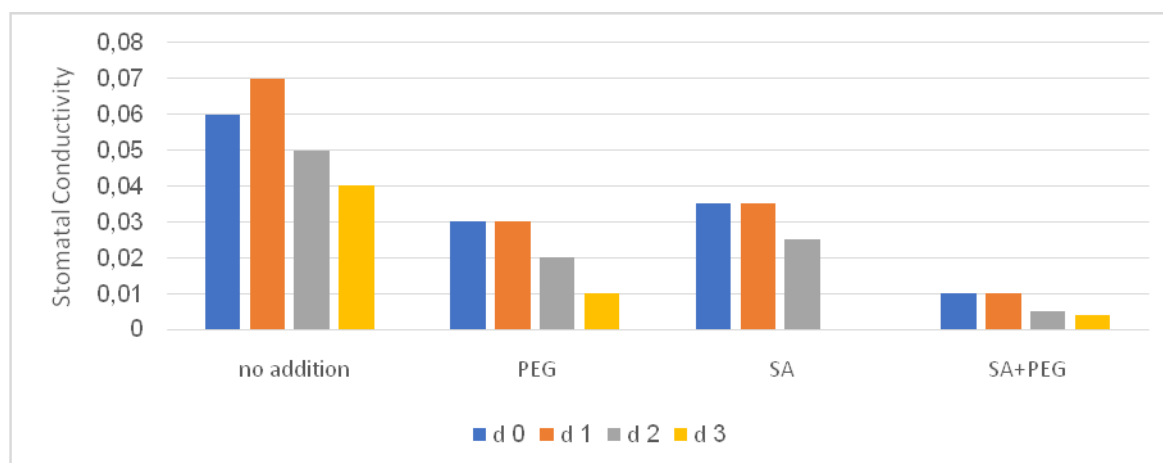

Figure 5: Effects of treatment for 3 day with 15% PEG-6000 (PEG), for 1 day with 0.5 mM hydroponic salicylic acid (SA) or for 1 day with SA followed by 3 day with PEG (SAPEG) at 22/20 8C on the stomatal conductivity in young maize (Norma hybrid) plants.

The measurements were carried out under light-saturated (PPFD1500 mmol m2 s 1) conditionsat 22 8C. (n5; vertical bar represents the least significant difference at the 5% level).

4 CONCLUSION AND DISCUSSION

It was previously shown that the pre-treatment of young maize plants with 0.5 mM salicylic acid increased their chilling tolerance [1]. A similar protective effect was achieved with the same concentration of other related compounds, such as acetyl-salicylic acid (aspirin) and benzoic acid [14]. It was also reported that growing tobacco plants on medium containing salicylic acid caused an increase in their thermolerance [9]. Protection from high temperature was also observed in mustard seedlings treated with salicylic acid [7] and potato microplants grown on acetyl-salicylic acid [10]. In all of these cases, increased antioxidant activity was shown, which was assumed to be responsible for the increased tolerance either to heat or cold [1,8,9].

Several data support the view that polyamines (putrescine and its derivatives) may have a function in the response to environmental stresses [16]. The first aim of the present study was to investigate whether polyamines might play a role in the increased cold tolerance caused by exogenous SA treatment. Both low temperature stress and SA pre-treatment at the growth temperature caused a significant increase in the putrescine level. About 3 days at 5 8C did not cause any further increase in the putrescine content in the SA treated plants. An increase in the putrescine level has been shown after several types of stresses, such as chilling in fruits and vegetables [17,26] or osmotic and salt stresses [19,20,27]. It was also shown that low temperature-induced photoinhibition also contributed to the increased putrescine level during chilling stress in maize [18]. A

marked putrescine accumulation was also observed in wheat varieties during low temperature hardening [21]. During chilling tolerance, putrescine was able to bind to antioxidant enzymes, such as superoxide dismutase, or to be conjugated to small antioxidant molecules, allowing them to permeate to the sites of oxidative stress within the cells [16].

It is assumed that polyamines inhibit chilling injury by retarding lipid peroxidation and preserving membrane integrity [28,29]. In zucchini squash, low temperature preconditioning was accompanied by increased spermidine and spermine contents [17]. In maize plants it was shown in a previous study that the spermidine content decreased during low temperature stress in the dark. In the light this decrease was preceded by a transient increase [18]. In the present study the spermidine content was the same in both cold and SA treated plants as in the untreated controls, but increased when the salicylic acid treatment was followed by low temperature stress. Spermidine and spermine might interact with membranes either by inhibiting the transbilayer movement of phospholipids [30], or by stabilizing molecular complexes of thylakoid membranes [31,32]. The results suggest that polyamines, i.e. the increased putrescine and spermidine level, may also contribute to the increased chilling tolerance induced by salicylic acid, although it would be hard to estimate the level of this contribution. Several changes reported due to SA treatments (e.g. increased antioxidant activity, polyamines) are also involved in protection against osmotic and salt stresses [16,20,33]. Therefore, drought stress was induced using PEG under conditions similar to those where SA induced chilling tolerance in maize. Both the SA and PEG treatments caused a slight decrease in the net photosynthesis. However, in plants which underwent both treatments, a dramatic decrease could be observed. This decrease correlated with the decrease in the stomatal conductivity. The electrolyte leakage did not change significantly either in PEG or SA treated plants under these conditions. However, in plants where SA treatment was followed by PEG treatment the electrolyte leakage significantly increased, showing serious membrane damage. The results suggest that the salicylic acid pre-treatment, which was able to increase the chilling tolerance of maize, was not effective against drought stress; in fact, the SA pre-treated maize plants suffered more from drought than the untreated ones. These results are in good agreement with experiments demonstrating that transgenic Arabidopsis plants (NahG) producing salicylate hydroxylase, which transforms SA to catechol, were better able to resist the oxidative damage generated by salt and osmotic stress than the wild type plants [34]. It can be concluded from the results that salicylic acid may increase the polyamine content, which may have a role in increased cold tolerance. However, the same SA treatment which was shown to increase chilling tolerance in maize caused increased sensitivity to drought in both maize and wheat.

5 ACKNOWLEDGEMENTS

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