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The effect of maize priming on germination characteristics, catalase and peroxidase enzyme activity, and total protein content under salt stress

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Abstract

We aimed to investigate the effect of maize priming on germination characteristics, catalase and peroxidase enzyme activity and foliar protein content under salt stress. A twofold experiment with a randomized factorial design and 3 replications was conducted. Maize characteristics such as final germination percentage (FGP), germination rate (GR), germination index (GI) and vigor index (VI), and weight of seedlings and radicals were recorded. Then, the effect of priming and salt stress on the antioxidant enzymes such as catalase and peroxidase and the total protein content were investigated in the fourth leaf. We found that salicylic priming and salt stress influenced FGP, GR, GI, VI, the fresh weight of seedlings and radicles, catalase and peroxidase enzyme activity and the total protein content. The highest amount of FGP, GR and GI were related to seeds primed with 1 and 2 mM salicylic acid planted under stress-free conditions. The seeds primed with 1 and 2 mM salicylic acid and placed in 1% salt stress showed the highest VI. Moreover, the highest fresh weight pertained to the seeds primed with 2 mM salicylic acid and placed in 2% salt condition. Seeds which were not pretreated with salicylic acid and were planted under salt stress of 1.5 g/kg wet soil demonstrated the highest catalase and peroxidase activity. The seeds in the control group (not treated with salicylic acid and NaCl) had the highest total protein content.

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Introduction

Saline soil is considered as a restrictive factor in agricultural areas around the world. In saline areas the plant's ability to absorb nutrients is influenced dramatically because of specific chemical conditions and high concentrations of elements such as sodium and chloride. In order to survive, the plants avail themselves of various morphological, physiological, and molecular mechanisms (Bohnert *et al.,* 1995).

Salt stress can negatively affect growth by increasing the concentration of various active oxygen in cells and damaging the plant's membrane lipids, proteins, and nuclic acids (Noctor & Foyer, 1998). The plant's antioxidants neutralize free radicals such as ascorbic acid, tocopherol and glutathione (Zhang & Kirkham, 1996). Seed priming is used to decrease such damages to the plant (Harris, 2005; Khodary, 2004). Priming refers to the seed treatment before sowing, in which the seeds pass the first stages of germination, but the radicle does not emerge because of insufficient amounts of absorbed water (Nascimento & Araga, 2004). This is a physiological mechanism which leads to higher rates of seed germination. In this method, the transfer of stored substances, activation and synthesis of some enzymes, RNA and DNA synthesis, generation of ATP, and cytoplasm membrane improvement are observed in the seeds (Hoseini and Kuchaki, 2009). However, whether the increase in germination rate will improve the subsequent growth and establishment of the plant it is yet to be investigated (Tari *et al.,* 2002). Therefore, other effective factors on plant growth should be considered to assess the effect of hydro-priming and osmo-priming on the physiological improvement of seeds.
The faster germination rate of primed seeds could be due to their increased enzyme activity. Enzymes are a group of proteins that have an activating role in the main metabolic routes of plants. They are identified as the most important proteins in the metabolic mechanisms of different organisms. Moreover, they can be studied in different organisms ranging from unicellular organisms to the most sophisticated creatures (Parhizgar, *et al.,* 2000). Peroxidase is one of the oxidoreductase enzymes which defends plants against external factors and contributing to detoxification of different forms of active oxygen (Marjamaa *et al.,* 2003). This enzyme also reflects the plants metabolic response to most stressors. Alterations in peroxidase activity are faster and more radical in strong trees compared with weaker ones (Parhizgar, *et al.,* 2000). Some researchers have found a relationship between alterations in enzyme activity and plant behavior. However, more studies should be done on enzymes sensitivity to specific environmental factors.

Salicylic acid is a natural compound used in the seed priming, and plays an essential role in the plant's resistance against environmental tension (Raskin, 1992). It can influence germination, ion exchange and transfer, penetrability of the membrane, photosynthesis and growth of plants (Khodary, 2004). Salicylic acid increased dry weight, germination percentages, chlorophyll (a+b) content, while reduced accumulation of proline and lipid peroxidation rates with increasing antioxiant activity. These results demonstrate that salicylic acid is very effective in strengthening the tolerance of chickpea (Asadi *et al.,* 2013). In conclusion seed priming by salicylic acid improved the germination, but this increase was not significant compared to control treatment (Habibi & Abdoli, 2013).

Motivation of the study is assess the effect of pre-treatment with salicylic acid on germination, growth, peroxidase and catalase enzyme activity, and foliar protein content of maize seedlings cultivated under salt stress.

Materials and methods

A two-fold experiment with a randomized factorial design with 16 treatments and 3 replications was conducted in the physiology laboratory of the Faculty of Agriculture of Azad University of Shiraz, Shiraz, southern Iran.

Pre-treatment with Salicylic Acid and Growth under Salinity Stress

Maize (*Zea mays* L.) variety 704 was disinfected for three minutes using 5% sodium-hypochlorite solution. The maize seeds were then washed by distilled water and soaked in the solutions with different concentration of salicylic acid (0, 1, 2, 3 mM) for 24 hours. After being washed by distilled water, the seeds were completely dried using on sterile paper and 50 seeds were transferred to the petri dish with a sterile paper at the bottom. Moreover, sodium chloride was used at different concentrations (0, 1, 2, 3 mM), 10ml for each petri dish. The dishes were sealed with parafilm and were transferred to the germinator at 25^{oc}. Germination was considered as at least 5 mm growth and emergence of radicles and seedlings. The number of germinated plants was counted every day after the onset of the experiment. Seven days into the experiment, the fresh weight of the radicles and seedlings were recorded. The other indices related to the seed germination were calculated as follows:

Final Germination Percentage (FGP):

FGP=

$$\frac{\sum \quad (\text{the number of germinated seeds up to the day}(i) * 100)}{\text{the total number of seeds}}$$

Germination Rate (GR):

GR= ∑ the number of germinated seed up to the day (i) / I

In which "I" is the number of days passed from the onset of the experiment.

Germination Index (GI):

GI= $(g_n \times i_1) + (g_{n-1} \times i_2) + ... + (n - (n_{-1}) \times i_n)$

In the above equation, "I" is the last day when all the seeds germinated Vigor Index (VI):

VI = [seedling length (cm) × germination percentage] / 100.

Biochemical Analysis of Response to Salinity Stress

In the next phase of the experiment, ten seeds pre-treated with 0, 1, 2, and 3 mM salicylic acid were planted in vases with a diameter of 25 cm and a height of 20 cm. The seeds were planted 2 cm deep into the soil. For each vase, soil, sand and manure were combined with the ratio of 6, 3, and 1, respectively. Different levels of salinity (0, 0.5, 1, 1.5 gr NaCl/kg soil) equivalent to electrical conductivity of 3.24, 5.92, 7.40, 10.20 dS/m were used.

In order to measure the enzymes' activity and the foliar protein content in the fourth leaf stage, some leaves were cut from each vase. The activity of the peroxidase enzyme was measured based on the method proposed by Ghanati and colleagues (2002). Twenty milligrams of the fresh tissue was powdered using liquid nitrogen and was concentrated at 0-4^{oc} in 0.02 M potassium phosphate buffer (pH: 6.8). The produced solution was centrifuged for 15 minutes at 2-4^{oc} at 12000 rpm. Enzyme activity was calculated using the spectrophotometer (Model LABoMeD, INC. UVD-2960), which consider the changes in the amount of absorption per milligram of protein. In so doing, adequate amount of enzyme concentrate, buffer solution, guaiocole with final molar mass of 28 and hydrogen peroxidase with final molar mass of 5 were added.

The method proposed by Cakmak & Horst (1991) was used to measure the catalase enzyme activity. 20 mg of the frozen sample was concentrated in 3ml of 25 M sodium phosphate (pH 6.8). The produced solution was centrifuged for 15 minutes at 4^{oc} at 1500 rpm and the zinc solution was added to measure the catalase enzyme activity. Hydrogen peroxidase decomposition was screened while the absorbance rate decreased at λ=240 nm and was recorded per each milligram of protein in the enzyme concentrate.

The amount of protein contained in the fourth leaf was calculated using the Bradford technique (1976). 1ml of Bradford solution and 100µl of the enzyme concentrate were combined completely and transferred to the spectrophotometer. The solution's absorbance rate was recorded at λ=595nm. The protein density was measured using the standard curve based on mg/g fresh tissue.

Statistical Analysis System (SAS) software and analysis of variance (ANOVA) were used to analyze the data. Mean comparisons were done using Duncan's multiple range tests at a significance level of 5%.

Results

Mean comparisons showed that the seeds primed with 1 and 2 mM concentrations revealed the highest FGP, GR, GI and VI. Moreover, the highest fresh weight for radicles and seedlings was achieved using 2 mM of salicylic acid as pre-treatment.

We also found that the seeds in the control group that were under no salinity stress had the highest FGP, GR, and GI compared with those under stress. As the sodium chloride concentration increased all of the above mentioned characteristics decreased. The highest radical VI (30.85) and fresh weight (0.024 g) was observed in the seeds placed in the 1% sodium chloride. However, the seeds grown in the 2% sodium chloride showed the highest fresh weight for the seedlings.

Table 2. Interaction effects of salicylic acid and sodium chloride on the evaluated traits.

salicylic acid (mM)	NaCl (%)	FGP (%)	GR (%)	GI	VI	fresh seedling weight (g)	fresh radicle weight (g)
0	0	85.33bc	426.70bc	287.00bc	21.55cdef	0.045i	0.018bcd
	1	79.33cd	396.70cd	280.70bc	34.10a	0.108de	0.020bc
	2	64.00fg	320.00fg	152.30ef	21.72cdef	0.122bc	0.018bcd
	3	47.33h	236.70h	121.00f	9.27g	0.088fg	0.011cde
1	0	94.00a	470.00a	335.70a	23.75bcd	0.058h	0.012cde
	1	88.67ab	443.33ab	293.70bc	35.25a	0.106de	0.019bc
	2	78.33cd	391.70cd	260.00cd	22.64bcde	0.111cde	0.013bcde
	3	77.67de	388.30de	275.20bc	24.91bc	0.114cd	0.007de
2	0	93.33a	466.70a	343.00a	26.51b	0.084fg	0.014bcde
	1	91.67ab	458.30ab	276.80bc	34.02a	0.127b	0.045a
	2	78.33cd	391.70cd	266.50bc	31.49a	0.142a	0.024b
	3	69.33f	346.70f	231.20d	2.66bcde	0.121bc	0.016bcde
3	0	89.67ab	448.30ab	299.70b	24.33bcd	0.077g	0.009cde
	1	70.33ef	351.70ef	228.80d	20.00def	0.102e	0.012cde
	2	60.33g	301.70g	184.80e	18.21ef	0.091f	0.018bcd
	3	57.00g	285.00g	166.80e	17.84f	0.082fg	0.006e

Means with the same letters in each column are not significantly different at 5% of probability level.

The interaction between different concentrations of salicylic acid and different salt stress conditions suggests that priming the seed with either 1 or 2 mM salicylic acid and avoiding the use of sodium chloride led to an increase in FGP, GR, and GI. Furthermore, hydro-priming and placing the seeds in 3% sodium chloride led to the lowest FGP, GR, and GI rates. Seeds treated with distilled water or primed with 1 or 2 mM salicylic acid had the highest GI, under salinity stress of 1%. Also, the fresh weight for the seeds primed with 2 mM salicylic acid and placed in the 2mM sodium chloride was higher compared with other conditions.

Furthermore, using distilled water as pre-treatment in salt-free conditions significantly decreased the fresh weight of the seedling. The interaction between different concentrations of salicylic acid and sodium chloride showed that the seeds primed with 2 mM salicylic acid could tolerate a sodium chloride concentration of up to 2% and had the highest fresh radicle weight. Increasing the concentration of salicylic acid to 3 mM led to a decrease in the fresh radicle weight. In fact, using this concentration of salicylic acid and the 3% sodium chloride resulted in the lowest fresh radicle weight.

ANOVA results show that pre-treatment with salicylic acid, salt stress, and their interactions have a significant effect on the catalase enzyme activity (P=0.01). Post-hoc analysis of data using Duncan's test shoed that the seeds placed in 1.5 mg NaCl/kg soil and not pre-treated with salicylic acid had the highest catalase enzyme activity. Priming the seeds with 2 or 3 mM salicylic acid under no salt stress yielded the lowest catalase activity.

As shown in table 1, the peroxidase enzyme activity was significantly influenced by the interaction between different concentrations of salicylic acid and sodium chloride (P=0.01). A salt stress equal to 1.5 mg/kg soil without pre-treatment with salicylic acid increased the activity of peroxidase enzyme. Priming the seeds with 1 or 2 mM Salicylic acid under no salt stress led to the lowest peroxidase enzyme activity.

Our findings also suggest that the interactions of different concentrations of salicylic acid and sodium chloride have a significant effect on the total protein content (P=0.01). The control group (untreated seeds) produced the highest total protein content. Moreover, it was shown that salt stress (1.5 mg/kg soil) and priming with different concentrations of salicylic acid decreased the total protein content to the lowest level in the experiment.

Table 2. Interacting effects of salicylic acid and sodium chloride on the evaluated traits.

salicylic acid (mM)	NaCl (mg/kg)	enzymes catalase activity	enzymes peroxidase activity	total protein
0	0	8.09e	172.90e	0.616a
	0.5	8.35e	181.70c	0.602bc
	1	18.72c	234.60b	0.598bc
	1.5	31.51a	293.50a	0.488g
1	0	7.07e	42.20k	0.605b
	0.5	6.34e	68.40j	0.592c
	1	8.91de	103.10i	0.577d
	1.5	12.21d	178.10d	0.485g
2	0	7.28e	164.10g	0.548f
	0.5	7.74e	169.40f	0.561e
	1	8.91de	161.35g	0.566de
	1.5	11.18d	144.70h	0.485g
3	0	9.48de	161.20g	0.531f
	0.5	18.80c	168.52f	0.550e
	1	23.22b	164.61g	0.577d
	1.5	26.11b	152.28gh	0.487g

Means with the same letters in each column are not significantly different at 5% of probability level.

Discussion

It can be inferred from the results that 1 or 2 mM salicylic acid increased FGP, GR, and GI in maize seedlings by decreasing the toxic and destructive effect of salt stress. The mentioned concentrations of salicylic acid had the highest influence on FGP and GR and increasing salicylic acid to 3 mM did not affect FGP and GR.

Previous studies have shown that priming the seeds with different concentrations of plant hormones increases FGP and GR under salt stress and no salt stress conditions (Hurly *et al.,* 1991; Lee *et al.,* 1998). According to Wang *et al.* (2006), salicylic acid is effective in eliminating the oxidative damages in the germination phase and increases the GR. Moreover, it has been found that salicylic acid increases the level of some plant hormones such as auxin and citokinin which are effective in activating germination. In contrast, high concentrations of auxin are reported to impede germination (Shakirova & Sahabutdinova, 2003). Thus, that the reduced FGP and GR in seeds exposed to 3M salicylic acid might be due to the high production of auxin.

Previous studies have also shown that priming is effective in increasing GR of some seedlings, which in turn contributes to their early establishment (Capron *et al.,* 2000; Foti *et al.,* 2002; Khan *et al.,* 2003). Duhal & Bradford (1990) concluded that priming used for tomatoes shorten the time span necessary for the activation of endosperm and increases the embryos ability to absorb water. Moreover, Hurly *et al.* (1991) reported that priming affects the physiological attributes such as sugar level, organic materials and ion concentrations in the seed, radicles, and leaves which finally results in higher GR and GI in inclement weather.

Results also show that priming with 2M salicylic acid under salt-free conditions enhances GR. GR can be used as an apt index of plantlet vigor. Higher GR actually shows a better function on the part of seedling.

Germination, as the first stage of plant development, may be negatively affected by environmental factors. Such factors have given rise to a number of studies conducted in salty soil where the salinity is a common problem for seed germination and early growth of the seedling (Kyungjin *et al.,* 1996). When a special type of seedling starts its enzyme activity and establishes itself in spite of undesirable salinity of the soil, higher density of the plant and improvement in its function will be observed (Asgari & Taghvayi, 1998).

In the shooting stage, among the investigated characteristics, some concentrations of salicylic acid which contributes to vigor index can be used as a pre-treatment to increase the plant tolerance against salinity.

Amid different concentrations of salicylic acid, concentrations of 1 and 2 mM yielded the highest seedling vigor. Researchers have found that primed seeds had a higher VI, which in turn accelerated their seedling growth (Basra *et al.,* 2005). Furthermore, early growth of seedling will save it from undesirable environmental conditions and increase the probability of its survival.

Although seeds primed with 1 and 2 molar concentrations in no salt stress had a higher GR, the highest fresh weight of the seedling was observed under 2% salt stress conditions. This suggests that high GR will not necessarily lead to a higher fresh weight and other factors will be effective as well. Faridudin *et al.*, (2003) reported that carbonic anhydrase and photosynthesis speed increased in mustard (Sinapis alba L.) treated by salicylic acid. Shekari *et al.,* (2010) found that priming could boost the number of mesophyll cells, concentrations of chloroplast, and the amount of chlorophyll and thus decrease light wastage, which will lead to higher fresh weight of the seedling.

The results show that priming with salicylic acid did not significantly affect the fresh weight of radicles. In other words, seed pre-treatment had a better effect on the growth of seedling than that of the radicle. Harris *et al.,* (1999) found that primed maize, rice, and white pea would improve germination and other related factors under salt stress conditions.

The metabolic changes such as modification in enzyme activities result from osmosis tension and ionic imbalance which are caused by salt stress (Bray, 1997). Furthermore, environmental tensions such as salt stress lead to oxidative tensions which impede the plant growth (Smirnoff, 1993). In our study, salt stress boosted the catalase activity while 1 and 2 mM salicylic acid had a negative effect and decreased the enzyme activity. This is because salicylic acid is a hormone-like combination which can play the role of an internal regulator in the plant defense mechanism (Szalai *et al.,* 2000).

The results show that salt stress enhances peroxidase enzyme activity which has a significant role in reacting against non-biological tensions such as salinity. Salicylic acid activates antioxidant enzymes directly or indirectly and can also function as an electron substrai for catalase and peroxidase. Janda *et*

al., (1999) reported that pre-treatment with salicylic acid enhances antioxidant activities in maize. Thus, it can be concluded that salicylic acid can counteract toxic and destructive effect of salt stress in the plant.
In our study, the control group (untreated seeds) produced the highest total protein content. Moreover, salt stress and priming with different concentrations of salicylic acid decreased the total protein content to the lowest level in the experiment. Free radicals produced under salt stress conditions may damage the proteins and decrease its content (Noctor & Foyer, 1998). Protein content is related to the discrepancy between its synthesis and decomposition. Several researchers have reported a decrease in the amount of protein and an increase in nitrate, ammonium, and free amino-acids under salt stress (eg. Yonis *et al.,* 1993). The decrease in the protein content in the salt stress condition can be due to a decline in nitrate, reductase, glutamine synthetaese, and 2-oxoglutrate aminotransferase enzyme activity.

Conclusion
In brief, the SA treatment reduced the damaging action of salinity on seedling growth and accelerated a restoration of growth processes.

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