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Salicylic acid induced changes in some physiological parameters in chickpea (*Cicer arietinum L.*) under salt stress

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Seed priming with hormones has been an efficient method for increasing seed vigor as well as seedling growth under Salinity conditions. The effect of seed presowing treatment with salicylic acid (1.5 mM and 3 mM) on growth and some physiological parameters under salt stress (NaCl 100 mM) in chickpea was studied. Results indicated that salinity caused a reduction in the germination percentages, chlorophyll (a+b) content and dry weight. Salinity also significantly increased the accumulation of proline and lipid peroxidation rates while salicylic acid treatment reduced lipid peroxidation content in chickpea. Results showed that exogenous of salicylic acid alleviated the toxic action induced by salinity. 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.

Key words: Salinity, Salicylic acid, Germination percentages, Chlorophyll (a+b) content, Proline
Abbreviations: SA, salicylic acid; MDA, malondialdehyde; ROS, reactive oxygene species; *Chl* a+b, chlorophyll (a+b)

Introduction

Salinity is the most important limiting factor for crop production and it is becoming an increasingly severe problem in the world (Ahmad *et al*., 2002; Bamidele *et al*., 2007; Bhadauria and Kumar, 2006; Nabipour *et al*., 2007; Hussein *et al*., 2008; Al-Busaidi *et al*., 2009; Asik *et al*., 2009; Brahim *et al*., 2009; Bybordi and Ebrahimian 2011; Nivas *et al*., 2011; Dkhil and Denden, 2012; Hussein *et al*., 2012). Salinity is a major problem in agriculture. The

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adverse effects of high concentration of salts for plants are due to the osmotic retention of water. The plants under salinity condition change their metabolism to overcome the changed environmental condition. Hasegawa *et al*. (2000) reported that proline is most common osmolyte in plants under stress conditions. Hernandez *et al*. (1995) reported that production of reactive oxygen species (ROS) is a major damaging factor in plants exposed to different environmental stresses, including salinity. In addition, Wang *et al*. (2001) reported that Salt stress-induced imbalance in the hormonal levels of plants is well known. Salinity at seedling stage reduction germination percentage, fresh and dry weight of shoots and roots. Recent studies show that SA is involved in the plant responses to salt and osmotic stress (Borsani *et al*., 2001).

Physiological treatments to improved seed germination and seedling emergence under various stress conditions have been intensively investigated in the past two decades (Bradford, 1986). Singh and Dara (1971) reported that presoaking seeds with optimal concentration of phytohormones has been shown to be beneficial to growth and yield of some crop species growth under saline conditions by increasing nutrient reserves through increased physiological activities and root proliferation. Salicylic acid (SA) has received much attention due to its association with economically important plant responses to diseases and other stresses. Koch *et al*. (2000); Borsani *et al*. (2001); Nemeth *et al*. (2002); Clarke *et al*. (2004) reported that salicylic acid involved in responses to abiotic stresses, such as ozone, salt and osmotic stress, drought and heat.

Farooq *et al*. (2006) investigated the possibility of seed invigoration by seed treatments with salicylate and ascorbate in coarse and fine rice. Results showed that ascorbate was more effective in vigor enhancement, salicylate also improved the germination rate and seedling growth. Therefore, this work was conducted to investigate the capability of salicylic acid to counteract the adverse effects of salinity stress on chickpea.

Materials and methods

The investigation was conducted in season 2011- 2012. The seeds of chickpea were obtained from Iran. The seeds were sterilized by using 30% hypochlorite for 5 minutes and then washed 3 times with distilled water. For priming, seeds were soaked in aerated solutions of salicylic acid (1.5 and 3 mM) for 24 h. After soaking period the seeds were air dried. Primed seeds were sown in plastic pots filled with soil composed of clay and sand (5 seeds in each pot). The plastic pots were divided into two sub-groups; the pots of the first subgroup were irrigated with normal water only to serve as control, while the pots of second sub-group were irrigated with 100 mM NaCl. Day and night lengths were 19/20 h and relative humidity was maintained at 65%. The plants

were uprooted 25 days after planting. Experimental treatment consisted of: control, 1.5 mM SA, SA, SA (1.5 mM) +NaCl (100 mM) and SA (3 mM) +NaCl (100 mM). Germination percentages was estimated after 14h using radicle protrusion (appearance of radicle 2 mM in length) as a criterion (Gill et al., 2003). The proline content of samples was measured according to Bates et al. (1973) method. Total chlorophyll (a+b) content was measured by chlorophyll meter (SPAD-502, Minolta Co. Japan) which is presented by SPAD value. Average of 3 measurements from different leaves was considered.

Oxidative damage to lipids was measured based on the method of Heath and Packer (1968). To determine dry weight; the freshly harvested roots and shoots were dried in an aerated oven at 80°C until constant weight. The samples were ground into fine powder and stored in sealed glasses at room temperature for the chemical analysis. Analysis of variance was performed on the data collected using the general linear model (GLM) procedure of the SPSS software (Version 16, IBM Inc.). The mean separation was conducted by tukey analysis in the same software ($p= 0.05$).

Results and discussions

The effect of different levels of SA on the dry matter, germination percentages, MDA and proline content and *Chl* a+b content are presented in table 1. The increasing SA levels from 1.5 mM to 3 mM increased germination percentages of test plants ($p<0.05$). Salt stress decreased seed germination percentage severely with 100 mM NaCl treatment but when SA and NaCl combined, an intermediate impact was observed in germination percentages (Table 1). Data of this investigation showed that 100 mM NaCl decreased dry weight (root and shoot) in comparison to control and other treatment, but SA levels increased dry weight (root and shoot) (Table 1, $p<0.05$). Results showed when SA and NaCl combined, induced increase of dry weight (root and shoot).

Seed treatment with 3 mM SA increased chlorophyll (a+b) content, while priming with 100 mM NaCl decreased chlorophyll (a+b) content but when SA and NaCl combined, an intermediate impact was observed in chlorophyll (a+b) content (Table 1, $p<0.05$) Under salt stress conditions, the proline and MDA concentration increased significantly (Table 1).The results showed that proline and MDA concentration decreased when plants were treated with 3 and 5 mM SA (Table 1, $p<0.05$). These results are in agreement with those obtained by other authors, showing that in wheat germination is significantly decreased by salinity (Ashraf and Rauf, 2001). Several workers reported that stimulating effects of SA on germination are concentration dependent (Rajjou *et al*., 2006 and Singh *et al*., 2010). Sakhabutdinova *et al*. (2003) reported that SA is germination stimulator. Al-Hakimi and Hamada (2001) reported that improved

germination rate and percentage by ascorbate and sodium salicylic acid treatments in wheat (*Triticum aestivum* L.). Results showed that priming with NaCl caused a considerable reduction in dry weight (root and shoot). The reduction in root and shoot development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings but SA levels could have significant effect on dry weight (shoot and root) so with SA increasing, dry weight increased, that shows salinity slightly effect on dry weight (shoot and root). Sakhabutdinova *et al.* (2003) reported that salicylic acid treatments maintain the IAA and cytokinin levels in the plant tissues, which enhanced the cell division and dry weight. Improved dry weights from the 100 $mg \cdot L^{-1}$ concentration of salicylic acid might be due to increased lateral growth (Metwally *et al*., 2003). In the present research, salt increased proline content in chickpea. Results showed that priming with SA caused a considerable increasing in proline and decreasing in MDA content. Sairam *et al*. (1998) reported that Increasing proline leads to increase in resistance to salt stress. Our results are in agreement with those obtained by Sahar *et al*. (2011) for *Salvia officianlis*. It is likely that the accumulation of free proline and total free amino acids could be one of the major mechanisms of salinity tolerance in chickpea. Salinity leads to decrease chlorophyll content that is because of increase chlorophyllas activity. Results showed that priming SA reduction chlorophyllas activity and increasing chlorophyll content. SA cause to pigment increasing and in some plants high SA concentration cause to chlorophyll content decreasing. In present study it was shown that exogenous SA decreased the negative effects of salinity in chickpea plants.

Table 1. Mean comparisons of dry weight (root and shoot), MDA, proline and *Chl a+b* in response to NaCl and SA and their interaction

Treatment	Germination percentages (%)	MDA (µmol/mg protein)	Proline (µmol.g^{-1} FW)	Chl a+b (SPAD reading)	Dry weight (g $plant^{-1}$) root	Dry weight (g $plant^{-1}$) shoot
Control	86	51.13	30.45	3.14	0.05	0.187
1.5 mM SA	90	30.45	33.14	3.89	0.055	0.201
3 mM SA	97	18.36	35.4	6.45	0.07	0.298
NaCl	46	142.36	70.09	1	0.022	0.09
SA (1.5 mM) +NaCl (100 mM)	72	70.68	87.36	2.09	0.038	0.105
SA (3 mM) +NaCl (100 mM)	80	49.36	103.58	3.74	0.059	0.02
F-test probabilities SA	0.02	0	0	0	0	0

Conclusion

In conclusion, the application of salicylic acid could alleviate the adverse effects of salinity on chickpea plants. However, further studies are imperative on the effects of salinity and other organic acids on certain other organisms so as to establish the role of these factors on the levels of endogenous enzymes.

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