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Alleviation of Salinity Stress in Spring Wheat by Hormonal Priming with ABA, Salicylic Acid and Ascorbic Acid

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ABSTRACT

Effects of hormonal priming with abscisic acid (ABA), salicylic acid (SA), or ascorbic acid on wheat (*Triticum aestivum* cv. Auqab - 2000) germination and seedling growth under normal (4 dS/ cm) and saline (15 dS/ cm) conditions were studied to determine their usefulness in increasing relative salt-tolerance. During germination test, most of treatments were effective in improving germination and seedling vigor of wheat during salinity stress. Seeds primed with 50 ppm ascorbic acid and 50 ppm SA not only improved final germination count but also reduced the germination time under saline conditions. Seedling raised from primed seeds with 50 ppm SA followed by 50 ppm ascorbic acid had significantly higher lengths and fresh and dry weight of shoot than other treated or non-primed seeds under non-saline and saline conditions. But all hormonal priming treatments decreased the electrolyte leakage of steep water as compared to that of non-primed seeds even after 12 h of soaking. Hormonal priming with 50 ppm SA induced maximum decrease in electrolyte leakage, while an increase in electrolyte leakage was observed by 10 ppm ABA and 100 ppm ascorbic acid. It is concluded that hormonal priming has reduced the severity of the effect of salinity but the amelioration was better due to 50 ppm SA and 50 ppm ascorbic acid treatments as these showed best results on seedling growth, fresh and dry weights under non-saline and saline conditions whereas hormonal priming with ABA as not effective in inducing salt tolerance under present experimental material and conditions.

Key Words: Hormonal priming; Salinity tolerance; Seedling vigor; Wheat seed

Abbreviations: Salicylic acid = SA, Abscisic Acid = ABA, Final germination percentage = FGP, Mean germination time = MGT, Electrical conductivity = EC, Reduction percentage of germination = RPG

INTRODUCTION

Salinity is one of the major and increasing problems in irrigated agriculture in Pakistan, particularly in wheat and rice grown areas. The areas affected by varying degrees of salinity are reported at around 6.3 M ha within Canal Command areas, of which half is cultivable or cultivated to some extent (Rafique, 1990). Ghassemi *et al*. (1995) estimated about 14% of irrigated land to be badly affected by salinity in Pakistan. The adverse effects of high concentration of salts for plants are due to the osmotic retention of water and to specific ionic effects on the protoplasm. Water is osmotically held in salt solutions, so as the concentration of salt increased water becomes less and less accessible to the plant.

Poor germination and seedling establishment are the results of soil salinity. It is an enormous problem adversely affecting growth and development of crop plants and results in to low agricultural production (Garg & Gupta, 1997). Wheat is grown on all type of soils and is classified as a moderate, salt tolerant crop (Mass & Hoffman, 1977). Yield losses on salt affected soils of Pakistan average about 64%. The effect of salinity at seedling stage of wheat range from reduction in germination percentage, fresh and dry weight of shoots and roots to the up-take of various nutrient ions.

Pre-sowing seed treatments have been shown to enhance stand establishment in non-saline areas (Khan, 1992) and have potential in saline areas as well (Ashraf & Ruaf, 2001; Basra *et al*., 2005). Prior to selecting these alternatives, it seems necessary to examine seed vigor enhancement techniques leading to better and synchronized stand establishment under stress conditions. Physiological treatments to improve seed germination and seedling emergence under various stress conditions have been intensively investigated in the past two decades (Bradford, 1986). It is thought that the depressive effect of salinity on germination could be related to a decline in endogenous levels of hormones (Debez *et al*., 2001). However, incorporation of plant growth regulators during presoaking, priming and other pre-sowing treatments in many vegetables crops have improved seed performance. Typical responses to priming are faster and closer spread of times to germination and emergence over all seedbed environments and wider temperature range of germination, leading to better crop stands, and hence improved yield and harvest quality, especially under suboptimal and stress condition growing conditions in the field (Halmer, 2004).

Plants produce proteins in response to abiotic and biotic stress and many of these proteins are induced by phytohormones such as ABA and salicylic acid (Jin *et al*., 2000). Salicylic acid is an endogenous growth regulator of phenolic nature, which influence a range of diverse processes in plants, including seed germination (Cutt & Klessig, 1992), stomatal closure (Larque-Saaveda, 1979), ion up-take and transport (Harper & Balke, 1981), membrane permeability (Barkosky & Einhellig, 1993), photosynthetic and growth rate (Khan *et al*., 2003). SA is also important signal molecule for modulating plant responses to environmental stress. SA is known to provide protection against a number of abiotic stresses (Senaratna *et al*., 2000).

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 (Singh & Dara, 1971). Concerted attempts have been made to mitigate the harmful effects of salinity by application of plant growth regulators (Datta *et al*., 1998). Thus the detrimental effects of high salts on the early growth of wheat seedlings may be reduced to some extent by treating seeds with the proper concentration of a suitable hormone (Darra *et al*., 1973).

To our knowledge, little research has been done on the use of SA, ABA and ascorbic acid as hormonal priming agent, however, these are abundantly used in stress conditions as a foliar application during different growth stages of crop plants. The present study is therefore, conceived with to investigate the effects of presoaking of wheat seeds in varying concentrations of ABA, SA and ascorbic acid upon their germination and subsequent growth under saline conditions. The research is important because it can reveal the role of hormones that impart stress resistance and provide insight in to the potential for wheat plants to adapt to saline conditions.

MATERIALS AND METHODS

Seed materials. Seeds of wheat (*Triticum aestivum* L.) cv. Uqab- 2000 were obtained from Punjab Seed Corporation, Faisalabad, Pakistan. Before the start of experiment, seeds were surface sterilized in 1% sodium hypochlorite solution for 3 min, then rinsed with sterilized water and air-dried.

Hormonal priming. Hormone solutions of appropriate concentrations were prepared. 250 g of seeds were soaked in 500 mL of aerated solution for 12 h and redried near to original weight with forced air under shade (Sundstrom *et al*., 1987).

Germination test. Germination potential of the wheat seeds was estimated in accordance with the International Rules for Seed Testing (ISTA, 1985). Four replicates of 25 seeds each were germinated in 12 cm diameter petri dishes at 25°C in growth chamber. 5 mL of saline solution of 15 dS/ cm was applied in each Petri dish to impose salinity stress, while distilled water with EC 4 $dScm^{-1}$ was applied for normal conditions. A seed was scored germinated when coleoptile and root lengths reached 2 - 3 mm. Counts of germinating seeds were made every 6 h, starting on the first day of imbibition, and terminated when maximum germination was achieved. During this, mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981):

$$MGT = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds, which were germinated on day D, and D is the number of days counted from the beginning of germination.

The reduction percentage of germination (RPG) or emergence (RPE) was calculated as quoted by Madidi *et al*. (2004);

$$RPG = (1 - Nx / Nc) \times 100$$

"Nx" is the number of germinated seedlings under salt treatments and "Nc" is the number of germinated seedlings under control.

Electrical conductivity of seed leachates. After washing in distilled water, 5 g seeds were soaked in 50 mL distilled water at 25ºC. Electrical conductivity of steep water was measured 0.5, 1.0, 1.5, 2.0, 6.0, 12.0 and 24.0 h after soaking using conductivity meter (Model Twin Cod B - 173) and expressed as μS/ cm (Basra *et al*., 2002).

The data collected was analyzed using the Fisher's analysis of variance technique under completely randomized block design (CRD) and the treatment means were compared by Least Significant Difference (LSD) test at 0.05 probability level (Steel & Torrie, 1984).

RESULTS

Hormonal priming had a significant effect on FGP, MGT and RPG under both normal and saline conditions (Fig. 1). Under normal conditions, maximum germination (98%) was achieved in seeds primed with 50 ppm ascorbic acid, which was statistically similar to all the remaining treatments including control except priming with 200 ppm salicylic acid and 100 ppm ascorbic acid (Fig. 1a). Under saline conditions, maximum germination was obtained in seeds primed with 50 ppm ascorbic acid and salicylic acid, which was statically similar to priming with 10 or 30 ppm ABA, 200 ppm ascorbic acid and 100 ppm acorbic acid. However, lowest MGT was achieved in seeds primed with 50 or 100 ppm ascorbic acid, 50 ppm salicylic acid and 30 ppm ABA followed by 10 ppm ABA treatment (Fig. 1b). During saline conditions, priming with 50, 100 or 200 ppm ascorbic acid along with 50 ppm salicylic acid and 30 ppm ABA showed lowest MGT as compared to control. However, maximum reduction in percentage was recorded in seeds primed with 200 ppm SA as compared to remaining hormonal treatments including control (Fig. 1c).

All the priming treatments showed a significant effect on shoot and root lengths and ratio under both conditions (Fig. 2). Maximum shoot length was attained in seeds

Fig.1. Effect of different priming techniques on (a) final germination (b) MGT and (c) RPG of wheat growing under normal and saline conditions. The vertical bars with different alphabets are statistically different indicating interactive effect of pre-sowing seed treatments and salinity.

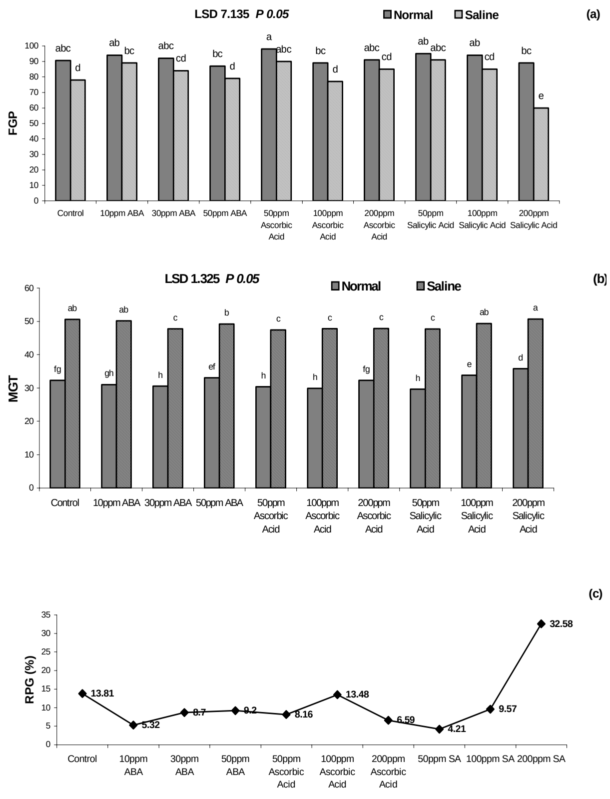
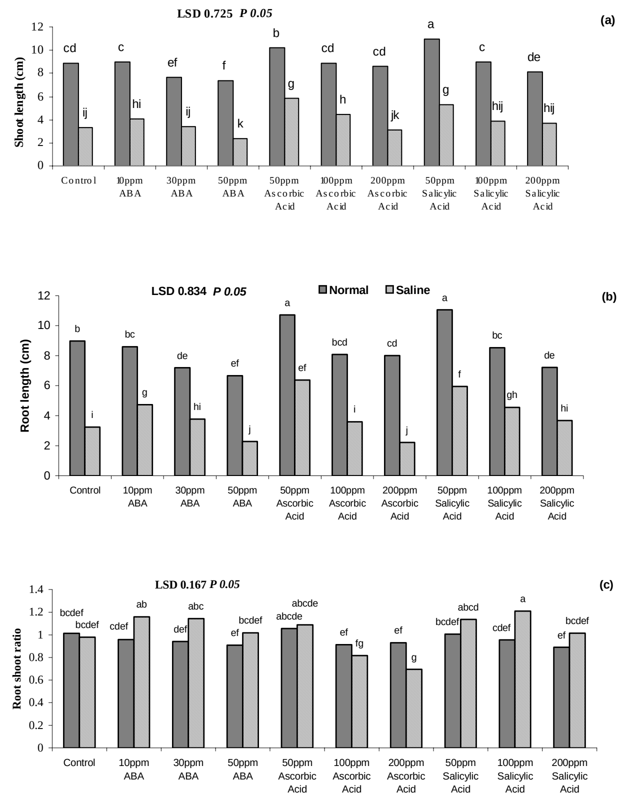


Fig.2. Effect of different pre-sowing seed treatments on root length (a), shoot length (b) and root shoot ratio (c) of wheat during germination test. The vertical bars with different alphabets are statistically different indicating interactive effect of pre-sowing seed treatments and salinity.



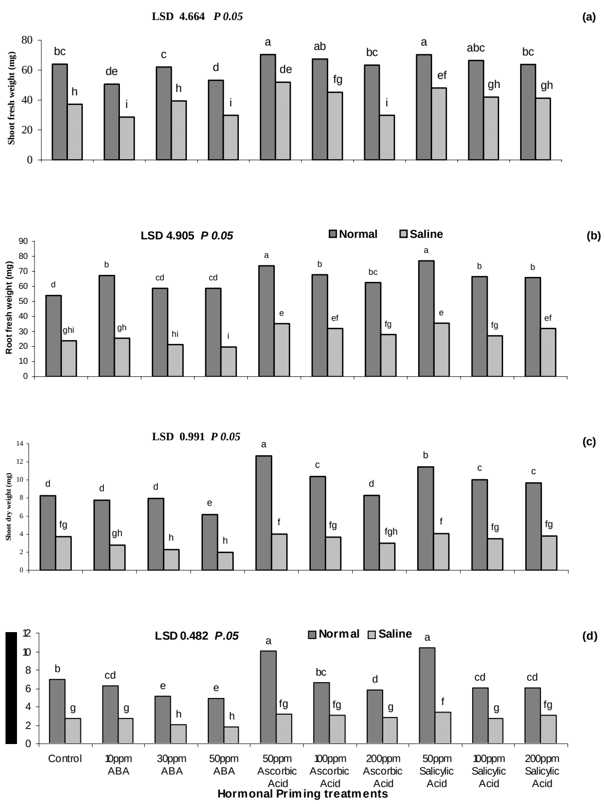
primed with 50 ppm salicylic acid and 50 ppm ascorbic acid, while 50 ppm ABA drastically effected under both normal and saline conditions (Fig. 2a). Priming with 50 ppm ascorbic acid and 50 ppm salicylic acid showed maximum root length as compared to control under both normal and saline conditions (Fig. 2b). During normal conditions, minimum shoot length was recorded in seeds treated with 50 ppm ABA, which was statistically non-significant with seeds treated with 30 ppm ABA and 200 ppm Salicylic acid. Under saline conditions, all the priming treatments had improved root length except priming with 50 ppm ABA and 200 ppm ascorbic acid as compared to control. Whereas root shoot ratio was significantly increased in wheat due to priming with 100 ppm salicylic acid as compared to control, which was statistically similar to 50 ppm ascorbic acid and salicylic acid, 10 or 30 ppm ABA under saline conditions (Fig. 2c).

All the priming treatments had significant effects on fresh and dry weight of shoot and root under both normal and saline conditions (Fig. 3). Shoot fresh weight was significantly increased in seedlings raised from seeds primed with 50 or 100 ppm ascorbic acid and 50 ppm SA under both conditions (Fig. 3a). Maximum root fresh weight was attained in seeds primed with 50 ppm of ascorbic acid and salicylic acid separately as compared to control under both normal and saline conditions (Fig. 3b). However, minimum root fresh weight was observed in seeds treated with 50 ppm ABA, which was statistically similar to hormonal priming (30 ppm ABA) and control.

Maximum shoot dry weight (12.67 mg) was achieved due to priming with 50 ppm ascorbic acid closely followed by priming with 50 ppm salicylic acid (11.42 mg) under normal conditions, while under saline condition, shoot dry weight was significantly decreased and all the priming treatments failed to improve dry weight of shoot (Fig. 3c). Minimum shoot dry weight was recorded in seeds primed with 30 or 50 ppm ABA.

Except priming with 50 ppm ascorbic acid and salicylic acid all the remaining treatments failed to improve root dry weight under normal conditions (Fig. 3d). During saline conditions, maximum root dry weight was attained due to priming with 50 ppm salicylic acid which was statistically similar to 50 or 100 ppm ascorbic acid and 200 ppm salicylic acid. Root dry weight was significantly

Fig. 3. Effect of different priming techniques on Shoot fresh weight (a), root fresh weight (b), root dry weight(c) and shoot dry weight (d) of wheat growing under normal and saline condition. The vertical bars with different alphabets are statistically different indicating interactive effect of pre-sowing seed treatments and salinity



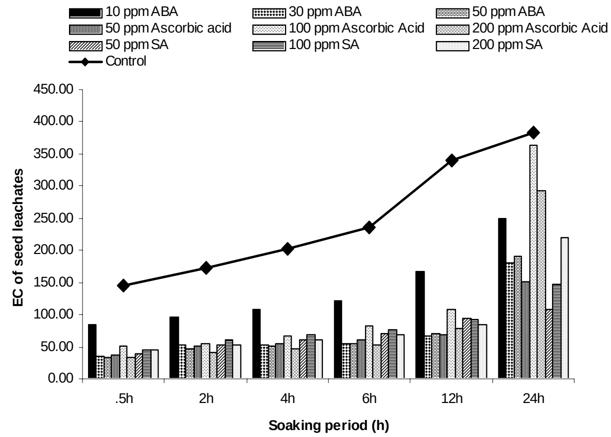
reduced due to priming with 30 or 50 ppm ABA as compared to control.

The electrolyte conductivity of seed leachates was significantly decreased with the application of hormonal priming treatments on wheat seeds (Fig. 4). Generally the electrolyte leakage was increased with increasing imbibition period including all treatments and control. After a longer period of imbibition ranging from 1 h to 24 h all the priming treatments lowered down the electrolyte leakage than control. An increase in electrolyte leakage was observed in seeds treated with 10 ppm ABA and 100 ppm ascorbic acid as compared to remaining treatments including control on all measuring periods. Maximum decrease in electrolyte leakage was induced by 50 ppm salicylic acid after 24 h of soaking period. Overall, all the priming treatments except 10 ppm ABA and 100 ppm Ascorbic acid were effective in decreasing electrical conductivity of seed leachates.

DISCUSSION

In present study, salt stress caused significant

Fig. 4. Effect of hormonal priming treatments on the electrical conductivity of seed leachates (μS cm^{-1} g^{-1}) in wheat cv. Auqab-2000



decreased in germination and seedling growth of wheat. These results are in agreement with those obtained by other authors, showing that in wheat germination is significantly decreased by salinity (Sarin & Narayanan, 1968; Ashraf & O'leary, 1997). It may be that NaCl reduced the rate of germination due to the reduced water potential and the resulting slower rate of imbibition. From present investigations, it is quite clear that seeds primed with various concentrations of ascorbic acid and salicylic acid proved to be effective in inducing salt tolerance at the germination stage in wheat. Hormonal priming with 50 ppm ascorbic acid and 50 ppm salicylic acid were more effective than other hormonal treatments. The results related to germination percentage can be related to earlier findings in which El-Tayeb (2005) found an improvement in seeds pretreated with SA solution than those of un-treated (controlled) seeds. These results are in consistent with those of Rajasekaran *et al*. (2002) and Shakirova *et al*. (2003), who showed a promotion in seed germination with SA application. Similary, Al-Hakimi and Hamada (2001) reported that grain soaking in ascorbic acid exerted some favorable effects on growth and transpiration of wheat seedlings counteracting the inhibitory effects of salinity stress. Working with wheat seeds, Roy and Srivastava (1999) observed that germination percentage, root and shoot length, root-shoot ratio and amylase activity decreased with increasing salinity, while seeds treated with 50 ppm ascorbic acid, 100 ppm $CaCl_2$, sodium benzoate and water decreased the negative effect of salinity. However ABA seed treatments failed to improve germination percentage and seedling growth (Fig. 1-3), which supports evidence that ABA inhibits germination in most of cases (Baskin & Baskin, 1998) as ABA is a generic stress hormone, which is known for its multiple functions.

Fresh and dry weights of roots and shoots decreased progressively due to salinity as compared to check (Fig. 3). These results are in agreement with those of Ghoulam *et al.*

(2001), who showed that salinity caused a marked reduction in growth parameters of shoots and roots of sugar beet plants but shoot fresh and dry weight and root fresh weight were increased in seedlings raised from seeds primed with 50 ppm salicylic acid, which confirms the results of El-Tayeb (2005) in barley and Gutie rrez *et al*. (1998) in soybean plants in response to salicylic acid treatment. Singh and Ushu (2003) also found that SA application increased the dry mass of wheat seedlings under water stress. It is supposed that the protective and growth promoting effect of SA are due to increased level of cell division with in the apical meristem of seedling root, which caused an increase in plant growth. Thus, it is well documented that SA treatment prevented decreased in IAA and cytokinin content completely, which reduced stress-induced inhibition of wheat growth. Also, high ABA levels were maintained in SA treated wheat seedlings, which provided the development of antistress reactions, for example, maintenance of proline accumulation (Sakhabutdinova *et al*., 2003). These observations are in consistent with those of Khodary (2004), who reported that SA increases the fresh and dry weight of shoot and roots of stress maize plants.

Maximum EC of seed leachates was recorded in untreated seeds (Fig. 4). All the seed treatments resulted in lower EC of seed leachates compared with check. Minimum EC of seed leachates was noted in seeds subjected to 50 ppm salicylic acid followed by 50 ppm ascorbic acid. The reduced EC of seed leachates from SA treated seeds might be related to induction of antioxidant responses that protect the plant from oxidative damage. An increase in electrolyte leakage was observed by 10 ppm ABA at all soaking periods, which was probably due to the loss of ability to reorganize cellular membranes rapidly and completely (McDonald, 1980).

Our experiments indicate that phytohormones play critical roles in plant responses to salinity and it can be concluded that hormonal priming with 50 ppm ascorbic acid and 50 ppm salicylic acid increase the ability of wheat to grow successfully under saline conditions whereas hormonal priming with ABA was not effective in inducing salt tolerance. Finally, in future, these hormonal priming treatments may be used for improving plant growth and yield in saline areas.

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