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Role of salicylic acid and ascorbic acid in the alleviation of salinity stress in wheat *(Triticum aestivum* L.*)*

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Abstract

Ascorbic acid is a major primary antioxidant, plays an important role in preserving the activity of enzymes. Salicylic acid is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants. This research was conducted to evaluate the effects of hormonal priming with ascorbic acid (AS) and salicylic acid (SA) Co(control), C1(2.5%AS), C2(5%AS), C3(2.5%SA) and C4(5%SA) on wheat (*Triticum sativum*) germination and seedling growth under normal (S_0) and saline NaCl,S1 (150 Mm), S2 (300 mM), S3 (150 Mm NaCl ratio $CaCl_2$) and S4 (300 mM NaCl ratio $CaCl_2$) in order to determine their usefulness in increasing relative salt-tolerance. Results showed that 300Mm (NaCl/1.8CaCl2) seed pretreatment by 2.5% Ascorbic acid can alleviate salinity stress by increasing proline and Glycinbetaien. Also priming with salcilic acid can decrease salinity stress 300Mm (NaCl and NaCl/1.8CaCl2) effects through increase superoxide dismutase. Salcilic acid can defense against ROS with superoxide dismutase. During germination test, most of treatments were effective in improving germination and seedling vigor of wheat during salinity stress.

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Introduction

Salicylic acid is a phenolic compound that acting as a ntioxidant defense system and regulate physiological and biochemical processes in plant(Babar *et al*., 2014). salicylic acid play an important role against abiotic stress in pea, barley, wheat, rice and sunflower(Khan *et al*., 2003). salicylic acid protect photosynthesis and in stomatal regulation of plant under salinity and drought stress(Arfan *et al*., 2007). ascorbic acid can be important in cellular oxidation-reduction reactions(Mohsen *et al*.,2013). El-Hifny and El-Sayed, (2011) reported that ascorbic acid significantly increased yield and its components of pepper plants and stimulating of plant growth (Mohsen *et al*.,2013)

Salinity is the major stress factor, which delimit crop plants cultivation, especially in developing countries. The adverse effect of salinity on plants may lead to disturbances in plant metabolism, which consequently lead to a reduction of the plant growth and productivity. Poor germination and seedling establishment are the results of soil salinity (Hernandez *et al.,* 2001). Salinity and water deficit induces accumulation of proline in seedlings. (Sakhabutdinova *et al*., 2003) on their study for the effect of salicylic acid on decreasing the damages of salt stress on wheat (*Triticum aestivum* L.) seedlings data suggested that proline is an important component in the spectra of salicylic acid -induced ABA-mediated protective reactions of wheat plants in response to salinity and water deficit, which contribute to a reduction of injurious effects of stress factors and acceleration of restoration processes during the period after action of stress, which might be a manifestation of the protective action of salicylic acid on wheat plants.

Shakirova showed that soaking of wheat (*Triticum aestivum* L.) seeds in 0.05 mM SA also reduced the damaging effects of salinity on seedling growth and accelerated the growth processes. The treatment of wheat plants with SA increased the level of cell division within the apical meristem of seedling roots, causing an increase in plant growth and elevated wheat productivity (Shakirova *et al.,* 2003).

Strong evidence exists that in different plants salt stress can induce accumulation of reactive oxygen species such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen (Lee *et al*., 2001). Elimination of ROS is mainly achieved by antioxidant compounds such as ascorbic acid, glutathione, thioredoxine and caroteniods, and by ROS scavenging enzymes e.g., superoxide dismutase, glutathione peroxidase and catalase (Noctor *et al*., 1998). Soaking wheat seeds in SA solution provided protection against not only drought, but also salinity stress (Hamada *et al*., 2001). L-Ascorbic acid serves as a co-factor for many enzymes and it contributes to the detoxification of reactive oxygen species (ROS) (Conklin and Barth, 2004). Ascorbic acid is a small, water-soluble anti-oxidant molecule which acts as a primary substrate in the cyclic pathway for enzymatic detoxification of hydrogen peroxide. Since proline is one of the important components of defense reactions of plants to salinity. Salicylic acid (SA) is generally present in plants in quantities of a few mg/g fresh mass or less, either in a free state or in the form of glycosylated, methylated, glucose-ester or amino acid conjugates. SA is a common plant-produced phenolic compound. Compounds in this group can function as growth regulators. In addition, SA could be included in the category of phytohormones. Exogenous application of SA may influence a range of diverse processes in plants, including seed germination, stomata closure, ion uptake and transport, membrane permeability, photosynthetic and growth rate (Tayeb, 2005). SA is also known as an important signal molecule for modulating plant responses to environmental stress (Senaratna *et al*., 2004). Therefore, the primary aim of this study was to characterize the influence of presowing seed treatments on plant defense system during salinity stress.

Materials and methods

Seeds of Wheat genotype N80–19 were surface sterilized with 5% (v/v) sodium hypochlorite solution

for 3 minutes to avoid fungal invasion followed by repeated washings with sterilized distilled water. Germination of the wheat seeds was assessed in accordance with the International rules for Seed Testing (ISTA, 1985). Four replicates of 50 seeds each were germinated in 12 cm diameter Petri dishes on Whatman No.1 filter paper at 20°C in a growth chamber. A sufficient volume (5 mL) under normal (S_0) and saline NaCl (150, 300 mM) and NaCl ratio $CaCl_2$ (150, 300 mM) conditions were added to submerge the seeds partially under saline environment. Water and saline solution requirements were checked daily and topped-up according to necessity. A seed was scored germinated when coleoptile and root lengths reached 2-3 mm. Seedlings were harvested after seven days and washed with deionized water after harvest. Five washed seedlings from each replication were separated into root and shoot for the determination of their fresh and dry weights. Dry weight was determined after oven drying the samples at 65°C. Pre-sowing Seed Treatments: ascorbate priming, seeds were soaked in 25 and 50 mg L-1 ascorbate solution for 12 h (Sundstorm *et al*.,1987) Also for Salicylic acid priming seeds were soaked in 25 and 50 mg L-1 ascorbate solution for 12 h.

After respective priming treatment for specific period, seeds were washed with distilled water (Khan *et al*., 2003). The seeds were dried back near to original weight on laboratory benches with forced air under shade for 48 hours. The laboratory temperature during the drying period was 27±2 oC. These seeds were packed in polythene bags and stored in a refrigerator 7±2 for further studies (Basra *et al*., 2003).

Determination of Catalase Enzyme Activities: Catalase activity in the seedling was estimated by the method as described by Beers and Sizer (Beers and Sizer 1952). Catalase activity level was determined by following the decrease in absorbance at 240 nm for 3 min by using spectrophotometer.

Determination of SOD Enzyme Activities: SOD activity was assayed by using the photochemical NBT method as described by Dixit *et al.* (Dixit *et al*., 2001). The photo reduction of NBT (formation of purple formazan) was measured at 560 nm and an inhibition curve was prepared against different volumes of extract. One unit of SOD was defined as that being present in the volume of extract that caused inhibition of the photo-reduction of NBT by 50 % (Afzal *et al*., 2006).

Determination of Proline: Proline was measured as described by Bates *et al.* (Bates *et al*., 1973). 100 mg of frozen plant material was homogenized in 1.5 ml of 3% sulphosalicylic acid and the residue was removed by centrifugation. 100 µl of the extract was reacted with 2 ml glacial acetic acid and 2 ml acid ninhydrin (1.25 g ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until dissolved) for 1 h at 100 °C and the reaction was then terminated in an ice bath. The reaction mixture was extracted with 1 ml toluene. The chromophore-containing toluene was warmed to room temperature and its optical density was measured at 520 nm. The amount of proline was determined from a standard curve in the range of 20–100 µg.

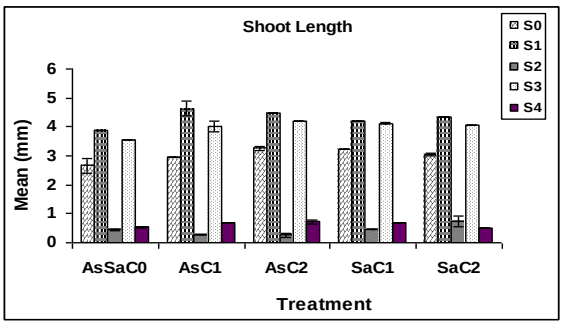
Glycine Betaine Content: The amount of GB was estimated according to the method of Grieve and Grattan. The plant tissue was finely ground, mechanically shaken with 20 ml deionized water for 24 h at 25°C. The samples were then filtered and filtrates were diluted to 1:1 with 2 NH_2SO_4. Aliquots were kept in centrifuge tubes and cooled in ice water for 1 h. Cold KI–I reagent was added and the reactants were gently stirred with a vortex mixture. The tubes were stored at 4°C for 16 h and then centrifuged at 10,000 rpm for 15 min at 0°C. The supernatant was carefully aspirated with a fine glass tube. The periodide crystals were dissolved in 9 ml of 1, 2-dichloroethane. After 2 h, the absorbance was measured at 365 nm using GB as standard. The data collected was analyzed using the Fisher's analysis of variance technique under Randomized Completely

Block Design (RCBD) and the treatment means were compared by Least Significant Difference (LSD) test at 0.05 probability levels (Steel and Torrie, 1984).

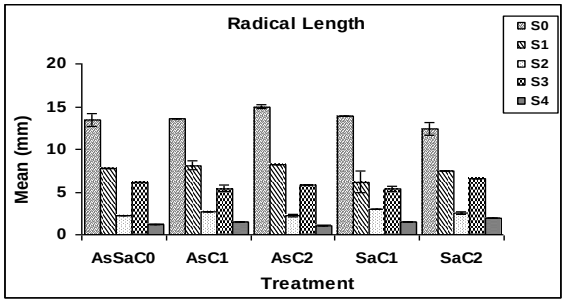
Results and discussion

Seeds pretreatment in 25% AS caused to increase shoot length in 150mM NaCl, while in 300Mm NaCl with 5% SA shoot length was highest. In 150 and 300Mm NaCl ratio to 1.8$CaCl_2$ salinity stress with 25% SA seed pretreatment shoot length increased (Fig1a). Damage effects of salinity (NaCl/1.8CaCl2) on root length higher than NaCl stress (Fig1b). Radical length of seedling that primed with 5% SA and AS in 300mM, NaCl ratio to 1/8 $CaCl_2$ increased (Fig1b). Number of radical increased in seed priming with Ascorbic acid and salicylic acid in (C1, C2, C3, and C4) with increasing salinity (Fig1c). At finally we found that low concentration of Ascorbic acid and Salicylic acid caused to increase number of root. In high salinity stress with NaCl solution (300mM) priming seed with 5% Salicylic acid caused to high number of root, but in high NaCl/1.8$CaCl_2$ solution, concentration of 2.5% Salicylic acid increased number of root (Fig1c). Root dry weight of seedling with seed priming AS in 150MmNaCl, 300mMNaCl and 300mMNaCl/1.8$CaCl_2$ is more effective than SA priming, but in 150mMNaCl/1.8$CaCl_2$ there was not different significantly between AS and SA (Fig2).

Afzal *et al.* founded that wheat seed priming AS and SA in salinity stress, root and shoot length, fresh and dry weight of seedlings were significantly increased, they believed that AS and SA priming has reduced the severity of salinity effects and ameliorate salinity damages (Afzal *et al.*, 2006). Janda showed that increase in the NaCl level and SA pre-treatment increased, the fresh and dry mass, in salt-stressed barley seedlings (Janda *et al.*, 2007). Seed priming AS5%, in salinity stress, root height were increased proline content of seedling increased significantly. In 300Mm NaCl salinity stress, high proline content of seedling was showed in AS 2.5% seed priming (fig 3b). Concerning this Hamada revealed that proline is one of the important components of defense reactions of plants to salinity it might be expected that pretreatment with SA contributes to accumulation of this amino acid under stress through maintaining an enhanced level of ABA in seedlings (Hamada *et al.*, 2000).



(a)



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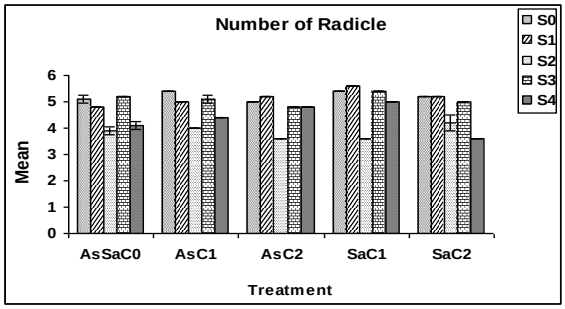


(c)

Fig. 1. (a) Shoot length (b) number of radical (c) radical length of seed priming barley with Ascorbic acid and salicylic acid in different concentration of salinity.

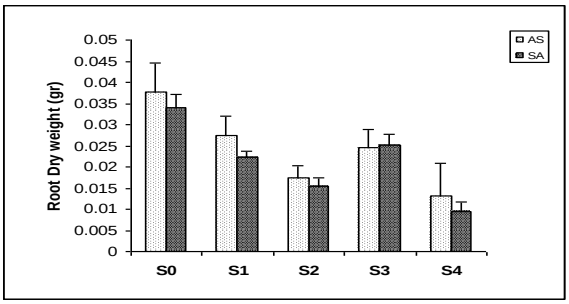
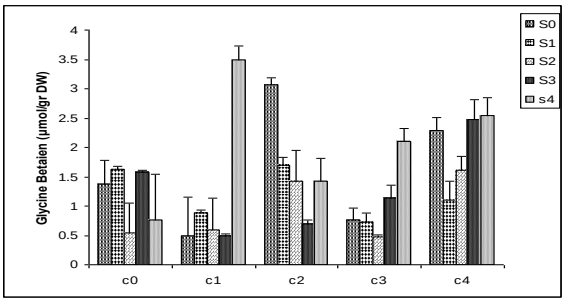
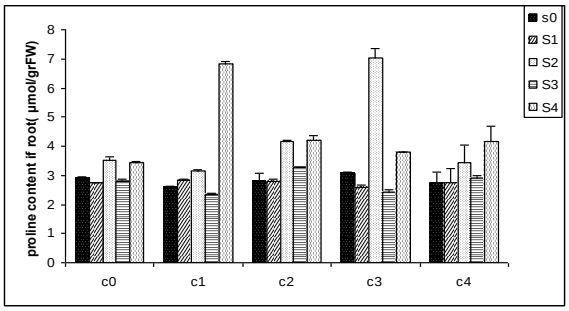


Fig. 2. Root dry weight (Cm) of seed priming barley with Ascorbic acid and salicylic acid in different concentration of salinity.

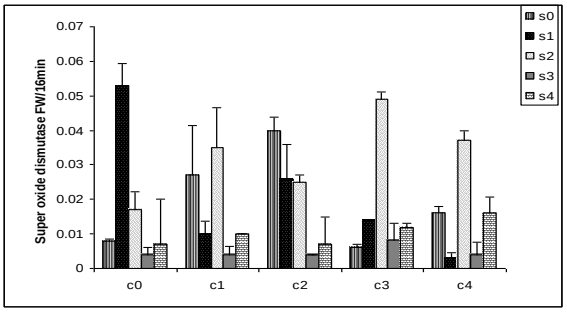
In 300Mm ($NaCl/1.8CaCl_2$) salinity with seed priming AS 2.5%, GB content of seedling increased significantly and in 300Mm NaCl salinity highest GB content was in seed priming AS 5%(fig 3a). It seems that tolerant mechanism in this concentration of seed priming, were high proline and GB content. Both of proline and GB content of seedling increased significantly in S4 with 2/5% AS priming (fig3a and 3b). Also SA pre-treatment provided protection against salinity in tomato plants, probably due to the accumulation of osmolytes, such as proline (Szepesi *et al.*, 2005).



(a)



(b)



(c)

Fig. 3. (a) Glycine betaien (b) proline (c) Super oxide dismutase content of root of barley in salinity stress.

Ascorbic acid can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduced H_2O_2 to water via ascorbate peroxidase reaction and is the major antioxidant that scavenges H_2O_2 (Chen and Gallie, 2004). In high salinity of NaCl with concentrations of priming such as C2, C3, C4, SOD enzyme activity increased, but seeds with SA 5% priming had high significantly SOD enzyme activity (fig3c). Sakhabutdinova showed that the stress-induced accumulation of active oxygen species and therefore, the level of SOD and peroxidase activity in the roots of young wheat seedlings pre-treated with SA, were significantly lower than in untreated plants, indicating that these enzymes contribute to the protective effect of SA on plants under conditions of stalinization (Sakhabutdinova *et al.*, 2004).

Germination rate correlated with root, shoot length and root, shoot dry weight. Basra *et al*, (2006) found that seed priming with SA improve the germination and early seedling growth in coarse and fine rice. Shoot length and number of root had significant negative correlation with SOD and prolin. Shoot and root length, number of root, shoot and root dry weight significantly negative correlated with proline (table1).

It is clear from the above results, that SA could be a very promising compound for the reduction of the abiotic stress sensitivity of crops, since under certain conditions it has been found to mitigate the damaging effects of various stress factors in numerous plant species (Janda *et al.*, 2007).

Conclusions

Finally we concluded that

1) Seed priming with salicylic acid and ascorbic acid can alleviate salinity stress by increasing osmolites and peroxidase enzyme.

2) In salinity stress, depends up on NaCl and $NaCl/1.8CaCl_2$, plants used different mechanisms.

3) In high concentration NaCl, seed priming 2.5%Ascorbic acid is more effective, but $NaCl/1.8CaCl_2$ seed priming 5% salicylic acid help to plant growth.

Table 1. Coefficient correlation of traits.

	ER	EU	Emax	shoot length	radicle length	n of radicle	D.W shoot	D.W radicle	SOD	Prolin	Glycin betaein
ER	1.000	**	**	*	**			*			
EU	-.449**	1.000	**								
Emax	.400**	-.504**	1.000		*						
shoot length	.245*	.051	.153	1.000	**	**	**	**	*	**	
radicle length	.395**	-.059	.282*	.630**	1.000	**	**	**		**	
n of radicle	.202	.066	.128	.837**	.666**	1.000	**	**	*	**	
D.W shoot	.194	.078	.042	.873**	.502**	.783**	1.000	**	*	**	
D.W radicle	.230*	.033	.086	.607**	.895**	.655**	.498**	1.000		**	
SOD	.065	-.131	.106	-.234*	.030	-.289*	-.275*	.032	1.000		
Prolin	-.206	.090	-.146	-.617**	-.481**	-.580**	-.426**	-.441**	.193	1.000	
Glycin betaein	.120	-.047	-.064	-.091	.052	-.078	.025	.040	-.003	.195	1.000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

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