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SALICYLIC ACID SEED PRIMING MODULATES SOME BIOCHEMICAL PARAMETRS TO IMPROVE GERMINATION AND SEEDLING GROWTH OF SALT STRESSED WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

Salicylic acid (SA) is a plant signaling molecule, which regulates various metabolic processes and involves in eliciting specific responses against abiotic/biotic stresses. Present study investigated the effect of SA on seed germination and seedling growth of salt stressed wheat. Wheat seeds were primed with water and two concentrations of SA (0.5 and 1.0 mM) for 12 h. For *In-vitro* experiment, seeds were soaked in 0, 50 and 100 mM NaCl solutions and seed germination and seedling growth parameters were studied. Primed seeds (both 0.5 and 1.0 mM SA) significantly improved seed germination, rate of germination, total chlorophyll, soluble sugars, proteins and phenolic content, as compared to non-primed and hydro-primed controls. For greenhouse experiment, hydro-primed (control) and SA primed (0.5 and 1.0 mM) seeds were grown in plastic pots and irrigated with 0 and 100 mM NaCl for 4 weeks. Plants emerged from SA primed seeds showed better response to salinity in which higher contents of photosynthetic pigments, soluble sugars, proteins and phenols contributed to enhanced growth and biomass production, as compare to control plants. Seed priming with 1.0 mM SA was found most effective to protect plants from damaging effects of salinity as compare to hydro-priming and 0.5 mM SA priming. Therefore, it is suggested that the priming of seeds with 1.0 mM SA can be adopted as a strategy to enhance wheat growth especially in salt affected soils.

Key words: Economical approach, Marginal lands, Salt tolerance, Seed pre-treatment.

Introduction

Salt stress is one of the major abiotic threats causing major reductions in growth and productivity of edible crops like wheat (Panta *et al.,* 2014; Mahboob *et al.,* 2017). Increasing population and severity of environmental stresses impose enormous pressure to agricultural production (Egemberdieva *et al.,* 2010). These pressures destroyed around 70% of the staple crop yield (Mantri *et al.,* 2012), affecting 925 million people with extreme hunger (Anon., 2010). Excessive salt in rhizosphere hamper various physiological and biochemical processes resulting severe crop loses. Salinity imposes osmotic stress and specific ion toxicity which causes disturbance in water relations, ionic imbalances, and nutritional disorders (Ashraf, 2004). In addition, it leads to photosystem malfunctioning due to stomatal and non-stomatal limitations and pigment disruption, which limits photosynthetic output and induce oxidative burst, which is potentially damaging to all kind of molecules and membrane bound systems (Dubey, 2005).

In order to survive in salt stressed conditions, plant synthesizes a variety of chemical substances as potential growth regulators including phytohormones, osmolytes, and antioxidants (Ashraf *et al.,* 2018). However, synthesis and accumulation of these substances depends upon type and quantum of applied stress. Variation in concentration of these substances directly effects salt tolerance ability and biomass production of growing plants. Therefore, exogenous applications of growth regulators were found effective to improve plant growth and yield production, under saline conditions (Arfan *et al.,* 2007, Azeem & Ahmad, 2011, Aziz *et al.,* 2018). Among these substances, salicylic acid (mono-hydroxy-benzoic acid, SA) is a naturally occurring, water soluble, hormone-like, non-enzymatic phenolic antioxidant, involved in various stress tolerance mechanisms (Janda *et al*., 2014). It is a vital signaling molecule that modulates plant responses against abiotic stresses including salinity (Nazar *et al.,* 2011; Li *et al.,* 2014). It involves in nutrient uptake and translocation, water and stomatal regulations and antioxidant defense (Arfan *et al.,* 2007). Furthermore, it also stimulates synthesis of photosynthetic pigments, quaternary ammonium compounds and upregulates the antioxidative system (Khan *et al.,* 2012; Miura & Tada, 2014). SA application improves seed germination and food manufacturing capabilities under saline environment (Khan *et al.,* 2012). SA mediated regulation of various physiological and biochemical processes indicated its role in plant stress tolerance (Abreu & Munne-Bosch, 2009; Ashraf *et al.,* 2018).

Wheat is a world's leading staple cereal crop, growing in several countries and shares a vital part in global agricultural economy. It is mainly used to feed humans all around the world and supplies 20% of the food calories. Pakistan is the 6th largest producer of wheat in the world and 3rd largest producer in Asia. Pakistan is producing more than 25 M tonnes of wheat annually with average arable land for wheat is more than 9 Mha. However, quickly depleating fresh water resources, soil salinization, agricultural malpractices and climat change effects are damging the productivity of this crop. On the otherhand a vast area of marginal/ degraded saline land

and brackish water resources are available, which could be utilized to bridge the evergrowing gap between demand and supply of wheat to feed over burgeoning population. Therefore, enabling this crop to be grown on such conditions and provide feasible economic outcomes is the need of the time. Owing to its role in salt stress tolerance and diverse involment in plants' metabolism, SA can be used in different ways to improve seed germination and growth of salt stressed wheat. In this study, we used SA in the form of seed priming agent and investigated its effect on two developmental stages of salt stressed wheat: 1) seed germination and 2) seedling growth.

Materials and Methods

Seed priming: Wheat seeds were obtained from the Crop Disease Research Institute, Pakistan Agriculture Research Council (CDRI-PARC), Karachi, Pakistan. Seeds were surface sterilized with 1% sodium hypochlorite (NaOCl) for 2 minutes and then thoroughly washed with sterilized water. Seed were soaked either in water (hydro-priming) or 0.5 and 1.0 mM SA solutions (SA priming), for 12 h. Seeds were collected from respective solutions, air dried in controlled and aseptic conditions and used for further experimentation.

Seed germination: Healthy and uniform seeds form all primed treatments were transferred into petri plates (10 seeds per plate), lined with double layer of filter paper. Petri plates were divided into three groups, moistened with 10 ml of 1) distilled water (non-saline control), 2) 50 mM NaCl solution and 3) 100 mM NaCl solution. Each group was further divided into four treatments, 1) non-primed 2) hydro-primed 3) primed with 0.5 mM SA and 4) primed with 1.0 mM SA. Each treatment had 5 replicates. Petri-plates were placed in growth incubating chamber (FTC-90I) at 28 ± 1ºC, (12 h light and 12 h dark) for 7 days. The protuberance of radical (2 mm) was considered as a mark of germination. After 7 days, seedlings (5 of each replicates) were kept in oven (70ºC for 48 h) for dry weight measurements. The remaining seedlings were kept refrigerated for the biochemical analyses. The plumule and radical lengths were measured and fresh weight, dry weight and biochemical parameters including total chlorophylls, total phenols, total proteins and total soluble sugars were estimated.

Greenhouse experiment: Ten seeds of three priming treatments (0, 0.5 and 1 mM SA) were sown in plastic pots containing quartz sand. Pots were placed in plastic trays and sub-irrigated with hoagland solution. In greenhouse experiment two saline treatmts, 1) sub-irrigated with Hoagland solution (non-saline) and 2) Hoagland solution with 100 mM NaCl, were used. Each group had 15 pots, which were further divided into three sub-groups on the basis of seed priming treatments 1) hydro-primed control, 2) 0.5 mM SA and 3) 1.0 mM SA. All pots were placed according to completely randomized design in a netted greenhouse. After four weeks of saline treatment, seedlings were harvested and growth and biochemical measurements were taken.

Seedling growth: Length, fresh weight and dry weight of seedlings of both experiments (*in-vitro* and greenhouse) were measured. Specific shoot length (SSL) and Specific root length (SRL) by Panuccio *et al.,* 2014, and leaf succulence by Abideen *et al.,* (2014) were also calculated using the following equations:

$$\text{SSL (cm g}^{-1}\text{ DW)} = \frac{\text{Shoot length}}{\text{Shoot dry weight}}$$

$$\text{SRL (cm g}^{-1}\text{ DW)} = \frac{\text{Root length}}{\text{Root dry weight}}$$

$$\text{Succulence (g H}_2\text{O g}^{-1}\text{ DW)} = \frac{\text{FW - DW}}{\text{DW}}$$

Estimation of total chlorophylls: Total chlorophylls was estimated using equation given by Arnon (1949). Fresh leaves (0.5g) were crushed in liquid nitrogen, homogenized in acetone (5 mL, 80%) and centrifuged (x 4000 rpm) for 10 minutes at 4ºC using refrigerated centrifuge (Hanil, micro 17-TR). Supernatant was separated carefully and absorbance was taken at 645, 663 and 480 nm.

Estimation of total soluble sugars: Fresh leaves (0.5g) were crushed in liquid nitrogen and homogenized in 10 ml distilled water. Homogenates were placed in water bath for 1 h at 100ºC. After cooling, extracts were filtered and supernatants were used for further estimation. Water extracts (0.1 ml) were mixed with Anthrone's reagent (0.5 mL) and shaken carefully. Samples were placed in water bath at 100ºC and after 30 minutes samples were immediately transferred in ice cold water bath. Finally, absorbance of the reaction mixture was taken at 630 nm. Total soluble sugars were determined using the method of Yemm & Willis (1954) against the standard cure of glucose.

Estimation of total protein content: Leaves (0.5 g) were crushed with liquid nitrogen and homogenized in 10 ml of chilled phosphate buffer (100 mM, pH 7.0). Homogenates were cold centrifuged for 10 min at 12000 rpm to separate leaf debris. Bradford's reagent (5 ml) was added in each leaf extract (1 ml) and incubated for 10 minutes in dark. Absorbance of the reaction mixture was recorded at 595 nm (Bradford, 1976). Protein content was estimated against standard curve of BSA.

Estimation of total phenols: Total phenolic content was determined in dry leaf samples using Swain & Hills (1959) method. Dried leaf powder (0.1 g) was mixed in 80% methanol (10 ml) and heated for 3 h at 40ºC. After heating, extract was centrifuged at 3000 rpm for 5 minutes and supernatant was recovered (Qasim *et al*., 2016). Leaf extract (1 ml) was mixed with 0.2 N Folin-Ciocalteu reagent (5 ml) and saturated sodium carbonate (4 ml) and incubated at room temperature for 90 minutes. Absorbance of the resulting blue colored solution was measured at 765 nm. Total phenols were estimated using gallic acid as reference standard.

Results

Seed germination and rate of germination: The two-way analyses of variance (ANOVA) showed significant (p< 0.001) effects of salinity, priming and their interaction on seed germination percentage and rate of seed germination of wheat (Table 1). Increasing salinity significantly (p<0.001) inhibited germination percent and rate of germination, while seed priming effectively alleviated germination inhibition at all salinities (Fig. 1). Irrespective of priming treatments, the lowest germination was found at highest salinity (100 mM NaCl). Whereas, treatment with both concentrations of SA (0.5 and 1.0 mM) significantly (p<0.001) improved seed germination and rate of germination at all salinities. The descending order of seed germination and rate of germination was as follows: SA 1.0 mM > SA 0.5 mM > hydro-prime > non-prime.

Table 1. Two way ANOVA shows the effect of salinity (S), priming (P) and their interaction (S x P) on final germination (%) and germination velocity of wheat seeds.

	Germination (%)	Germination velocity
Salinity	468.870***	13474.648***
Priming	112.411***	5152.636***
Salinity x Priming	6.096***	281.028***

The F values are given with significance level in superscript at p<0.001

Seedling growth *(In vitro):* The two-way ANOVA showed significant (p<0.001) effects of salinity, priming and their interaction on seedling growth parameters (Table 2). Increasing salinity significantly (p<0.001) inhibited length, fresh weight and dry weight of root and shoot, while seed priming effectively improved all growth parameters (Fig. 2). At all salinities, both concentrations of SA (0.5 and 1.0 mM) significantly increased the length and biomass (fresh and dry) of wheat seedlings however, maximum improvement was found in 1.0 mM SA, as compared to non-prime controls.

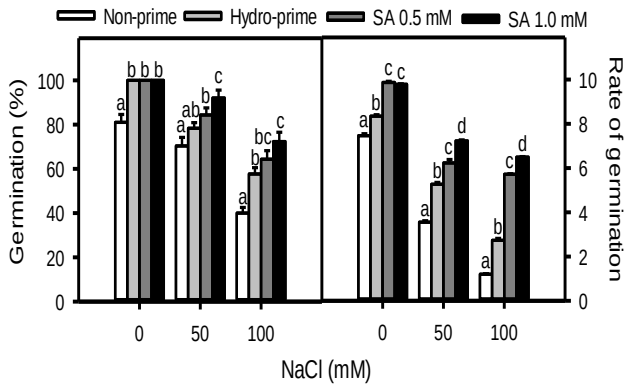

Fig. 1. *In-vitro* germination percentage and rate of germination of wheat primed at various concentrations of salicylic acid growing at different salinity levels.

Effect of SA on biochemical parameters *(In vitro):* The two-way ANOVA showed significant (p<0.001) effects of salinity, priming and their interaction on all biochemical parameters of wheat seedlings (Table 2). A significant (p<0.001) reduction in total chlorophylls and proteins were observed, while total soluble sugars and phenols were significantly enhanced with increasing salinity. Seed priming effectively improved total chlorophylls, proteins, phenols and soluble sugars (Fig. 3). The maximum amount of total chlorophylls and proteins were found in 1 mM SA treatment, under both non-saline and saline conditions, whereas maximum amount of soluble sugars and phenols were also recorded at 1 mM SA under 100 mM NaCl conditions.

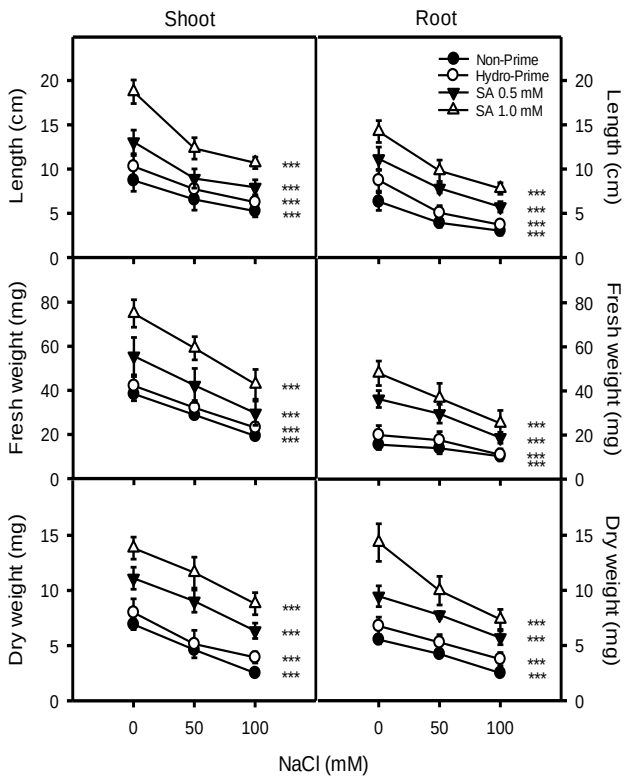

Fig. 2. *In vitro* vegetative growth parameters of wheat primed at various concentrations of salicylic acid growing at different salinity levels (FW and DW represents fresh weight and dry weight respectively).

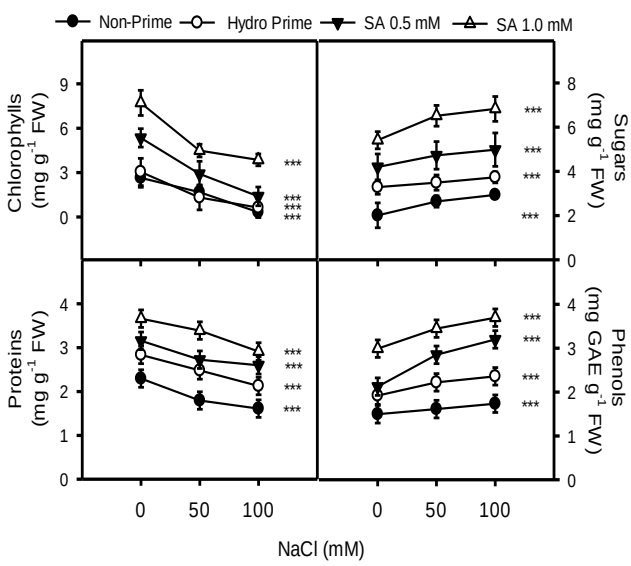

Fig. 3. *In vitro* biochemical parameters of wheat primed at various concentrations of salicylic acid growing at different salinity levels.

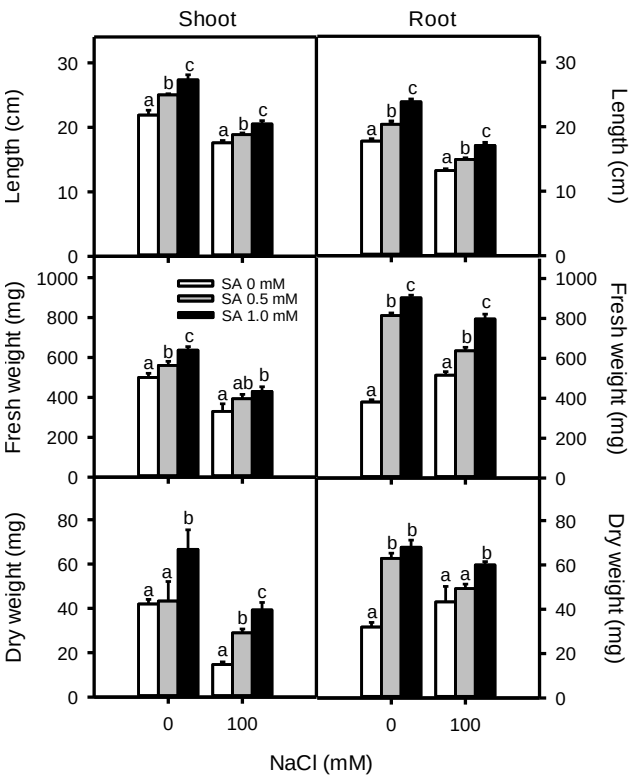

Fig. 4. Vegetative growth parameters of wheat primed at various concentrations of salicylic acid growing in green house at different salinity levels.

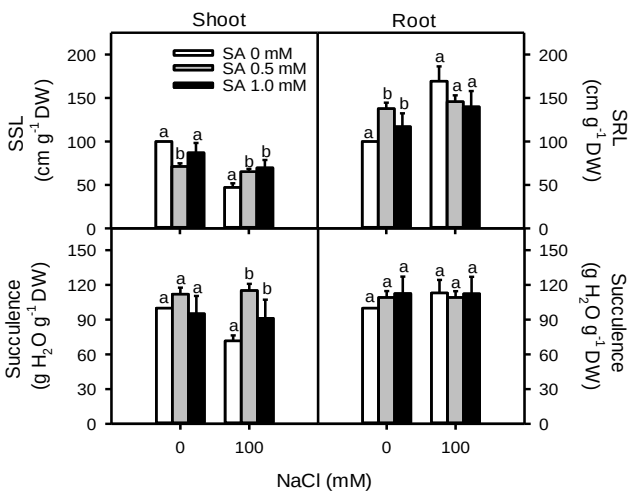

Fig. 5. Effect of SA treatment on percent change (with respect to hydro-primed non-saline control) in specific shoot length (SSL), specific root length (SRL) and succulence (shoot and root) of wheat seedlings, grown at 0 and 100 mM NaCl.

Greenhouse experiment

Effect of SA on vegetative growth: The two-way ANOVA showed significant (p<0.001) effects of salinity, priming and their interaction on all growth parameters of wheat seedlings, grown under greenhouse conditions (Table 2). Significant (p<0.001) reduction in seedling length, specific shoot length (SSL) and shoot succulence was observed with onset of salinity, while seed priming effectively improved these parameters (Figs. 4 and 5). Seedlings treated with SA showed better growth with

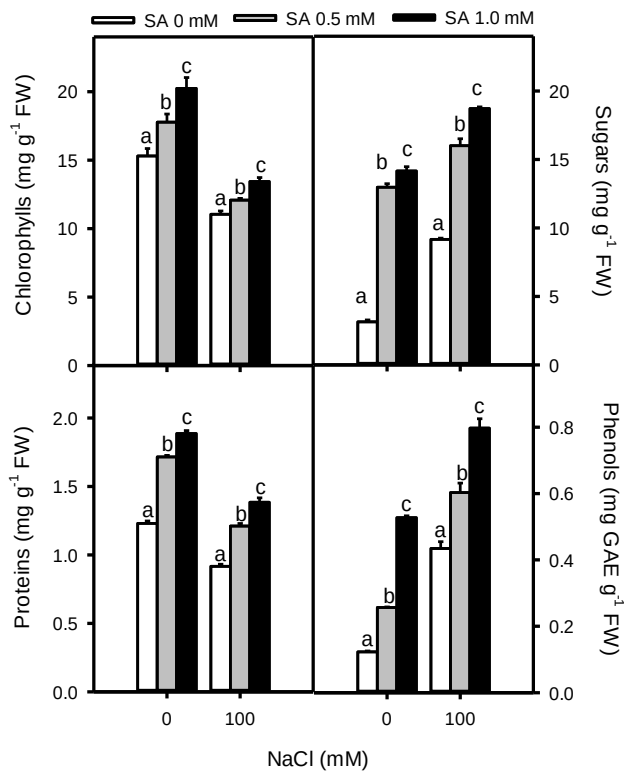

Fig. 6. Biochemical parameters of wheat primed at various concentrations of salicylic acid growing in green house at different salinity levels.

higher biomass (fresh and dry), SSL, SRL and succulence, under all saline conditions. However, maximum length and fresh and dry weights of seedlings were found in 1.0 mM SA (Fig. 4; Table 2). Interestingly, SA (1.0 mM) priming not only alleviates salt induced growth inhibition, but it enhanced growth of wheat seedlings almost equal to (in case of shoot) or even higher (in case of root) than non-saline controls.

Effect of SA on biochemical parameters: The two-way ANOVA showed significant (p<0.001) effects of salinity, priming and their interaction on all biochemical parameters of wheat seedlings (Table 1). Total chlorophyll and protein contents were significantly (p<0.001) decreased with onset of salinity, while total soluble sugars and phenols were significantly increased at 100 mM NaCl salinity. Seed priming (especially 1.0 mM SA) effectively improved total chlorophylls, proteins, total soluble sugars and phenols under both non-saline and saline conditions (Fig. 6).

Discussion

This study investigated the role of salicylic acid (SA) priming on seed germination and seedling growth of wheat, under saline conditions. Early plant growth is considered most important phase for plants, particularly crops. In this study, results illustrate the salt induced reduction in seed germination, seedling growth and biochemical parameters *in-vitro* and in greenhouse conditions. In the presence of excessive salts, seeds are unable to uptake enough amount of water due to higher osmotic potential, which reduce and/or delaye its germination process (Kaya *et al*., 2006;

Hayat *et al*., 2010 Tayyab *et al*., 2016). Data reveals that SA seed priming effectively normalized the adverse effects of salt stress and enhanced seed germination and rate of germination. SA is known to induce number of metabolic and biochemical changes in germinating seeds including, hydrolysis of nutrient assimilates and activation of enzymes related to inititate germination and/or breaking dormancy (Farooq *et al*., 2013). For instance, Shakirova (2007) observed higher germination rate and better seedling growth of wheat when treated with SA. Other studies also illustrated the role of SA treatment in promoting crop growth under stress conditions (Khodray, 2004; El-Tayeb; 2005; Mahboob *et al*., 2015).

SA seed priming markedly increased height and biomass (fresh and dry) of root and shoot of wheat seedlings, under control as well as salt stress conditions. Further, SA pretreatment is reported to alleviate stress injuries and improving growth of wheat (Al-Hakimi & Hamada, 2001). Hayat *et al*., (2005) reported improvement in fresh and dry weights and number of leaves of wheat by exogenous application of SA. Further, SA and related group of compounds are reposrted to enhance leaf area and dry mass of plants (Khan *et al*., 2003). Several other studies also illustrated the application of SA in promoting plant growth under stress conditions (El-Tayeb; 2005; Mahboob *et al*., 2015). For instance, Maiz seeds primed with SA showed better tolerance and improvement of various growth parameters such as height, fresh and dry biomass of root and shoot, under salt stress (Khodray, 2004).

Higher medium salinity hampers the photosynthetic capacity and chlorophyll synthesis of plants (Eleiwa *et al*., 2011; Mahboob *et al*., 2016). In this study, similar decline was found in photosynthetic pigments when seedlings exposed to NaCl, as reported in other crops like rice (Cha-Um *et al*., 2010), tomato (Taffouo *et al*., 2010) and wheat (Mahboob *et al*., 2017). The common reason of chlorophyll reduction is increase synthesis and/or activity of chlorophyllase enzyme (Moharekar *et al*., 2003). However, SA priming showed increase in photosynthetic pigments compared to controls under saline conditions. Our results are in aggremnt with Hayat *et al*., (2005), who observed that exogenous application of SA increased the content of photosynthetic pigments in wheat seedlings. Other reports on a number of cereal crops including barley, rice, wheat and maize also suggested that exogenously applied SA effectively increased photosynthetic rate by improving chlorophyll contents (Pancheva *et al*., 1996; Maibangsa *et al*., 2000; Khan *et al*., 2003; Khodary, 2004). However, alteration of photosynthetic pigments mainly dependant on the SA concentration and plant species. Higher concentration SA also reduced chlorophyll content as reported in wheat and mung bean seedlings (Moharekar *et al.*, 2003). Therefore, the selection of appropriate concentration of SA for exogenous application is crucial for plant growth performance. Previously, it was reported that SA concentration higher than 1 mM caused reduction in photosynthetic rate (Pancheva *et al.*, 1996). SA treated plants evidently enhanced the plant photosynthetic activity, which ultimately increased the amount of carbohydrate production. Present study showed a significant effect of SA on carbohydrate contents of wheat seedlings. Polysaccharides and soluble sugar content of plants increased with SA application (Luo *et al*., 2014). Higher level of soluble sugars contributes to the osmotic regulations (as osmoregulators or osmolytes, Shakirvora, 2007). However, seedlings emerged from non primed seeds showed lower level of soluble sugars with poor osmotic adjustment, as reported in Maize (Khodray, 2004).

Salinity significantly decreased the protein content of wheat leaves, which was significantly improved by SA priming as compared to non-prime controls. Salt stressed plants increase free amino acid content for osmotic adjustment and reduce protein content by induction of several proteases. However, plant accumulates stress responsive proteins, which contributes in salt tolerance mechanisms and ameliorate deleterious effects of salinity (Ali *et al*., 2012). Agastian *et al*., (2000) found increased soluble proteins in salt stressed wheat. In *H. vulgare* genotypes, SA application improved stress tolerance by enhancing the amount and activity of several metabolic enzymes and apoplastic proteins (Mutlu *et al*., 2013). SA also protects ultra-structure of proteins (Kang *et al*., 2012) and upregulates the synthesis of some stress proteins, chaperone, heatshock proteins etc. (Ding *et al*., 2002).

Table 2. Two way ANOVA shows the effect of salinity (S), priming (P) and their interaction (S x P) on different growth and biochemical parameters of wheat, grown under petri plates as well as in soil.

	Plates			Soil		
	Salinity (S)	**Priming (P)**	**S x P**	**Salinity (S)**	**Priming (P)**	**S x P**
Shoot L	73.374^{***}	72.963^{***}	3.350^{***}	544.860^{***}	96.415^{***}	9.418^{***}
Shoot FW	55.362^{***}	52.076^{***}	0.949^{ns}	244.500^{***}	34.813^{***}	1.224^{ns}
Shoot DW	74.691^{***}	100.924^{***}	0.497^{ns}	81.256^{***}	32.535^{***}	2.884^{***}
Root L	99.376^{***}	82.767^{***}	1.695^{***}	1093.670^{***}	293.505^{***}	14.333^{***}
Root FW	33.199^{***}	62.162^{***}	2.814^{***}	38.715^{***}	897.342^{***}	141.655^{***}
Root DW	55.683^{***}	77.467^{***}	3.092^{***}	0.949^{ns}	19.500^{***}	4.581^{***}
Phenols	780.316^{***}	3428.785^{***}	69.481^{***}	1231.300^{***}	641.288^{***}	6.267^{***}
Proteins	988.291^{***}	2302.383^{***}	15.135^{***}	2090.646^{***}	1197.012^{***}	43.237^{***}
Sugars	3.782^{*}	106.191^{***}	2.852^{***}	1213.635^{***}	2345.316^{***}	43.828^{***}
Chlorophylls	69.470^{***}	64.606^{***}	2.195^{***}	583.692^{***}	83.634^{***}	10.138^{***}

L-Length, FW- Fresh weight, DW- Dry weight

The F values are given with significance level in superscript

Plants generally respond under stressful conditions by increasing indigenous secondary metabolites particularly the phenolic compounds to regulate the salt tolerance mechanisms (Agati and Tattini, 2010). The production of phenolic compounds in response to salt stress is involved in neutralizing harmful oxidants and protecting biomolecules from oxidative chain reaction (Pollastri and Tattini, 2011). SA is a phenolic compound and a plant signaling molecule, which regulates various metabolic processes and involves in eliciting specific responses against abiotic/biotic stresses like salinity. Increasing level of phenolic compounds with SA application indicates the role of SA in salt tolerance capability of wheat. Besides being used as an antioxidant agent, SA also mediates antioxidative enzyme defense system (Shirasu *et al*., 1997; EL-Tayeb, 2005). SA induced stress tolerance is supported by increased activity of phenylalanine ammonia lyase (PAL) and accumulation of phenolic compounds (Wen *et al*., 2008).

Conclusions

Salicylic acid effectively improves seed germination and seedling growth of wheat. Therefore, priming of seeds with 1 mM SA is recommended to get high output of wheat cultivation, especially under saline conditions. In addition, this approach can be used to get edible crops from theoretically unproductive saline/ marginal and degraded lands, which not only helps to shrinks the gap between demand and supply of food crops but also improves socio-economic status of poor farmers.

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