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Does exogenous application of salicylic acid improve growth and some key physiological attributes in sunflower plants subjected to salt stress?

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Summary

To appraise the effect of foliar-applied salicylic acid (SA) on growth and some key physiological attributes in sunflower plants under salt stress, a greenhouse experiment was conducted. Two sunflower lines (Hysun-33 and SF-187) were subjected to non-saline (control) and saline regimes (120 mM NaCl). After 14 days of initiation of salt treatment plants of both sunflower lines were supplied with varying levels (100, 200 and 300 mg L^{-1}) of SA applied foliarly to all sunflower plants exposed to normal or saline substrates. After 21 days of SA application, data for growth (shoot biomass), photosynthetic pigments (chlorophyll *a* and *b*), water relation components, and accumulation of proline and mineral nutrients were recorded. Salt stress adversely affected the growth, chlorophyll pigments, water relations and contents of some key mineral nutrients, while increased the amount of proline and leaf and root Na^+ as well as Cl^- contents in both sunflower lines. Foliar-applied SA improved growth, chlorophyll *a* and *b* pigments, leaf turgor potential, and leaf and root Ca^{2+} concentrations. Of all salicylic acid levels used in the present study, 200 and 300 mg L^{-1} were found to be relatively more effective than the other levels in improving chlorophyll *a* and *b* pigments, leaf turgor potential, leaf and root Ca^{2+} concentration, while the other attributes remained unaffected due to SA application. Of both sunflower lines, Hysun-33 had higher amounts of photosynthetic pigments and essential nutrients than did SF-187.

Introduction

Plants exposed to saline environments experience four basic problems such as reduced water potential, accumulation of toxic ions (Na^+, Cl^-) and their harmful effects on various physiological and biochemical processes of the plant, thereby decreasing the absorption of some essential mineral nutrients such as K^+ and Ca^{2+}, hormone imbalance, and production of reactive oxygen species (ROS) (Munns, 2005; Ashraf, 2009; Ashraf et al., 2010). Availability of nutrients to plants depends on the functioning of membrane transporters that mediate the translocation of nutrients from the soil into the plant thereby compartmentalizing them into the cells, tissue and organs (Tester and Davenport, 2003; Epstein and Bloom, 2005). For example, Ca^{2+}-ATPases (Ca^{2+}-pumps) regulate low cytoplasmic Ca^{2+} levels in plants (Geisler et al., 2000). Salt tolerant cultivars absorb toxic ions to a lesser magnitude as compared to salt susceptible ones. The uptake of ions in higher quantities results in burning symptoms of leaves and ultimately leading to plant death (Munns et al., 1983; Flowers and Flowers, 2005; Akram et al., 2009, 2010).

In view of a number of reports it is now evident that soil pH, composition and concentration of elements, climatic conditions, soil textural class, quality of irrigation waters and type of plant species affect the nutrient dynamics and their absorption by plants under saline conditions (Grattan and Grieve, 1999; Zhu, 2003; Munns, 2005; Akram et al., 2010). For instance, high soil Na^+ impairs the Ca^{2+} activity in the external medium, thereby restricting its availability in plants e.g., in *Celosia argentea* (Carter et al., 2006), *Setaria verticillata* (Horst et al., 2006), and maize (Suarez and Grieve, 1988). It is well established that mineral nutrients acting synergistically or antagonistically may imbalance the nutrition of plants under saline environments. The deficiencies of most of the nutrients commonly occur due to higher absorption of Na^+ and Cl^- by plant tissues (Grattan and Grieve, 1999; Subbarao et al., 2003; Hu et al., 2005; Ashraf et al., 2010). In view of these reports, it is likely that reduction in uptake of mineral nutrients under saline conditions may occur due to Na^+-induced blockage or reduced activity of membrane ion transporters. Similarly, potassium uptake is perturbed by salinity thereby resulting in a reduced K^+/Na^+ ratio (Izzo et al., 1991; Subbarao et al., 1990). However, high K^+/Na^+ ratio in plants under saline conditions has been suggested as an important selection criterion for salt tolerance (Shah et al., 1987; Reynolds et al., 2005).

Plant hormones affect plant growth in multifarious ways affecting a number of physiological/biochemical processes in plants subjected to biotic and abiotic stresses (Reymond and Farmer, 1998; Steduto et al., 2000; Ashraf et al., 2008, 2010). Salicylic acid (SA) is one such plant growth regulators, which participate in the regulation of a number of physiological events taking place in the plant (Dolatabadian et al., 2008; Ashraf et al., 2010). SA regulates some key plant functions such as stomatal functioning (Aldesuquy et al., 1998), ion uptake and transport (Glass, 1975; Kaydan et al., 2007), photosynthesis (Noreen and Ashraf, 2008), water relations (Barkosky and Einhellig, 1993), production rate and content of anthocyanin and chlorophyll (Khurana and Maheshwari, 1980). It also increases growth (Arfan et al., 2007; Ashraf et al., 2010), and up-regulates antioxidative system (Noreen et al., 2009). All these functions have a significant role in plant tolerance to salinity (Noreen and Ashraf, 2008; Noreen et al., 2009; Dolatabadian et al., 2008; Abreu and Munne-Bosch, 2009; Ashraf et al., 2010). Furthermore, SA can greatly perturb the trans-membrane electrochemical potential of mitochondria and the ATP-dependent proton gradient of tonoplast-enriched vesicles (Macri et al., 1986).

In view of all the above reports, the principal objective of the present study was to appraise whether varying levels of exogenously applied SA could alleviate the adverse effects of salt stress on photosynthetic pigments, water relations and ion accumulation in sunflower plants. Furthermore, some key biochemical processes influenced by SA were identified that could be used as selection criteria in sunflower under saline conditions.

Materials and methods

To assess the influence of foliar-applied salicylic acid to offset the salt-induced adverse effects on chlorophyll pigments, water relation components and accumulation of some key inorganic nutrients in sunflower (*Helianthus annuus* L.), an experiment was conducted at the Botanical Garden of the University of Agriculture, Faisalabad, Pakistan under the growth conditions as described earlier (Noreen and Ashraf, 2008). Two sunflower lines (Hysun-33 and SF-187)

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were subjected to two saline regimes i.e., 0 and 120 mM NaCl prepared in the modified full strength Hoagland's nutrient solution. Different concentrations (100, 200 and 300 mg L^{-1}) of salicylic acid ($C_7H_6O_3$) were prepared and pH of the solutions was adjusted at 5.5. Salicylic acid was applied exogenously in combination with 0.1 percent (v/v) tween-20 as a surfactant to ensure spreading of the applied solution on the leaf surface so as to attain maximal penetration into the leaf tissues. The plants were harvested 21 days after the foliar application of SA at the vegetative stage and data for following attributes were recorded:

Photosynthetic pigments

Chlorophylls *a* and *b* were appraised following Arnon (1949). The leaf samples (0.2 g each) were prepared by cutting the leaves into small pieces having 0.5 cm size. The extraction of the leaf samples was done in 80% acetone at -4 °C for 24 h. The extracted material was centrifuged at 10, 000 x *g* for 5 min. The absorbance of the supernatant was recorded by using a UV-Visible spectrophotometer (Model Hitachi-U 2001, Tokyo, Japan) at 645 and 663 nm against a blank containing 80% acetone.

Leaf water potential (Ψ_w)

A Scholander type water potential measuring system (Cook and Sons, Birmingham, England) was used to measure leaf water potential. A fully expanded youngest leaf from the top was cut from each plant and measurements were made following Scholander et al. (1965).

Leaf osmotic potential (Ψ_s)

The leaf osmotic potential was measured following Wilson et al. (1980). The leaf material was kept in polypropylene micro-centrifuge tubes containing capacity of 2 cm^3. The tubes were frozen at -45 °C for one week. The material was thawed and homogenized with a tissue grinder. Then the homogenized material was centrifuged at 8000 x *g* for 4 min. A 10 µL was used in a Wescor-5500 vapor pressure osmometer for measuring osmotic potential.

Leaf turgor potential (Ψ_p)

The leaf turgor pressure was determined using the following equation:

$$\Psi p = \Psi w - \Psi s$$

Relative water content (RWC)

The fully expanded leaves were collected randomly from each plant from each replicate. After washing the samples were weighed and these values referred to as initial readings. Then, the leaf samples were placed in distilled water for 3 h in the dark at room temperature. The turgid leaves were blotted dry and weighed. After weighing, the material was oven-dried at 80 °C for 24 h. RWC of the leaves was appraised as described by Jones and Turner (1980) using the following formula:

$$RWC\ (\%) = [(f.\ wt. - d.\ wt.)/(t.\ wt. - d.\ wt.)] \times 100$$

where f.wt, d.wt, and t.wt are the fresh, oven dry, and turgid weights, respectively.

Free proline content

Free proline was determined following Bates et al. (1973). Leaf tissue (0.5 g) was ground with 10 mL of 3% (w/v) sulfo-salicylic acid solution. The sample filtrate containing 2.0 mL was reacted with 2.0 mL acid ninhydrin solution (1.25 g ninhydrin mixed with 30 mL glacial acetic acid), 20 mL (6*M* orthophosphoric acid) and 2.0 mL glacial acetic acid. The reaction was done at 100 °C, and terminated in an ice bath. A continuous stream of air was passed through the reaction mixture added with 4 mL toluene. The absorbance of the supernatant was read at 520 nm using a spectrophotometer (Model Hitachi U 2001 Tokyo, Japan). Proline concentration was worked out using the following formula:

Free proline (µmol g^{-1} fresh weight) = Proline (µg ml^{-1}) x Volume of toluene (mL)/(Mol. wt. of proline (g mol^{-1}) x Leaf tissue fresh weight (g)

Analysis of nutrients

The oven-dried plant material (0.1 g) was ground and passed through a 40-mesh screen for chemical analysis. A digestion mixture consisting of H_2SO_4 and H_2O_2 was used to digest the material. The chemical analysis of different ions (K^+, Ca^{2+}, Na^+) was carried out following Wolf (1982).

Determination of Na^+, K^+ and Ca^{2+}

Different concentrations of standards for sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) were prepared. The standard curves of these ions were drawn using a flame photometer (Jenway-PFP7, ELE Instrument Co, Ltd. England). The samples were run for the determination of Na^+, K^+ and Ca^{2+} and the values of the ions in the unknown samples were worked out using the appropriate standard curves.

Determination of chloride (Cl^-)

The chloride content was determined by extracting plant material (0.5 g) with 10 mL distilled de-ionized water. The material was heated at 80 °C till the volume was reduced to half. The volume of the solution was again made to 10 mL with distilled water. Chloride Analyzer (Model 926, Sherwood Scientific Ltd, Cambridge, UK) was used to determine the chloride content in the extracted samples.

Statistical analysis of data

Analysis of variance (ANOVA) of the data for all attributes was worked out using the COSTAT computer package (Cohort, Berkely, California). The least significance difference test was employed following Snedecor and Cochran (1980) to assess whether mean values differed significantly.

Results

Data presented in Tab. 1 for percent inhibition in shoot fresh and dry biomass showed that although salt stress caused a marked reduction in shoot biomass, foliar-applied salicylic acid reduced the salt-induced inhibition in these growth attributes in both hybrid lines of sunflower. Of all SA levels, 200 and 300 mg L^{-1} were relatively more effective in improving growth measured in terms of shoot biomass.

Addition of salt (NaCl) to the root growing medium markedly reduced chlorophyll *a* in both hybrid lines. However, values of chlorophyll *a* were found similar in both hybrid lines (Tab. 2). The foliar spray of 200 or 300 mg L^{-1} SA caused a significant improvement in chlorophyll *a* content in both sunflower hybrid lines grown under saline and non-saline regimes (Fig. 1). However, under salt treatment, foliar applied 300 mg L^{-1} SA significantly improved chlorophyll *a* in line SF-187, while, 200 mg L^{-1} of SA in hybrid line Hysun-33 (Fig. 1). Salt stress significantly reduced chlorophyll *b* in both hybrid lines (Tab. 2; Fig. 1). Line Hysun-33 maintained greater

Tab. 1: Percent inhibition in shoot biomass due to salt stress (120 mM NaCl) and percent improvement due to foliar-applied varying levels of salicylic acid of two lines of sunflower.

Salicylic acid (SA)	Percent (%) inhibition under saline conditions							
	Shoot fresh weight				Shoot dry weight			
	SF-187		Hysun-33		SF-187		Hysun-33	
0 mg L^{-1}	39.47		50		24.4		42.1	
100 mg L^{-1}	40.8		49.9		27.3		45.4	
200 mg L^{-1}	41.4		46.5		47.9		41.0	
300 mg L^{-1}	38.6		44.08		36.2		37.6	
	Percent (%) improvement due to foliar-applied SA under normal and saline conditions							
	Shoot fresh weight				Shoot dry weight			
	SF-187		Hysun-33		SF-187		Hysun-33	
	Control	Saline	Control	Saline	Control	Saline	Control	Saline
100 mg L^{-1}	12	9.47	4.44	4.75	9.5	13.2	8.2	1.9
200 mg L^{-1}	26.13	22.08	4.62	11.9	15.7	20.6	11.4	9.8
300 mg L^{-1}	14.24	15.93	10.5	23.6	2.53	13.7	16.8	25.9

content of chlorophyll *b* than that in SF-187 under saline conditions. Salicylic acid improved chlorophyll *b*. Of all SA levels, 300 mg L^{-1} was found better than the others. The chlorophyll *a/b* ratio of the sunflower hybrid lines was not influenced by salinity. However, there were significant differences between the hybrid lines in terms of chlorophyll *a/b* ratio.

Leaf water potential (Ψ_w) of both sunflower lines was significantly reduced due to salinity, and more marked being in line SF-187 (Tab. 2; Fig. 1). Exogenous application of varying levels of SA did not change the leaf water potential of sunflower hybrid lines under non-stressed conditions, whereas, under salinity stress, foliar application of all varying levels of SA caused a significant decrease (more negative increase) in leaf water potential, particularly, in line Hysun-33 (Fig. 1).

Imposition of salinity had a significant reducing effect on leaf osmotic potential (Ψ_s) of both sunflower lines (Tab. 2; Fig. 1). Lines differed only under saline conditions. Line SF-187 had lower leaf osmotic potential than that in line Hysun-33 under salt-stressed conditions. Foliar spray of SA did not affect leaf osmotic potential of the hybrid lines under non-stress conditions, whereas under saline regimes, all SA levels caused a decrease in leaf osmotic potential but only in line SF-187.

Leaf turgor potential (Ψ_p) remained almost unaltered due to imposition of salt stress (Tab. 2; Fig. 1). Both hybrid lines did not significantly differ in leaf turgor pressure. Foliar-applied 200 or 300 mg L^{-1} SA resulted in enhanced leaf turgor potential of salt stressed plants of line SF-187, whereas the reverse was true in line Hysun-33.

Relative water content (RWC) of sunflower plants was significantly decreased because of raising salinity of the growth medium ($P \leq 0.05$) (Tab. 2; Fig. 2). Moreover, both lines also differed significantly in this parameter. Foliar-applied SA did not significantly alter leaf RWC in the salt-stressed or non-stressed plants of both lines. Values for RWC did not vary significantly due to exogenous application of SA under salt-treated and untreated plants.

Proline content differed significantly due to different salt and SA treatments. However, both sunflower lines did not differ significantly in proline content. Moreover, there were non-significant interactions between salinity, SA or hybrid lines. Proline content increased with increasing levels of SA under saline and non-saline conditions. Line Hysun-33 maintained higher content of proline compared to that in line SF-187. Both lines, Hysun-33 and SF-187, contained higher content of proline under salt stress than that under normal conditions (Tab. 2; Fig. 2).

Concentrations of Na^+ in the leaves and roots increased significantly due to imposition of salt stress in the growth medium (Tab. 2). Both lines did not show difference in leaf Na^+, whereas they differed significantly in root Na^+. Exogenous application of different levels of SA had a slight decreasing effect on Na^+ content in the leaf tissues of salt stressed plants of both lines, particularly after foliar application of 300 mg L^{-1} SA (Fig. 2). On the other hand, foliar-applied SA at different concentrations produced inconsistent pattern of Na^+ accumulation by the roots of sunflower hybrid lines (Fig. 2).

The addition of NaCl to the growth medium resulted in a significant reduction in root K^+ content of the hybrid lines. Both sunflower lines also differed significantly in this physiological attribute ($P \leq 0.05$). The accumulation of K^+ by the leaf or root tissues was not affected significantly due to foliar spray of SA. However, a slight improvement in this ion content was observed in both hybrid lines under saline and non-saline regimes. The SA application resulted in a slight increase in the uptake of K^+ by the root tissues in line Hysun-33 as compared to that in line SF-187 under NaCl treatment (Fig. 2).

The concentration of Ca^{2+} in the leaves of Hysun-33 was higher than that in line SF-187 grown under non-saline conditions. Contrarily, the pattern of accumulation of Ca^{2+} in the leaves of both lines was inconsistent under salinity stress (Tab. 2; Fig. 3). However, accumulation of Ca^{2+} in the roots of line SF-187 was elevated as compared to that in line Hysun-33 under salt-stressed and non-stressed conditions. Foliar application of 100 mg L^{-1} SA resulted in increased uptake of Ca^{2+} by leaf tissues of line Hysun-33 under salt-stressed conditions. Furthermore, foliar spray of 200 and 300 mg L^{-1} SA resulted in a maximal increase in root Ca^{2+} in the sunflower hybrid lines under salt stress and non-stress substrates (Fig. 3).

The plants grown under NaCl-treated regime showed a significant effect on the uptake of Cl^- by leaves and roots of the sunflower hybrid lines under salt-stressed and non-stress regimes (Tab. 2). There was no significant difference in both lines in leaf or root Cl^- content (Fig. 3). Furthermore, accumulation of Cl^- by leaf or root tissues was little affected by foliar application of SA under saline or non-saline

Tab. 2: Analyses of variance of the data (Mean squares) for chlorophyll pigments, water relation components, free proline and concentrations of inorganic nutrients in two sunflower (*Helianthus annuus* L.) hybrid lines when varying levels of salicylic acid were applied as a foliar spray to 24-day old plants subjected to normal or saline conditions.

Sources of variation	df	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a/b* ratio	Leaf water potential	Leaf osmotic potential
Salt (S)	**1**	3.21***	0.82***	0.284ns	2.024***	2.043***
Hybrid lines (HBL)	**1**	0.095ns	0.027*	0.825*	0.007ns	0.157**
Salicylic acid (SA)	**3**	0.246*	0.009ns	0.394ns	0.035***	0.018ns
S x HBL	**1**	0.054ns	0.006ns	0.002ns	0.073***	0.288***
S x SA	**3**	0.021ns	0.009ns	0.065ns	0.007ns	0.023ns
HBL x SA	**3**	0.06ns	0.003ns	0.087ns	0.064***	0.018ns
Salt x HBL x SA	**3**	0.084ns	0.002ns	0.148ns	0.005ns	0.003ns
Error	**48**	0.068	0.005	0.184	0.005	0.016
		Leaf turgor potential	**Relative water content**	**Leaf free proline**	**Leaf Na^+**	**Root Na^+**
Salt (S)	**1**	0.0004ns	2635.4***	51.12***	2053.2***	4856.3***
Hybrid lines (HBL)	**1**	0.099*	141.6**	0.004ns	3.376ns	481.3**
Salicylic acid (SA)	**3**	0.039ns	26.0ns	2.416**	8.471ns	77.61ns
S x HBL	**1**	0.071ns	1.28ns	1.062ns	0.083ns	549.3**
S x SA	**3**	0.013ns	13.52ns	0.08ns	6.74ns	104.8ns
HBL x SA	**3**	0.095*	29.43ns	0.064ns	5.83ns	47.41ns
Salt x HBL x SA	**3**	0.01ns	16.18ns	0.527ns	1.365ns	25.84ns
Error	**48**	0.023	14.91	0.561	3.757	63.42
		Leaf K^+	**Root K^+**	**Leaf Ca^{2+}**	**Root Ca^{2+}**	**Leaf Cl^-**
Salt (S)	**1**	7255.8***	4863.7***	73.68***	454.9***	4728.3***
Hybrid lines (HBL)	**1**	24.47ns	111.2*	2.939ns	44.76**	7.548ns
Salicylic acid (SA)	**3**	108.7*	28.22ns	2.925ns	65.48***	13.52ns
S x HBL	**1**	16.72ns	3.478ns	1.67ns	11.41ns	27.59ns
S x SA	**3**	12.48ns	5.03ns	11.81***	1.767ns	15.33ns
HBL x SA	**3**	2.898ns	10.0ns	2.574ns	7.422ns	7.317ns
Salt x HBL x SA	**3**	10.7ns	6.70ns	9.32**	3.299ns	1.453ns
Error	**48**	30.05	23.68	1.462	5.13	24.96
		Root Cl^-	**Leaf K^+/Na^+**	**Root K^+/Na^+**	**Leaf Ca^{2+}/Na^+**	**Root Ca^{2+}/Na^+**
Salt (S)	**1**	2499.0***	782.4***	57.87***	23.87***	7.49***
Hybrid lines (HBL)	**1**	2.074ns	1.64ns	6.54***	0.226ns	2.02***
Salicylic acid (SA)	**3**	8.56ns	4.44ns	0.234ns	0.232ns	0.26**
S x HBL	**1**	25.6ns	2.99ns	9.37***	0.097ns	1.387***
S x SA	**3**	5.67ns	2.026ns	0.154ns	0.317ns	0.038ns
HBL x SA	**3**	5.1ns	2.756ns	0.232ns	0.123ns	0.05ns
Salt x HBL x SA	**3**	3.18ns	1.503ns	0.127ns	0.199ns	0.069ns
Error	**48**	8.55	1.647	0.296	0.132	0.053

*, **, *** = significant at 0.05, 0.01 and 0.001 levels.
ns = non-significant

regimes.

Salt stress had a significant decreasing effect on leaf and root K^+/Na^+ ratios of both sunflower lines. In contrast, under saline conditions, at 200 mg L^{-1} of SA a slight increase in K^+/Na^+ ratio was observed in cv. SF-187. Foliar-applied varying levels of salicylic acid showed inconsistent results for these ionic ratios (Tab. 2; Fig. 4).

Leaf and root Ca^{2+}/Na^+ ratios were significantly ($P \leq 0.001$) influenced by salt stress. A non-significant effect of exogenously applied salicylic acid was observed on leaf Ca^{2+}/Na^+ ratio, while root Ca^{2+}/Na^+ was increased due to SA application. Of all SA levels, 300 mg L^{-1} was highly effective for improving tissue Ca^{2+}/Na^+ ratios in both sunflower hybrids. Of both sunflower hybrids, SF-187 was better than Hysun-33 in this attribute (Tab. 2; Fig. 4).

Discussion

Salicylic acid (SA) being a vital plant growth regulator as well as an antioxidant (RASKIN et al., 1992) has a potential to allay the salt-

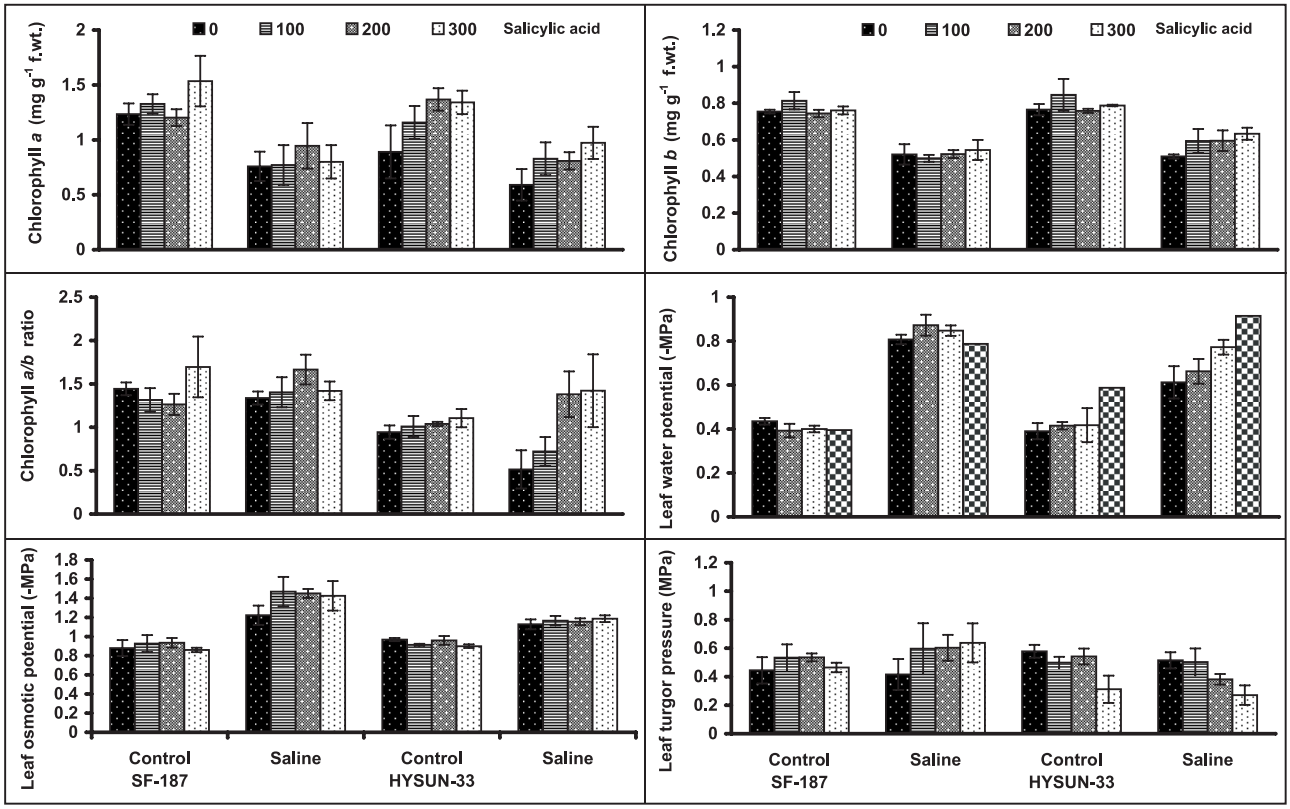

Fig. 1: Chlorophyll *a* and *b* contents, chlorophyll *a/b* ratio, and leaf water, osmotic and turgor potentials of two sunflower (*Helianthus annuus* L.) hybrid lines when varying levels of salicylic acid were applied as a foliar spray to 24 day-old plants subjected to normal or saline conditions (Mean ± S.E.; $n = 4$).

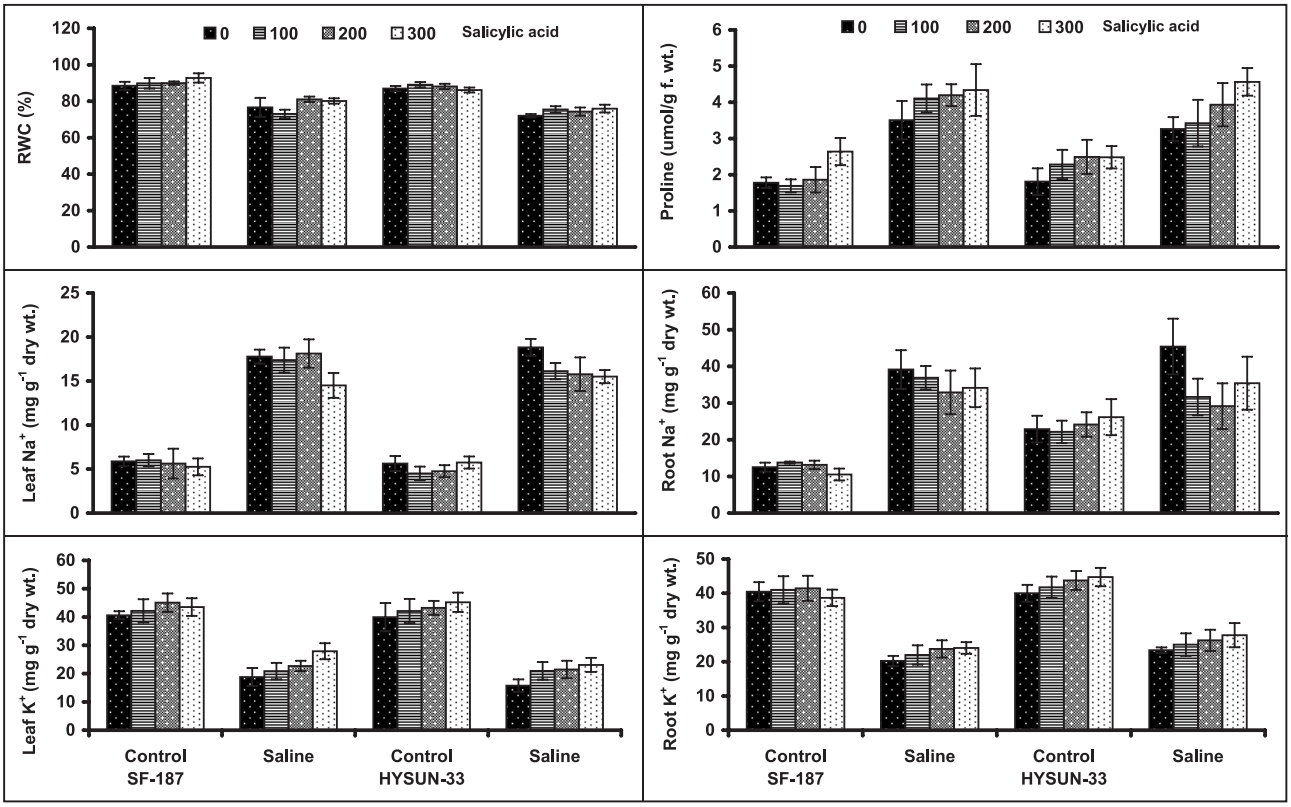

Fig. 2: Relative water content, proline accumulation, and leaf and root Na^+ and K^+ contents of two sunflower (*Helianthus annuus* L.) hybrid lines when varying levels of salicylic acid were applied as a foliar spray to 24 day-old plants subjected to normal or saline conditions (Mean ± S.E.; $n = 4$).

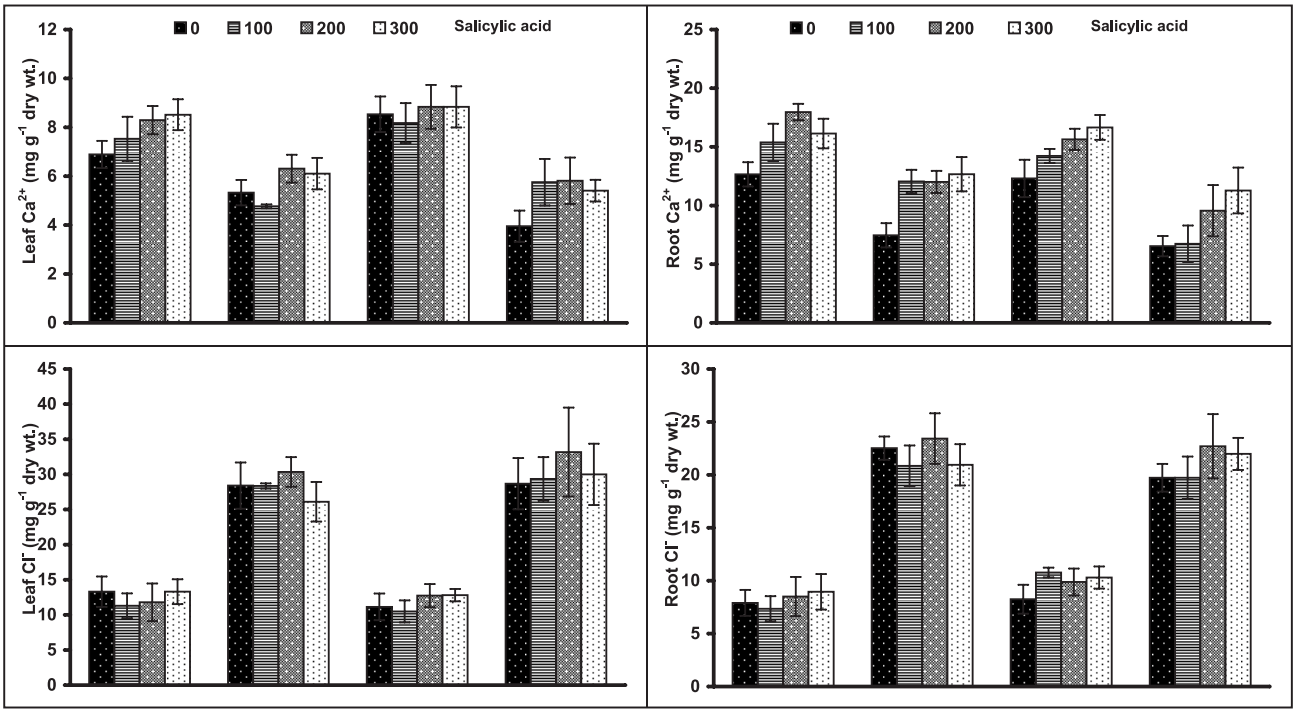

Fig. 3: Leaf and root Ca^{2+} and Cl^- accumulation in two sunflower (*Helianthus annuus* L.) hybrid lines when varying levels of salicylic acid were applied as a foliar spray to 24 day-old plants subjected to normal or saline conditions (Mean ± S.E.; $n = 4$).

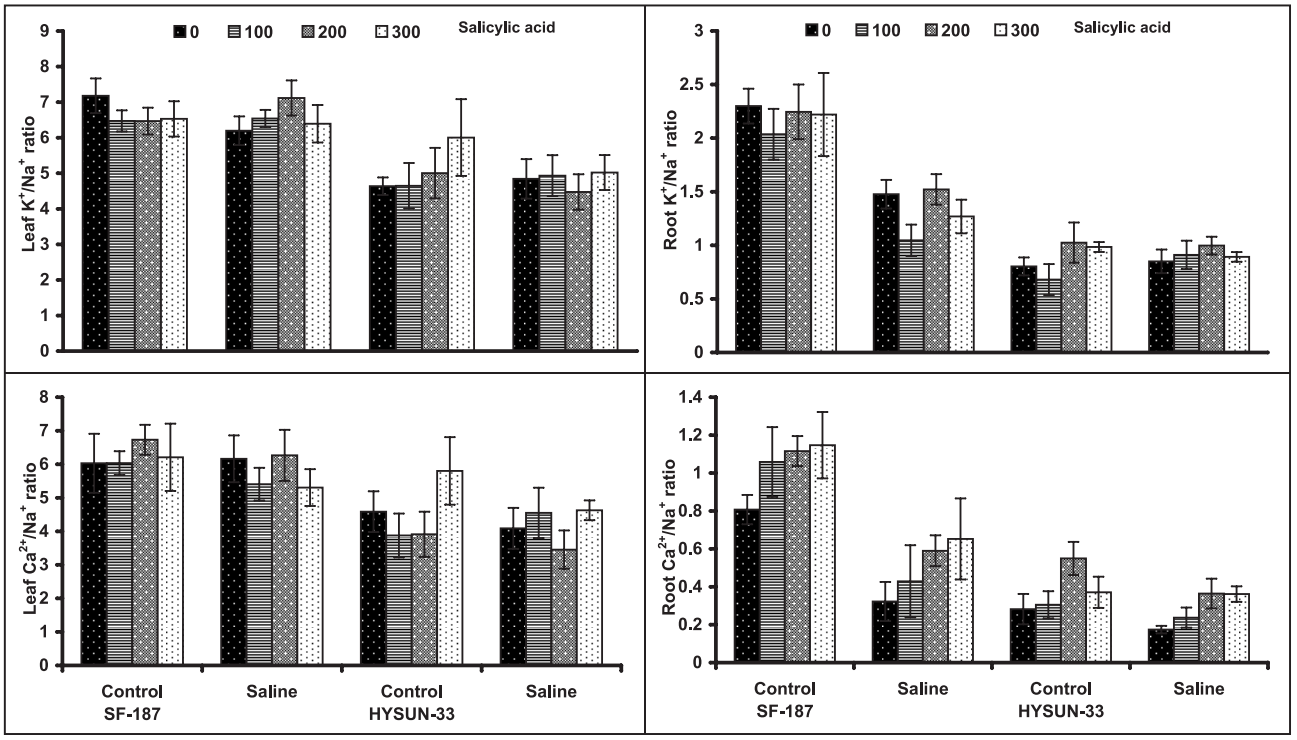

Fig. 4: Leaf and root K^+/Na^+ and Ca^{2+}/Na^+ ratios of two sunflower (*Helianthus annuus* L.) hybrid lines when varying levels of salicylic acid were applied as a foliar spray to 24 day-old plants subjected to normal or saline conditions (Mean ± S.E.; $n = 4$).

induced harmful effects on crop growth and development (El-Tayeb, 2005; Arfan et al., 2007; Ashraf et al., 2010). Photosynthetic pigments such as chlorophyll *a* and *b* are chief components of photosystems driving the mechanism of photosynthesis and hence growth in terms of biomass production or seed yield. In the present study, the contents of photosynthetic pigments was slightly improved with SA application. These findings are similar to those of Ghai et al. (2002) who showed a considerable improvement in chlorophyll contents due to foliar applied SA (200 mg L^{-1}). Similarly, Fariduddin et al. (2003) reported that *Brassica juncea* plants sprayed with

10^{-5} M of SA showed 20 percent higher chlorophyll than those sprayed with water only. By contrast, no change in chlorophyll content was observed in corn and soybean plants exogenously supplied with acetyl salicylic acid (KHAN et al., 2003). In the present study, a positive correlation of photosynthetic efficiency (*A*) with chlorophyll *a* or chlorophyll *b* (r = 0.416**; 0.436**, respectively) has been observed. Such relationships between *A* and chlorophyll contents have earlier been reported by FARIDUDDIN et al. (2003). They found that exogenous SA application caused an improvement in photosynthetic capacity in salt stressed *Brassica juncea* plants in association with improved chlorophyll content. The reduction in chlorophyll contents may occur due to acceleration in chlorophyll degradation or reduction in chlorophyll synthesis. However, it has been reported that imposition of salt stress causes deterioration in the structure of chloroplast e.g., thylakoid membranes and plastids due to direct Na^+ toxicity and cellular oxidative damage (MITTLER, 2002). In the present study, a strong negative correlation between leaf Na^+ and each of leaf chlorophyll *a*, chlorophyll *b*, or *A* (r = -0.610***; -0.804*** and -0.527*** respectively) has been observed.

Maintenance of plant water status or osmoregulation is a vital physiological process for maintaining optimal plant growth (TAIZ and ZEIGER, 2006). In the present study, leaf water relation parameters, leaf turgor, water and osmotic potentials, and relative water content of both sunflower lines were adversely affected by salt stress. Application of 200 or 300 mg L^{-1} SA improved leaf relative water content (RWC), but decreased leaf osmotic potential and leaf Ψ_w. Moreover, leaf RWC and leaf Ψ_p of the salt stressed plants of line SF-187 were higher than those in the leaves of line Hysun-33. Leaf osmotic potential of the salt stressed plants of SF-187 was also reduced due to SA application. These findings are in agreement with an earlier study (ASHRAF, 1989) in which leaf turgor potential increased with a concomitant decrease in osmotic potential in blackgram (*Vigna mungo*) under salt stress. Decrease in osmotic potential may occur due to accumulation of inorganic and/or organic solutes. Of various organic solutes, glycinebetaine, amino acids mainly proline, and soluble sugars play important roles in osmotic adjustment (HASEGAWA et al., 2000; ASHRAF and FOOLAD, 2005, 2007). Among inorganic solutes, K^+ and Na^+ are considered very important because they also play a vital role in osmoregulation, but Na^+ is potentially damaging when compared either with K^+ or organic solutes (SUBBARAO et al., 2001; TESTER and DAVENPORT, 2003). However, exogenous application of SA decreased leaf Na^+ content in the salt stressed plants of both sunflower lines, but leaf K^+ remained almost unaffected in both shoots and roots of both lines due to foliar-applied SA application. If relationships are worked out between leaf Ψ_s and each of proline, leaf Na^+, or leaf K^+ (leaf OP vs leaf proline; leaf OP vs leaf Na^+; leaf OP vs leaf K^+, r = 0.625***; 0.652***; -0.649***), it is amply clear that leaf Na^+ and leaf proline had a marked contribution in osmoregulation. Increased accumulation of proline in the salt stressed plants of both sunflower lines is similar to the findings of SHAKIROVA et al. (2003) who reported that SA-treated wheat seedlings showed enhanced accumulation of free proline. This can be further supported by the inference drawn by ASHRAF and HARRIS (2004) that under salt stress, higher accumulation of proline is one of the vital strategies of plants for causing osmotic adjustment, particularly in the presence of high cytosolic Na^+. In view of the results from the present study and all these reports, it can be suggested that although exogenous SA reduced accumulation of Na^+ in the leaves, its concentration is high enough to cause toxic effects, so increased accumulation of proline in the salt stressed sunflower plants reduced the toxic effect of Na^+ during osmotic adjustment. The results for accumulation of different mineral ions in the root and shoot tissues of both sunflower lines showed enhanced accumulation of Na^+ and Cl^- accompanied with a decline in K^+ and Ca^{2+} in both sunflower lines under saline substrate. These results support the viewpoint that plants subjected to saline substrate are prone to specific ion toxicity, ionic imbalance, and nutrient deficiency (ASHRAF, 1994, 2004). However, exogenously applied SA reduced leaf Na^+ of both sunflower lines. These results support the earlier findings of EL-TAYEB (2005) in which SA application resulted in reduced Na^+ in the leaves of barley seedlings under salt stress. In contrast, K^+ accumulation remained almost unchanged due to SA application. However, root or leaf Ca^{2+} content of salt stressed plants of both sunflower lines increased due to exogenously applied SA. These results support the earlier findings of KAWANO and MUTO (2000) who observed that SA enhanced the cytosolic Ca^{2+} level in tobacco cell suspension culture. In view of these results and some published reports available in the literature, it is suggested that exogenous SA application might have elevated cytosolic Ca^{2+} that acted as a second messenger taking part in a multitude of physiological responses including expression of osmotic responsive genes (PARDO et al., 1998) and antioxidant enzymes (CHEN and LI, 2001; AGARWAL et al., 2005). To appraise salt tolerance, K^+/Na^+ and Ca^{2+}/Na^+ ratios are considered as potential selection criteria, however, in the present study salt stress significantly decreased the leaf and root K^+/Na^+ ratios, while Ca^{2+}/Na^+ ratios were not influenced in sunflower plants and foliar-applied salicylic acid showed inconsistent results for these ionic ratios.

In conclusion, salt stress adversely affected the growth, chlorophyll pigments, water relations and contents of some key mineral nutrients, while increased the amount of proline and leaf and root Na^+ as well as Cl^- contents in both sunflower lines. Of both sunflower lines, Hysun-33 had higher amounts of photosynthetic pigments and essential nutrients than did SF-187. Foliar-applied SA improved growth, chlorophyll *a* and *b* pigments, leaf turgor potential, and leaf and root Ca^{2+} concentrations. Of all salicylic acid levels, 200 and 300 mg L^{-1} were found to be relatively more effective than the other levels in improving chlorophyll *a* and *b* pigments, leaf turgor potential, leaf and root Ca^{2+} concentration, while the other attributes remained unaffected due to SA application.

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