

ALLEVIATION OF ADVERSE EFFECTS OF SALT STRESS ON SUNFLOWER (*HELIANTHUS ANNUUS* L.) BY EXOGENOUS APPLICATION OF SALICYLIC ACID: GROWTH AND PHOTOSYNTHESIS

SIBGHA NOREEN AND *MUHAMMAD ASHRAF*

Department of Botany, University of Agriculture, Faisalabad 30840, Pakistan

Abstract

The present study was conducted to assess whether exogenously applied SA as a foliar spray could ameliorate the adverse effects of salt stress on sunflower plants. Two lines of sunflower (Hisun-33 and SF-187) were grown under normal or saline (120 mM NaCl) conditions. Different levels of salicylic acid (0, 100, 200, 300 mg L⁻¹) were applied as a foliar spray. Salt stress reduced the growth of both lines, but both cultivars were equally responsive to the stress. Application of 200 mg L⁻¹ of SA caused an increase in biomass of both lines under non-saline or saline conditions. Photosynthetic rate of both cultivars increased due to SA applied foliarly both under control and saline conditions, particularly in line SF-187. Furthermore, increase in growth of both cultivars due to exogenously applied SA may have been due to changes in photosynthesis. However, SA applied as a foliar spray did not change the sub-stomatal CO₂ suggesting that stomatal factors were not the major controlling factors for photosynthesis. Overall, the adverse effects of salt stress could be alleviated by exogenous application of SA.

Introduction

Salt stress is known to perturb a multitude of physiological processes including photosynthesis. For example, a significant reduction in photosynthesis was found in *Brassica* spp. (Nazir *et al.*, 2001), and wheat (Raza *et al.*, 2006). However, degree of salt-induced reduction in photosynthetic capacity depends on amount of photosynthesizing tissue (leaf area), photosynthetic pigments, stomatal and non-stomatal factors that affect the CO₂ assimilation (gas exchange and metabolism) (Dubey, 2005). Salt-induced osmotic stress as well as sodium toxicity trigger to the formation of reactive oxygen species (ROS) such as superoxide (O₂^{•-}), hydrogen peroxide (H₂O₂), hydroxyl radical (OH), and singlet oxygen (¹O₂), which can damage mitochondria and chloroplasts by disrupting cellular structures (Mittler, 2002). To scavenge ROS, plants synthesize different types of antioxidant compounds or activate key antioxidant enzymes (Mittler, 2002). In addition, salt tolerance has been found to be positively associated with a more efficient antioxidant system (Mittler, 2002). In view of these reports, it was suggested that salt tolerance could be induced by enhancing antioxidant capacity of plants.

SA is water-soluble antioxidant compound that can also regulate plant growth (Aberg 1981). It also has a role in abiotic stress tolerance such as drought tolerance in wheat (Singh & Usha, 2003), and salt tolerance in wheat (Sakhabutdinova *et al.*, 2003; Shakirova *et al.*, 2003). Ameliorative effect of SA on growth of crop plants under abiotic stress conditions may have been due to its role in nutrient uptake (Glass 1974), water relations (Barkosky & Einhelling 1993), stomatal regulation (Larque-saavedra, 1979; Arfan *et al.*, 2007),

* Corresponding author: ashrafbot@yahoo.com

photosynthesis and growth (Khan *et al.*, 2003; Arfan *et al.*, 2007). In contrast, while working with maize, Németh *et al.* (2002) reported that exogenously applied SA through the rooting medium caused growth inhibition. Likewise, Borsani *et al.* (2001) demonstrated that transgenic *Arabidopsis* plants (*NahG*) producing salicylate hydroxylase, which transforms SA to catechol (thus lower concentration of endogenous SA in transgenic plant than that in wild type plant), were better able to resist the oxidative damage caused by salt and osmotic stress than the wild type plants. These reports clearly show that SA cannot induce abiotic stress tolerance in all types of plants or in other words the effectiveness of SA in inducing stress tolerance depends upon type of species or concentration of SA applied. Therefore, an experiment was conducted to determine whether foliar applied SA could induce salt tolerance in sunflower plants and to draw relationships between growth and photosynthetic capacity to elucidate mechanism associated with improved salinity tolerance in sunflower due to exogenously applied SA.

Materials and Methods

An experiment was conducted under greenhouse conditions at the Department of Botany, University of Agriculture, Faisalabad Pakistan. Achenes of sunflower (SF-187 and Hisun-33) were obtained from the Regional Office of Pakistan Seed Council Faisalabad, Pakistan. During the growth period mean day temperature $30.6 \pm 5.1^\circ\text{C}$, night temperature $18.3 \pm 7.6^\circ\text{C}$, relative humidity (RH) 35.9 ± 6.5 and the day length from 8 to 11 hours were recorded. Achenes of sunflower (*Helianthus annuus* L) were surface sterilized in 5% sodium hypochlorite solution for 10 minutes before using them for experimentation. Ten sunflower achenes were directly sown in each plastic pot of 28 cm diameter containing 12 kg well-washed sand, but after germination, seedlings were thinned to three of almost uniform size. The experiment was arranged in a completely randomized design with four replicates. All pots were irrigated with full strength Hoagland's nutrient solution for 18 days after which NaCl treatments in Hoagland's nutrient solution were begun. The NaCl treatments used were 0 or 150 mM in full strength Hoagland's nutrient solution. Salt solution was applied in aliquots of 50 mM every day. Two liters of a treatment solution was applied to each pot after every week, however, moisture content of the sand was maintained daily by adding 200 ml distilled water to each pot. Different concentrations of salicylic acid (SA) (M. wt. = 138.1) [(0, 0.1% Tween 20 solution; 100, 200 and 300 mg L⁻¹ in 0.1% Tween-20 {polyoxyethylene sorbitan monolaurate} solution)] were applied as a foliar spray at the vegetative stage. Tween-20 solution was used as a surfactant to ensure penetration of SA into leaf tissues. A constant volume (5 ml/plant) of the solution was sprayed on all pots with a manual sprayer. The plants were sprayed once on the leaves early in the morning. The plants were harvested three weeks after foliar application of SA at the vegetative stage and data for fresh biomass were recorded. The plants were then oven-dried at 65°C for 72 h and dry biomass recorded. However, before harvesting the plants following physiological parameters were also measured:

Gas exchange characteristics: Measurements of gas exchange attributes were made on 2nd intact leaf from the top of each plant using an ADC LCA-4 portable infrared gas analyzer (Analytical Development, Hoddesdon, UK). These measurements were recorded from 10:30 to 12:30 h with the following specifications/adjustments of the leaf chamber:

leaf surface area, 11.25 cm^2 ; water vapor pressure into the chamber ranged from 6.0 to 8.9 mbar, ambient CO_2 concentration, $352 \mu\text{mol mol}^{-1}$; temperature of the leaf chamber varied from 28.4 to 32.4 °C; leaf chamber gas flow rate (U), $251 \mu\text{mol s}^{-1}$; molar flow of air per unit leaf area (U_s) $221.06 \text{ mol m}^{-2} \text{ s}^{-1}$; RH of the chamber 41.2 %; PAR (Q_{leaf}) at the leaf surface at noon was up to $918 \mu\text{mol m}^{-2} \text{ s}^{-1}$; ambient pressure was 98.8 kPa.

Statistical analysis: The experiments were set up in a completely randomized design (CRD) with four replicates. Analysis of variance of all parameters was computed using the Costat computer package. The least significance difference between the mean values was calculated following Snedecor & Cochran (1980).

Results

Salt stress caused a significant reduction in shoot fresh and dry weights of both sunflower lines (Fig 1). However, shoot fresh and dry weights of both lines were appreciably increased due to exogenous application of salicylic acid under both stressful

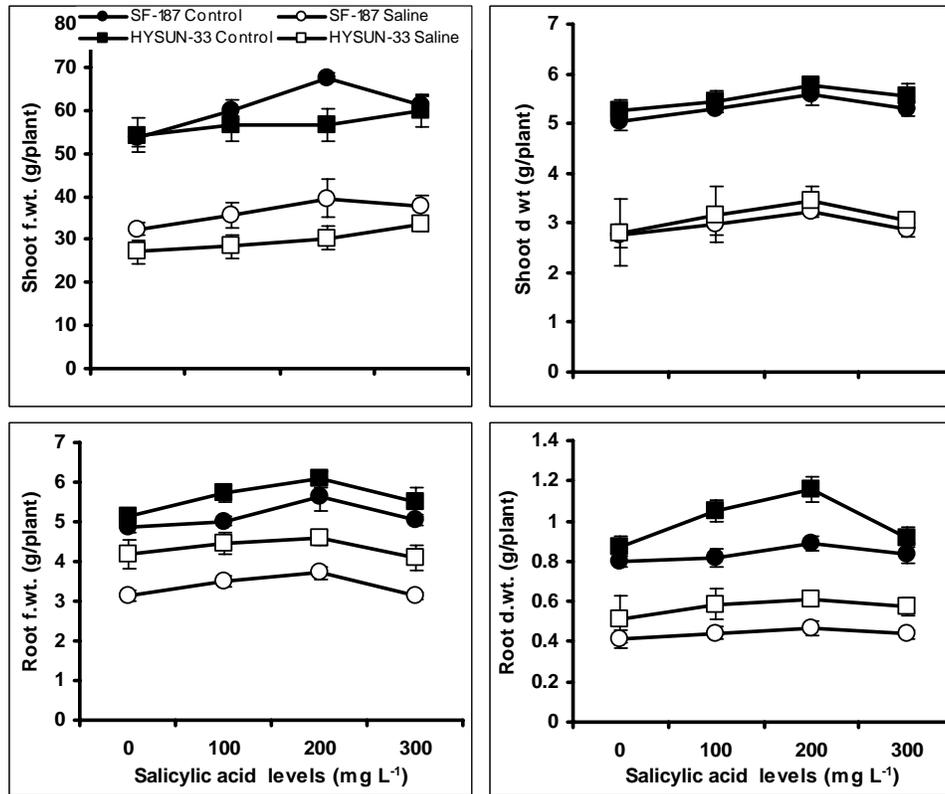


Fig. 1: Fresh and dry weights of shoots and roots of salt stressed and non-stressed plants of two lines of sunflower when different concentrations of SA were applied at the vegetative stage as a foliar spray.

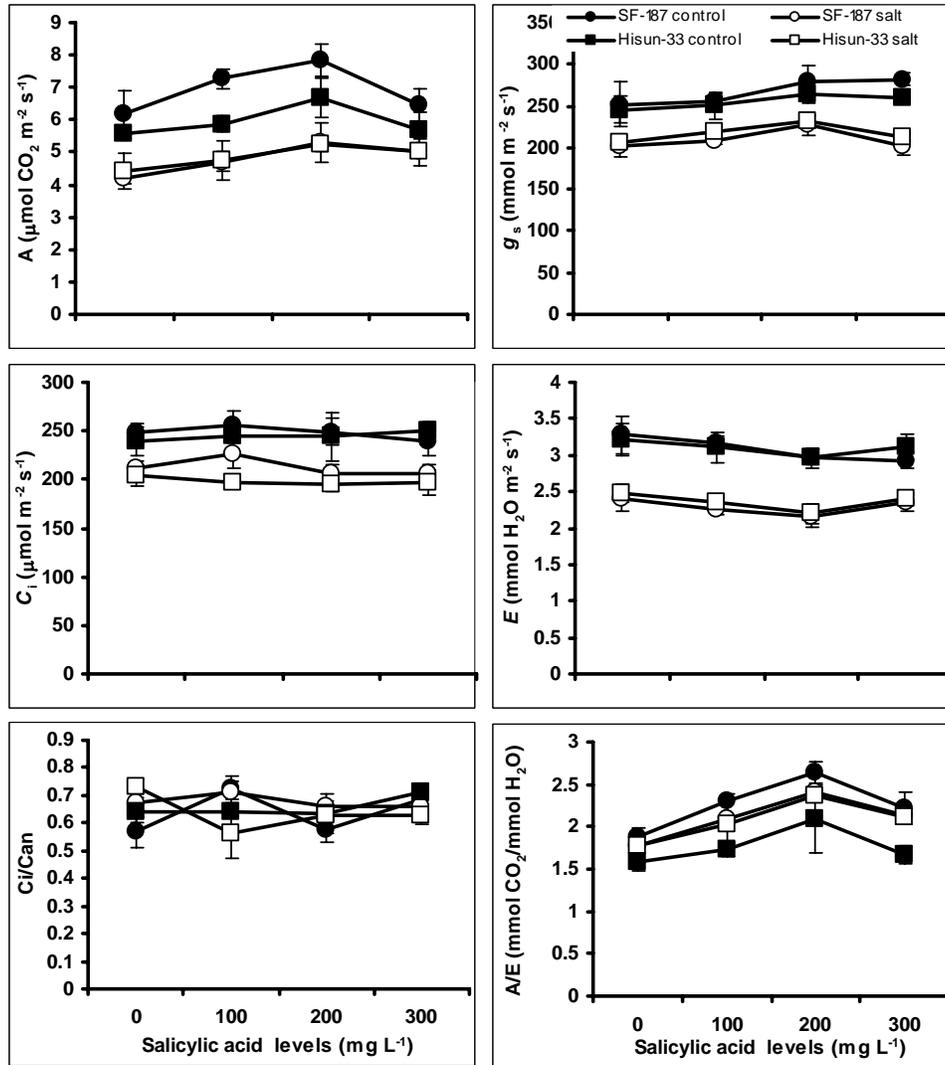


Fig. 2: Gas exchange characteristics of salt stressed and non-stressed plants of two lines of sunflower when different concentrations of SA were applied as a foliar spray at the vegetative stage.

and non-stressful conditions. However, line SF-187 had higher shoot fresh weight than that of Hisun-33, particularly under saline conditions. A maximum increase in shoot fresh weight was recorded at 200 mg L⁻¹ of SA. In contrast, difference in shoot dry weights between sunflower lines was non-significant (Fig. 1). Root fresh and dry weights of both lines decreased due to imposition of salt stress. However, line Hysun-33 produced higher fresh and dry root biomass compared to line SF-187 under saline conditions.

Salt stress reduced net CO₂ assimilation rate (P_N), transpiration rate (E), stomatal conductance (g_s), and sub-stomatal CO₂ (C_i) of both sunflower lines. However, water use efficiency ($WUE = P_N/E$) and C_i/C_a was not significantly affected due to salt stress. Exogenous application of 200 mg L⁻¹ increased net CO₂ assimilation rate of both lines under both non-saline and saline conditions. However, line SF-187 showed higher net assimilation rate under saline conditions, whereas under saline conditions differences between the lines were non-significant (Fig 2). Similarly, stomatal conductance was also improved due to foliar applied SA in stressed and non-stressed plants of both lines, particularly at 200 or 300 mg L⁻¹ of SA level (Fig 2). However, the lines did not differ in this gas exchange attribute. In contrast, transpiration rate of both lines remained almost unchanged due to foliar applied SA under both non-saline and saline conditions. Sub-stomatal CO₂ (C_i) also remained unaffected due to SA applied as a foliar spray in both salt stressed and non-stressed plants of both lines, except in salt stressed SF-187 plants where 100 mg L⁻¹ SA caused a significant increase in C_i . The interactive effects showed that water-use-efficiency (WUE) [P_N/E] was significantly affected by salicylic acid and salinity (Fig. 2). Generally WUE increased due to SA applied as a foliar spray in both salt stressed and non-stressed plants of both sunflower lines, particularly at 200 mg L⁻¹ SA level.

Discussion

From the results of the present study, it is obvious that salt stress reduced plant growth of both sunflower cultivars. However, exogenous application of SA improved the plant growth in salt stressed plants of both cultivars. These results are in agreement with those of El-Tayeb (2005) and Arfan *et al.* (2007) who reported that exogenous foliar application of SA ameliorated the adverse effects of salt stress on growth of barley and wheat, respectively. Similarly, foliar application of SA also caused increase in biological yield of wheat under water stress (Singh & Usha, 2003). Thus suggests that SA-induced enhancement in growth may also promote crop yield. Since salt stress limits plant growth by adversely affecting various physiological and biochemical processes including photosynthesis, antioxidant capacity, and ion homeostasis (Ashraf, 2004), it is suggested that SA-induced enhancement in growth of salt stressed plants might have been due to SA-induced changes in these biochemical or physiological processes. For example, El-Tayeb (2005) reported that SA-induced increase in growth could be related to enhanced activity of antioxidants that protect the plants from oxidative damage. Therefore, photosynthesis which is a major controlling factor for plant growth and yield (Natr & Lawlor, 2005) might have been increased due to SA application. In the present study, foliar application of SA caused considerable enhancement in net photosynthetic rate under salt stress, particularly at 200 mg L⁻¹ SA level. This increase in photosynthetic rate due to exogenously applied SA was in agreement with some earlier studies in which it was found that exogenously applied SA increased the photosynthetic rate in different crops, e.g., barley (Pancheva *et al.*, 1996), rice (Maibangsa *et al.*, 2001), soybean (Kumar *et al.*, 2000; Khan *et al.*, 2003), wheat (Singh & Usha, 2003), and maize (Khan *et al.*, 2003; Khodary, 2004). Furthermore, a positive relationship between photosynthetic rate and growth has been observed, which suggests that SA-induced changes in photosynthetic rate might have contributed in growth enhancement under saline conditions. Similar relationship between growth and photosynthetic capacity was found in different crops e.g., *Brassica* spp. (Nazir *et al.*, 2001), wheat (Arfan *et al.*, 2007).

In the present study, exogenously applied SA caused increase in stomatal conductance, but it did not change the sub-stomatal CO₂. These findings suggest that SA applied as a foliar spray reversed the salt-induced stomatal closure. This argument can be supported by the findings of Rai *et al.* (1986) that SA application can reverse the stomatal closure induced by ABA. However, SA-induced increase in *A* and *g_s* with no change in sub-stomatal CO₂ (*C_i*) suggests that stomatal limitations were not the controlling factors for photosynthesis as was previously observed in *Capsicum annum* (Bethke and Drew, 1992). However, it can be suggested that foliar spray with SA might have affected certain metabolic factors in carbon uptake or fixation including Rubisco enzyme concentration and activity, and/or photosynthetic carbon reduction (PCR) cycle as previously suggested by Khodary (2004) and Arfan *et al.* (2007).

In conclusion, the adverse effects of salt stress on the growth of sunflower can be mitigated by foliar spray of SA. However, effectiveness of SA in alleviating the adverse effects of salt stress was dose dependent.

Acknowledgement: The work presented here is a part of Ph.D thesis of the first author.

References

- Al-Hakimi, A.M.A. 2001. Alleviation of the adverse effects of NaCl on gas exchange and growth of wheat plants by ascorbic acid, thiamine and sodium salicylate. *Pak. Biol. Sci.* 4:762-765.
- Arfan, M., H.R. Athar and M. Ashraf. 2007. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *J. Plant Physiol.*, 6(4): 685-694.
- Ashraf, M. 2004. Some important physiological selection criteria for salt-tolerance in plants. *Flora*, 199:361-376.
- Barkosky R. R., and F. A. Einhelling. 1993. Effect of salicylic acid on plant water relationship. *J. Chem. Ecol.* 19: 237-247.
- Borsani, O., V. Valpuesta and M.A. Botella. 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. *Plant Physiol.* 126:1024-1034.
- Dubey, R.S. 2005. Photosynthesis in plants under stressful conditions *In* Photosynthesis handbooks. 2nd Ed. M. Pessarakli C.R.C. Press, New York. p. 717-718.
- El-Tayeb, M.A. 2005. Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regul.*, 45:215-224.
- Ghoulam, C.F., F. Ahmad, and F. Khalid, 2001. Effect of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Env. Exp., Bot.*, 47:139-150.
- Glass, A.D.M. 1974. Influence of phenolic acid on ion uptake. III. Inhibition of potassium absorption. *J. Exp. Bot.*, 25: 1104-1113.
- Khan, W., Prithiviraj, B. and D. Smith. 2003. Photosynthetic response of corn and soybean to foliar application of salicylates. *J. Plant Physiol.* 160:485-492.
- Khodary, S.E.A. 2004. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. *Intl. J. Agri. Biol.*, 6: 5-8.
- Larque,-Saavedra, A. 1978. The antitranspirant effect of acetylsalicylic acid on *Phaseolus vulgaris*. *Physiol. Plant.* 43: 126-128.
- Larque-Saavedra, A. 1979. Stomatal closure in response to acetalsalicylic acid treatment. *Z. Pflanzenphysiol.*, 93: Pp. 371-375.
- Liu,C; J. Zhan, Y. Yong, B. Yu Cuiben and F. Yu-Long. 1999. Effects of salicylic acid on the photosynthesis of apple leaves. *Acta Hort. Sinica*, 26: 261-262.

- Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.*, 7: Pp. 405-410.
- Natr L, and D.W. Lawlor. 2005. Photosynthetic plant productivity. In *Photosynthesis Handbook*, (2nd Ed). M. Pessarakli. C.R.C. Press, New York, USA. p. 501-524.
- Nazir, N., M. Ashraf and R. Ejaz. 2001. Genomic relationships in oilseed Brassicas with respect to salt tolerance-photosynthetic capacity and ion relations. *Pak. J. Bot.*, 33: 483-501.
- Nemeth, M., T. Janda, E. Horvath, E. Paldi and G. Szalai, 2002. Exogenous salicylic acid increases polyamine content but may decrease drought tolerance in maize. *Plant Sci.* 162: Pp. 569-574.
- Ormaetxe, I., Escudero, P. R., Arrese-Igor, C. and M. Becana., 1998. Oxidative damage in pea plants exposed to water deficit or paraquat. *Plant Physiol.* 116: 173-181.
- Rajasekaran, L.R., T.J. Blum. 1999. New plant growth regulators protect photosynthesis and enhance growth under drought of jack pine seedlings *J. Plant Growth Regul.* 18: 175-181.
- Raskin, I. 1992. Role of salicylic acid in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 43: 439-463.
- Rawson, H.M. 1986. Gas exchange and growth in wheat and barley grown in salt. *Aust. J. Plant Physiol.* 13:475-489.
- Raza, S.H., H.R. Athar, and M. Ashraf. 2006. Influence of exogenously applied glycine betaine on photosynthetic capacity of two differently adapted wheat cultivars under salt stress. *Pak. J. Bot.* 38:341-351.
- Sakhabutdinova, A.R., D.R. Fatkudinova, M.V. Bezrukova and F.M. Shakirova 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulg. J. Plant. Physiol.*, Special Issue, 21: 314-319.
- Shakirova, F.M., A.R. Sakhabutdinova, M.V. Bezrukova, R.A. Fatkhutdinova and D.R. Fatkhutdinova. 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci.*, 164: 317-322
- Singh, B. and K Usha. 2003. Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul.*, 39:137-141.
- Snedecor, G. W. and G. W. Cochran. 1980. *Statistical Methods*, 7th edition. The Iowa State University Press, Ames, Iowa.

(Received for publication 21 April, 2008)

