Research Article

Influence of Foliar Application of Osmoprotectants to Ameliorate Salt Stress in Sunflower (*Helianthus annuus* L.)

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Abstract | Osmoprotectants are highly soluble, neutral and non-toxic chemical compounds which have low molecular weight. These molecules help the plants to regulate osmotic adjustment and to enhance abiotic stress tolerance in plants. This study was aimed to examine mitigation effects of different osmoprotectants (salicylic acid, ascorbic acid, proline and their admixture) @ 200 mgL^{-1} on sunflower (*Helianthus annuus* L.) grown under saline environment (150 mM NaCl). The results showed that salinity stress (150 mM) resulted into significant decrease in biomass accumulation of sunflower up to 50%, while it was improved by 80% in control by the foliar application of osmoprotectants. Chlorophyll (Chl-*a*, chl b and total chlorophyll) contents were also decreased significantly due to saline environment; however, there was improvement of 288.5%, 162.5% and 141.0% by 200 mgL^{-1} spray of proline. The amount of hydrogen peroxide and Malondialdehyde were elevated by 170.3% and 68.7%, respectively while enhanced by 54% and 47.4% by spray of proline under salinity. Significant reduction in K^+ uptake with concurrent increase in Na^+ content was observed under NaCl salt stress, while the foliar application of proline increased the amount of K^+ at vegetative growth under stress and non-stress conditions. The biological yield, physiological, and oxidative activity of sunflower crop could be enhanced by aerial spray of proline at the rate of 200 mgL^{-1} under saline environment.

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Introduction

Globally, the production of oilseed crops has attained third position following cereals and legumes crops for staple and dietary consumption (Damude and Kinney, 2008). Among other oilseed crops, sunflower (*Helianthus annuus* L.) is a high valued crop, which contains 48% oil content and is a rich source of vitamins (A, D, E, and K). In Pakistan, sunflower shares 0.14 million hectors land and having yield potential yield of 0.17 billion tons. Pakistan is net importer of edible oil seeds and oils, which suffices 80% of the total requirement, while 20% is met through local production. More than 2.50 million tons of edible oil is imported at the cost of PKR 190.58 billion to meet the national demand (GoP, 2016). On the other hand, the consumption of edible oil is 12.4 kg $capita^{-1}$ $Annum^{-1}$, which is the highest among the regional countries. The scenario shows that sunflower crop could be a potential and prospective oil seed crop to increase local oil production.

Sunflower crop is considered as a moderately salt tolerant crop (Steduto et al., 2000), however, its yield is drastically reduced at salinity level of EC_e 2.5 dSm^{-1} (El-Kader et al., 2006). Beyond 10.0

dSm^{-1}salinity level the plants yield decreases by 50% (Pitman and Lauchli, 2002). Plants which grow on these soils accumulate the toxic ions like Na^+, Cl^- in their cell (Acosta-Motos et al., 2017), which lead oxidative stress through increase in (reactive oxygen species (ROS) production (Sharma et al., 2012). The cumulative effects of these stresses result in alteration of biochemical and molecular composition (Schroeder et al., 2013). The growth and development of plants is impacted negatively in terms of reduction in leaf size, stem, and root organs, lowering in water use efficiency (Farooq et al., 2009), decreased photosynthetic efficiency and translocation of nutrients from rhizosphere to upper plant portions (Zhang et al., 2014) ultimately results in low productivity (Saleem et al., 2011). Under the adverse growing environment, plant strives to withstand by adapting certain mechanisms, *i.e.* synthesis of low molecular weight compatible osmoprotectants (proline, salicylic acid, ascorbic acid, glycinebetaine) at their cellular level (Mahboob et al., 2016). These osmoprotectants adjust osmotic balance by protecting cellular membranes and scavenging ROS production due to stressful conditions (Gill and Tuteja, 2010). The external application of osmoprotectants, such as ascorbic acid, proline and salicylic acid reinforce the mechanisms to cope with salt stress for maintenance of growth and development (Noreen and Ashraf 2008, 2010; Noreen et al., 2012, 2018; Sing et al., 2015).

The naturally occurrence of ascorbic acid (AsA) in chloroplast works as substrate for ascorbate peroxidase enzymes against oxidative damage for continuation of photosynthetic machinery (Saeidi-Sar et al., 2013). The beneficial effects are evidenced in increased in biomass production, ionic ratio and photosynthetic pigments leading to improved antioxidant enzymatic efficiency (El-Afry et al., 2018).

Proline is instantly produced and accumulated in plant system, in response to different abiotic stresses (Mahboob et al., 2016; Noreen et al., 2018). It rescues the sub-cellular organelles by mitigating the obnoxious effects of over production of ROS (Ashraf and Foolad, 2007). Under saline environment, the foliage shower of proline alleviated oxidative stress and led to improved physiological processes for continuation of growth and development in arable crops like maize (Hussein et al., 2007), rice (Deivanai et al., 2011), wheat (Mahboob et al., 2016), sunflower (Khan et al., 2014) and cotton (Noreen et al., 2013).

Salicylic acid (SA) is a phyto-hormone and produced endogenously in the plant system (Hamida and Shaddad, 2010), which works as a signaling molecule (Rajeshwari and Bhuvaneshwari, 2017). SA helps to regulate physiological processes, improving the efficiency of photosynthetic machinery, maintaining ionic balance, with concurrent mitigating obnoxious effects caused by abiotic stresses in plant system (Arfan et al., 2007; Farooq et al., 2009; Sing et al., 2015). Due to climate change, either intermittent or long term persistence of adversity of weather conditions the disruption in the growth and development is occurred to a larger proportion than the normal environment. Thus, a research was done to check effect of osmoprotectants on sunflower under saline state.

Materials and Methods

This study was conducted at Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan-Pakistan during the first week of February 2017. The average day/night temperature was 21°C/7°C, humidity 64%, wind pressure 1015mbar and pan evaporation rate 67mm was recorded during February 2017. The seeds of sunflower (*Helianthus annuus* L. var. "Hysun-33") were used for experimentation. Ten kg sand was filled-in plastic pots having diameter of 24.5cm × 28.0cm depth. The treatments were consisted of (a) foliar spray of osmoprotectants (SA, ASA and proline and their admixture at the rate of 0 (control) and 200 mgL^{-1}) and (b) two levels of NaCl salt stress [0mM (control) 150 mM (NaCl)] and organized in completely randomized design (CRD) with four replicates. Ten seeds were dibbled in each pot and thinning was to three plants per pot after complete germination. Hoagland's nutrient solution (Hoagland and Arnon, 1950) was applied regularly to meet the nutritional requirement. The salinity level was developed by dissolving neutral NaCl salt in one litter of modified full strength Hoagland's Nutrient Solution. The salinity stress was developed step-wise from 50 mM to final 150 mM NaCl by adding 2.5 litters of Hoagland's Nutrient Solution to each pot on consecutive treatments in order to leach out extra salts from sandy pots and to maintain level of stress.

The spray solution of SA, ASA and proline and their admixture was prepared with Tween-20 (0.1%). The plants were sprayed early in the day using hand-held pump containing 15 ml of osmoprotectant solution

in two splits with an interval of six days. Plants were harvested after 15 days of second spray and were used to record different morphological and biochemical attributes.

Morphological attributes

The uprooted plants were washed with distilled water and data for root and shoot lengths and root and shoot fresh weights was recorded immediately after harvest. The plant material (root and shoot) were placed in oven at 70°C for 72 hours and root and shoot dry weights were recorded.

Chlorophyll contents

Chlorophyll (*a*, *b* and total) contents were analyzed using Arnon (1949) method. 0.1g fresh leaf material was extracted in 10 ml of 80% acetone. The extract was centrifuged in at 8000 rpm for 10 minutes. The absorbance of supernatant was recorded at 645 and 663 nm with spectrophotometer. The chlorophyll contents were calculated using following equations.

$$Chlo\text{-}a = 12.7 \times OD663 - 2.69 \times OD645 \times (V \times W)/1000$$
$$Chlo\text{-}b = 22.9 \times OD645 - 4.68 \times OD663 \times (V \times W)/1000$$

Where;
V is volume of acetone (10ml) and W is the weight of leaf sample (0.1g).

Enzyme extraction

For enzyme extraction, 0.5 g of fresh leaves were homogenized with 5 ml of 50 mM pre-cooled sodium phosphate buffer (pH 7.8) placed in ice bath. The homogenate was centrifuged at 12000 rpms for 12 minutes at 4°C. The supernatant (enzyme extract) was used for the estimation of total soluble protein, total free amino acids and antioxidant enzyme activities.

Total soluble protein contents: Total soluble protein contents (TSP) were estimated following the method of Bradford (1976). For each sample, 0.1 µl of enzyme extract was mixed with 5 ml of Bradford reagent and placed for 15 minutes at 30°C in test tubes. A test tube containing distilled water in place of enzyme extract mixed with Bradford served as blank which was used as reference. Absorbance was recorded at 595 nm with spectrophotometer.

Total free amino acid contents: Total free amino acid contents were estimated following Hamilton and Van slyke (1943) method. 0.5 ml of enzyme extract was taken into 25 ml glass beaker along with 0.5 ml of pyridine (10%) and 0.5 ml of ninhydrin (2%) solution. A beaker containing all reacting solutions and distilled water in place of enzyme extract served as blank which was used as reference. These beakers were water bathed at 90°C for 30 minutes. Volume of each sample beaker was raised to 25 ml with distilled water. The absorbance of colored samples was recorded at 570 nm using spectrophotometer.

Catalase: Activities of Catalase (CAT) was assayed following Aebi (1984) method. The final volume (3 ml) of the reaction mixture in test tubes contained 2.8 ml of 50 mM phosphate buffer, 0.1 ml of 300 mM H_2O_2 and 0.1 ml enzyme extract. The reaction was initiated by adding enzyme extract to the reaction mixture. A test tube containing all reacting solutions and distilled water in place of enzyme extract served as blank which was used as reference. Changes in absorbance of the reaction solution due to decomposition of H_2O_2 was observed at 240 nm using spectrophotometer over one-minute time scan. The activity of CAT was expressed as U mg^{-1} protein min^{-1} where U represents the mmoles units (U) of H_2O_2 decomposed during reaction time.

Peroxidase: The activity of peroxidase (POD) was determined by guaiacol oxidation method (Chance and Maehly, 1955). The final volume (3 ml) of reaction mixture in test tubes contained 2.7 ml of 50 mM phosphate buffer (pH 7.0), 0.1 ml of 1.5% guaiacol, 0.1 ml of 300 mM H_2O_2, and 0.1 ml of enzyme extract. A test tube containing all reacting solutions and distilled water in place of enzyme extract served as blank which was used as reference. Changes in absorbance of the reaction solution, due to guaicol oxidation, was observed at 470 nm using spectrophotometer over one-minute time scan. The activity of POD was expressed as U mg^{-1} protein min^{-1} where U represents the extension of mmoles units (U) of guaiacol during reaction time.

Hydrogen peroxide

Hydrogen peroxide (H_2O_2) content was estimated according to Velikova et al. (2000). For estimation of H_2O_2 fresh leaves (0.25 g) were homogenized in 5 ml 0.1% TCA in pestle and mortar. The homogenized samples were centrifuged at 12000 rpm for 15 minutes. Reaction solution contained 0.5 ml potassium phosphate buffer, 1 ml potassium iodide solution and 0.5 ml enzyme extract poured in test tube. This

mixture was vortexed and reading was taken 390 nm by using spectrophotometer.

Malondialdehyde

The contents of malondialdehyde (MDA) were observed by Carmak and Horst (1991) method with minor modifications. Leaf samples of 1.0 g was homogenized in 3 ml of 0.1% (w/v) trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 12000 rpm for 15 minutes and supernatant was removed. 3 ml of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA was added to 0.5 ml of the supernatant. The mixture was heated at 95°C in water bath for 50 minutes. The reaction was stopped by immediate cooling the tubes in ice. Then the samples were centrifuged at 8000 rpm for 10 minutes and the absorbance of the supernatant was read at 532 and 600 nm using spectrophotometer.

Determination of mineral elements

Digestion mixture: The Na^+ and K^+ contents in roots and shoots were determined by Wolf (1982) method. For this purpose, digestion mixture was prepared by adding 0.42 g of Se and 14 g of $LiSO_4.2H_2O$ to 350 ml of H_2O_2. This mixture was slowly added to 420 ml of conc. H_2SO_4 keeping flask in ice bath. The reagent so prepared was stored at 2°C and was used for the digestion of plant material.

Digestion method: Oven dried plant samples of leaf and root (0.1 g) were taken in digestion flasks and 2 ml of digestion mixture was poured into the digestion flasks and were incubated overnight at 25°C. On the next day flasks were placed onto the hot plate and was warmed at 250°C until fume formation occurred. Heating was continuously supplied for 30 minutes. Digestion flasks were removed from hot plate and 0.5 ml of perchloric acid ($HClO_4$) was carefully poured into each flask and heating process was continued for about 2 hours at 250°C until discoloration of the mixture. This digested material was filtered and final volume was raised up to 50 ml into volumetric flasks, which was used for determination Na^+ and K^+ contents in samples.

Na+ and K+ ion estimation: The contents of sodium (Na^+) and potassium (K^+) in samples of root and shoot were determined by flame photometer. The ionic concentrations were calculated by comparison with standard curve.

Statistical analysis

Data were analyzed using a computer-based program SPSS-20 and Duncan's Multiple Range Test (DMRT) for comparison purpose (Steel et al., 1966). Data were presented contains means ± standard error (SE) of four replicates (n=4) for each treatment.

Results and Discussion

Data for biological yield differed significantly ($p<0.05$) due to imposition of 150 mM NaCl salt stress (Table 1). Nevertheless, the foliar spray of SA, AsA, proline and admixture on sunflower at the rate of 200 mgL^{-1} enhanced significantly ($p<0.05$) effects the growth and development of crop under both untreated check and saline environment. The biological yield decreased up to 50% under saline conditions. At the earliest stage of growth, the biomass production at the seedling stage is affected to a greater degree compared to the later stages of growth. The reduction in biomass production is mainly due to low osmotic potential and diminishing of cell division and expansion (Bastista-Sanchez et al., 2015). The lower biomass accumulation is an outcome of reduced efficiency of photosynthetic machinery coupled with decreased production of compatible osmoprotectants in the plant system (Nawaz et al., 2017), which is directly correlated with growth and developments (Sharma et al., 2012).

Chlorophyll (Chl-*a*, Chlo-*b* and total Chlo) were significantly ($p<0.05$) affected under 150 mM NaCl salt stress in sunflower. Chlorophyll content reduced to the extent of 56.8% over the untreated check (Table 1). On the other hand, the exogenous application on sunflower of different osmoprotectants caused improvement of chlorophyll constituents. Out of these antioxidants, the foliar spray of proline evidenced in increased amount of total chlorophyll, chl-*a* and chl-*b* by 288.5%, 162.5%, and 141.0%, respectively under saline environment over water sprayed crop. The salt stress negatively affects the amount of total chlorophyll, chl 'a' and chl 'b' resulting in reduction of healthiness of plants (Jan et al., 2016). The amount of chlorophyll constituents was negatively reduced due to greater generation of ROS and also excessive excitation of energy by photosynthetic system (Mancarella et al., 2016). In most of the cases, the amount of production of osmoprotectants is very minimal, which have a little ability to address the external threats (Noreen and Ashraf, 2010). Thereby, the increased amounts of compatible osmoprotectants

Table 1: *Influence of exogenous application salicylic acid (SA), ascorbic acid (AsA), proline (Pro) and their admixture on shoot fresh and dry weights (g), root fresh and dry weights (g), chlorophyll contents on sunflower plants grown under salt stress environment.*

Parameters	Salinity Level (mM, NaCl)	Untreated Check Distilled Water	Salicylic acid 200 mgL^{-1}	Ascorbic acid 200 mgL^{-1}	Proline 200 mgL^{-1}	SA+SA+Pro 200 mgL^{-1}
Shoot f. wt. (g)	0	28.27 ± 0.865^{a}	34.85 ± 0.854^{b}	32.68 ± 0.975^{c}	38.635 ± 2.058^{b}	32.173 ± 0.887^{a}
	150	14.20 ± 1.042^{a}	21.81 ± 0.862^{b}	20.35 ± 0.724^{b}	26.035 ± 1.329^{c}	22.08 ± 0.928^{b}
Shoot d. wt. (g)	0	7.425 ± 0.407^{a}	9.451 ± 0.352^{b}	8.458 ± 0.554^{ab}	10.869 ± 0.447^{c}	8.458 ± 0.554^{ab}
	150	4.783 ± 0.333^{a}	6.250 ± 0.732^{ab}	5.648 ± 0.365^{a}	7.350 ± 0.512^{b}	6.050 ± 0.613^{ab}
Root f. wt. (g)	0	8.540 ± 0.288^{a}	10.75 ± 0.745^{b}	9.850 ± 0.267^{b}	12.843 ± 0.234^{c}	10.39 ± 0.342^{b}
	150	4.450 ± 0.420^{a}	7.225 ± 0.179^{b}	6.950 ± 0.359^{b}	8.231 ± 0.412^{c}	6.685 ± 0.445^{b}
Root d.wt. (g)	0	1.838 ± 0.276^{a}	2.500 ± 0.125^{bc}	2.275 ± 0.128^{abc}	2.700 ± 0.105^{c}	2.200 ± 0.105^{ab}
	150	0.935 ± 0.054^{a}	1.333 ± 0.082^{bc}	1.225 ± 0.099^{bc}	1.450 ± 0.075^{c}	1.175 ± 0.099^{b}
Chl.'a'(mg/g f.wt.)	0	1.363 ± 0.134^{a}	1.531 ± 0.079^{ab}	1.699 ± 0.028^{bc}	1.889 ± 0.099^{c}	1.584 ± 0.134^{ab}
	150	0.437 ± 0.035^{a}	0.746 ± 0.148^{b}	1.050 ± 0.131^{c}	1.148 ± 0.041^{c}	0.788 ± 0.075^{b}
Chl. 'b' (mgg^{-1} f.wt.)	0	0.104 ± 0.003^{a}	0.140 ± 0.018^{b}	0.154 ± 0.006^{bc}	0.169 ± 0.007^{c}	0.113 ± 0.007^{a}
	150	0.052 ± 0.006^{a}	0.074 ± 0.006^{ab}	0.088 ± 0.008^{b}	0.127 ± 0.012^{c}	0.056 ± 0.005^{a}
Total Chl.	0	0.099 ± 0.006^{a}	0.120 ± 0.006^{b}	0.190 ± 0.002^{d}	0.208 ± 0.011^{d}	0.162 ± 0.012^{c}
	150	0.038 ± 0.009^{a}	0.076 ± 0.014^{b}	0.108 ± 0.010^{c}	0.148 ± 0.006^{d}	0.093 ± 0.011^{bc}

Means ± SE followed by different letters differ significantly (95% probability level) according to Duncan's Multiple range test (SPSS-20).

are ought to be augmented through external application of osmoprotectants to avoid the attack of external stresses (Korkmaz et al., 2015). Foliar spray of osmoprotectants at early stages of growth enhances chlorophyll constituents and biological yield under saline condition (Akhtar et al., 2015). There are evidences that exogenous application of proline and salicylic acid at lower level of concentration could mitigate the negative effects of ROS produced by salinity (Roy et al., 2014) resulting into increased chlorophyll content (Dolatabadlian et al., 2008).

The quantum of total soluble proteins (TSP) and total free amino acids (TFAA) were expressively affected and enhanced under 150 mM NaCl salt stress in sunflower. However, foliar spray of different osmoprotectants balanced the amount of TSP and TFAA in control and treated plants (Table 1). It was observed that TSP contents in sunflower were salinity reduced to3.8 % as compared to control. Out of different osmoprotectants, proline was found more efficacious in enhancing TSP contents by 76.9% under water sprayed conditions. Furthermore, TFAA's were enhanced by 44% and 33%, respectively under non-saline and saline conditions (Figure 1). The greater accumulation of osmoprotectants in plant system resort to make the ionic and osmotic adjustments to minimize the external risks posed to growth and development of plants (Darwesh, 2013; Akhtar et al., 2015).

The results of this study showed that a substantial increase in catalase (CAT) by 68.7% and peroxidase (POD) by 69.2% was observed in sunflower plants under salinity treatment. Exogenous application of osmoprotectants caused reduction in CAT and POD contents, while these were enhanced under water sprayed conditions. Out of the osmoprotectants, the aerial application of proline (200 mgL^{-1}) caused increase in CAT and POD contents under unsprayed conditions. Overall, the spray of admixture of different osmoprotectants reduced the amount of CAT and POD under saline environment in sunflower (Figure 1). Under this study proline chemical was found to be more effective in accumulating higher amount of osmoprotectants to address increased production of CAT and POD under salt stress. The addition of proline resulted into greater production of antioxidants enzymes. These results also in agreement with those of Kibria et al. (2017), who found that proline chemical was found more effective compared with other chemicals under study. Cuin and Shabala (2007) reported that proline chemical was highly effective in scavenging the damage caused by overproduction of ROS. This phenomenon occurred due to reduction in lipid peroxidation in membrane jointly with enhanced antioxidant enzyme system (CAT, POD, and SOD) in plant system (Hoque et al., 2007).

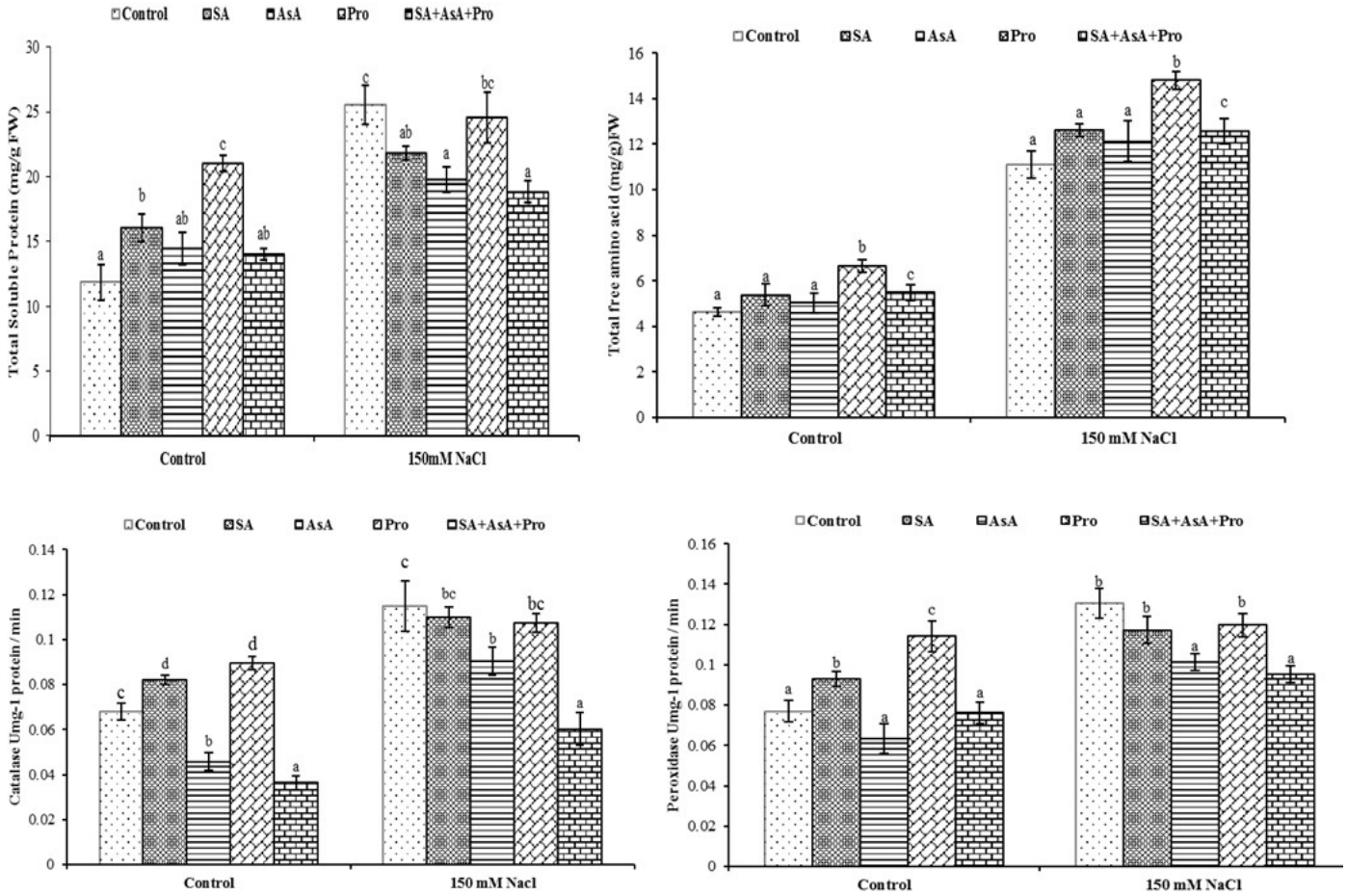

Figure 1: *Influence of foliar application of salicylic acid (SA), ascorbic acid (AsA), proline (Pro) and their admixture on total soluble protein contents (mg/g FW), total free amino acid contents (mg/g FW), catalase (CAT) (Umg^{-1} protein/min) and peroxidase (POD) (Umg^{-1} protein/min) contents of sunflower grown under control and saline conditions. Different letters differ significantly (95% probability level) according to Duncan's Multiple range test (SPSS 20).*

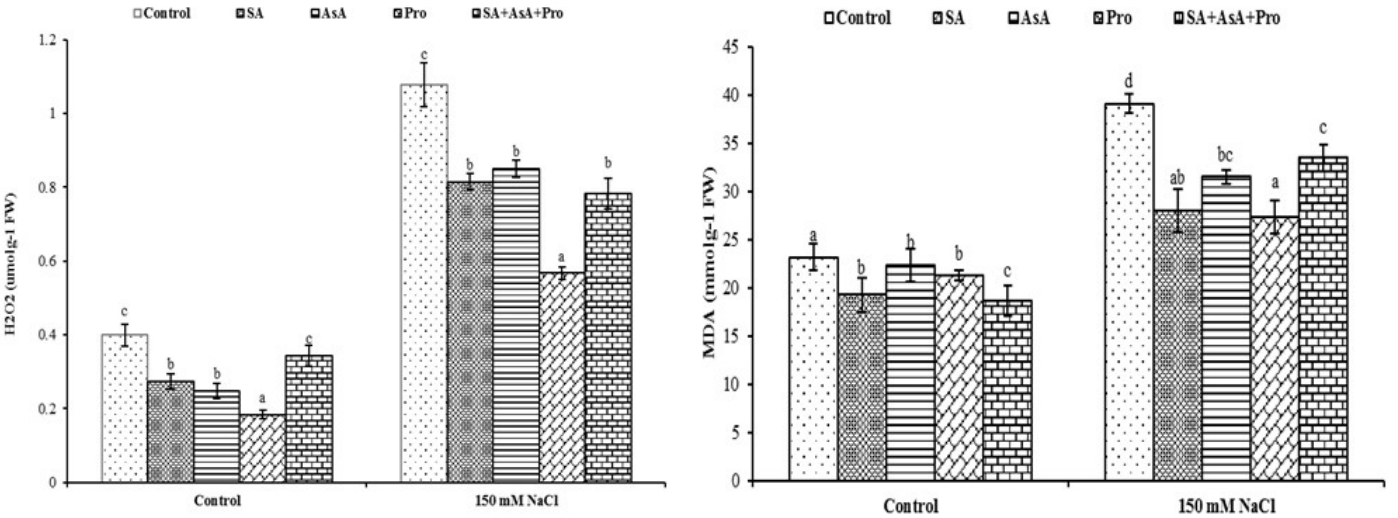

Figure 2: *Influence of foliar application of salicylic acid (SA), ascorbic acid (AsA), proline (Pro) and their admixture on H_2O_2 (umolg^{-1} FW) and MDA contents (mmolg^{-1} FW) contents of sunflower grown under control and saline conditions. Different letters differ significantly (95% probability level) according to Duncan's Multiple range test (SPSS-20).*

Similarly, amount of Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents were also enhanced significantly ($p<0.05$) enhanced in response to salinity stress in sunflower. Resultantly, the values of H_2O_2 and MDA were enhanced by 170.3% and 68.7%, respectively. Contrarily, exogenous application of all osmoprotectants including admixture reduced the amount of H_2O_2 and MDA. However, the foliar spray of proline on sunflower reduced it by 54.6%. Moreover, SA also resulted in reducing the amount of

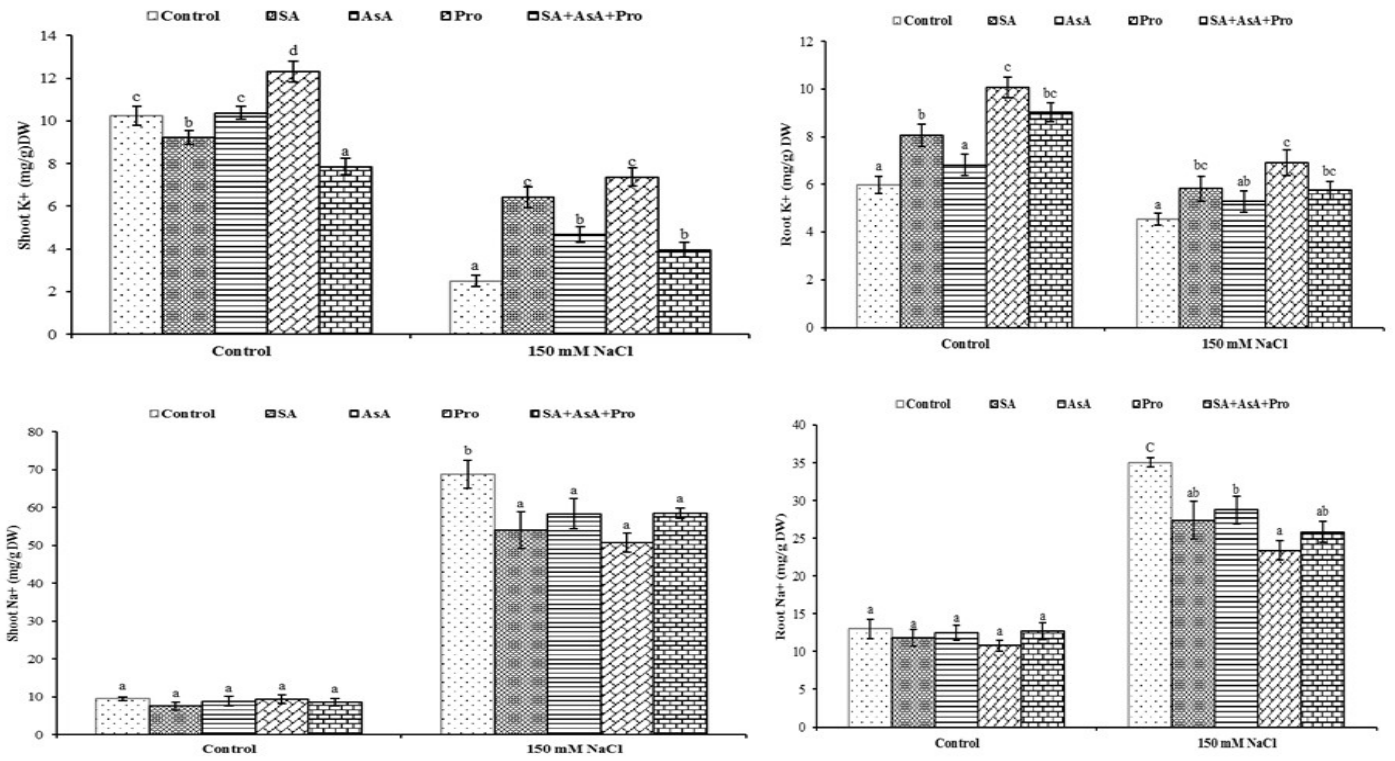

Figure 3: *Influence of foliar application of salicylic acid (SA), ascorbic acid (AsA), proline (Pro) and their admixture on shoot and root K^+ and Na^+ contents (mg/g DW) contents of sunflower grown under control and saline conditions. Different letters differ significantly (95% probability level) according to Duncan's Multiple range test (SPSS-20).*

MDA by values of 16.8% and 29.9% under treated and control conditions (Figure 2). Various researchers (Leyva et al., 2011) also reported that amount of H_2O_2 was enhanced due to lipid peroxidation. Thereby, generation of ROS could be detoxified by external addition of certain osmoprotectants (Chaudhury et al., 2017). The lipid peroxidation also deteriorated stability of cell membranes (Elkahoui et al., 2005).

It was observed that ionic constituents of sunflower were pointedly affected by 150 mM NaCl salt stress. The salt stress reduced content of K^+ in shoot and root organs by percentage of 75.6% and 24.0% with simultaneous increase in Na^+ content by 623.8% and 169.3% shoot and root organs, respectively. The decrease in K^+ and increase in Na^+ was pronounced in shoot rather than in root organ (Figure 3). On the other hand, the foliar application of various osmoprotectants along with admixture resulted in increase in K^+ content while conversely reduction in Na^+ content in sunflower. Apart from other chemicals, foliar spray of proline was found to be more effective in enhancing K^+ content by 20% and 194.6% in shoot organ, while, 68.7% and 52.41% in root organ respectively (Figure 3). SA (200 mgL^{-1}) reduced Na^+ by 20.3% in shoot organ compared to 26.2% by spray of proline under salt-stress conditions. Moreover, the application of proline also caused reduction in Na^+ by 17.2% and 33.3% in shoot organ under non-saline and saline conditions, respectively. Statistical analysis also revealed that ionic contents were altered *i.e.*, reduction in K^+ with concurrent increase in Na^+ ion content under salt-stress. The increased contents of Na^+ deteriorated plasma membrane and exuded greater amount K^+ from the cell (Negrao et al., 2017). Increased amount of osmoprotectants in the plant system would result in sharp increase in K^+ with the simultaneous reduction in Na^+ contents (Mahboob et al., 2016). There are evidences that exogenous application of proline resulted in increased amounts of K, N, P and Ca contents, conversely reduction in Na^+ and Cl^{-1} contents (Negrao et al., 2017).

Conclusions and Recommendations

The greater efforts are needed to enhance the productivity of oilseed crops as to narrow down the gap between local production and import to meet the national edible oil requirements. The biological yield, physiological and biochemical parameters of sunflower were reduced under salinity state, while were improved by aerial spray of SA, proline and ASA in sunflower crop. The cultivation of sunflower would be an added value towards achieving the national goal. The proline chemical mitigated the negative salinity constraint by scavenging the overproduction

of reactive oxygen species by reinforcing the amount of content of osmoprotectants in the plants. The higher production of sunflower crop under salt-stress could be augmented by foliar application of proline at the rate of 200 mgL^{-1}.

Novelty Statement

The finding of this study revealed that exogenous application of various osmoprotectants viz, salicylic acid, proline and ascorbic acid has produced improved salt tolerance mechanism in sunflower plants which ultimately enhanced biological yield, antioxidant activity and development of sunflower plants under saline condi-tion.

Author's Contribution

Sibgha Noreen: Designed and conducted the experiment, wrote the manuscript
Sumrina Faiz: Conducted the experiment and data collection.
Muhammad Salim Akhter: Helped in statistical analysis of data .
Kausar Hussain Shah: Helped in editing the manuscript

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