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Does Exogenous Application of salicylic acid induce salt stress tolerance in potentially high-yielding modern wheat cultivars?

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SALINITY is one of the abiotic stresses that affect negatively wheat productivity across the globe. In Pakistan, wheat is a cash crop. Probably, exogenous application of salicylic acid could be helpful to induce salt stress resistance in possibly high-yielding modern wheat cultivars in Pakistan (Ujala 2016 and Akbar 2019). A pot experiment of a complete randomized design was therefore conducted in sand-filled plastic containers. These pots were planted with wheat seeds at the experimental area of the Department of Botany, Government College Women's University Faisalabad, Pakistan, during the winter season of 2021-2022. Two levels of NaCl [0 and 150mM] were applied along with Hoagland's nutrient solution at 14-day interval and two levels of salicylic acid [0 and 100 mg L^{-1}] were applied to wheat leaves after 28 DAS. All treatments were replicated 4 times. Plants were sampled after 3 weeks of foliar application to estimate morphological and biochemical parameters. Salinity significantly lessened shoot and root fresh/dry weights. Also, it diminished soluble protein, shoot K^+ ion, and shoot K^+/Na^+ ratio in the two wheat cultivars when grown under saline conditions compared to control. On the other hand, salinity stress significantly raised the levels of catalase, superoxide dismutase, ascorbic acid, total phenolic, glycine betaine, hydrogen peroxide, malondialdehyde and shoot Na^+ ion. Exogenic application of SA raised the activities of catalase, peroxidase, superoxide dismutase, ascorbic acid, soluble protein, phenolic, glycine betaine, shoot calcium, shoot K^+/Na^+ ratio and this consequently enhanced shoot and root (fresh and dry) weights of the two wheat cultivars under salt stress conditions and also under the non-stressful condition. In conclusion, exogenous application of SA was more effective for both wheat cultivars to acclimatize under saline condition. The wheat cultivar Akbar-2019 revealed better performance than Akbar 2019 in most morphological characteristics of wheat grown in salt stress conditions.

Keywords: Salicylic acid, stress, wheat, cultivars, resistance.

1. Introduction

Wheat (*Triticum aestivum* L.) is the premium staple food crop worldwide (Farid *et al*. 2014; Hussein *et al*. 2022; Farid et al. 2023; Saad *et al*. 2023; Abd El-Aty *et al.* 2024). In 2019, about 220 million acres of global farmlands were cultivated with wheat, yielding

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771.72 tons of grains (FAO 2019). These grains represent a significant portion of the daily caloric intake (21%) for over 4.5 billion people (Saddiq *et al.* 2021).

Abiotic factors like heat, salt, and drought may significantly reduce wheat production (Yadav *et al.* 2020; Abd El-Aty *et al*. 2024; Elmasry and Abd El-Rady 2024; Elshaboury *et al.* 2024; Rashwan *et al.* 2024). In particular, salinity, declines the production of variety of crops all over the world (Lalarukh and Shahbaz 2020a; Lalarukh *et al*. 2022e), especially in arid and semi-arid regions, which represents more than 30% of the world arable lands (Shrivastava and Kumar 2015). Together with drought stress, the percentage of loss in plant productivity owing to salinity and drought stresses rises up to 50% (Gupta *et al*. 2022). Therein, grown plants suffer from nutrient deficiency, water shortage and ion toxicity (Lalarukh and Shahbaz 2020b),

Ionic stress is brought on when plants absorb too much Na^+ and Cl ions, which slows down normal metabolic activities. A considerable decrease in cytosolic K^+ occurs in conjunction with increasing absorption of Na^+ and Cl, thus affecting several metabolic activites within plant cells and their viability (Rubio et al. 2020).

Salt stress exhibits both primary and secondary impacts on the grown plants (Yang and Guo 2018). Osmotic, oxidative stress and ionic effects are among the primary consequences (Taha *et al*. 2020), whereas nutritional and hormonal irregularities, as well as the oxidative stress, are considered secondary effects (Amjad *et al*. 2021a; Dianatmanesh *et al*. 2022). Plants under saline stress increase plants' internal water potential while lessen cell turgor (Gupta *et al*. 2022).This in turn lowers plant capacity to absorb more water from soil (Lu *et al*. 2023) and at the same time prevents water loss via regulating stomatal conductance (Yang and Guo 2018). It is worth noting that prolong salinity lessens plants capacity to ingest CO_2 via closing stomata (Gupta *et al*. 2022; Zörb *et al*. 2022). Overall, salt stress interferes with cellular homeostasis by denaturing proteins and nucleic acids, initiating lipid peroxidation, and increasing ROS (Siddiqui et al. 2017; Seleiman et al. 2020).

A phenolic substance called salicylic acid (SA), naturally exists in plant tissues, may function as an endogenous hormone regulator against biotic and abiotic stress (Jayakannan *et al*. 2015; Khan *et al*. 2015; Mushtaq *et al*. 2021; Gupta *et al*. 2023). Yet, its mode of action is not well recognized (Torun *et al*. 2022). Maybe, SA activates the ascorbate-glutathione pathway (AsA-GSH), which increase plant resistance to tolerate ion imbalance (Kaya *et al*. 2020). Additionally, SA modulates plant salt tolerance via maintaining redox homeostasis (Hediji *et al*. 2021; Lalarukh *et al*. 2022c), up-regulating SOD and hydrogen peroxide scavenging enzymes such catalase peroxidase, and ascorbate peroxidase (Hasanuzzaman *et al*. 2017; Rehman *et al*. 2022) and also encourages the accumulation of osmoprotectants (Costa *et al*. 2022).

Numerous research (Amirinejad *et al*. 2017; Kaya *et al*. 2020) have illustrated that adding SA to fruit and vegetables, including bell peppers, can lower salt stress (Mimouni *et al*. 2016). Thus, the current study investigates the mechanisms beyond the effects of SA on increasing wheat tolerance under salt stress conditions. This includes the assessment of different morphological and biochemical variations in two wheat cultivars in salt stress conditions. Specifically, we anticipate that wheat plants growing under salinity stress suffer considerably i.e. significant increases occurred in reactive oxygen species which lead to concurrent reductions in the anabolic rate of stressed plants and therefore their growth parameters decreased considerably (Hypothesis 1). Spraying plants with SA enhances both enzymatic and non-enzymatic antioxidants, which may induce salt stress tolerance in wheat to lessen reactive oxygen species and consequently improve plant growth (Hypothesis 2). Also, it increases safely total soluble protein and organic osmolytes which may lessen the impacts of Na influx in plant roots and its translocation to shoots (Hypothesis 3).

2. Materials and Methods

A pot experiment was therefore conducted to assess the impacts of foliar spray of salicylic acid to alleviate salt stress on wheat plants.

2.1 Materials of study

Seeds of wheat (Akbar-2019 and Ujala-2016) were supplied by Ayub research center Faisalabad, Pakistan. Fine sand was collected from the Botanical Garden of the Government College Women University Faisalabad then left overnight and *0.05 M* sulfuric acid to displace all cations; thereafter, washed several times with distilled water to remove acid traces. This investigation was carried out at the research site of the Government College Women University Faisalabad, Pakistan during 2021-2022. Portions of fine acid-washed sand were packed in 32

plastic pots were of 12 ×11 inch width-length then arranged under greenhouse conditions in a completely randomized design (CRD) with four replicates. Ten seed of wheat were sown per pot (32 pots for each cultivar) and every 14 days Hoagland solution was applied to half number of pots (16 pots, control) while the other half (16 pots) received both Hoagland solution + 150 mM NaCl solution (1 L). Half the pots, comprising the control and saline media, were sprayed with salicylic acid (0 and 100 mg L^{-1}) after.28 days of sowing (DAS), while the other half was sprayed with distilled water. Three weeks later (42 days of cultivation) , 5 healthy plants were selected randomly for morpho-physiological and biochemical analyses.

2.2 Plant growth parameters

Plants were washed with tap water then distilled water, divided into roots and shoots and their fresh weights were recorded in grams (g) using an electric balance. After 48 hours in an oven at 65-70 degrees Celsius, the dry biomasses of roots and shoots were determined.

2.3 Enzymatic antioxidants

A pre-chilled mortar and pestle were used to grind the plant material. For estimating enzymatic antioxidants, 10 mL of PB (50 mM, pH 7.8) was added to 0.5 g fresh leaves then kept at 4^{o}C. Prior to enzymatic determinations, the mixture was blended then centrifuged at 12000 for 20 minutes. To determine enzymatic antioxidants like catalase, peroxidase, and glutathione peroxidase, supernatant was obtained and kept at -20°C.

Catalase and peroxidase

Enzyme activities of catalase as well as peroxidase were determined according to Maehly and Chance method (1955). One mL of H_2O was added to 1.9 mL (5.9 m*M*) phosphate buffer in a cuvette (pH 7.8). Then, 0.1 mL of enzyme extract was added to the mixture to initiate the reaction. To determine catalase activity, the absorbance of reaction mixture was measured at 20-second intervals for two minutes. Absorbance at 240nm was noted with the help of spectrophotometer (Model SM1200; Randolph, NJ, United States). In case of peroxidase determination, 100 L enzyme extract was mixed with (750 L) phosphate buffer, (100 L) guaiacol (20 mM), and 100 L hydrogen peroxide in a cuvette (40 mM). Peroxidase activity was assessed by noting fluctuation in absorbance at 470 nm every 20 seconds for 3 minutes. One unit of catalase and peroxidase was equated to a 0.01 unit/minute change in absorbance.

Superoxide dismutase (SOD)

Pursuit of superoxide dismutase was determined according to Giannopolitis and Ries (1977) technique as follows: 400 mL of distilled water and 250 mL of phosphate buffer (pH =7.8) were mixed in a cuvette. Methionine (100 mL), N-butyl thiocyanate (50 mL), enzyme extract (50 mL), and riboflavin (50 mL) were added to this mixture, and then kept for 15 minutes in presence of fluorescent light. A spectrophotometric analysis of absorbance for this mixture was conducted at 560 nm (Ultraviolet visible). An activity unit for superoxide dismutase is clear as quantity of enzyme essential to reduce NBT photo-reduction by 50% relative to control (mixture devoid of enzyme extract).

2.4 Non-enzymatic antioxidants

Ascorbic acid (ASA) content

Mukherjee and Choudhuri (1983) estimated ascorbic acid levels as follows 2 g of fresh samples were mixed with 6% trichloroacetic acid (TCA), filtered, and centrifuged at 1000 g for 20 minutes. A droplet of thiourea and four milliliters of the extract were combined with two milliliters of a 2 percent dinitrophenyl-hydrazine (10 percent in 70 percent ethanol). After 15 minutes of heating in a water bath, solution was left to cool at room temperature before being treated with 5 mL of 80 percent (v/v) H_2SO_4.

Total phenolic

Total phenolic was determined as outlined by Julkenen-Titto (1985) method, as follows: freshly selected leaves (0.1 g) were blended in 2 ml of 80% acetone and spun at 10,000 x g for 15 minutes. At -20°C, the supernatant was maintained. Then, 100 mL of leaf extracts (supernatant) was mixed with 2 mL of distilled water and 0.5 mL of phenol (Folin-Ciocalteau) in a test tube. Moreover, 2.5 mL of 20 percent Na_2CO_3 and 5 ml of distilled water were added, and the solution was then mixed viciously for 5 to 10 seconds. After 20 minutes, the combination's absorbance at 750 nm was tested.

Organic osmolytes

Organic osmolytes were extracted from the dried samples of wheat shoots by ethanol (80%) as outlined by Lalarukh *et al*. (2022a), then filtered using Whatman # 2 filter paper. Afterwards, total soluble sugar was determined in plant extracts spectrophotometrically after being treated with fresh prepared anthrone chemical reagent then heated in a water bath at 90-95 degrees Celsius for 20 min (Yoshida *et al*. 1976). Glycine Betaine content was assessed spectrophotometrically at 365 nm after being mixing with 0. 2*N* HCl and potassium tri-iodide (Grieve and Gratan's 1983). Total soluble proteins were measured by spectrophotometer at 570 nm after being stained using Bradford reagent dye (Bradford 1976).

Free proline

Fresh shoot portions (equivalent to 0.1 g) were added to 2 mL of aqueous sulphosalicylic acid (3%), then crashed, centrifuged, and mixed with one mL of each of Ninhydrin reagent and glacial acetic acid. Thereafter, the mixture was heated in a water bath for 10 min, cooled and measured at 520 nm (Bates *et al*. 1973)

Total free amino acids

The total free amino acid contents were measured using Hamilton and Van-Slyke's (1973) methodology. 0.1 g of fresh leaf was added to a test tube, together with 2 mL of buffer. One mL of the leaf extract was then added to 1 mL of a 10% pyridine solution and 1 mL of a ninhydrin solution. For 30 minutes, test tubes were kept in the hot water bath. The entire test tube volume increased to 25 mL, and the spectrophotometer's reading was taken at 570 nm.

2.5 Reactive oxygen species

Hydrogen peroxide

Using mortar and pestle, 0.1g of fresh leaf was mashed with 1mL of 0.1 percent TCA. The extract was then centrifuged for 15 minutes at 12000 rpm. Put 1mL of KI, 0.5ml of supernatant, and 0.5mL of pH 7.0 phosphate buffer in test tubes. A spectrophotometer measured the absorbance at 390 nm after the mixture was vortexed (Velikova *et al*. 2000).

Malondialdehyde (MDA)

Malondialdehyde constituents of wheat leaves were determined following the Heath and Parker, (1968) technique, i.e. 10 mL of (5 percent w/v) TCA were added to 0.5 g of wheat shoot fresh weight, then heated for 30 minutes in a water bath at 95°C, and centrifuged. Thereafter, one mL of the supernatant was combined with 4 mL of Thiobarbituric Acid (0.5 percent), made in 20 percent TCA. The absorbance was checked at both 600 and 532 nm after being cooled in ice.

2.6 Nutrient ions (K^+, Na^+ and Ca^{2+})

Plant shoots and roots (equivalent to 0.1 g dried biomass) were mixed together with 2 mL of the digestion mixture (Wolf 1982) and flasks were incubated at room temperature for an entire nigh. Thereafter, flasks were heated to 150 degrees Celsius before receiving 0.5 mL of H_2O_2 (35 percent) and digestion continued at 250° C until became colorless. In volumetric flasks, digest volumes were sustained up to 50 mL and filtered , then K^+ was assessed via flame photometer (PFP7 flame, USA), while Ca^{2+} and Na^+ ions were measured by Atomic Absorption Spectrum (AAS; Shimadzu instruments, Inc., Spectra AA-220, Kyoto, Japan).

2.7 Statistical analyses

Data was forwarded to the analysis of variance (ANOVA) technique, and the difference between treatment means was evaluated using the COSTAT program. Figures were plotted using SigmaPlot 10.

3. Results

3.1 Plant growth parameters

Significant reductions ($P < 0.01$) occurred in fresh and dry weights of wheat shoots and roots for the two cultivars under study when they were grown under saline condition versus those not subjected to salinity stress (Fig. 1A-D).

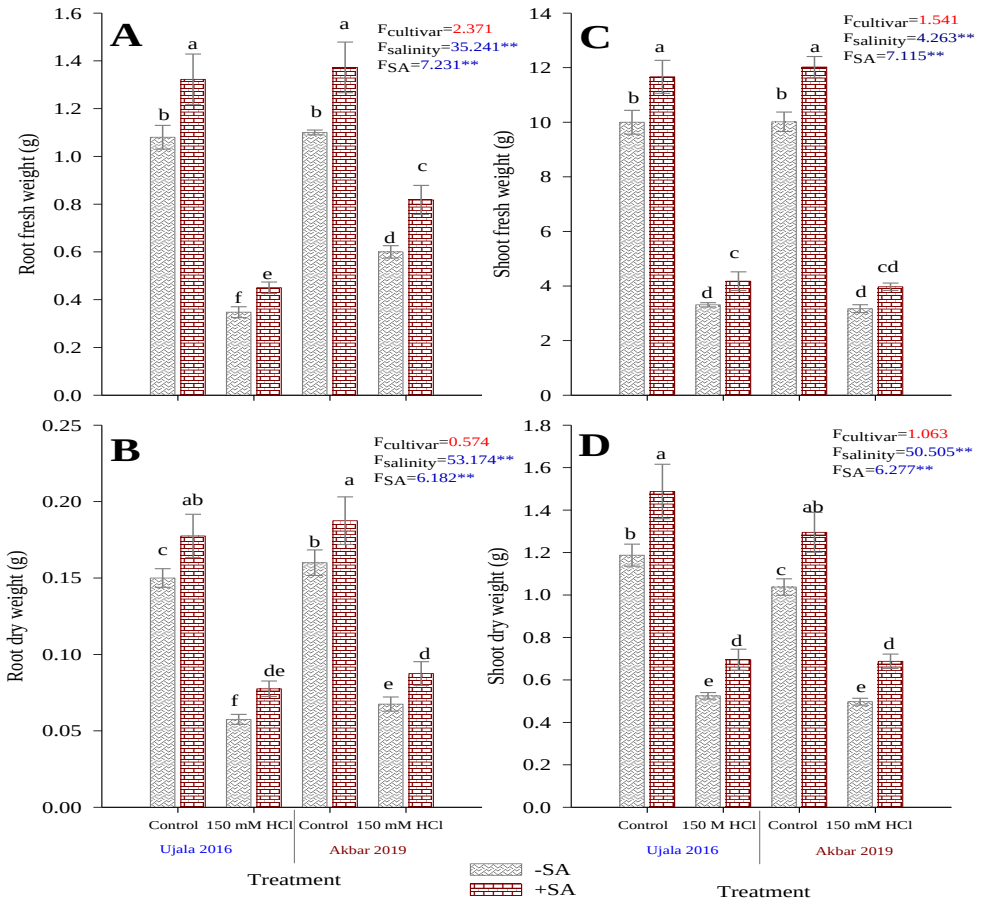
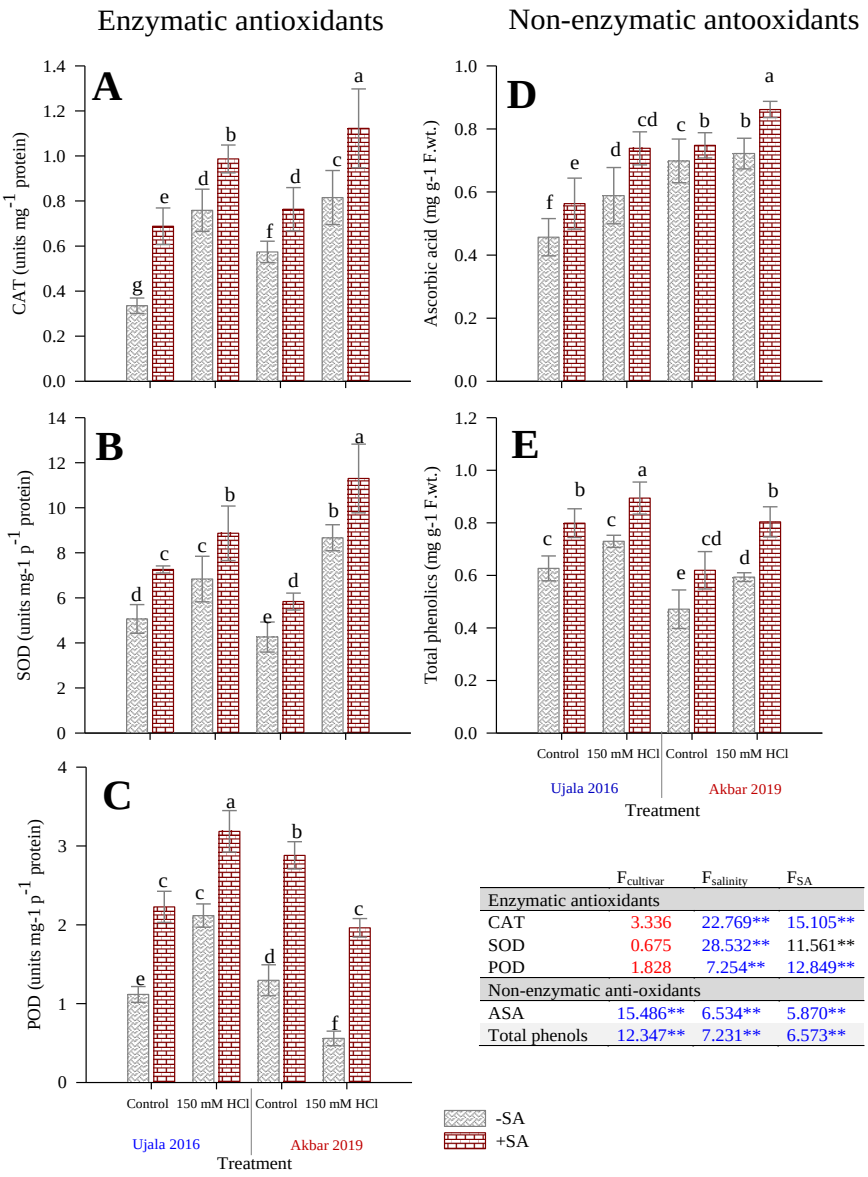


Fig. 1. Fresh and dry weights of wheat shoots and roots as affected by salicylic acid foliar application. Similar letters indicate no significant variations among treatments.

Also, exogenous use of salicylic acid (SA) displayed significant enhancement ($P < 0.01$) in the investigated growth parameters for both stressed and non-stressed plants. This result signifies the important role of SA for enhancing plant growth and also presents SA as an effective safe additive for ameliorating salinity stress. On the other hand, wheat cultivars depicted non-significant variations on these attributes.

3.2 Enzymatic and non-enzymatic antioxidants

The conditions of salinity elevated significantly enzymatic oxidants ($P < 0.05$) in wheat shoots, i.e. catalase, polyphenol oxidase and superoxide (Fig 2).



	$F_{cultivar}$	$F_{salinity}$	F_{SA}
Enzymatic antioxidants			
CAT	3.336	22.769**	15.105**
SOD	0.675	28.532**	11.561**
POD	1.828	7.254**	12.849**
Non-enzymatic anti-oxidants			
ASA	15.486**	6.534**	5.870**
Total phenols	12.347**	7.231**	6.573**

Fig. 2. Enzymatic (catalase CTA, super oxide dismutase SOD and polyphenol oxidase POD) and non-enzymatic antioxidants (ascorbic acid (ASA and total phenols (TP) as affected by salicylic acid foliar application. Similar letters indicate no significant variations among treatments.

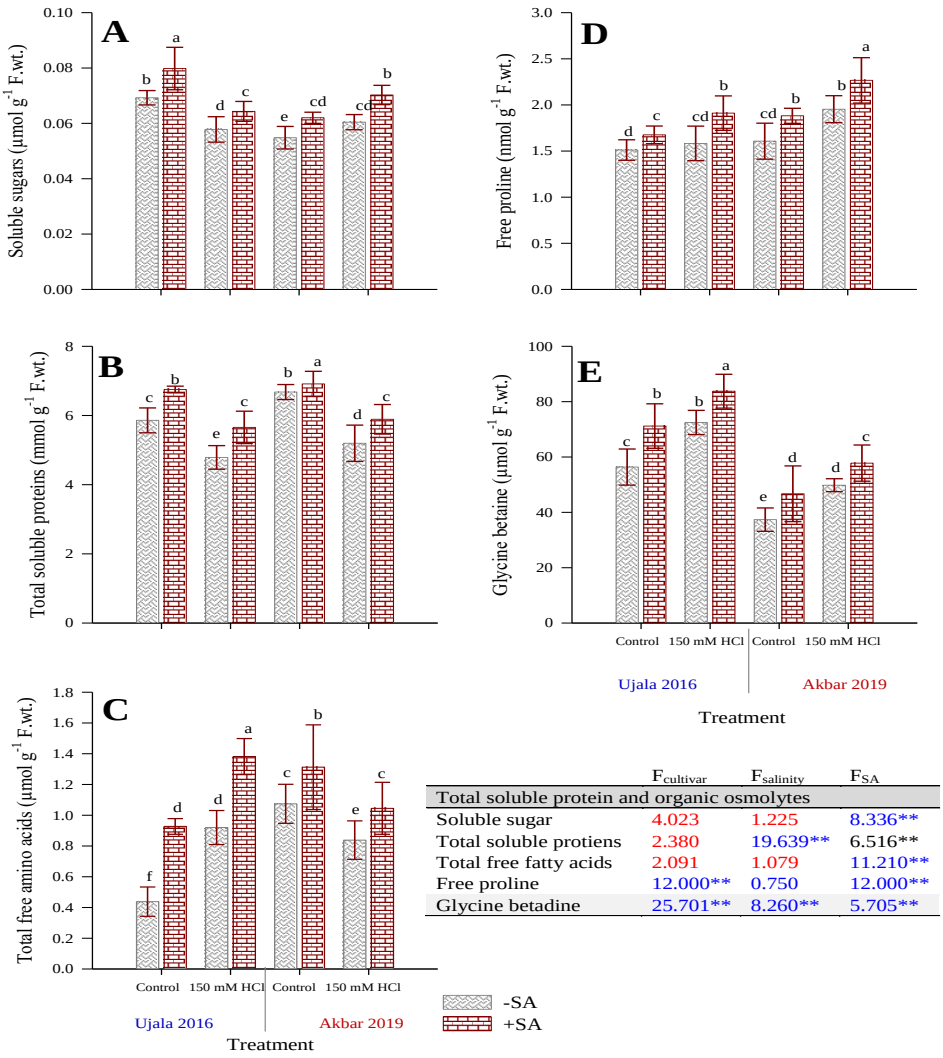
Likewise, significant increase occurred in the non-enzymatic antioxidants (ascorbic acid (AsA) and total phenols) owing to such stressful conditions. Increases is ASA were higher in Akbar 2019 cultivar versus Ujala 2016 yet total phenols were higher in Ujala 2016 cultivar.

Foliar application of salicylic acid raised significantly all enzymatic and non-enzymatic antioxidants in wheat shoots even under non-stressful conditions. The responses of the two cultivars under investigation to this foliar application seemed to be comparable

3.3 Total soluble protein and organic osmolytes

Total soluble proteins were low in shoots of the two wheat cultivars that were grown on a saline media versus the corresponding ones cultivated under non-saline condition ($P<0.05$, Figure 3). Shoot contents of proteins upraised significantly ($P<0.05$) after spraying plants with salicylic acid. It seems that variations

among cultivars were almost not detectable in this concern.



	$F_{cultivar}$	$F_{salinity}$	F_{SA}
Total soluble protein and organic osmolytes			
Soluble sugar	4.023	1.225	8.336**
Total soluble protiens	2.380	19.639**	6.516**
Total free fatty acids	2.091	1.079	11.210**
Free proline	12.000**	0.750	12.000**
Glycine betadine	25.701**	8.260**	5.705**

Fig. 3. Total soluble protein and organic osmolytes in wheat shoots as affected by salicylic acid (SA) foliar application. Similar letters indicate no significant variations among treatments.

On the other hand, salinity conditions raised significant free proline and glycine betaine contents within wheat shoots (Fig 3 D and E). Further increases occurred in these organic osmolytes after salicylic acid application as a foliar spray. Overall, Akbar exhibited higher levels of free proline ($P < 0.01$) under saline and non-saline conditions compared to Ujala; however the levels of glycine betaine were higher in Ujala 2016.

Regarding soluble sugar content in wheat shoots as well as total free amino acid content, no significant variations were observed on such parameters when plants were subjected to salinity stress conditions; anyway, their organics raised significantly when plants were sprayed with salicylic acid, with no significant variations among cultivars.

3.4 Reactive oxygen species

Hydrogen peroxide increased significantly in wheat shoots subjected to salinity stress (Fig 4A). Likewise, malondialdehyde (MDA) increased significantly

under these conditions (Fig 4B). The increases in H_2O_2 were more pronounced in stressed Akbar cultivar, while stressed Ujala cultivar expressed higher MDA content. When these two cultivars were sprayed with SA, significant reductions occurred in their contents. Even under the non-stressful conditions, H_2O_2 and MDA contents lessened noticeably in SA sprayed plants versus the non-sprayed ones. It seems that H_2O_2 content in plant tissues did not vary between the two cultivars of study, but MDA exhibited higher contents in Uhala 2016 than in Akbar 2019.

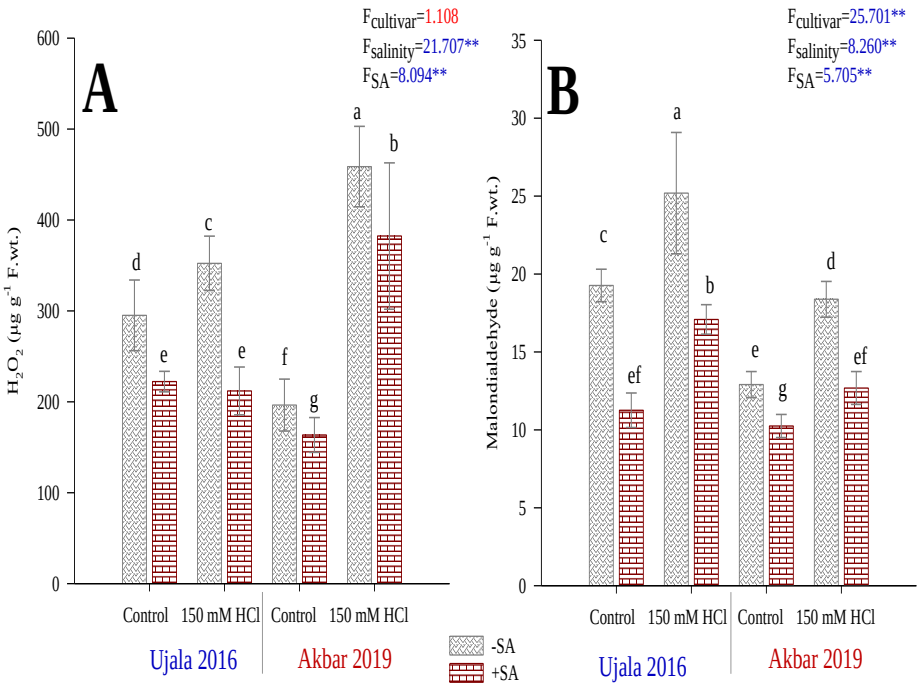
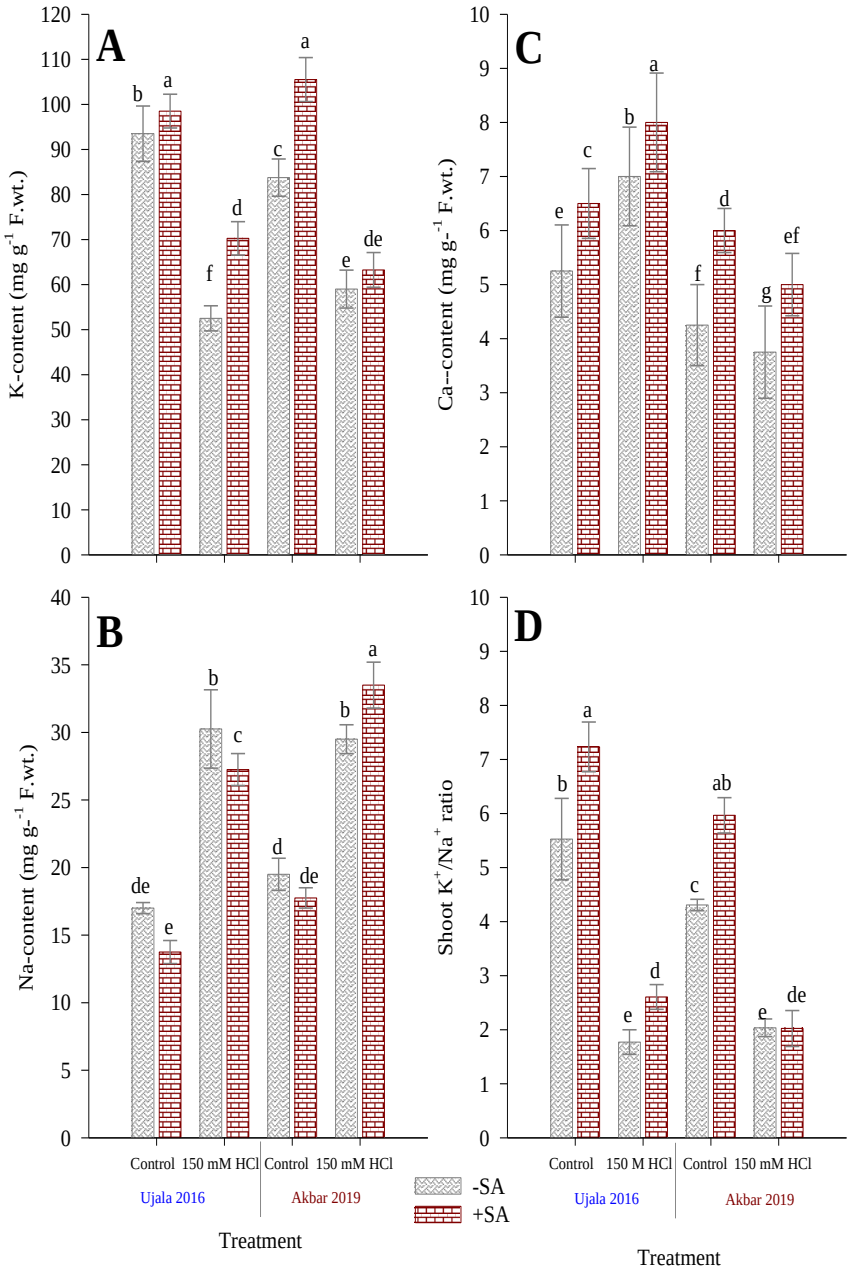


Fig. 4. Reactive oxygen species in wheat shoots as affected by salicylic acid (SA) foliar application. Similar letters indicate no significant variations among treatments.

3.5 Shoot Ionic analysis

K-content in shoots of non-stressed plants decreased significantly (P<0.05) under salinity stress conditions (Fig 5A). On the contrary, Na content increased extensively (P<0.05) in shoots of stressed plants (Fig 5B). Therefore, the ratio between K/Na in shoots decreased in stressed plants versus non-stressed ones (P<0.05, Fig 5D).

Foliar application of SA raised significantly K content in the shoots of the two cultivars while lessened sodium content. This in turn raised K/Na ratio. A point to note is that Ujala 2016 exhibited higher K/Na ratio than Akbar cultivar while variations in K and Na in shoots were not significant between these two cultivars.



	$F_{cultivar}$	$F_{salinity}$	F_{SA}
K in shoots	0.037	65.825**	8.427**
Na in shoots	3.260	62.406**	6.362**
K/Na in shoots	6.902**	178.227**	15.408**
Ca in shoots	13.045**	0.665	5.986**

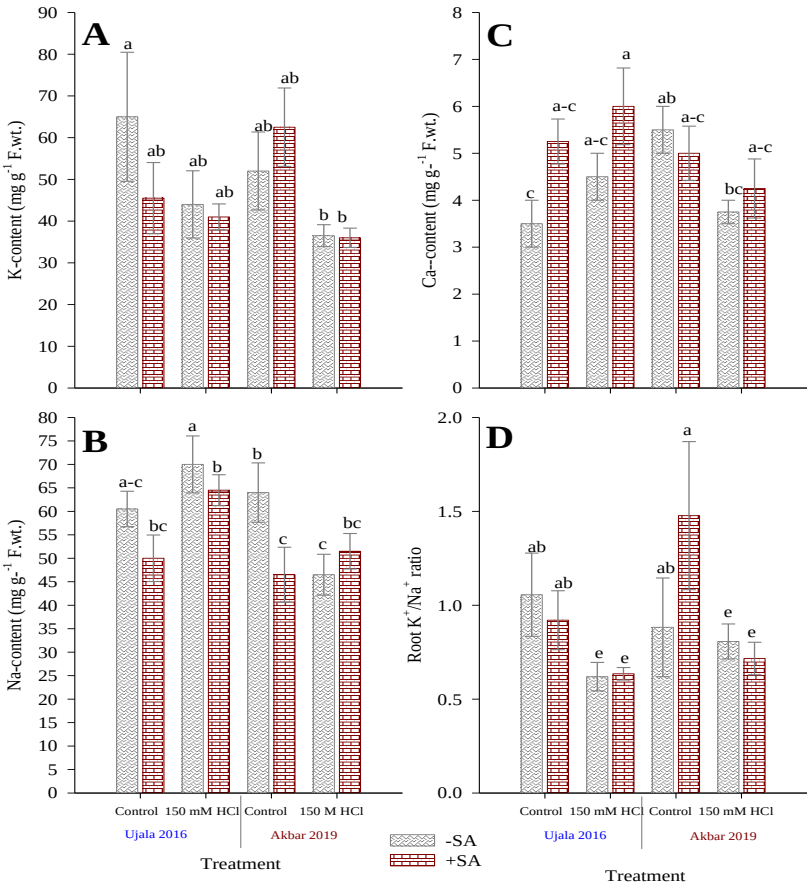
Fig. 5. Ionic contents in wheat shoots as affected by salicylic acid (SA) foliar application. Similar letters indicate no significant variations among treatments.

Salinity caused an increase in calcium within the shoot of Ujala cultivar, while decreased Ca in shoots of Akhbar. Probably, Ca was incorporated in effective mechanisms to ameliorate salinity stress in

plants of Akhbar cultivar (plant biomass improved significantly, as shown in Fig 1); thus its soluble content decreased notably. Exogenous treatment of salicylic acid showed a slight increase ($P<0.05$) in shoot calcium of both wheat cultivars under saline and control conditions compared to water spray.

3.6 Root Ionic analysis

Salinity along with salicylic acid exhibited complicated impacts on soluble contents of sodium, potassium and calcium within wheat roots of both cultivars (Fig 6 A, B and D). Even for K/Na, no obvious trend could be deduced (Fig 6C). It seems as if dynamic changes occurred in wheat roots between these three nutrients in face of salinity stress, so no definite trends were noticed in their concentrations in roots. Generally, Na content increased in roots of Ujala 2016 than the corresponding content in Akhbar 2019.



	$F_{cultivar}$	$F_{salinity}$	F_{SA}
K in roots	0.126	7.937**	0.272
Na in roots	6.870**	0.682	4.189
K/Na in roots	1.323	7.560**	0.459
Ca in roots	0.231	0.231	4.333*

Fig. 6. Ionic contents in wheat roots as affected by salicylic acid foliar application. Similar letters indicate no significant variations among treatments.

4. Discussion

4.1 Impacts of salinity stress on wheat growth

Salinity represents an abiotic stress factor threatening global food security. Our results revealed that this stress lessened considerably wheat shoots and roots (fresh and dry weights). These findings were documented by many researchers, i.e. in strawberry (Yaghubi *et al.* 2016) and sunflower (Lalarukh and Shahbaz 2018; Lalarukh et al 2022 b& d). Under such stressful conditions, plants exhibited lower K contents in their tissues while recorded significant increases in Na contents. These results agree with Alamri *et al.* (2020), Lotfi et al. (2020) and Yaseen *et al.* (2021) who found pronounced drop in essential nutrients (N and K) when *Brassica juncea* L. seedlings were subjected to elevated Na^+ concentrations. On the other hand, wheat plants

suffer from excess hydrogen peroxide and DMA in their tissues. This is one of the main impacts of salt stress on grown plants (Singh and Dwivedi 2018; Torut and Akbulut 2020; Yu *et al.* 2020) which results in ROS build-up (Al Kharusi *et al*. 2019). Accordingly, plants augment formation of enzymatic antioxidants like catalase, peroxidase and SOD to cope with this stress. Similarly, Jini and Joseph (2017) and Lalarukh and Shahbaz (2020a) noticed significant increases in CAT and other enzymatic antioxidant activity in sunflower and rice when grown plants were subjected to salt stress. Kazemi *et al.* (2020) also observed that blueberries enhanced phenolic content to improve their resistance when subjected to saline stress.

In our case, Ujala 2016 cultivar induced further increases in formation of total phenols while Akbar 2019 induced more the synthesis of ASA. Plants also synthesize more organic osmolytes to induce salt stress tolerance under these adverse conditions. These osmolytes increase osmo-protectants of plant cells to protect them against harms that occurred under salinity hazards (Choudhary et al. 2023). Based on the above results, the first hypothesis becomes valid.

4.2 Effect of SA on induction of plant tolerance towards salinity stress

SA is an endogenous growth regulator that controls stomatal conductivity, ion uptake by roots, and other physiological processes for plants under stressful conditions (Mozafari *et al*. 2018). In our research, ascorbic acid (ASA) and total phenols increased substantially when salicylic acid was sprayed on wheat plants. This result agrees with the findings of Hossain *et al*. (2021) and Sogoni et al. (2021). Also, this foliar additive acts as an antioxidant signal molecule that removes ROS, e.g. malondialdehyde (Wang *et al*. 2018; Torun 2019) and H_2O_2 under salt stress conditions (Torun 2019) via induction of oxygen radical scavenging enzymes (Liu and Wang 2012) such as CAT (Faried *et al*. 2017), POD and SOD activities (Solanki Mital *et al*. 2018). These results confirm the second hypothesis.

Another mechanism for SA is to alleviate salinity stress through enhancing accumulation of various metabolites within plant tissues, such as sugars and amino acids (Iqbal, 2015; Liu *et al*. 2016; El-Esawi *et al*. 2017; Raza *et al.* 2023). Free proline is an example of amino acid metabolites that was detected in high concentrations in tissues of *Cucumis sativus* and *Solanum lycopersicum* when being subjected to salinity stress (Mimouni *et al*. 2016). This product is thought to be responsible of increasing PSII efficiency (Youssef *et al*. 2018).

Regarding nutritional status of grown plants under salinity stress, Yang *et al*. (2020) found that SA application improved the nutritional status of wheat and pearl millet plants. For example, SA raised potassium level in plant cells (strawberry) (Faghih *et al*. 2017), while lessened Na uptake (Ghadakchiasl *et al*. 2017). Accordingly, SA raised K/Na ratio (Abdelraouf *et al*. 2017; Haider *et al*. 2021); and this in turn lessened Na toxicity (Lalarukh and Shahbaz 2020b; Lalarukh *et al*. 2022a). Salicylic acid also improved absorption of Ca^{2+} ions by roots and maintained its level high in shoot. This calcium is thought to be incorporated in root development to mitigate some of the detrimental consequences of salt stress (Pirasteh-Anosheh *et al*. 2021).

In our study, stressed plants had lower protein content; yet, this protein increased progressively upon spraying plants with SA. Similar results were found on wheat (Alsahli *et al*. 2019; Amjad *et al*. 2021b), tomatoes (Arbaoui and Belkhodja 2018), and Faba beans have also been found (Anaya *et al*. 2017).This may also explain the significant increases that took place in plant biomass when sprayed with SA foliar application. Accordingly, the third hypothesis becomes valid.

5. Conclusion

Results from the present study clearly revealed the positive effect of salicylic acid foliar application on morpho-physiological and Biochemical features of two wheat cultivars (Ujala 2016 and Akbar, 2019) as a safe and effective approach for increasing plant tolerant to salt stress. This spray had a remarkable influence on plant physiology, i.e. induction of enzymatic and non-enzymatic antioxidants as well as osmolytes to overcome salinity stress conditions. This in turn increased significantly plant growth. Wheat cv. Akbar-2019 seemed to be more tolerant to salt stress [150mM] than Ujala 2016. Therefore, the adverse impact of salt stress on morpho-physiological parameters can be mitigated via the foliar application of salicylic acid [100 mg L^{-1}] on wheat.

Conflicts of interest: There are no conflicts to declare.

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5. References

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