REGULATING EFFECTS OF EXOGENOUS SALICYLIC ACID APPLICATION ON WHEAT GROWTH UNDER SALINE AND HEAT STRESS CONDITIONS

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Abstract. Salicylic acid (SA) has emerged as an excellent phyto-hormone to improve stress tolerance in plants. However, no information is available about the role of SA under combined SS and HS. Therefore, this study was performed to underpin the potential role of SA to mitigate SS and HS in wheat crop. The study was comprised of different treatments: control, SS (8 dS m^{-1}), HS (imposed at flag leaf stage) and combination of SS (8 dS m^{-1}) and HS and different levels of SA: control, 50 mM, 100 mM and 150 mM. Salinity and HS substantially decreased the growth, and yield traits of wheat that was linked with increased electrolyte leakage (EL), malondialdehyde (MDA) and hdrogen peroxid (H_2O_2) production, sodium (Na^+) and chloride (Cl^-) accumulation and decrease in relative water contents (RWC), photosynthetic pigments, total soluble proteins (TSP) and free amino acids (FAA) accumulation and nutrient uptake. Nonetheless, foliar applied SA particularly 150 mM appreciably improved the growth and yield of wheat crop by increasing RWC, antioxidant activity soluble sugars, proline, TSP, FAA accumulation, nutrient uptake and decreasing EL, electrolyte leakage, MDA and H_2O_2 and restricting the entry of toxic ions. Therefore, these findings suggested that SA could improve the growth and yield of wheat under SS and HS stresses by improving physiological activity, photosynthetic pigments, antioxidant activity and accumulation of osmolytes.

Keywords: *antioxidants, photosynthetic pigments, nutrient homeostasis, wheat, yield*

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Introduction

Abiotic stresses are a major threat to agriculture and they are negatively affecting crop growth and productivity globally. The yield losses are projected to increase in the future owing to rapid global warming, an increase in the intensity of abiotic stresses and a reduction in the availability of fertile soils (Subashchandrabose et al., 2013). The major challenge of current and future agriculture is increasing food supply to meet the rising food needs of the soaring population, therefore, reducing the effects of abiotic stresses is a major concern globally (Boscaiu and Fita, 2020). Salinity stress (SS) is a serious abiotic stress posing a serious challenge to crop productivity and global food security (Fang et al., 2021; Rasheed et al., 2021). Globally 7% of world's soils (1 billion hectares) are salt affected (Hopmans et al., 2021) and it is expected that half of the arable lands of the world will be salinized by the end of 2050 owing to poor application of fertilizers, irrigation and industrial development (Ivushkin et al., 2019). Soil salinity impairs nutrient and water uptake and induce oxidative stress that decreases the nitrogen metabolism and photosynthetic rate thereby resulting in substantial in growth and cell apoptosis (Ismail et al., 2014; Pompeiano et al., 2016). The increase in reactive oxygen species (ROS) owing to SS damage protein, DNA and cause cell death besides this, excessive ROS also negatively affect the photosynthesis (Jiang et al., 2021). Moreover, SS also damage the structure of chloroplast, disturbs nutrient homeostasis and reduces membrane stability which in turn increases the loss of important solutes and reduces plant growth (Li et al., 2015; Yamane et al., 2018; Altaf et al., 2022).

Global warming is a serious concern across the globe which needs dire attention to secure future food security. Rapid global warming has increased the ambient temperature which is also posing serious threat to plant growth and our environment. Temperature is an important environmental variable that affects various physiological processes in plants (Ashraf and Harris, 2013). However, increase in temperature beyond certain level negatively affect plant functioning and cause significant growth and yield losses (Ashraf and Harris, 2013). It has been recorded that for each centigrade increase in seasonal temperature can cause yield losses more than 17% (AL-Shoaibi, 2020). Heat stress (HS) has been reported to negatively affect seed germination, canopy growth, photosynthetic efficiency and chlorophyll fluorescence (Guidi et al., 2019; Yi et al., 2019; Rasheed et al., 2021). The exposure of plants to high temperature (HS) reduces chlorophyll synthesis and accelerates chlorophyll degradation which causes a remarkable reduction in photosynthesis and plant growth (Ashraf and Harris, 2013). Heat stress also induces ROS production that damages the plant membrane, DNA, proteins, and also induce cell death (Hassan et al., 2021).

To improve crop productivity and meet food needs; plant tolerance against diverse stresses must be improved. To date, most of studies are conducted to study the plant responses against single stress conditions (Prasch and Sonnewald, 2015; Aycan et al., 2021), at field level. However, these stresses occur together and result in a massive reduction in crop (Nahar et al., 2022) and plants also show different responses against combined stresses as compared to single stress (Rasmussen et al., 2013; Zhang and Sonnewald, 2017). Combined stresses are more lethal to plants and they can cause more growth and yield losses as compared to single (Pandey et al., 2017; Cohen et al., 2021). Therefore, it is mandatory to reduce the deleterious impacts of these abiotic stresses on plants. The application of plant growth regulator has emerged as an excellent tool to mitigate the adverse effects of abiotic stress.

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Salicylic acid (SA) is an important phyto-hormone and signaling molecule that substantially improve stress tolerance. The application of SA improves the salinity tolerance through improvement in physiological parameters and antioxidant activities (Sheteiwy et al., 2019; Bukhat et al., 2020). SA also improves chlorophyll synthesis, RWC, proline and soluble sugars accumulation and hormones (Indole acetic acid: IAA and gibberellin: GA) synthesis and reduces ethylene synthesis thereby improve plant growth under SS (Sultan et al., 2021). Moreover, SS also activate osmo-regulation system that reduces adverse effects of ROS on membrane structure, metabolic activity and maintain ionic homeostasis (Abdoli et al., 2020; Xu et al., 2022). In case of heat stress SA also maintains membrane stability, efficiency of PS-II, and plant ability to perform normal carbon assimilation for maintaining normal growth (Ananieva et al., 2002; Fan et al., 2022). Moreover, SA also reduced the ROS production and maintains photosynthetic rate by reducing the damage to PS-II (Wang et al., 2012).

The role of SA against SS and HS is well reported however, there is no information available about the role of SA to mitigate the toxic effects of combined SS and HS. Therefore, we speculated that SA could improve the SS and HS in wheat by modulating photosynthetic capacity, anti-oxidant activity, nutrient homeostasis and osmolytes accumulation. Therefore, this study was performed to determine the role of SA on plant growth, physiological traits, photosynthetic pigments, nutrient homeostasis and antioxidant activity.

Materials and methods

Experimental details

The present study was executed at the wire of the University of Agriculture Faisalabad (UAF) Pakistan to determine the protective role of SA in mitigating the salinity and heat stress and a combination of both stresses on wheat crop. The soil for filling of pots was taken from 1 to 10 cm depth from Agronomy farm and pots were filled with soil and silt ratio of 3:1. The information regarding the soil properties is given in *Table 1*.

Table 1. *Physiochemical properties of soil used in study*

pH	7.86
Organic matter	1.13%
EC	0.99 d ms^{-1}
Total nitrogen	0.66%
Available phosphorus	6.78 ppm
Available potassium	155 ppm

The pots have a diameter of 28 cm and were filled with 8 kg soil and the sowing of the wheat crop was done in the third week of November. Wheat variety Faisalabad 2008 was used as the test crop and 12 seeds of wheat were sown at a depth of 1 cm in each pot. After germination 10 plants were retained in each pot and extra plants were removed. The irrigation was applied to pots according to crop needs weeds were manually uprooted and no attack of insect pests was observed during the study. The pots were fertilized with urea (1.56 g), and di-ammonium phosphate (DAP: 1.64 g) to fulfill the nutrient needs of the crop. The urea contained 46% N and DAP contained 18% N and 46% phosphorus.

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Experimental treatments

The experiment was comprised of different treatments: control, SS (8 dS $^{m-1}$), HS (heat stress was imposed at flag leaf stage), and a combination of SS (8 dS m^{-1}) and HS and different levels of SA: control, 50 mM, 100 mM, and 150 mM. The salinity stress was imposed at the time of sowing, foliar application of SA was done at the flag leaf stage (BBCH, 2) and HS was imposed at the booting stage (BBCH, 4). The experiment was conducted in a completely randomized design with a factorial arrangement having three replications. Each treatment has three pots and in total complete experiment contained 48 pots. The desirable level of SS was obtained by using given below formula (1):

$$\text{Salt req.}\,(\text{g/kg}) = \frac{Tss \times mol.weight \times saturation(\%)}{100 \times 100} \quad \text{(Eq.1)}$$

The concentration of TSS was determined with given below equations. In give below Equation (2) EC_1 is the original EC of soil whereas EC_2 was that EC that has to be attained.

$$TSS = (EC2 - EC1) \times 10 \quad \text{(Eq.2)}$$

The soil saturation percentage (SP) was determined with given below equation. For determination of SP the soil paste was made by adding distilled and allowed for 2 hours in order to reach equilibrium. Then, extract was obtained and afterwards soil was oven dried and SP was determined as Equation (3):

$$Saturation\ (\%)\ \frac{loss\ in\ soil\ weight\ on\ drying}{weight\ of\ soil\ after\ drying} \times 100 \quad \text{(Eq.3)}$$

Data collection

Growth traits

After 15 days of application of SA three plants were up-rooted carefully from each pot. The plants were carefully up-rooted and roots were cut from the shoots to determine their fresh and dry weights. Similarly, roots and shoots of these three plants were used for determination of fresh and dry weights.

Photosynthetic pigments

The concentration of chlorophyll and carotenoids contents in wheat plants was determined by the method of Lichtenthaler (1987). In 80% methanol solution; 0.5 g fresh leaf sample was homogenized by using pestle and mortar. Then the extract was centrifuged and filtrate was obtained. Later on absorbance was recorded at 663, 645 and 480 nm wavelengths with spectrophotometer (Hitachi U-2001, Tokyo, Japan), to determine the chlorophyll-a, chlorophyll-b and carotenoid contents.

Electrolyte leakage and relative water contents

For determination of EL; fresh leaf samples were harvested washed and placed in vials in a rotary shaker (25°C) after 24 hours first EC1 was taken and then leave samples were autoclaved for 20 minutes and second EC2 was taken and EL was measured with the

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following equation: For determination of RWC fresh leaf samples were taken from plants and weighed to determine fresh weight (FW) and leaf samples were dipped in water for 24 h and turgid weight was taken. Later on, samples were removed and oven dried and dry weight (DW) was taken and RWC was determined with the following formula: RWC = (FW-DW)/(TW-DW) × 100. For determination of anthocyanin 0.5 g plant sample was homogenized in 5 ml potassium phosphate and extract was obtained. Later on, extract was centrifuged for 15 min and then absorbance was recorded at 535 nm to determine anthocyanin contents.

Antioxidant activities

For determination of catalase (CAT) activity test tube contained 100-µL of extract, H_2O_2 and buffer solution was prepared and absorbance was noted at 240 nm to determine the CAT activity (Aebi, 1984). In order to determine peroxidase (POD) activity; we took H_2O_2 (0.1 ml) phosphate buffer (2.7 ml) and shake well and after that 0.1 ml enzyme extract and 0.1 ml of guicol was added in extract and absorbance was noted at 470 nm (Zhang, 1992). For determination of ascrobate peroxide (APX) activity test tubes contained 100 µL enzyme extract, 100 µl ascorbate, 100 µL H_2O_2, and 2.7 m potassium buffer was prepared and absorbance was taken at 290 nm (Nakano and Asada, 1981). Lastly for determination of ascorbic acid leaf samples were homogenized with 10% trichloroacetic acid (5 ml) and centrifuged for 10 minutes. Later on, DTC regent (0.5 ml) was added in 2 ml of supernatant and incubation was done for 3 hours. After that samples were cooled rapid and then added drop wise 2 of sulfuric acid in shacked slightly. Then solution was left for 30 minutes and absorbance was noted at 520 nm (Mukherjee and Choudhuri, 1983).

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) determination

H_2O_2 concentration was assessed by the method of Velikova et al. (2000). 0.5 g plant sample was grinded in 5 ml of trichloroacetic acid (TCA) and centrifuged. Then IM potassium iodide (KI) and 100 µl potassium phosphate buffer (PPB) was added in supernatant of crude extract and maintained at room temperature for 30 minutes and later on absorbance was recorded at 390 nm for determination of H_2O_2 concentration. 0.5 g leaf sample was homogenized in 5 ml trichloroacetic acid (TCA) and centrifuged for 15 min at 12,000 rpm. Then 5 ml of thiobarbituric acid (TBA) added in the supernatant and mixture was heated for 20 minutes and then cooled rapidly at 4°C with the help of an ice bath and absorbance was recorded at 532 nm.

Osmo-regulating compounds

For determination of total soluble proteins (TSP) frozen leaf sample was grinded in 5 ml phosphate buffer then centrifuged at 14000 rpm for 15 min at 4°C. Then the plant sample treated with 2 ml Bradford reagent and this mixture was allowed for 15-20 min and absorbance was recorded at 595 nm (Bradford, 1976). In case of free amino acids (FAA) 1 ml of crude extract was homogenized with buffer, and poured into test tube and added 1 ml pyridine with 1 ml ninhydrin in it. Afterward, samples were placed in water bath for 30 minutes at 90°C and volume of these test tubes was maintained to 25 ml by adding dH2O in it and absorbance noticed at 570 nm (Hamilton and Van Slyke, 1943). In case of proline contents, we took 0.5 g of wheat plant samples and extracted with 10 ml of 3% sulpho-salicylic acid and centrifuged for 10 minutes at 1000 rpm. Later on

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supernatant was mixed with acid-ninhydrin and placed in water bath for 30 minutes and absorbance was noted at 520 nm (Bates et al., 1973). For determination TSS 1-2 drops of obtained supernatant was placed on prism of refractometer and brix concentration of was obtained to determine TSS.

Ionic concentration

For determination of nutrient concentration plant samples (root and shoot) were oven dried and then ground to make the powder. Afterward, 0.5 g plant samples were digested by using the mixture of acids. Sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) concentration in plant samples was determined with flame photometer ((Jenway PFP-7, Burlington, NJ, USA) whereas concentration of chloride (Cl) was determined with chloride analyzed (model 926, Sherwood Scientific, Cambridge, UK).

Yield traits and statistical analysis

The plants present in pots were taken to determine, spike length, grains/spike and spikelet's/spike. The spikes of all plants were threshed and weighed to determine grain yield/pot and 1000 grain weight. The data obtained from the traits were statistically analyzed using Fisher's Analysis of Variance (ANOVA) technique and significance among treatments was checked by LSD test (at 5% probability level) (Steel et al., 1997).

Results

Growth traits

The results showed that SS and HS induced deleterious impacts on the growth traits of wheat (*Table 2*). The growth traits including root and shoot biomass were significantly decreased under both SS and HS as compared to control. The SFW and SDW showed a reduction of 20.73% and 21.46% under combined SS and HS as compared to control (*Table 3*). Similarly, RFW and RDW also decreased by 15.97% and 14.75%, respectively under combined SS and HS as compared to control. The foliar application of SS markedly improved the growth traits under SS and HS (*Table 3*). The application of 150 mM SA improved the SFW and SDW by 24.43% and 12.96% whereas the application of a similar level of SA improved the RFW and RDW by 43.07% and 38.57% respectively (*Table 2*). SS and HS and the combination of both these stresses also significantly reduced the PH and LPP, however, the application of SA improved the PH and LPP than control (*Table 3*).

Photosynthetic pigments and anthocyanin contents

The results showed that SS and HS negatively affected the photosynthetic pigments of wheat plants (*Table 2*). The chlorophyll (a and b) was significantly reduced under stress conditions and maximum reduction was noticed under a combination of SS and HS followed by HS and SS alone as compared to control (*Table 4*).

Carotenoids also showed a similar trend and maximum reduction in carotenoid contents was recorded in when plants were exposed to combined SS and HS followed by HS and SS alone as compared to the control (*Table 4*). The application 150 mM SA markedly improved chlorophyll and carotenoids under both stresses as compared to control and other levels of SA (*Table 4*). Anthocyanin activity was increased with the increasing concentrations of applied/induced stresses.

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Table 2. *ANOVA sources, and significance in growth traits of wheat*

Sources	DF	PH	SFW	SDW	RFW	RDW	LPP	Chl-a	Chl-b	Cart.	Anth.	RWC	EL
Treatments (T)	3	135.24**	9.50**	5.24**	7.76**	4.89**	13.58**	0.063*	0.160*	0.036**	6.88**	149.65**	1691.41*
Foliar (SA)	3	86.68**	2.50**	0.53**	3.41**	3.24**	6.47**	0.052*	0.95**	0.14**	87.38**	93.32**	144.62**
T×SA	9	0.732*	0.057*	0.013*	0.032**	0.001**	0.86*	0.002*	0.001*	0.001*	0.119**	1.28**	1.58*

PH: plant height, RL: root length, RFW and RDW are root fresh and dry weight, LPP: leaves per plant, SFW and SDW are shoot fresh and dry weights. Chl and EL indicate chlorophyll and electrolyte leakage. Cart and Anth are carotenoid and anthocyanin. * and ** indicates significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

Table 3. *Effect of salicylic acid (SA) application on the growth traits of wheat grown under salinity and heat stress*

Treatments	SA application	PH (cm)	SFW (g)	SDW (g)	RFW (g)	RDW (g)	LPP
Control	Control	66.50d±0.92	5.77d±0.25	3.49d±0.11	4.71bc±0.032	1.98b±0.014	3.33cde±0.22
Control	50 mM	68.00c±0.44	6.14c±0.18	3.60c±0.028	4.79b±0.059	2.04b±0.022	4.33bc±0.14
Control	100 mM	70.66b±0.26	6.84b±0.22	3.70b±0.14	4.89b±0.042	2.17a±0.019	5.33ab±0.25
Control	150 mM	73.16a±0.32	7.18a±0.14	3.81a±0.13	4.98a±0.039	2.22a±0.021	6.33a±0.19
SS: 8 dSm^{-1}	Control	60.50k±0.51	4.78h±0.33	2.86g±0.29	4.04f±0.025	1.65de±0.014	2.66defg±0.12
SS: 8 dSm^{-1}	50 mM	62.50ij±0.49	5.07f±0.29	3.03f±0.10	4.16e±0.019	1.72d±0.022	3.00def±0.20
SS: 8 dSm^{-1}	100 mM	64.00fg±0.87	5.43e±0.14	3.29e±0.21	4.22d±0.045	1.75d±0.015	3.66cd±0.10
SS: 8 dSm^{-1}	150 mM	66.66d±0.72	5.76d±0.22	3.48d±0.19	4.35d±0.022	1.84c±0.029	3.33cde±0.095
HS	Control	60.50k±0.65	3.99l±0.20	2.32j±0.23	3.50i±0.020	1.44f±0.032	2.00fg±0.16
HS	50 mM	62.83hi±0.50	4.39j±0.34	2.44i±0.25	3.69h±0.017	1.45f±0.020	2.66defg±0.20
HS	100 mM	64.33f±0.28	4.66i±0.12	2.65h±0.17	3.71h±0.025	1.49f±0.044	2.00fg±0.33
HS	150 mM	66.66d±0.14	4.92g±0.22	2.89g±0.33	3.89g±0.022	1.60e±0.028	3.66cd±0.19
SS+HS	Control	58.16l±0.54	4.22k±0.25	1.91l±0.19	2.68l±0.032	1.22h±0.022	1.66g±0.25
SS+HS	50 mM	61.83j±0.35	4.66i±0.14	2.44i±0.21	2.80k±0.060	1.34g±0.014	2.33efg±0.32
SS+HS	100 mM	63.33gh±0.43	4.91g±0.19	2.27j±0.26	2.92k±0.055	1.36g±0.034	3.00def±0.25
SS+HS	150 mM	65.16e±039	5.08f±0.24	2.32j±0.10	3.06j±0.051	1.40f±0.014	3.33cde±0.20

PH: plant height, SFW: shoot fresh weight, SDW: shoot dry weight, RFW: root fresh weight: RDW: root dry weight, LPP: leaves per plant. The presented value above is mean of three replicates with S.E. (±) and different letters with each value showing significance at 0.05 P level

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Table 4. *Effect of salicylic acid (SA) application on the chlorophyll, carotenoids and anthocyanin contents and RWC and EL of wheat grown under salinity and heat stress*

Treatments	SA	Chl-a	Chl-b	Carotenoids	Anthocyanin	RWC (%)	EL (%)
Control	Control	0.57d±0.012	1.51c±0.016	0.51d±0.014	3.00j±0.55	78.33e±2.22	31.50k±156
Control	50 mM	0.60c±0.009	1.64b±0.018	0.57c±0.020	3.75i±0.40	81.00d±1.56	29.00l±1.44
Control	100 mM	0.62b±0.014	1.73a±0.014	0.60b±0.016	1.46e±0.49	83.66bc±1.78	27.00m±0.8
Control	150 mM	0.67a±0.022	1.80a±0.020	0.65a±0.019	4.84h±0.60	87.00a±2.23	25.00n±0.76
SS: 8 dSm^{-1}	Control	0.48g±0.011	1.27ef±0.025	0.40gh±0.029	5.87g±0.45	78.50e±0.99	40.00h±2.00
SS: 8 dSm^{-1}	50 mM	0.57de±0.08	1.31e±0.029	0.45e±0.030	6.07g±0.98	80.50d±1.27	37.00i±1.12
SS: 8 dSm^{-1}	100 mM	0.61c±0.012	1.42d±0.010	0.50d±0.014	7.19f±0.82	82.66c±2.56	34.00j±0.98
SS: 8 dSm^{-1}	150 mM	0.63b±0.017	1.50c±0.012	0.56c±0.015	7.37ef±0.75	84.50b±2.22	31.50k±1.78
HS	Control	0.41i±0.020	1.07h±0.015	0.39gh±0.010	7.98de±0.55	74.16h±1.56	49.83d±1.78
HS	50 mM	0.52f±0.022	1.15gh±0.018	0.42fg±0.008	8.43cd±0.19	76.66fg±1.44	46.66e±1.39
HS	100 mM	0.56e±0.026	1.21e±0.018	0.45ef±0.014	8.97bc±0.65	78.16e±1.87	44.66f±1.12
HS	150 mM	0.59c±0.022	1.31e±0.024	0.47e±0.017	9.44b±0.60	80.00d±1.90	42.50g±1.56
SS+HS	Control	0.33j±0.014	0.84j±0.022	0.25k±0.025	9.11bc±0.78	72.00i±1.52	60.16a±1.56
SS+HS	50 mM	0.43h±0.015	0.97i±0.016	0.28j±0.022	9.58b±0.22	74.50h±2.23	57.00b±2.00
SS+HS	100 mM	0.52f±0.018	1.08h±0.014	0.34i±0.020	10.65a±0.36	76.00g±2.00	54.00c±1.99
SS+HS	150 mM	0.57de±0.02	1.15gh±0.20	0.39h±0.029	11.09a±0.40	77.66ef±1.91	50.00d±1.90

RWC: relative water contents, EL: eltrolyte leakage. Chl-a: chlorophyll a, Chl-b: chlorophyll b. The presented above is mean of three replicates with S.E. (±) and different letters with each value showing significance at 0.05 P level

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The application of SA anthocyanin concentration by 34%, 13.84%, and 61.42% at 50, 100, and 150 mM as compared to control (*Table 4*).

Relative water contents and electrolyte leakage

RWC were decreased under all stresses and a decrease of 5.01%, 2.63% and 7.94% in RWC, was recorded at 8 dS^{-1} of SS, HS and combination of SS and HS (*Table 2*). The foliar application of SA markedly increased RWC and an increase of 11.16%, 16.36% and 22.04% were recorded at 50-, 100- and 150-mM SS (*Table 4*). EL was significantly increased under stress conditions and an increase of 12.03%, 6.52% and 17.89% in EL was recorded at SS, HS and combination of both stresses (*Table 4*). By using SA, the EL was significantly deceased and a reduction of 35.57%, 83.63% and 49.14% in EL was recorded at 50-, 100- and 150-mM SA application (*Table 4*).

Osmo-regulating compounds

The results indicated that SS and HS and a combination of both these stresses significantly reduced the TSP and FAA (*Table 5*). TSP was decreased by 14.02%, 6.27%, and 23.06% at SS, HS, and their combination as compared to control (*Table 6*). Similarly, FAA was also decreased by 12.28%, 6.23%, and 18.71% at SS, HS, and their combination as compared to control (*Table 7*). The different levels of foliar applied SA significantly increased the TSP and FAA (*Table 7*) and a linear increase was observed with increasing SA concentration. TSP increased by 9.04%, 30.87%, and 53.14% whereas FAA was increased by 7.21%, 20.77%, and 44.36% at 50, 100, and 150-mM SS applications (*Table 7*). Proline and SS showed a different trend and their concentration was significantly increased under both stresses (*Table 7*). The maximum increase in proline and TSS was recorded under a combination of SS and HS followed by HS and SS. The foliar-applied SA also increased the synthesis of proline and TSS and maximum increase was recorded with application of 150 mM SA, followed by 100 and 50 mM SA application, respectively (*Table 7*).

Antioxidant activities

The activity of all the antioxidants was significantly increased under stress conditions they were further increased by application SA (*Table 5*). An increase of 11.45%, 5.20%, and 20.83% in CAT activity was recorded at SS, HS, and a combination of both these stresses (*Figure 1*). Similarly, POD activity was also increased by 32%, 70%, and 83.76% at SS, HS, and a combination of both these stresses as compared to control (*Figure 1*). The various levels of SA also significantly increased both CAT and POD activities. The activity CAT was increased by 36.60%, 10.71%, and 42.85% whereas POD activity was increased by 10.69%, 5.61%, and 16.57% at 50, 100, and 150-mM concentrations of SA (*Figure 1*). APX and ascorbic acid also showed an increasing trend under stressed conditions (*Figure 1*). An increase of 4.14%, 1.62%, and 6.37% in APX was noted at 8 dS^{-1} of SS, HS, and their combination (*Figure 1*). Similarly, foliar-applied SA also increased APX activity by 18.11%, 9.05%, and 25.34% at 50, 100 and 150-mM concentrations of SA (*Figure 1*). The activity of ascorbic acid was increased by 22.44%, 6.41%, and 17.86% at SS, HS, and their combination. Moreover, foliar-applied SA (50, 100, and 150 mM) increased the ascorbic acid activity by 42.92%, 17.1%, and 58.01% compared to control (*Figure 1*).

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Table 5. *ANOVA sources, and significance in growth traits of wheat*

Sources	DF	TSP	FAA	Proline	TSS	CAT	POD	AsA	APX	MDA	H_2O_2
Treatments (T)	3	3.51^{**}	2.024	0.045^{*}	4.33^{**}	0.088^{**}	9.47^{**}	2.93^{**}	16.01^{*}	1.44^{*}	0.85^{**}
Foliar (SA)	3	25.1^{**}	14.82	0.033^{*}	23.1^{**}	0.624^{**}	0.84^{*}	6.47^{**}	307.63^{*}	10.93^{*}	9.44^{**}
T×SA	9	0.099^{**}	0.039	0.002^{*}	0.012^{**}	0.005^{*}	0.013^{*}	2.66^{**}	0.67^{*}	0.013^{*}	0.013^{*}

TSP and FAA are soluble proteins and free amino acids while TSS is total soluble sugars. CAT and POD indicates catalase and peroxidase, and AsA and APX are ascorbic acid and ascorbate peroxidase, and H_2O_2 is hydrogen peroxide. * and ** indicates significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

Table 6. *ANOVA sources, and significance in growth traits of wheat*

Sources	DF	Root Na	Shoot Na	Root K	Shoot K	Root Cl	Shoot Cl	Root Ca	Shoot Ca	Root Mg	Shoot Mg
Treatments (T)	3	5.33^{**}	6.44^{*}	14.54^{**}	13.67^{*}	4.44^{**}	3.89^{*}	20.33^{**}	16.55^{*}	18.87^{*}	15.63^{*}
Foliar (SA)	3	72.22^{**}	72.3^{**}	218.44^{**}	206.22^{*}	65.41^{**}	69.21^{**}	289.12^{*}	333.44^{*}	378.55^{*}	323.33^{**}
T×SA	9	0.112^{*}	0.021^{*}	0.56^{*}	0.44^{*}	0.212^{**}	0.49^{*}	0.57^{*}	0.89^{*}	0.72^{*}	0.89^{**}

Na: sodium, K: potassium, Cl: chloride and Mg: magnesium. * and ** indicates significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

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Table 7. *Effect of salicylic acid (SA) application on the TSP, FAA, proline and soluble sugars of wheat grown under salinity and heat stress*

Treatments	SA application	TSP (mg/g FW)	FAA (mg/g FW)	Proline (mg/g FW)	TSS (mg/g FW)
Control	Control	5.78d±0.092	5.37d±0.024	0.58e±0.017	10.72m±0.23
Control	50 mM	6.21c±0.062	5.56c±0.020	0.60de±0.029	11.11l±0.14
Control	100 mM	6.86b±0.074	5.77b±0.026	0.65d±0.022	11.56k±0.29
Control	150 mM	7.20a±0.082	6.04a±0.017	0.66d±0.018	11.75k±0.12
SS: 8 dSm^{-1}	Control	4.84h±0.051	4.84g±0.009	0.62d±0.034	12.70j±0.22
SS: 8 dSm^{-1}	50 mM	5.07f±0.056	5.07e±0.017	0.66d±0.041	12.92j±0.42
SS: 8 dSm^{-1}	100 mM	5.43e±0.082	5.43d±0.022	0.67d±0.026	13.28i±0.20
SS: 8 dSm^{-1}	150 mM	5.75d±0.078	5.75b±0.015	0.71c±0.022	13.87h±0.19
HS	Control	3.99k±0.065	3.99j±0.017	0.69c±0.018	14.52g±0.26
HS	50 mM	4.39j±0.061	4.39i±0.019	0.72c±0.029	14.98f±0.20
HS	100 mM	4.66i±0.045	4.66h±0.025	0.75c±0.030	15.55f±0.32
HS	150 mM	4.95g±0.54	4.95f±0.029	0.81bc±0.041	16.50e±0.35
SS+HS	Control	2.08o±0.056	2.50n±0.031	0.80b±0.026	17.12d±0.50
SS+HS	50 mM	2.96n±0.043	2.96m±0.023	0.84b±0.022	17.87c±0.44
SS+HS	100 mM	3.38m±0.052	3.38l±0.045	0.88b±0.036	18.30b±0.42
SS+HS	150 mM	3.79l±0.044	3.79j±0.041	0.94a±0.019	19.33a±0.29

TSP: total soluble proteins, FAA: free amino acid, TSS: total soluble sugars. The presented value above is mean of three replicates with S.E. (±) and different letters with each value showing significance at 0.05 P level

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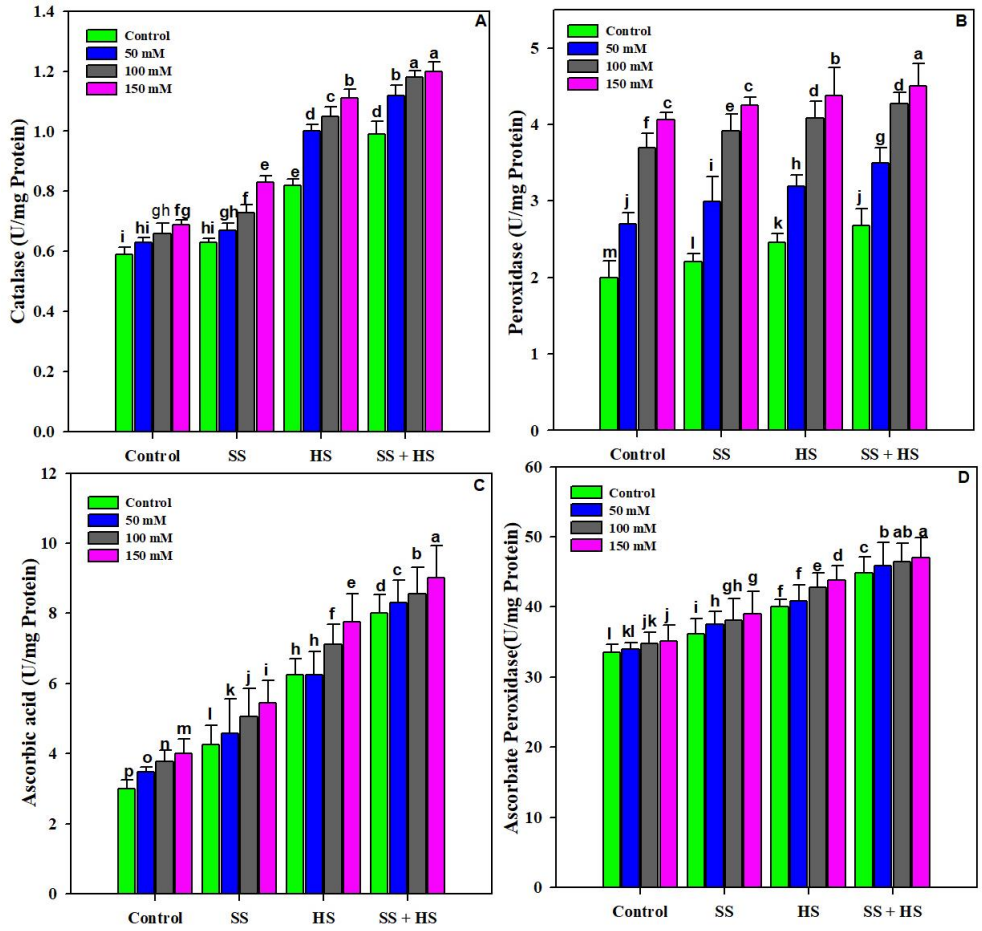

Figure 1. *Effect of salicylic acid (SA) application on the antioxidant activity of wheat grown under salinity and heat stress. The presented value above give bars is mean three replicates with S.E. (±) and different letters with each value showing significance at 0.05 P-level*

H_2O_2 and MDA concentration

H_2O_2 concentration was substantially increased under stressed conditions and an increase of H_2O_2 concentration was increased by 10.69%, 4.27% and 16.84% at SS, HS and their combination (*Figure 2*). The different levels of foliar applied SA reduced H_2O_2 concentration in wheat plants. The H_2O_2 was decreased 28.18%, 7.44% and 45.5% at 50, 100- and 150-mM concentrations of SA as compared to control (*Figure 2*). MDA concentration was also increased by 24.29%, 7.92% and 37.5% at SS, HS and their combination (*Figure 2*). Nonetheless, SA application reduced the MDA concentration by 9.46%, 4.33% and 15.97% at 50-, 100- and 150-mM concentrations respectively (*Figure 2*).

Nutrient concentration

The results showed that concentration of all nutrients was significantly affected by the SS and HS. The concentration of Na and Cl was considerably increased under saline conditions (*Table 6*). The foliar applied SA also significantly affected the Na and Cl concentration and it reduced the Na and Cl accumulation in plants. The concentration of K was also significantly affected owing to stress conditions. The maximum reduction in

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K accumulation in plant parts were noted under combination of SS and HS followed by HS and SS. Similarly, Ca and Mg concentration was significantly reduced under stress conditions and maximum reduction was noted under combined SS and HS (*Table 8*). Nonetheless, foliar applied SA appreciably increased the Ca and Mg concentration under all stress conditions as compared to control (*Table 8*).

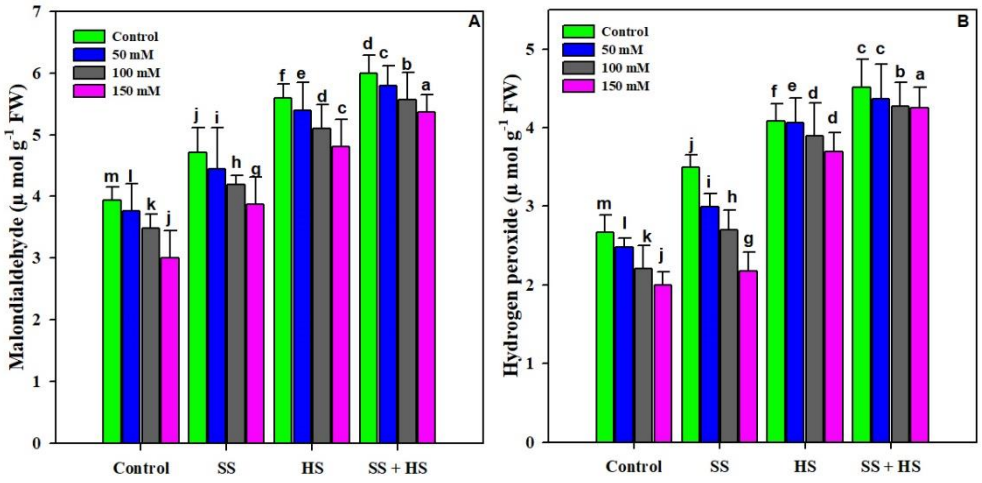

Figure 2. *Effect of salicylic acid (SA) application on MDA and H_2O_2 concentration of wheat grown under salinity and heat stress. The presented value above give bars is mean three replicates with S.E. (±) and different letters with each value showing significance at 0.05 P level*

Yield traits

The results of our study showed that SS, HS, and a combination of both stresses negatively affected the yield traits of wheat crops (*Table 9*). The results indicated that the maximum reduction in yield traits (spike length, grains/spike, TGW and SL, and GY) was recorded under combined SS and HS followed by HS and SS (*Table 10*). The application of SA appreciably improved the yield traits of wheat crop. The foliar applied SA (150 mM) mitigated the adverse effects of both stresses and appreciably improved the yield traits of wheat crop (*Table 10*).

Discussion

Salinity and heat stress are two co-occurring major abiotic stresses negatively affecting crop growth and productivity. Therefore, it is crucial to understand the response of plants to these abiotic stresses (Kakar et al., 2019; Wu et al., 2021). The seedling growth is considered to be sensitive to these stresses. According to a study SS and HS caused a substantial reduction in growth (*Table 2*) owing to increased ROS production, EL leakage, reduced photosynthetic efficiency, and chlorophyll synthesis and disturbed the nutrient homeostasis (*Table 3*) therefore, resulting in a significant reduction in growth traits of wheat (Rana et al., 2019; Ferguson et al., 2020). Besides this reduction in growth and biomass under combined SS and HS was linked with the accumulation of toxic ions (Na+ and Cl-) and reduction in uptake of Ca, K, and Mg (Alexieva et al., 2001; Chaitanya et al., 2003).

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Table 8. *Effect of salicylic acid (SA) application on Na, K and Cl concentration in different plant parts of wheat grown under salinity and heat stress*

Treatments	SA application	Root Na	Shoot Na	Root K	Shoot K	Root Cl	Shoot Cl
Control	Control	2.12g±0.06	2.72f±0.088	22.33c±0.56	27.70d±0.77	3.10g±0.15	2.66g±0.087
Control	50 mM	1.97g±0.24	2.49f±0.077	25.90b±0.30	33.37c±1.03	2.87g±0.032	2.43g±0.050
Control	100 mM	1.92g±0.044	2.46f±0.023	25.07b±0.72	35.83b±0.34	2.79g±0.013	2.43g±0.018
Control	150 mM	1.84g±0.23	2.36f±0.019	27.97a±0.62	37.60a±0.62	2.70g±0.049	2.30g±0.043
SS: 8 dSm^{-1}	Control	19.57bc±0.02	21.93b±0.37	15.92fgh±0.88	15.17gh±0.34	31.20b±1.13	26.27b±0.92
SS: 8 dSm^{-1}	50 mM	17.33d±0.52	19.80c±0.45	16.23fg±0.37	17.80f±0.42	28.07cd±0.47	24.70cd±0.35
SS: 8 dSm^{-1}	100 mM	15.30e±0.20	18.00de±0.31	18.17e±0.32	18.57ef±0.49	26.43ef±0.55	21.67e±0.67
SS: 8 dSm^{-1}	150 mM	13.00f±0.75	17.27e±0.29	20.03d±0.20	19.80e±0.45	25.53f±0.65	19.07f±0.46
HS	Control	2.17g±0.017	2.78f±0.11	14.90gh±i0.70	13.63ij±0.34	3.17g±0.14	2.73g±0.049
HS	50 mM	2.01g±0.052	2.50f±0.80	15.43fgh±0.46	15.13gh±0.39	2.94g±0.010	2.54g±0.030
HS	100 mM	1.98g±0.088	2.45f±0.048	16.43f±0.32	17.43f±0.30	2.85g±0.019	2.50g±0.027
HS	150 mM	1.85g±0.029	2.40f±0.026	18.33e±0.42	18.33f±0.36	2.77g±0.022	2.43g±0.025
SS+HS	Control	21.23a±0.043	23.03a±0.74	14.03i±0.29	12.67j±0.44	33.03a±0.68	28.60a±0.51
SS+HS	50 mM	20.40ab±0.58	21.27b±0.64	14.67hi±0.20	14.10hi±0.22	29.13c±0.90	25.83bc±0.33
SS+HS	100 mM	19.40bc±0.86	18.53d±0.41	15.50fgh±0.10	15.10gh±0.23	28.13cd±0.20	23.70d±0.65
SS+HS	150 mM	18.07cd±1.12	17.63de±0.58	16.23fg±0.24	15.63g±0.35	27.47de±0.49	21.27e±0.54

The presented value above is mean of three replicates with S.E. (±) and different letters with each value showing significance at 0.05 P level

Table 9. *ANOVA sources, and significance in growth traits of wheat*

Sources	DF	SL	SLPS	GPS	TGW	GY
Treatments (T)	3	2.74^{**}	20.33^{**}	18.33^{*}	16.55^{*}	20.45^{*}
Foliar (SA)	3	6.33^{**}	412.11^{**}	353.72^{*}	289.11^{*}	278.56^{**}
T×SA	9	3.45^{**}	1.67^{*}	1.14^{*}	1.29^{*}	2.23^{*}

SL and SLPS indicate spike length, and spikelets per spike, and GPS, TGW and GY indicates, grains per spike, thousand grain weight, and grain yield. * and ** indicates significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

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Table 10. *Effect of salicylic acid (SA) application on yield traits of wheat grown under salinity and heat stress*

Treatments	SA application	SL	SLPS	GPS	TGW	GY
Control	Control	9.04d±0.043	41.21c±0.23	46.33bc±0.3	38.67c±0.82	35.67d±0.36
Control	50 mM	9.39c±0.12	42.00bc±0.26	48.33b±0.39	41.33b±0.34	38.00c±0.58
Control	100 mM	9.96b±0.14	43.28ab±0.98	51.00a±0.56	43.00b±0.58	41.00b±0.99
Control	150 mM	10.19a±0.10	44.45a±1.15	52.33a±1.45	45.67a±1.45	44.00a±1.15
SS: 8 dSm^{-1}	Control	8.24fg±0.061	32.03g±0.64	38.67f±0.56	31.67e±0.88	31.33 fgh±0.33
SS: 8 dSm^{-1}	50 mM	8.40f±0.029	33.77f±0.72	41.67e±0.66	34.00d±0.64	32.00fg±0.67
SS: 8 dSm^{-1}	100 mM	8.67e±0.082	36.10e±0.45	43.33de±1.2	34.67d±0.88	33.33ef±0.42
SS: 8 dSm^{-1}	150 mM	8.83e±0.035	38.90d±0.49	44.67cd±0.5	37.33c±0.56	35.33de±0.32
HS	Control	7.72j±0.020	21.73k±0.87	28.00i±1.20	28.67f±0.32	28.33jkl±0.99
HS	50 mM	7.83ij±0.029	23.83j±1.15	31.67h±0.67	29.00f±0.42	29.00ijk±1.09
HS	100 mM	7.93h±0.058	26.63i±1.00	33.33h±0.88	31.33e±0.78	31.00ghi±0.43
HS	150 mM	8.10gh±0.032	29.47h±1.58	36.00g±1.15	33.00de±0.92	33.33ef±0.59
SS+HS	Control	7.08m±0.052	15.43m±1.06	19.67l±0.39	22.00h±0.25	24.67m±0.82
SS+HS	50 mM	7.21lm±0.05	17.87l±0.30	22.00kl±1.0	22.33h±1.01	26.33lm±0.78
SS+HS	100 mM	7.39kl±0.039	18.97l±0.55	23.00jk±0.4	25.67g±0.33	27.00kl±0.59
SS+HS	150 mM	7.51k±0.046	20.53k±1.00	25.00j±0.92	27.00fg±0.65	29.67hij±0.66

The presented value above is mean of three replicates with S.E. (±) and different letters with each value showing significance at 0.05 P level. SL: Spike length, SLPS: spikelets per spike, GPS: grains per spike, TGW: thousand grain weight, GY: grain yield

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This complex relationship between growth and nutrient uptake under combined stresses was due to opposing signaling induced by combined stresses (Nahar et al., 2021).

The foliar applied SA appreciably improved the growth of wheat (*Table 2*) due to less MDA, H_2O_2, and EL accumulation (Alsahli et al., 2019) and improved chlorophyll contents (*Table 5*), antioxidant activities (*Figure 2*) and increased uptake of Ca, Mg and K (*Table 3*) therefore, improved wheat growth under SS. Under SS and HS plants with supplied with foliar spray of SA showed lower MDA levels which indicates that plants had better membrane integrity and therefore they had better growth as compared to non-treated plants (Younis et al., 2021; Wang et al., 2022). Further, the foliar application of SA also protects the photosynthetic apparatus from the deleterious impacts of HS and SS and maintains better chlorophyll synthesis and photosynthetic efficiency thus ensuring better plant growth (Zheng et al., 2013). Additionally, SA abated the reduction in flag leaf area under HS and delayed the leaf senescence which also contributes toward better photosynthetic efficiency under SS conditions (Fan et al., 2022). RWC plays an important role in maintaining the balance between water to leaf tissue and rate of transpiration. Both stresses reduced the leaf RWC, however, foliar application of SA maintained better RWC and prevented EL leading to an appreciable increase in plant growth under stress conditions (Sultan et al., 2021).

The high availability of salts and HS impairs the photosynthetic process by disturbing the photosynthetic pigments (Wise et al., 2004) and reducing the performance of RuBP and ROS scavengers (Nahar et al., 2022). We also found that SS and HS significantly reduced chlorophyll synthesis which can be attributed to a reduction in enzyme activity involved in the synthesis of chlorophyll (Dutta et al., 2009). However, the application of SA appreciably improved chlorophyll synthesis which could be linked with improved antioxidant activity and an increase in the activity of enzymes involved in the synthesis of chlorophyll (Gurmani et al., 2018). The increase in chlorophyll contents by SA can improve the photosynthetic rate and therefore, plant growth under combined SS and HS (Arfan et al., 2007). Besides this SA also improves the photosynthetic rate by safeguarding the chloroplast structure and regulating the antioxidant system balance in the chloroplast, thus it lays the foundation for better accumulation of assimilates and delays the leaf senescence and ensures better performance under combined stresses. Additionally, SS also abated the decrease in stomata number which resulted in better CO_2 fixation and assimilate production leading substantial reduction in yield losses (Wakabayashi et al., 2012; Liu et al., 2013).

Salinity and HS significantly reduced the TSP and FAA and increased the TSS and proline accumulation (*Table 7*). However, foliar-applied SS significantly improved the synthesis of TSP which could be attributed to SA-induced protein kinase synthesis (El-Tayeb, 2005; Fahad and Bano, 2012). SA-mediated increase in TSP improved the photosynthetic capacity and yield (*Table 10*). The increase in TSS is considered a means to improve stress tolerance and in our experiment accumulation of TSS was trigged by both HS stresses and application of SA. Yuan et al. (2020) noted that TSS was significantly increased in tomato plants growing under SS. Proline has broader functions in plants linked with energy regulation and antioxidant activity (Launay et al., 2019). Salinity and HS significantly increased the proline accumulation possibly due to an increase in activity of P5CS. This increase in proline synthesis was substantially increased by SA application which could be due to an increase in P5CS activity. Likewise, different authors also noted that SS and a combination of SS + HS significantly improved proline accumulation (Rivero et al., 2014). The increase in proline accumulation following SA

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application improved the chlorophyll synthesis, RWC, and antioxidant activities and reduced the MDA and H_2O_2 accumulation thus maintaining better growth under SS + HS.

The increase in proline accumulation following SA application improved the chlorophyll synthesis, RWC, and antioxidant activities and reduced the MDA and H_2O_2 accumulation thus maintaining better growth under SS + HS. The higher antioxidant activity is considered an important means to tolerate SS and HS (Sehar et al., 2021). Antioxidant plays a major role in stress tolerance and in the present study we noted that the activity of antioxidants (APX, CAT, POD, and ascorbic acid) was significantly increased which protected the wheat plants from the toxic effects of SS + HS. SA application might increase cell signaling and therefore improve antioxidant activities and minimize the deleterious impacts of stress conditions (Uddin et al., 2014). Likewise, Syeed et al. (2011) and Liu et al. (2021) also found that SS application improved the APX and SOD activity which destroyed the H_2O_2 and protect the plants from the SS + HS-induced oxidative stress. The exogenous supply of SA facilitates the detoxification of H_2O_2 by enhancing the APX, POD and CAT activity thereby improving the plant performance by increasing photosynthetic efficiency, osmolytes accumulation, and chlorophyll synthesis (Xu et al., 2006; Li et al., 2011). Many other authors also found a substantial reduction in ROS production owing to an increase in antioxidant activity following SA under SS and HS (Hameed et al., 2016; Wassie et al., 2020).

Nutrient homeostasis plays an important role in stress tolerance and in present study we noted that Na^+ and Cl^- accumulation were significantly increased under SS, however, HS showed a minor effect on Na^+ and Cl^- accumulation (*Table 11*). Salinity induced excessive Na^+ accumulation that increased ROS production which in turn increased MDA accumulation, EL, and degraded protein (Gao et al., 2015) (*Table 4*). Additionally, excessive Na negatively affects cytosolic activity resultantly reduced carbon assimilation and induced premature leaf senescence (Suzuki et al., 2014). Further, SS and HS also increased EL owing to the efflux of K (*Table 9*), however, foliar application SA reduced EL and helped the plants to retain K to counter-balance excessive Na (Jayakannan et al., 2015). K^+ uptake was significantly reduced under both SS and HS and this reduction could be linked with an increase in K+ efflux however, this remains to be confirmed.

Under SS and HS, potassium channel (GORK) mediated increase in K^+ efflux owing to activation of ROS (Demidchik et al., 2010). ROS could promote the K^+ efflux which can be reduced by the application of osmoprotectants (Cuin and Shabala, 2007), and in the current study, SA also improved the K^+ uptake by decreasing its efflux (Chérel and Gaillard, 2019). The results showed that SS and HS significantly reduced the uptake of Ca, Mg, and K (*Tables 8 and 11*) and the application of SS appreciably increased the uptake of these nutrients. SA application promotes the uptake of Ca^{2+}, K^+, and Mg^{2+} owing to its inhibitory effect on excessive Na^+ accumulation and its ability to reduce the competitive effect of Na^+ on K^+ and keep excessive Na^+ from replacing Ca^{2+} on the membrane binding site (Ghassemi-Golezani and Farhadi, 2021). SA-mediated increase in Mg^{2+} uptake increased the chlorophyll synthesis, therefore, maintaining better photosynthesis under stress conditions (Xu et al., 2022). In the present study, both 6stresses caused a serious reduction in wheat yield owing to enhanced H_2O_2 and MDA accumulation (*Figure 1*) and reduced chlorophyll synthesis, RWC, carotenoid contents (*Table 4*), TSP, and FAA (*Table 7*) and uptake of beneficial nutrients. Further application of SA improved the yield and yield traits of wheat by favoring a significant increase in chlorophyll synthesis, physiological activity, antioxidant performance, osmolyte accumulation, and nutrient uptake (Xu et al., 2022).

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Table 11. *Effect of salicylic acid (SA) application on Ca and Mg concentration in different plant parts of wheat grown under salinity and heat stress*

Treatments	SA application	Root Ca	Shoot Ca	Root Mg	Shoot Mg
Control	Control	56.90cd±1.25	66.17d±0.72	40.93def±0.37	53.37c±1.60
Control	50 mM	58.67c±0.49	70.47c±0.50	44.13bc±0.87	56.90b±1.23
Control	100 mM	61.80b±0.70	74.77b±0.37	46.23b±1.07	57.70b±1.47
Control	150 mM	65.47a±0.57	77.63a±1.58	50.67a±0.85	62.00a±0.94
SS: 8 dSm^{-1}	Control	45.70f±0.79	51.80hi±0.65	36.17hij±1.24	41.47fg±1.73
SS: 8 dSm^{-1}	50 mM	50.17e±0.34	55.10g±0.32	40.13ef±1.11	45.43de±1.15
SS: 8 dSm^{-1}	100 mM	54.27d±0.95	58.27f±0.40	42.67cd±0.93	46.53d±1.30
SS: 8 dSm^{-1}	150 mM	56.27cd±1.20	63.07e±0.67	44.47bc±0.63	50.51c±1.24
HS	Control	42.37f±1.31	49.43ij±0.70	34.10jk±0.88	34.87ij±0.98
HS	50 mM	45.67f±1.88	52.97gh±1.35	36.57hi±0.69	37.60hi±0.82
HS	100 mM	45.87f±0.91	55.27g±1.54	40.10ef±0.73	39.43gh±0.93
HS	150 mM	50.17e±0.35	58.30f±1.45	42.23cde±0.52	43.03ef±1.15
SS+HS	Control	39.87g±1.10	43.57k±0.78	32.20k±0.55	31.43k±1.55
SS+HS	50 mM	41.13g±0.58	47.73j±0.62	34.47jk±0.69	32.53jk±0.80
SS+HS	100 mM	42.60g±0.86	51.30hi±0.52	37.00gh±0.60	37.33hi±0.72
SS+HS	150 mM	46.50g±1.15	53.40gh±0.63	39.20fg±0.49	41.07fg±0.63

The presented value above is mean of three replicates with S.E. (±) and different letters with each value showing significance at 0.05 P level

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Conclusion

In conclusion, salinity in combination with heat stress significantly hampered wheat growth and productivity by altering photosynthetic pigments, physiological activity, nutrient homeostasis, and osmolyte accumulation. However, the foliar-applied SA offset the deleterious effects of SS and HS and markedly increased wheat growth and yield. SA-mediated increase in growth and yield was linked with improved antioxidant activities, nutrient homeostasis, and osmolyte accumulation. Therefore, foliar spray of SA could be an effective measure to reduce the deleterious impacts of salinity and heat wheat. However, more studies are direly needed to optimize the SA concentration for wheat under different soil and climatic conditions before making its use on a large scale.

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