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RESEARCH PAPER

Evaluating the effect of exogenous application of salicylic acid on heat stress tolerance in wheat (*Triticum aestivum* L.)

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Abstract

Heat stress is a major abiotic constraint that impairs photosynthesis, disrupt cellular homeostasis, and reduce grain yield in wheat. Salicylic acid (SA), an important signalling molecule, enhances tolerance to several abiotic stresses, but its role in improving wheat performance under heat stress remains insufficiently explored. Therefore this research study was was conducted to evaluate the effect of exogenous salicylic acid (SA; 5 mM) on heat stress tolerance in wheat (*Triticum aestivum* L., cv. FSD-2002). Plants were exposed to >32 °C after 45 days of seedling growth and treated with SA as a foliar spray, soil supplementation, or both. ANOVA showed significant ($p < 0.05$) improvements in all growth and yield traits. Under control conditions, shoot and root lengths increased from 59 and 33 cm in untreated plants to 87 and 52 cm with combined SA treatment, while under heat stress they rose from 49 and 26 cm to 71 and 45 cm, respectively. Shoot and root fresh weights improved from 5.6 and 1.5 g to 8.7 and 3.1 g under control, and from 4.5 and 1.3 g to 7.7 and 2.2 g under stress. SA enhanced physiological traits, raising SPAD values from 34.2 to 46.1 (control) and 27.5 to 39.4 (stress), and leaf area from 21.5 to 35.6 cm^2 and 16.4 to 29.3 cm^2, respectively. Yield traits improved markedly: spikes from 8 to 15, spikelets from 20 to 35, grains per spike from 35 to 52, and 1000-grain weight from 35 to 59 g under control; corresponding stressed values rose from 5 to 11 spikes, 12 to 26 spikelets, 22 to 38 grains, and 30 to 51 g. Mineral nutrition was enhanced, with shoot calcium increasing from 8.1 to 11.5 mg/g DW (control) and 7.2 to 9.8 mg/g DW (stress), sulphate from 6.9 to 11.1 and 6.1 to 9.6 µg/g DW, and phosphate from 7.5 to 10.3 and 7.0 to 9.7 µg/g DW. These findings demonstrate that SA plays a key role in strengthening heat stress tolerance in wheat by sustaining biomass production, improving mineral nutrition, and preserving chlorophyll and photosynthetic capacity under thermal stress. © 2025 The Author(s)

Keywords: Chlorophyll retention, Heat stress, Mineral nutrition, Physiological tolerance, Salicylic acid, Wheat, Yield attributes

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Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most important staple cereals, providing a major share of daily caloric and protein intake for a large proportion of the global population (Iqbal et al., 2018; Abbas & Shafique, 2019; Mehmood et al., 2020). Its adaptability to diverse agro-climatic regions and central role in food security make the improvement of its productivity a continual priority in agricultural research. Ensuring stable wheat production, particularly under increasing environmental stresses such as heat, remains essential for sustaining global food systems and supporting rural livelihoods (Alamgeer et al., 2022; Dinsa & Balcha, 2024). Among major abiotic stress factors, heat stress has gained more attention due to its irreversible damages to plant growth and development (Battisti & Naylor, 2009). The environmental conditions are constantly changing, that causes an increase in average temperature that is very damaging to agricultural crops (Noroz et al., 2021; Omokhafe et al., 2024). The atmospheric temperature is continuously increasing up to 0.27 °C per decade (Zhao et al., 2017; Raza et al., 2019; Malhi et al., 2021; Forster et al., 2025). Heat shock is a sudden extreme high temperature (>32 °C) for short period of time (three-four days), while chronic heat stress consists of moderate temperature (20-30°C) for a longer duration (Corbellini et al., 1997). Heat stress causes many damages during reproductive stages (Larkindale & Knight, 2002) and disrupts ions and osmotic homeostasis at cellular levels. It affects photosynthesis and alters those genes that maintain protein homeostasis required for stability of deoxyribonucleic acid (DNA) (Abdelrahman et al., 2017). Heat stress often affects grain yield, biomass production, and thousand kernel weights (Barma, 2005; Rahman, 2009). The rate of plant metabolic processes, the production of fruits and grain are mainly influenced by temperature (Tandel et al., 2025).

The high temperature at the reproductive stage of wheat causes a severe decline in photosynthetic efficiency and leaf area, reduces shoot mass and sugar contents of kernels, and decreases water use-efficiency (Shah & Paulsen, 2003). Functions of chloroplast blocked due to ROS formation during high temperature stress (Apel & Hirt, 2004). Under high-temperature stress tillering capacity shows an association with overall plant biomass (Samad, 1994; Mann, 1994). Chlorophyll retention, leaf senescence, photosynthetic rate and canopy temperature depression are main indicators of heat tolerance (Rahman, 1996; Reynolds et al., 1997). In heat stress response pathway, the macromolecules phosphorylation and de-phosphorylation plays an important role. These macromolecules serve as primary heat sensors in plant heat-stress responses (Abdelrahman et al., 2017). The highly conserved pathways for heat stress response involves the major signaling pathways (1) the epigenetic mediated small-RNA modifications of histone proteins, (2) heat stress-induced transcriptional regulations of heat shock protein families (molecular chaperones), (3) Ca^{+2} depending on kinases (Ohama et al., 2017).

A phenolic compound salicylic acid (SA) regulates many physiological processes in plants (Raskin, 1992). SA also stimulates expression of many genes (Morris et al., 2000) and increases the pathogen protection by synthesizing pathogen-related proteins (Metraux, 2001). SA increases the plant tolerance under abiotic stress such as salinity (Shakirova & Bezrukova, 1997), water deficit (Bezrukova et al., 2001) heat stress (Senaratna et al., 2000), metal (Zhang et al., 2015), and osmotic (Alavi et al., 2014). SA induced the synthesis of heat shock proteins (Burkhanova et al., 1999) and helped in lectins accumulation in wheat (Shakirova & Bezrukova, 1997). SA regulates photosynthesis, nitrogen, and proline metabolisms and provides protection against harmful pollutants (Khan et al., 2010; 2013; 2014; Harrison, 2012). SA has its part in encoding molecular chaperone (Jumali et al., 2011). SA is involved regulation, expression, and activation mitogen-activated protein kinase (MAPK) (Chai et al., 2014) and modulates defense mechanisms (Herrera-Vasquez et al., 2015). To reduce heat-induced changes and to reset their cellular activities SA regulates the signaling cascades (Zandalinas et al., 2017). Proline and ABA contents are increased by foliar applications of SA (Shakirova et al., 2003).

Wheat (*Triticum aestivum* L.), is cultivated to a wide range of eco-climatic conditions in six continents, making it the world's second most important staple food. Around 36% of the world's population relies solely on wheat to meet their calorie needs (Ortiz et al., 2008). Heat stress mostly occurs in those crops which were planted late and causes main decrease in yield at grain filling stage (Saunders, 1988). The wheat is less adjusted to warmer temperature than other main crops (rice or maize). High temperature stress has drastic effects on wheat yield, and it decreases almost 15% of worldwide wheat production (Qin et al., 2008). The terminal heat occurs when temperature is high during reproductive development. The range of optimum temperature for grain filling is between 19 °C to 22 °C for wheat (Porter & Gawith, 1999). The threshold temperature for wheat is almost 26 °C. Daily mean temperature indicates a detectable reduction in growth at post-anthesis stage (Stone & Nicolas, 1994). Heat stress affects final grain weight by shortening the grain filling duration (Dias & Lidon, 2009). In Mexico the yield of wheat crops is reduced by 10% for every 1 °C increase in nighttime temperature (Lobell et al., 2005). The range of temperature for well-defined maturation stage is ranged from 12 to 22 °C. At the anthesis stage, elevated temperatures disrupt flower formation and arrangement. Temperatures above 30 °C during floret formation can reduce the number of fertile spikes, decrease the number of grains per spike, and lower individual grain weight, thereby negatively affecting overall yield.

Many stress-related genes and different transcription factors are involved in heat tolerance. Some specific wheat species at moderate temperatures are capable of inducing transcription and did not reduce net assimilation. In various wheat varieties polyamine signaling occurs during stress acclimation processes (Qin et al., 2008). The production of heat shock proteins are main responses of high temperature stress which may also regulate transcriptional control (Hasanuzzaman et al., 2013), boost up the carbohydrates formation and its breakdown (Wang et al., 2012), triggers alternate signaling mechanisms and improves the anti-oxidative systems (Zhou et al., 2019). SA has a more significant role in hypersensitive responses because it plays a key role in signaling pathways (Kawano et al., 1998). SA also takes part in calcium signaling (Kawano et al., 1998) and stimulates the growth in wheat and improves the plant dry mass, chlorophyll contents, sodium/potassium ratio (Kaydan et al., 2007) and leaf area (Khan et al., 2003). Different plant growth regulators like abscisic acid, auxin, gibberellic acid, jasmonic acid, cytokinins, salicylic acid, ethylene, brassinosteroids, nitric oxide and polyamines play significant role to reduce the effects of heat stress (Clarke et al., 2009; Khan et al., 2013; Song et al., 2013). Keeping in view these facts, the present study was conducted to investigate the heat stress tolerance potential of wheat with exogenous application of salicylic acid under heat stress.

Materials and Methods

To determine the effect of heat stress on wheat plant, a pot experiment was conducted in Old Botanical Garden of University of Agriculture, Faisalabad during the year 2020-21. Heat stress was applied after 45 days of seedlings. After that, treatment of salicylic acid (SA) was applied to mitigate the effects of heat stress. Wheat (*Triticum aestivum* L.) variety FSD-2002 was used as experimental plant. Wheat plants were treated with salicylic acid (SA) applied either solely or in combination as a foliar spray (0.5 mM) and soil supplementation (0.5 mM) to evaluate its effects on physiological and biochemical attributes. The wheat variety FSD2002 was sown in pots with three replicates per treatment. One set of plants was shifted to a glasshouse for heat stress, after that SA (5 mM) treatment was supplied. Growth parameters were measured 20 days after SA application, including shoot and root length, shoot and root diameter, fresh and dry biomass, number of leaves, leaf area per plant, and number of tillers per plant. Fresh samples were also collected and preserved for biochemical analyses. The physiological parameters were also recorded, including leaf photosynthetic pigments, soluble phenolics, flavonoids, anthocyanin, sodium, potassium, calcium, nitrates, Phosphates and sulphates, and predetermined protocols. The remaining plants were again harvested for maturity parameters.

Parameters studied

Shoot and root length (cm) of plant was measured by using a scale meter. Shoot length was measure from the base of the shoot to the tip of the shoot. While root length was measured from shoot end to root tip. Shoot and root fresh weight (g) for three plants per treatment was measured by using average values. After harvest plant were kept in oven at 65 °C for 48 hours. After 4 hours dry weight (g) was calculated by using analytical balance. Leaves number per plant was counted for each replicate. In order to examine number of roots per plant, the number of roots mainly emerging from stem of each plant was counted manually and after that mean value of replicates was calculated. Number of tillers of all three replicates was examined by counting tillers emerging from main stem. Stem and root diameter was measured with the help of Vernier calliper. Number of spikes was examined by counting number of spikes per plant for three replicates. Spike length (cm) of every spike per replicate were measured by measuring tape, then mean value was taken. Grains were collected and counted from every spike of plants then mean values were obtained. To determine the number of grains per plant, all grains from each spike were counted manually. The 1000-grain weight (g) was estimated by weighing 100 grains from each replicate and multiplying the value by 10.

Leaf area (cm^{-2})

Area of leaf (cm^{-2}) was calculated by measuring length and width of leaf (Yu et al., 2020):

Total area of leaf = Maximum length of leaf × Maximum width of leaf × Correction factor
Where (C. F) = 0.75

Harvest index

In order to find harvest index, biological yield, economic yield and husk weight were calculated. Biological yield means weight of dry straw without grains, while economic yield means number of grains per plant. Following formula was used to calculate harvest index:

$$\text{Harvest index} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

Determination of calcium contents

Yoshida et al. (1976) method was used for determination of calcium contents. Calcium contents were determined by using flame photometer (Sherwood model, 410 UK). Standard series (10, 20, 30, 40 and 50 ppm) were made for this purpose.

Determination of sulphate (SO_4^{2-}-S) contents

Sulphate content was measured with the help of Tendon (1993) method. For this purpose, 5 mL of digestive samples solution was taken out in test tubes then 0.5 mL 6 N H_4Cl and 0.5 mL of gum acacia were added. After that samples were vortexed, and 0.25 g of barium chloride crystals were added in prepared solution and reading was noted with spectrophotometer at 440 nm.

Determination of soluble phosphate (PO_4^{3-}-P) contents

For the determination of soluble phosphate, method of Yoshida et al. (1976) was used. For 1 mL of extract 2 mL of 2N HNO3 was used, after that volume was maintained up to 8 mL by using distilled water. Then, 1 mL of molybdate-vanadate reagent was added, and sample was diluted up to 10 ml. Spectrophotometer was used to measure the absorbance at 420 nm.

Statistical analysis

All collected data were subjected to analysis of variance (ANOVA) using the statistical software Statistix 8.1 to determine the significance of treatment effects. Means of all measured parameters were compared using the Least Significant Difference (LSD) test at a 5 % probability level ($p \leq 0.05$) (Steel & Torrie, 1980).

Results

Shoot and root length (cm)

Analysis of variance showed a significant effect ($p < 0.05$) of salicylic acid (SA) application and heat stress on shoot length, along with a significant interaction between the two factors. Under control (non-stress) conditions, shoot length was lowest in untreated plants (59 cm), but increased significantly with the application of SA. Plants treated with foliar SA spray had a shoot length of 68 cm, while those receiving soil supplementation reached 78 cm. The maximum shoot length under normal conditions was observed in plants treated with the combination of foliar spray and soil supplementation, reaching 87 cm. Under heat stress, shoot length declined across all treatments. The lowest shoot length was observed in the untreated control (49 cm), followed by SA spray alone (52 cm). Plants treated with SA supplementation under heat stress recorded a shoot length of 69 cm, while the combined SA treatment still resulted in the highest shoot length under stress conditions (71 cm) (Fig. 1a). Under control (non-stressed) conditions, root length was lowest in untreated plants (33 cm). The application of foliar SA spray increased root length to 39 cm, while soil supplementation further enhanced it to 47 cm. The combined treatment of foliar spray and soil supplementation produced the highest root length, reaching 52 cm. Under heat stress conditions, root length decreased across all treatments. The lowest value was observed in the control plants (26 cm), followed by SA spray (30 cm), then SA supplementation (38 cm). The combined application of foliar and soil-applied SA once again showed the highest root length under stress, reaching 45 cm (Fig. 1b).

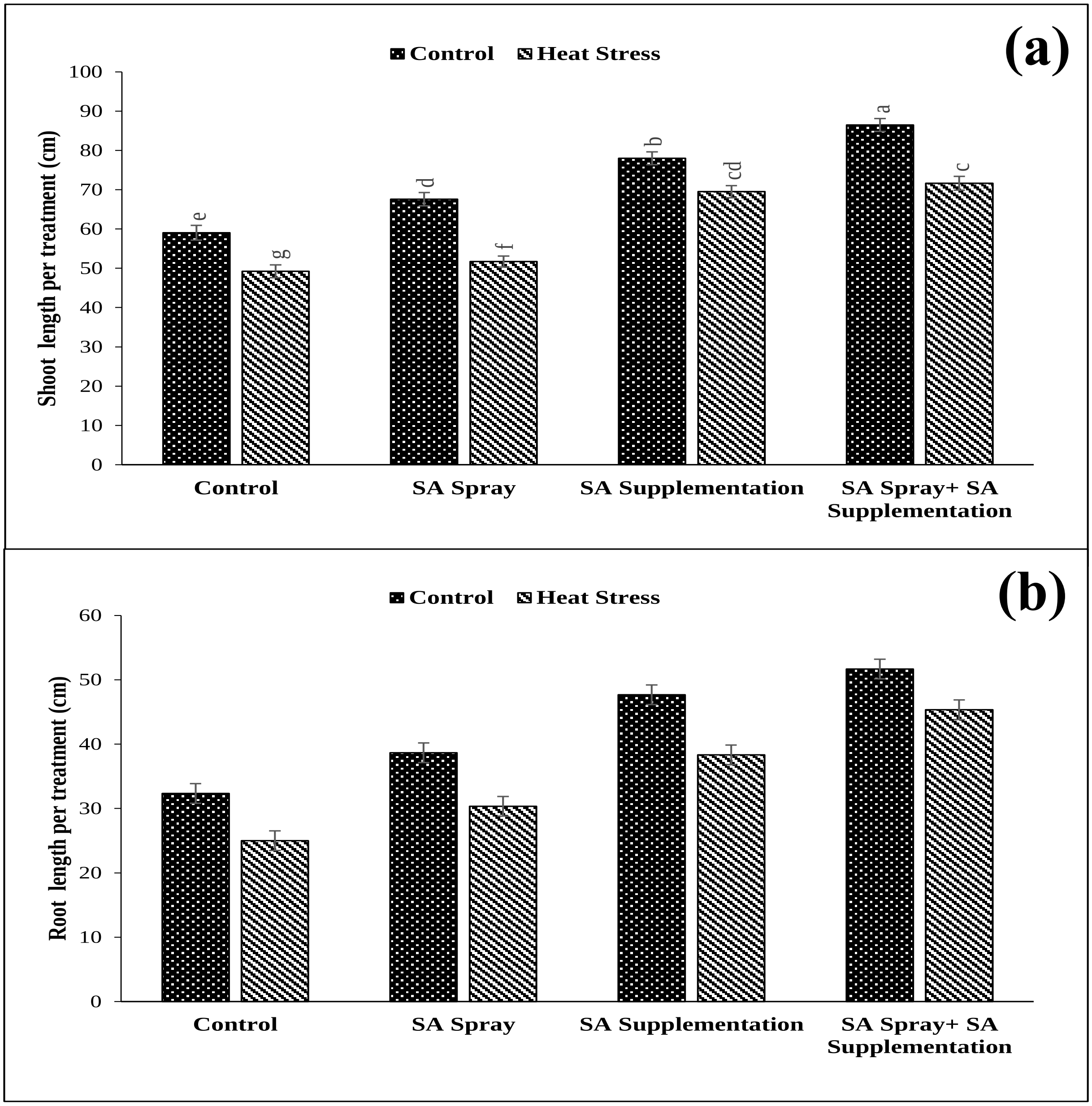

Fig. 1 (a) Effect of exogenous application of SA spray, supplementation and their combination on shoot length (cm) of wheat under heat stress (b) Effect of exogenous application of SA spray, supplementation and their combination on root length (cm) of wheat under heat stress. Bars represent mean ± SE (n = 3). Different letters indicate significant differences ($p < 0.05$) among treatments.

Shoot and root fresh weight (g)

Under normal conditions, the lowest shoot fresh weight was observed in the untreated control plants (5.6 g). Fresh weight increased progressively with SA treatments: SA spray improved shoot weight to 6.6 g, SA supplementation to 7.5 g, and the highest value was recorded with the combined foliar and soil application of SA (8.7 g). In plants exposed to heat stress, shoot fresh weight was generally reduced, but SA treatment helped mitigate the decline. The control plants under stress had the lowest shoot weight (4.5 g), while plants treated with SA spray reached 6.5 g, and SA supplementation improved it slightly to 6.9 g. The combined treatment again resulted in the highest shoot fresh weight under stress, recorded at 7.7 g. The statistical analysis revealed a significant effect ($p < 0.05$) of salicylic acid (SA) application, heat stress, and their interaction on root fresh weight of wheat plants. Under control conditions, root fresh weight was the lowest (1.5 g). Foliar application of SA resulted in a noticeable increase (2.1 g), while SA supplementation through the growth medium further enhanced root biomass (2.6 g). The combined treatment of SA spray and supplementation recorded the maximum root fresh weight (3.1 g). Exposure to heat stress markedly reduced root fresh weight across all treatments. The lowest root fresh weight (1.3 g) was observed in the heat-stressed control plants. However, exogenous application of SA mitigated the negative effects of heat stress, as evident from higher root fresh weights in SA spray (1.6 g), SA supplementation (2.0 g), and combined spray + supplementation treatments (2.2 g). The combined treatment under heat stress still maintained significantly greater root mass compared with the untreated control, indicating a synergistic effect of dual SA application in improving heat stress tolerance (Fig. 2b).

Shoot and root dry weight (g)

The statistical analysis revealed a significant ($p < 0.05$) main effect of salicylic acid (SA) application and heat stress on shoot dry weight of wheat plants, while their interaction was found to be non-significant ($p > 0.05$). Under normal conditions, shoot dry weight was the lowest in the control treatment (2.9 g). Application of SA as a foliar spray increased shoot dry weight to 3.7 g, while SA supplementation through the growth medium further improved it to 4.1 g. The highest shoot dry weight was observed in plants receiving both SA spray and medium supplementation (4.4 g). Heat stress resulted in reduction in shoot dry weight across all treatments. The minimum value (2.3 g) was recorded in the heat-stressed control plants. However, exogenous application of SA partially mitigated this decline, with shoot dry weights of 3.1 g in the foliar spray treatment, 3.4 g in the supplementation treatment, and 3.8 g in the combined SA spray + supplementation treatment. Although heat stress suppressed biomass accumulation, SA-treated plants maintained significantly higher shoot dry weights compared with untreated controls, suggesting a protective role of SA in sustaining shoot growth under thermal stress (Fig. 3a). Under normal growth conditions, root dry weight was the lowest in the control plants (0.78 g). Foliar application of SA increased root dry weight to 0.92 g, while SA supplementation through the medium further enhanced it to 1.05 g. The highest value (1.48 g) was recorded in plants receiving both foliar spray and medium supplementation of SA. Heat stress reduced root dry weight across all treatments. The lowest value (0.63 g) was observed in the heat-stressed control plants. However, the application of SA effectively alleviated this reduction, with values of 0.82 g in the SA spray treatment, 0.89 g in the SA supplementation treatment, and 0.95 g in the combined SA spray + supplementation treatment. Although heat stress negatively affected root biomass accumulation, SA-treated plants exhibited significantly higher root dry weights than untreated controls, confirming the protective role of SA in maintaining root growth under thermal stress (Fig. 3b).

Shoot and root diameter (mm)

The statistical analysis revealed a significant ($p < 0.05$) effect of salicylic acid (SA) application and heat stress on shoot diameter, along with a significant ($p < 0.05$) interaction between the two factors. Under normal conditions, the lowest shoot diameter (0.65 mm) was recorded in the control plants. Foliar application of SA significantly increased shoot diameter to 0.87 mm, while SA supplementation through the growth medium further enhanced it to 0.91 mm. The highest value (0.96 mm) was observed in plants treated with a combination of SA spray and supplementation. Exposure to heat stress reduced shoot diameter across all treatments. The heat-stressed control plants exhibited the smallest diameter (0.55 mm). However, plants treated with SA maintained higher diameters under stress, recording 0.75 mm in SA spray, 0.79 mm in SA supplementation, and 0.90 mm in the combined SA spray + supplementation treatment. Despite the overall decline due to heat stress, SA-treated plants consistently showed thicker shoots compared to untreated controls, suggesting a protective effect of SA in maintaining stem robustness under elevated temperature conditions (Fig. 4a). The statistical analysis revealed a significant ($p < 0.05$) effect of salicylic acid (SA) application and heat stress on root diameter, whereas the interaction between the two factors was non-significant ($p > 0.05$). Under normal conditions, the lowest root diameter (0.58 mm) was recorded in the control plants. Foliar application of SA increased root diameter to 0.73 mm, while SA supplementation through the growth medium further enhanced it to 0.80 mm. The maximum root diameter (0.88 mm) was observed in plants treated with both SA spray and supplementation. Heat stress reduced root diameter across all treatments. The heat-stressed control plants exhibited the smallest root diameter (0.48 mm). However, plants treated with SA maintained greater diameters, recording 0.66 mm under SA spray, 0.72 mm under SA supplementation, and 0.77 mm under combined SA spray + supplementation. Although heat stress negatively affected root thickness, SA application effectively alleviated the reduction, indicating its protective role in maintaining root structural integrity and development under adverse temperature conditions (Fig. 4b).

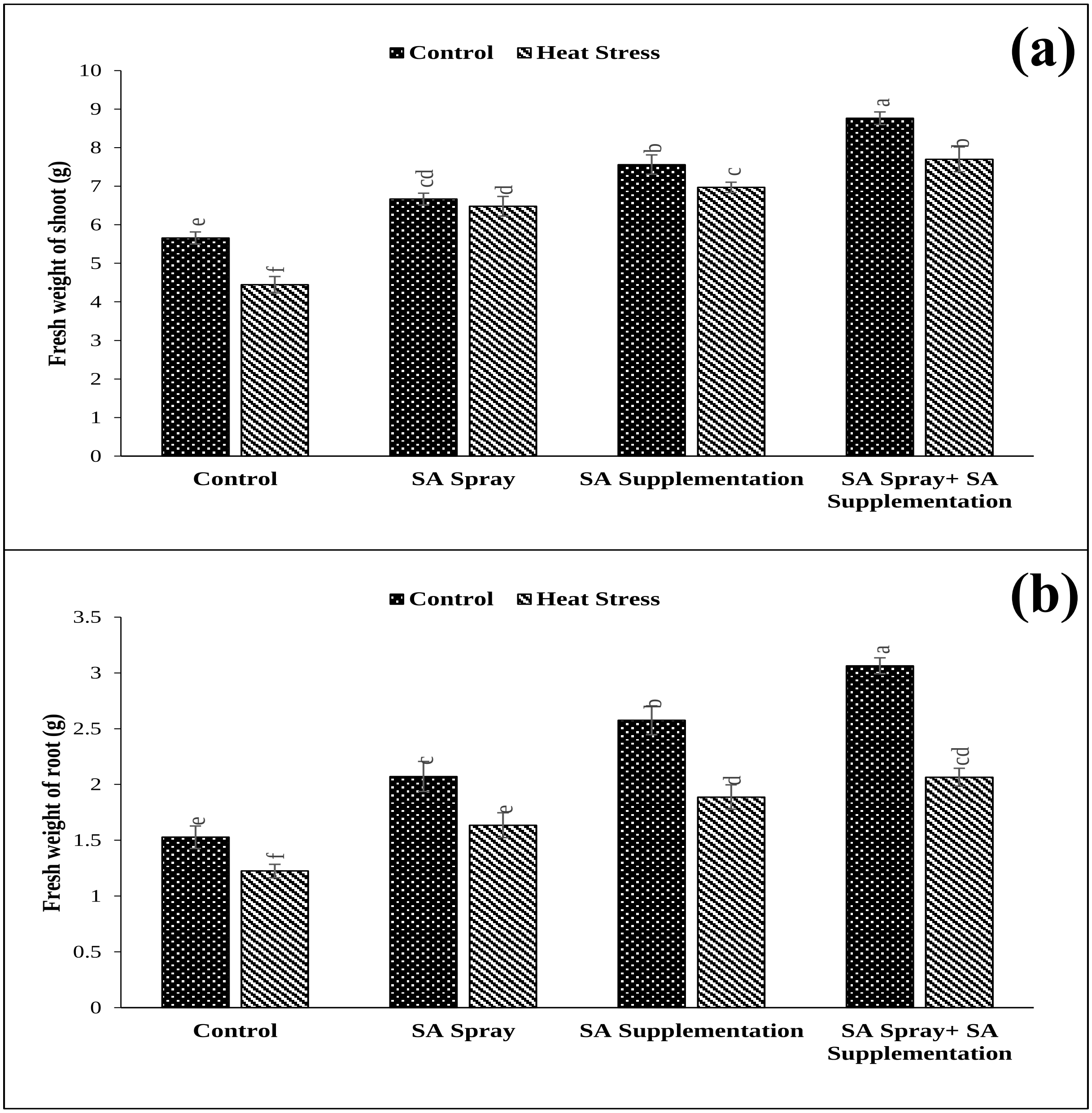

Fig. 2 (a) Effect of exogenous application of SA spray, supplementation and their combination on shoot fresh weight (g) of wheat under heat stress (b) Effect of exogenous application of SA spray, supplementation and their combination on root fresh weight (g) of wheat under heat stress

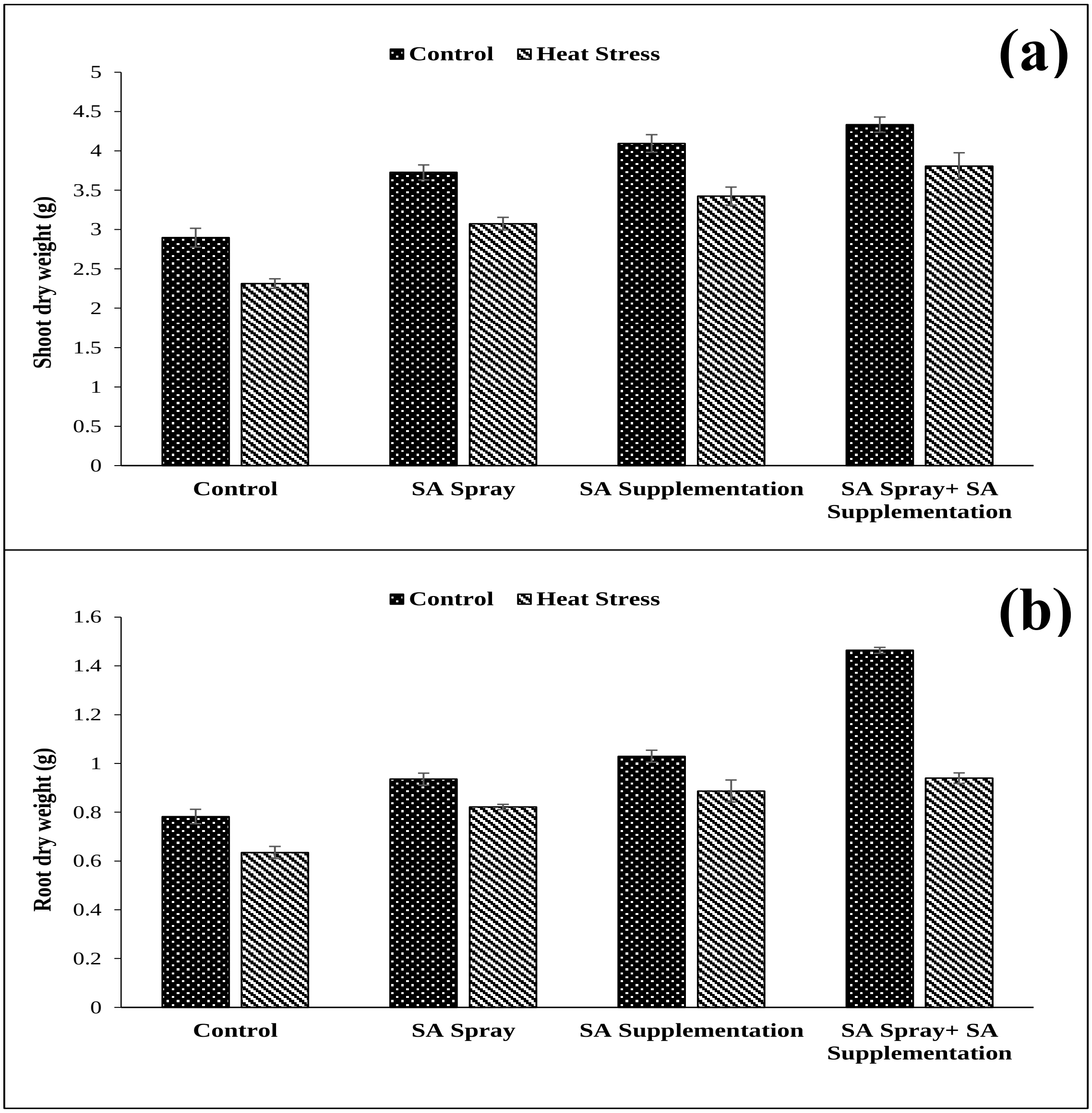

Fig. 3 (a) Effect of exogenous application of SA spray, supplementation and their combination on shoot dry weight (g) of wheat under heat stress (b) Effect of exogenous application of SA spray, supplementation and their combination on root dry weight (g) of wheat under heat stress. Bars

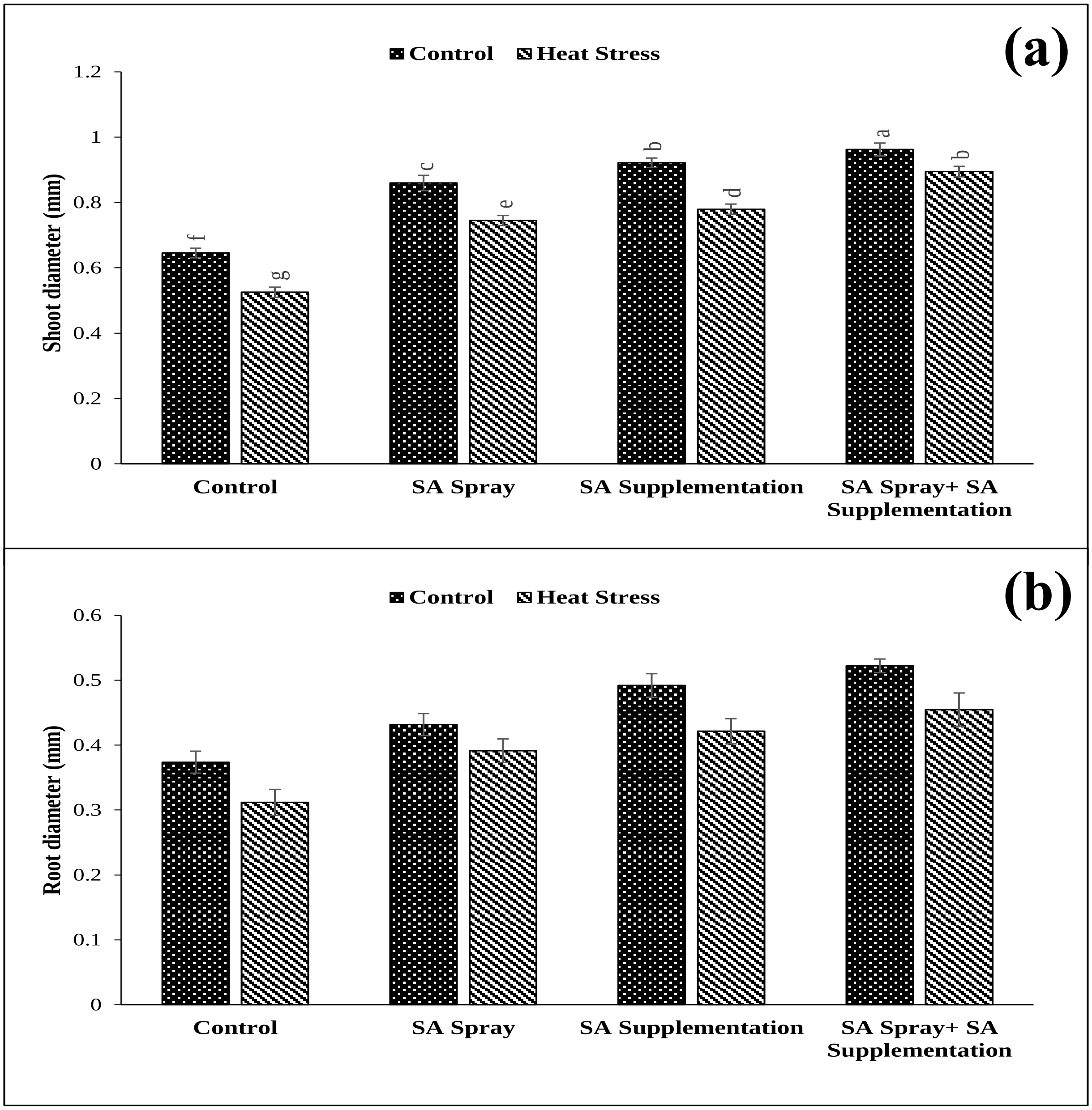

Fig. 4 (a) Effect of exogenous application of SA spray, supplementation and their combination on shoot diameter (mm) of wheat under heat stress (b) Effect of exogenous application of SA spray, supplementation and their combination on root diameter (mm) of wheat under heat stress. Bars represent mean ± SE (n = 3).

Number of roots

Under normal growth conditions, the control plants produced the lowest number of roots (12 roots per plant). Foliar application of SA increased the number of roots to 16, while SA supplementation through the growth medium further enhanced root proliferation to 19. The combined treatment of SA spray and supplementation resulted in the highest number of roots (22 per plant), indicating a synergistic effect of dual SA application in stimulating root initiation and development. Heat stress significantly reduced the number of roots across all treatments. The heat-stressed control plants showed the lowest root number (9 roots per plant). However, SA-treated plants exhibited partial recovery, with 13 roots under foliar SA spray, 15 roots under SA supplementation, and 17 roots under the combined SA spray + supplementation treatment. Although heat stress adversely affected root development, SA application effectively mitigated this reduction, demonstrating its potential role in promoting root growth and maintaining plant vigor under thermal stress conditions (Fig. 5).

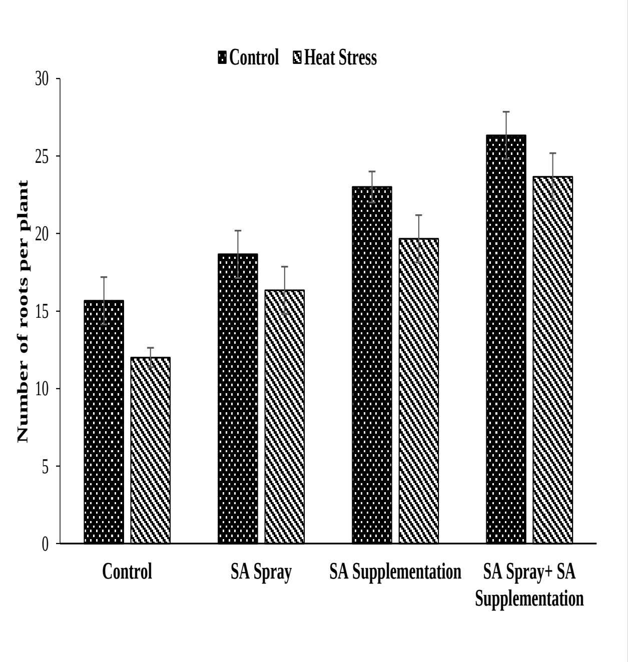

Fig. 5 Effect of exogenous application of SA spray, supplementation and their combination on number of roots of wheat under heat stress. Bars represent mean ± SE (n = 3).

Number of tillers

Under normal growth conditions, the control plants produced the lowest number of tillers (3.8 tillers per plant). Foliar application of SA increased the number of tillers to 5.1, while SA supplementation through the growth medium further enhanced it to 5.8 tillers per plant. The highest number of tillers (6.4) was recorded in plants receiving both SA spray and supplementation, indicating that combined SA application most effectively promoted tiller formation under optimal conditions. Heat stress significantly reduced the number of tillers across all treatments. The heat-stressed control plants had the lowest number (2.7 tillers per plant). However, SA applications alleviated this decline, with 3.9 tillers under SA spray, 4.4 tillers under SA supplementation, and 5.2 tillers under the combined SA spray + supplementation treatment. Although heat stress negatively affected tiller formation, exogenous SA improved tiller number even under stress, suggesting its regulatory role in maintaining shoot initiation and growth under adverse thermal conditions (Fig. 6).

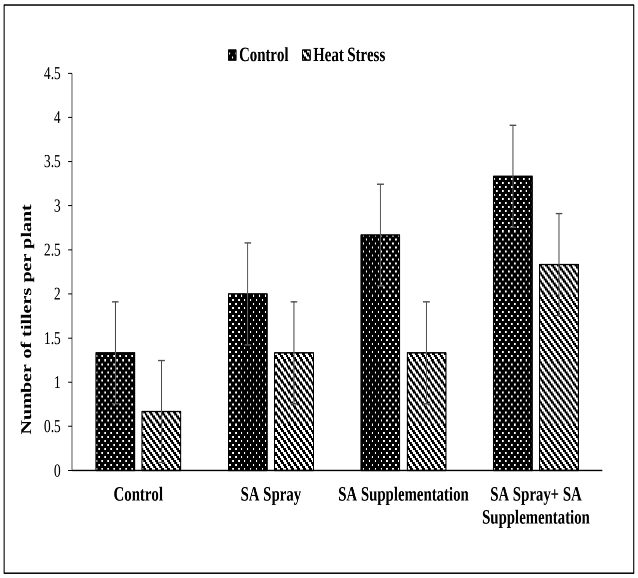

Fig. 6 Effect of exogenous application of SA spray, supplementation and their combination on number of tillers of wheat under heat stress. Bars represent mean ± SE (n = 3).

Leaf area (cm^2)

Under normal growth conditions, the control plants exhibited the lowest leaf area (21.5 cm²). Foliar application of SA increased leaf area to 27.8 cm², while SA supplementation through the growth medium further enhanced it to 31.2 cm². The combined treatment of SA spray and supplementation recorded the highest leaf area (35.6 cm²), indicating a strong synergistic effect of dual SA application on foliage expansion. Under heat stress, a marked reduction in leaf area was observed across all treatments. The heat-stressed control plants showed the lowest leaf area (16.4 cm²). However, plants treated with SA maintained larger leaf areas, recording 22.5 cm² with foliar SA spray, 25.7 cm² with SA supplementation, and 29.3 cm² with combined SA spray + supplementation. Despite the overall decline under heat stress, SA application significantly mitigated leaf area reduction, demonstrating its protective role in sustaining photosynthetic surface and vegetative growth under elevated temperature conditions (Fig. 7).

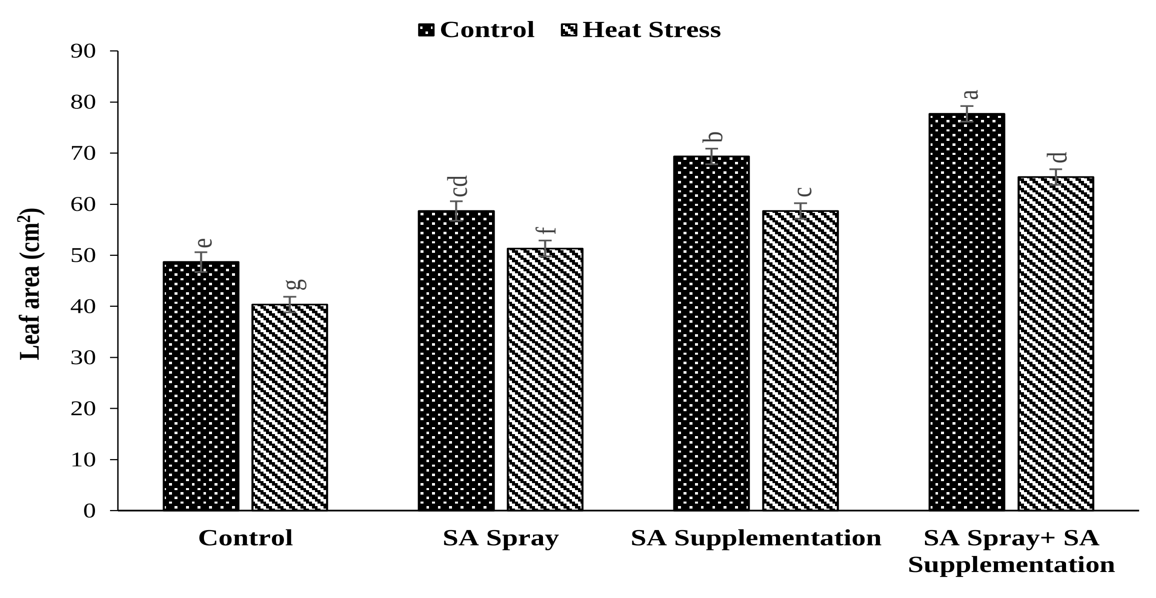

Fig. 7 Effect of exogenous application of SA spray, supplementation and their combination on leaf area (cm^2) of wheat under heat stress. Bars represent mean ± SE (n = 3). Different letters indicate significant differences ($p < 0.05$) among treatments.

SPAD value

The statistical analysis revealed a significant ($p < 0.05$) effect of salicylic acid (SA) application and heat stress on SPAD values, along with a significant ($p < 0.05$) interaction between the two factors. Under normal conditions, the control plants exhibited the lowest SPAD value (34.2). Foliar application of SA increased chlorophyll content to 39.6, while SA supplementation through the growth medium further enhanced it to 42.8. The combined treatment of SA spray and medium supplementation recorded the highest SPAD value (46.1), indicating that dual SA application effectively promoted chlorophyll retention and synthesis. Under heat stress, SPAD values declined markedly across all treatments. The lowest value (27.5) was recorded in the heat-stressed control plants. However, plants treated with SA maintained relatively higher SPAD readings, with 32.9 under foliar SA spray, 36.2 under SA supplementation, and 39.4 under the combined SA spray + supplementation treatment. Although heat stress reduced chlorophyll content, exogenous application of SA effectively mitigated this decline, suggesting its role in protecting the photosynthetic apparatus and stabilizing chlorophyll pigments under high temperature stress.

Spike development traits

The statistical analysis revealed a significant effect ($p < 0.05$) of exogenous salicylic acid (SA) application and heat stress on the number of spikes, while the interaction between these factors was non-significant ($p > 0.05$). Under control conditions, the number of spikes was lowest in untreated plants (8 spikes/plant) and increased with exogenous SA application via foliar spray (12 spikes/plant), medium supplementation (13 spikes/plant), and the combination of both spray and medium supplementation (15 spikes/plant). Under heat stress, the number of spikes decreased overall, with the lowest value observed in the control (5 spikes/plant). Foliar SA spray (7 spikes/plant) and medium supplementation (8 spikes/plant) partially mitigated the stress effect, while the combination of SA spray and medium supplementation produced the highest number of spikes (11 spikes/plant) under heat stress (Fig. 8a). Statistical analysis revealed a significant effect ($p < 0.05$) of exogenous salicylic acid (SA) application and heat stress on the number of spikelets, with a highly significant interaction between these factors ($p < 0.01$). Under control conditions, the number of spikelets was lowest in untreated plants (20 spikelets/plant) and increased with SA foliar spray (28 spikelets/plant), medium supplementation (30 spikelets/plant), and the combination of SA spray and medium supplementation (35 spikelets/plant). Under heat stress, the number of spikelets decreased overall, with the lowest value observed in the control (12 spikelets/plant). Foliar SA spray (18 spikelets/plant) and medium supplementation (20 spikelets/plant) partially alleviated the negative effects of heat stress, while the combination of SA spray and medium supplementation produced the highest number of spikelets (26 spikelets/plant) under heat stress (Fig. 8b). Statistical analysis showed a significant effect ($p < 0.05$) of exogenous salicylic acid (SA) application and heat stress on the number of grains per spike, while the interaction between these factors was non-significant ($p > 0.05$). Under control conditions, the number of grains per spike was lowest in untreated plants (35 grains/spike) and increased with SA foliar spray (45 grains/spike), medium supplementation (48 grains/spike), and the combination of SA spray and medium supplementation (52 grains/spike). Under heat stress, the number of grains per spike decreased overall. The lowest values were observed in control plants (22 grains/spike), followed by plants receiving SA foliar spray (30 grains/spike) and medium supplementation (32 grains/spike), while the combination of SA spray and medium supplementation resulted in the highest grain number (38 grains/spike) under heat stress (Fig. 8c). Statistical analysis revealed a significant effect ($p < 0.05$) of exogenous salicylic acid (SA) application and heat stress on spike length, with a significant interaction between these factors ($p < 0.05$). Under control conditions, the spike length was lowest in untreated plants (8.5 cm) and increased with SA foliar spray (10.2 cm), medium supplementation (10.5 cm), and the combination of SA spray and medium supplementation (11.8 cm). Under heat stress, spike length decreased overall. The lowest values were observed in control plants (6.3 cm), followed by SA foliar spray (7.5 cm) and medium supplementation (7.8 cm), while the combination of SA spray and medium supplementation resulted in the highest spike length (9.2 cm) under heat stress (Fig. 8d).

Number of grains per plant

Under control conditions, the minimum number of grains per spike (41) was recorded in untreated plants. Foliar application of SA increased the grain number to 50, while SA supplementation in the growth medium further improved it to 59. The highest value (65) was observed with the combined SA spray + SA supplementation treatment. Under heat stress, a similar trend was observed though overall grain number was reduced. The lowest value (38) was recorded in the stressed control, followed by SA spray (44) and SA supplementation (51). The combined treatment again produced the highest grain number (58), indicating partial mitigation of heat-induced yield losses through SA application (Fig. 9).

1000-grain weight (g)

Under control conditions, the lowest 1000-grain weight (35 g) was observed in untreated plants. Foliar application of SA increased this value to 47 g, while SA supplementation in the growth medium further improved it to 53 g. The maximum weight (59 g) was recorded under combined SA spray and medium supplementation treatment. Under heat stress, 1000-grain weight decreased across all treatments but followed a similar trend. The minimum value (30 g) was observed in the stressed control plants, while foliar SA spray improved grain weight to about 41 g. SA supplementation under stress further enhanced the weight to 45 g, and the combined treatment again produced the highest value (51 g) (Fig. 10).

Harvest index

Statistical analysis indicated a significant ($p < 0.05$) effect of both heat stress and exogenous salicylic acid (SA) application on the harvest index, along with a significant ($p < 0.05$) interaction between these two factors. Under control conditions, the harvest index was lowest (29) in untreated plants. Foliar application of SA improved it to 34, while SA supplementation in the growth medium further increased the value to 37. The maximum harvest index (41) was recorded under the combined SA spray and medium supplementation treatment. Under heat stress conditions, a decline in harvest index was evident across all treatments. The lowest value (27) was observed in the stressed control plants, followed by SA spray (29) and SA supplementation (31). The combination of SA spray and medium supplementation again resulted in the highest harvest index (32) among stressed plants (Fig. 11).

Calcium contents of shoot and root (mg/g DW)

Under control conditions, the calcium content was lowest (8.1 mg/g DW) in untreated plants. Foliar application of SA slightly increased calcium content to 9.0 mg/g DW, while SA supplementation in the growth medium further improved it to 10.0 mg/g DW. The highest calcium accumulation (11.5 mg/g DW) was observed under the combined SA spray + medium supplementation treatment. Under heat stress, calcium content decreased slightly across all treatments but followed a similar pattern. The lowest value (7.2 mg/g DW) was observed in stressed control, followed by SA spray (7.8 mg/g DW) and SA supplementation (8.4 mg/g DW). The combined treatment again produced the highest calcium content (9.8 mg/g DW) (Fig. 12a). Statistical analysis revealed a significant ($p < 0.05$) effect of both exogenous salicylic acid (SA) application and heat stress on calcium contents of roots, whereas their interaction remained non-significant ($p > 0.05$). Under control conditions, the calcium content of roots was lowest (6.5 mg/g DW) in untreated plants. Foliar application of SA increased calcium concentration to 7.4 mg/g DW, while SA supplementation in the growth medium further improved it to 8.2 mg/g DW. The highest root calcium content (9.3 mg/g DW) was observed under combined foliar and medium supplementation treatment. Under heat stress, a general reduction in calcium accumulation was recorded across all treatments. The minimum value (5.8 mg/g DW) occurred in stressed control, while SA spray (6.6 mg/g DW) and SA supplementation (7.2 mg/g DW) improved calcium retention moderately. The combination of SA spray and medium supplementation again resulted in the highest calcium content (8.4 mg/g DW) (Fig. 12b).

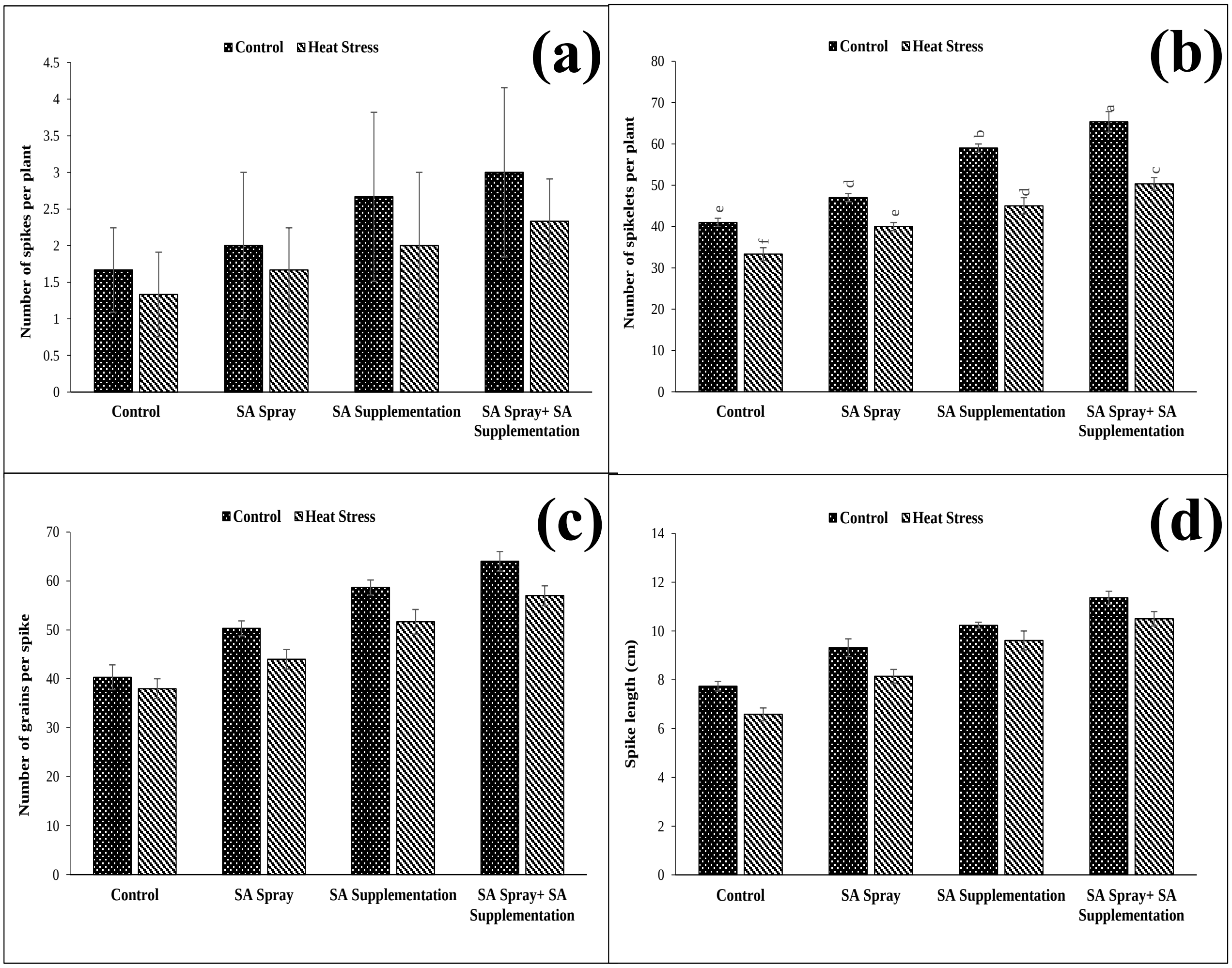

Fig. 8 (a) Effect of exogenous application of SA spray, supplementation and their combination on number of spikes of wheat under heat stress (b) Effect of exogenous application of SA spray, supplementation and their combination number of spikelets of wheat under heat stress (c) Effect of exogenous application of SA spray, supplementation and their combination on grain per spike of wheat under heat stress (d) Effect of exogenous application of SA spray, supplementation and their combination on spike length (cm) of wheat under heat stress. Bars represent mean ± SE (n = 3). Different letters indicate significant differences ($p < 0.05$) among treatments.

Sulphate contents of shoot and root (µg/g DW)

The statistical analysis revealed a significant effect ($p < 0.05$) of exogenous salicylic acid (SA) application and heat stress, along with a significant interaction ($p < 0.05$) between these two factors. Under control conditions, the lowest sulphate content was recorded in untreated plants (6.9 µg/g DW). Foliar spray of SA increased the sulphate concentration to 8.1 µg/g DW, while SA supplementation in the growth medium further enhanced it to 9.5 µg/g DW. The maximum sulphate content (11.1 µg/g DW) was observed in plants that received both foliar spray and medium supplementation of SA. Under heat stress, a noticeable decline in sulphate contents was observed across all treatments. The lowest value (6.1 µg/g DW) occurred in the heat-stressed control plants, followed by 7.2 µg/g DW in the SA spray treatment and 8.8 µg/g DW in the SA supplementation treatment. The highest value under heat stress (9.6 µg/g DW) was again recorded in plants treated with the combined SA spray and supplementation (Fig. 13a). Under control conditions, the lowest root sulphate content was observed in untreated plants (7.2 µg/g DW). Foliar spray of SA increased sulphate concentration to 8.5 µg/g DW, while SA supplementation in the growth medium further enhanced it to 10.6 µg/g DW. The maximum sulphate content (12.0 µg/g DW) was recorded in plants that received both foliar spray and medium supplementation of SA. Under heat stress, sulphate contents in roots declined compared with the corresponding control treatments. The lowest value (6.4 µg/g DW) was recorded in the heat-stressed control, followed by 7.4 µg/g DW in the SA spray treatment and 9.2 µg/g DW in the SA supplementation treatment. The highest root sulphate content under heat stress (10.4 µg/g DW) was found in plants treated with both foliar spray and medium supplementation of SA (Fig. 13b).

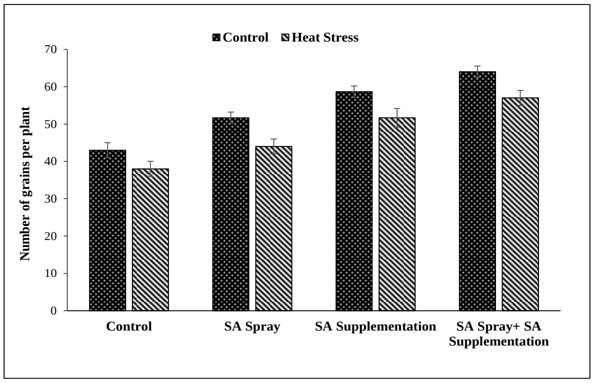

Fig. 9 Effect of exogenous application of SA spray, supplementation and their combination on grain per plant of wheat under heat stress. Bars represent mean ± SE (n = 3).

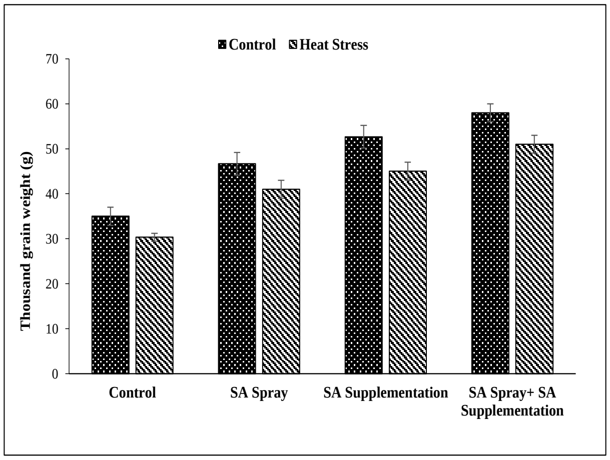

Fig. 10 Effect of exogenous application of SA spray, supplementation and their combination on 1000-grain weight (g) of wheat under heat stress. Bars represent mean ± SE (n = 3).

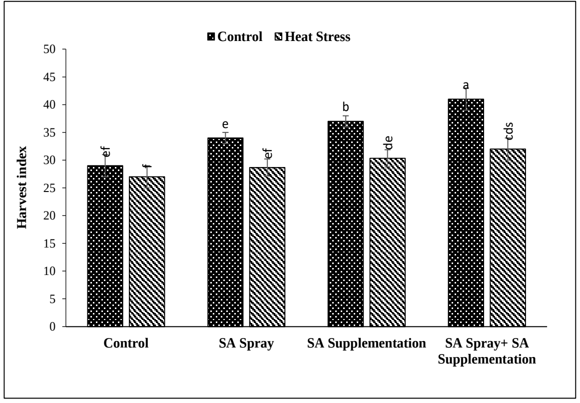

Fig. 11 Effect of exogenous application of SA spray, supplementation and their combination on harvest index of wheat under heat stress. Bars represent mean ± SE (n = 3). Different letters indicate significant differences ($p < 0.05$) among treatments.

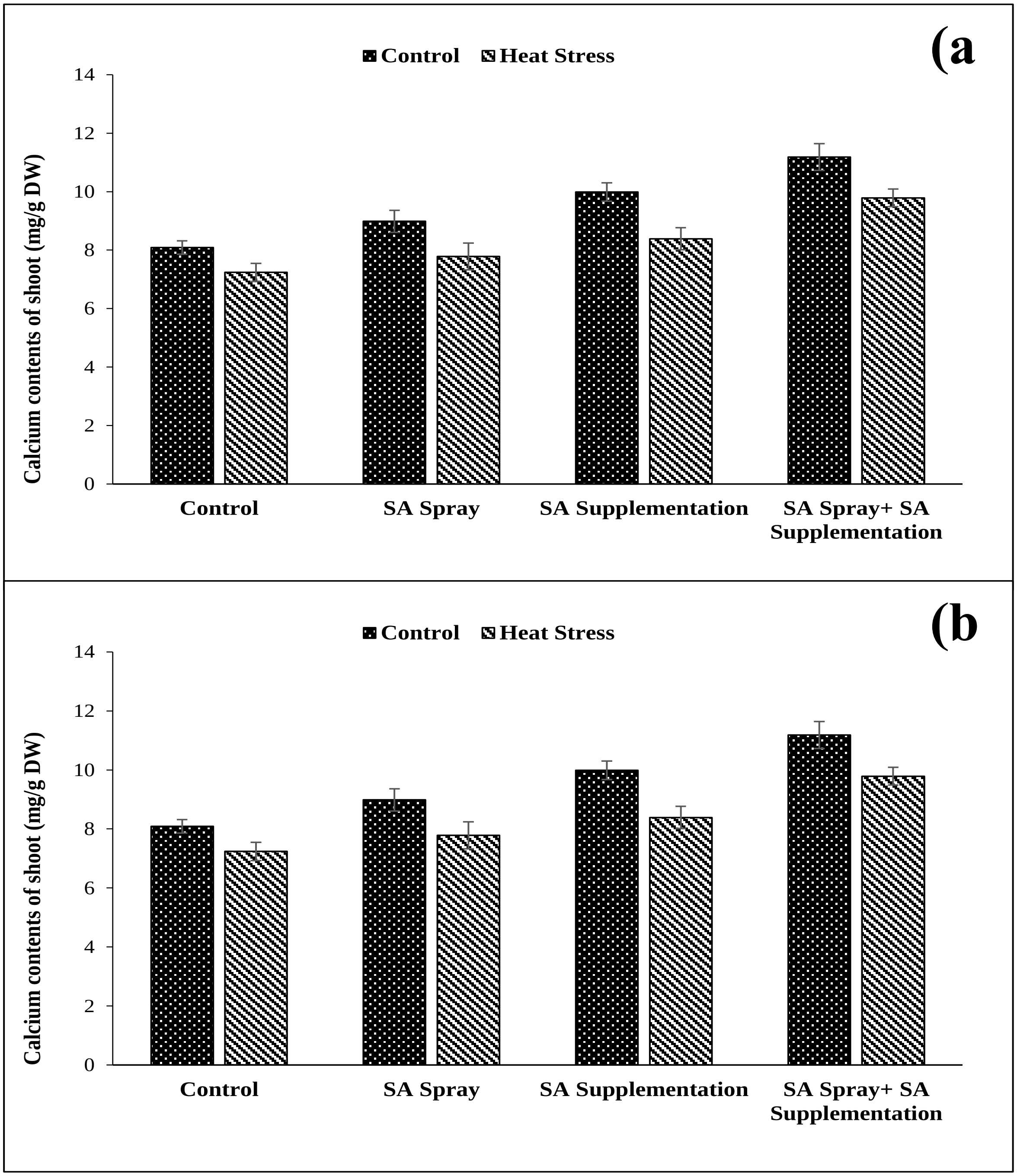

Fig. 12 (a) Effect of exogenous application of SA spray, supplementation and their combination on calcium contents (mg/g) of shoot of wheat under heat stress (b) Effect of exogenous application of SA spray, supplementation and their combination on calcium contents (mg/g) of root of wheat under heat stress. Bars represent mean ± SE (n = 3).

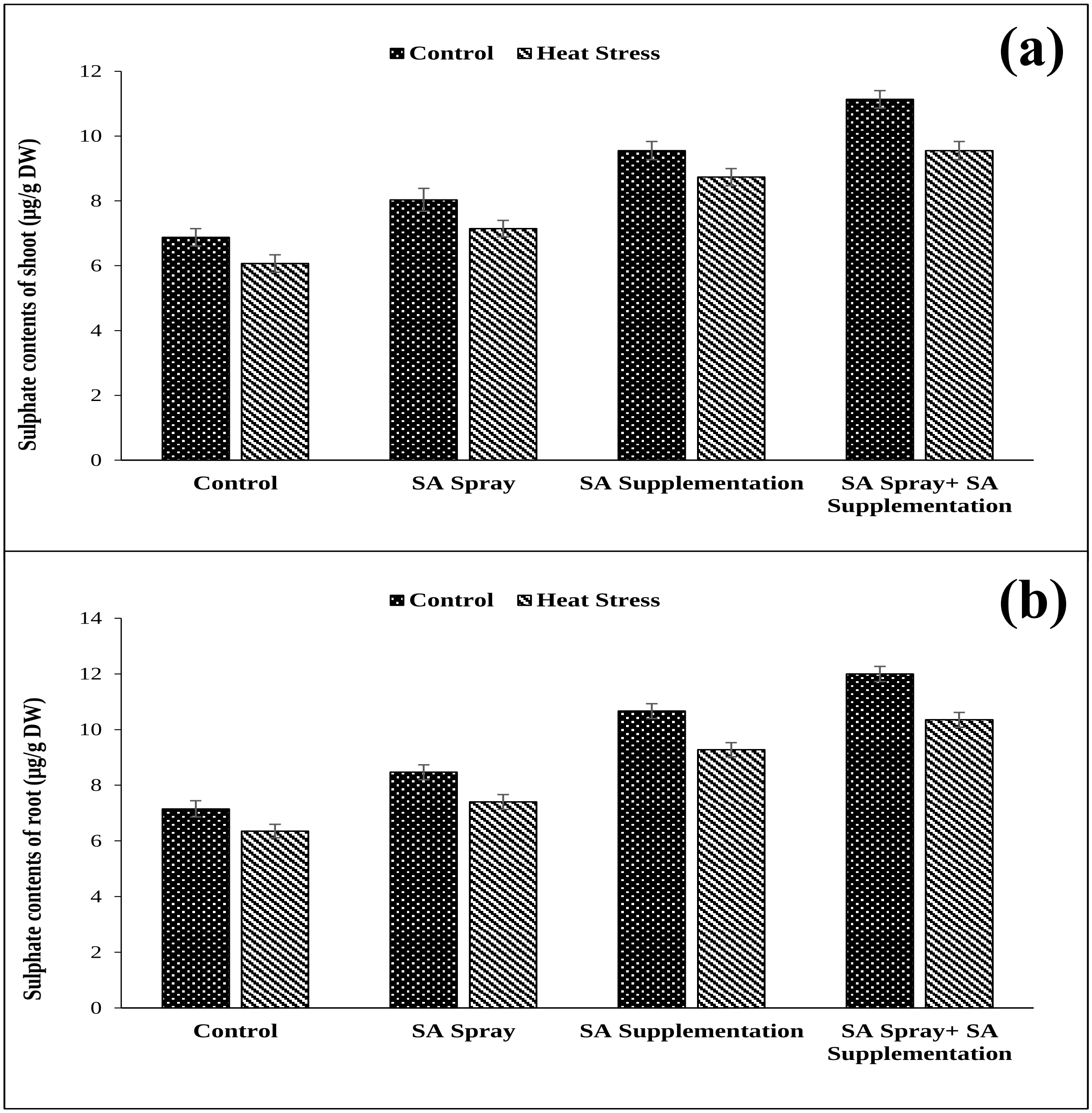

Fig. 13 (a) Effect of exogenous application of SA spray, supplementation and their combination on sulphate content of shoot (µg/g DW) of wheat under heat stress (b) Effect of exogenous application of SA spray, supplementation and their combination on sulphate content of root (µg/g DW) of wheat under heat stress. Bars represent mean ± SE (n = 3).

Phosphate contents of shoot and root (µg/g DW)

The statistical analysis revealed a significant ($p < 0.05$) effect of both salicylic acid (SA) treatments and heat stress on shoot phosphate contents, while their interaction effect was non-significant ($p > 0.05$). Under control conditions, the phosphate content of the shoot was the lowest (7.5 µg/g DW). Foliar application of SA resulted in a moderate increase (8.7 µg/g DW), whereas SA supplementation through the growth medium further enhanced phosphate accumulation (9.6 µg/g DW). The combined treatment of foliar spray and medium supplementation showed the highest phosphate concentration (10.3 µg/g DW). Under heat stress, a similar trend was observed but with slightly reduced phosphate levels in all treatments. The lowest phosphate content was recorded in the heat-stressed control plants (7.0 µg/g DW), followed by SA spray (8.0 µg/g DW), SA supplementation (9.0 µg/g DW), and SA spray + supplementation (9.7 µg/g DW). Overall, exogenous SA applications partially mitigated the heat-induced reduction in phosphate contents of shoots, with combined treatment proving most effective (Fig. 14a). The statistical analysis indicated a significant ($p < 0.05$) effect of salicylic acid (SA) treatments, heat stress, and their interaction on the phosphate contents of roots. Under control conditions, root phosphate content was the lowest (7.6 µg/g DW). Foliar application of SA led to a significant increase (8.8 µg/g DW), while SA supplementation in the growth medium further elevated phosphate levels (9.7 µg/g DW). The combined treatment of SA spray and medium supplementation recorded the highest phosphate accumulation in roots (10.5 µg/g DW). Under heat stress, a general decline in phosphate contents was observed across all treatments. The lowest phosphate content was recorded in the heat-stressed control plants (7.2 µg/g DW), followed by SA spray (7.5 µg/g DW), SA supplementation (8.6 µg/g DW), and the SA spray + supplementation treatment (9.2 µg/g DW). Despite the stress-induced reduction, exogenous application of SA particularly in combination form effectively mitigated the adverse effects of heat stress on phosphate uptake and accumulation in roots (Fig. 14b).

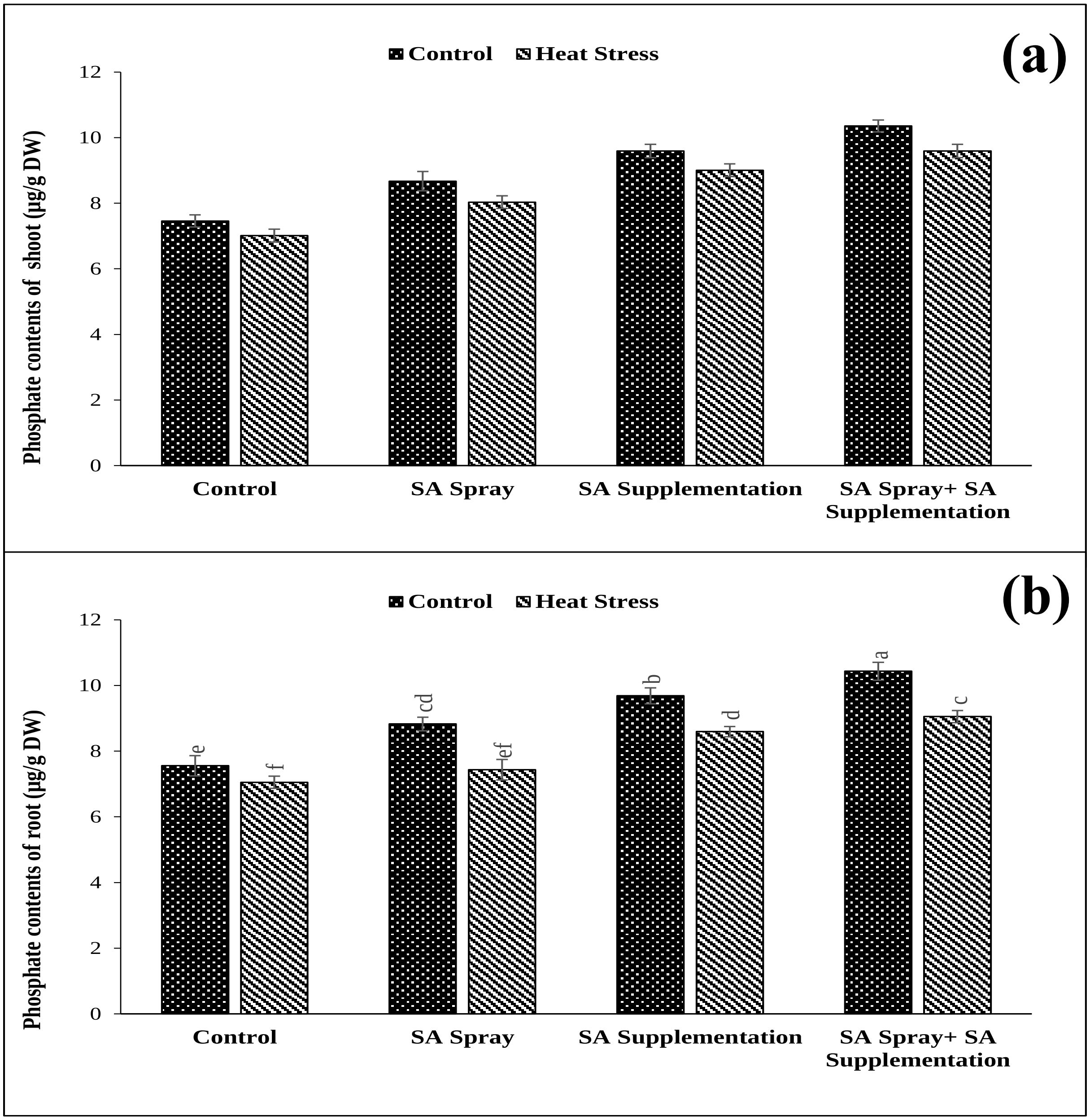

Fig. 14 (a) Effect of exogenous application of SA spray, supplementation and their combination on phosphate content of shoot (µg/g DW) of wheat under heat stress (b) Effect of exogenous application of SA spray, supplementation and their combination on phosphate content (µg/g DW) of root wheat under heat stress. Bars represent mean ± SE (n = 3). Different letters indicate significant differences ($p < 0.05$) among treatments.

Discussion

The increase in temperature causes heat stress to plants, which negatively impacts the growth parameters and caused drastic effect on various biochemical attributes. Our results revealed that morphological and yield attributes decreased under stress conditions. Different growth parameters such as shoot fresh and dry weight, height of plants, number of leaves, leaf area were severely affected under heat stress. Same results were reported by Shah & Paulsen (2003); Plaut et al. (2004); Essemine et al. (2010) by studying wheat as a model plant. Our results were also confirmed by Larkindale and Knight (2002); Khan et al. (2003); Wahid et al. (2007); Bidabadi et al. (2012); Li et al. (2023) in different crops such as arabidopsis, soybean, musatrd, banana, potato, tobacco, maize and tomato. In our study, foliar and soil supplementation of SA produced a significant ($p \leq 0.05$) improvement in plant phenology, including earlier flowering and maturity under heat stress.

Our findings reported that the increase in root fresh weight following salicylic acid (SA) application indicates that SA plays a vital role in maintaining root growth and metabolic activity under both normal and heat-stressed conditions. Heat stress is known to impair root development by disrupting cellular homeostasis, reducing water uptake, and impairing carbohydrate translocation. However, the observed improvement in root biomass with SA treatments suggests that exogenous SA alleviates these adverse effects, possibly by enhancing antioxidant defense, stabilizing membranes, and improving osmotic adjustment. The combined treatment of foliar spray and medium supplementation proved most effective, implying that simultaneous local (foliar) and systemic (root zone) delivery of SA provides a synergistic protection mechanism. Similar findings have been reported in wheat and other crops where SA application enhanced root growth under heat or drought stress by promoting hormonal balance, particularly maintaining auxin and cytokinin levels, and stimulating root elongation and lateral root formation. Thus, the enhanced root fresh weight under combined SA treatments in the present study reflects an improved stress tolerance and better resource acquisition capacity, ultimately supporting plant resilience under thermal stress.

The observed enhancement in shoot dry weight following salicylic acid (SA) application indicates that SA plays a significant role in sustaining shoot growth and biomass production under both normal and heat-stressed conditions. Heat stress commonly disrupts photosynthetic efficiency, accelerates leaf senescence, and inhibits assimilate partitioning, leading to reduced shoot biomass. In the present study, exogenous application of SA alleviated these negative effects, likely by improving photosynthetic performance, maintaining chlorophyll content, and stabilizing cellular membranes. The greater effectiveness of the combined SA spray and supplementation treatment suggests that dual application routes ensure better absorption and systemic distribution of SA within plant tissues, resulting in stronger stress mitigation. Previous studies in wheat and other cereals have similarly demonstrated that SA enhances shoot growth under abiotic stresses by promoting antioxidant enzyme activities, improving osmotic regulation, and maintaining hormonal balance particularly through modulation of abscisic acid and cytokinin levels. Therefore, the increased shoot dry weight under SA treatments in the current study reflects improved physiological resilience and metabolic stability, which collectively support plant performance under heat stress conditions.

The increase in root dry weight following salicylic acid (SA) application indicates that SA contributes to maintaining root biomass and physiological activity under both normal and heat-stressed conditions. Heat stress often impairs root development by inhibiting cell elongation, protein synthesis, and water uptake, ultimately leading to reduced dry matter accumulation. However, the enhanced root dry weight observed with SA treatments in this study suggests that SA mitigates these adverse effects, likely through activation of antioxidant defense mechanisms, stabilization of cellular membranes, and maintenance of root metabolic activity. The combined application of SA spray and supplementation proved most effective, implying a synergistic response due to improved systemic availability of SA within plant tissues. Similar results have been reported in wheat and other cereal crops, where SA enhanced root biomass under abiotic stresses by modulating hormonal signaling (particularly auxins and cytokinins), improving osmotic adjustment, and sustaining nutrient uptake. Thus, the increased root dry weight under SA treatments reflects improved physiological resilience, enabling plants to sustain growth and biomass accumulation even under heat stress conditions.

Our results showed that applications of this compound had improved the growth of wheat plants. Same results were also reported by investigators such as Khan et al. (2013); Kumar (2014). They concluded that applications of SA in two different forms i.e. supplementation and foliar spray significantly help to accelerate the growth of wheat plants regarding untreated and control plants. Zhou et al. (2019) also explained that SA foliar application increased plants' growth and yield attributes under control or stress condition. Same results were also obtained in our study. The order of improvement was SA spray + SA medium supplementation > SA medium supplementation > SA spray > control under both control and stress conditions

Plants under heat stress had lower photosynthetic rate. Similar results were reported by Zahedi & Jenner (2003). They used wheat as an experimental plant and used application of SA. They noted that photosynthetic parameters increased with SA as compared to the control plants, irrespective of stress treatments. Same results were found by Farooq et al. (2011); Meng et al. (2013); AbdElgawad et al. (2015); Rakic et al. (2015); Salehi-Lisar and Bakhshayeshan-Agdam (2016) who also reported that plants which were treated with SA showed higher content of photosynthetic pigments as compared to the control plants. Concentration of carotenoid was significantly inhibited by heat stress. SA foliar application had enhanced the carotenoid content especially when applied in soil as compared with untreated plants. Same results were reported by Janda et al. (2019) who found that the application of phenolic compounds specially SA help to maintain the carotenoid concentration in plant tissues. Supplementation with foliar application of SA under stress conditions showed a significant increase in AsA content, which indicated that SA helps to increase antioxidant activities in plants during stress conditions (Sangwan et al., 2022). Resulted reported that phenolics content, soluble sugars and SPAD value of chlorophyll had increased in those plants on which SA were applied. According to the Liu et al. (2017); Kousar et al. (2018) SA increased the total phenolic content, soluble sugars and SPAD value of chlorophyll.

High temperature stress reduced the uptake of essential nutrients. There was high uptake of ions in those plant on which SA applications was applied as compared to the other treatments under stress conditions. Similar result was concluded by Kaydan et al. (2007); Nazar et al. (2015). They reported that SA application enhanced the absorption of essential ions under normal and stress conditions. In conclusion, SA treatment both in spray and supplementation forms significantly improved the wheat yield and physicochemical properties under heat stress by modulating the production of antioxidants. Calcium (Ca^{2+}) plays a vital structural and signaling role in plants, being essential for maintaining cell wall stability, membrane integrity, and regulation of stress-responsive pathways. In the present study, both shoot and root calcium contents were significantly affected by salicylic acid (SA) application and heat stress, while their interaction remained non-significant. Overall, SA application enhanced calcium accumulation under both control and heat stress conditions, whereas heat stress caused a general decline in calcium levels in both organs. Under normal growth conditions, calcium accumulation was higher in shoots compared to roots, reflecting its preferential transport and limited phloem mobility. This trend aligns with earlier studies reporting higher Ca^{2+} deposition in above-ground tissues due to xylem-based translocation (White & Broadley, 2003; Hirschi, 2004).

The foliar application and medium supplementation of SA both improved calcium contents in shoots, with the combined treatment being most effective. The enhancement of calcium accumulation by SA may be attributed to improved membrane permeability, enhanced transpiration-driven ion uptake, and upregulation of calcium transporters (Reddy et al., 2004). SA has also been reported to promote root system development and ion absorption efficiency, contributing indirectly to increased calcium transport to shoots. Under heat stress, a marked reduction in both shoot and root calcium contents was recorded, which is consistent with previous findings that high temperature disturbs ion homeostasis and disrupts calcium signaling pathways (Yurina, 2023). Heat-induced impairment of root membrane integrity likely limits calcium uptake, while increased transpiration and oxidative stress further reduce calcium retention in cellular compartments. However, plants treated with SA maintained relatively higher calcium levels compared to untreated controls, suggesting that SA partially alleviated stress-induced ionic imbalance. This protective effect may result from SA-mediated stabilization of plasma membrane H^{+}-ATPase activity, reduced lipid peroxidation, and improved antioxidant defense (Nazar et al., 2011; Khan et al., 2015).

Root calcium content followed a similar trend but with lower absolute values than shoots. The combined SA spray and medium supplementation significantly enhanced root calcium concentration under both temperature regimes. This improvement could be associated with SA's role in enhancing root growth, increasing root hair density, and stimulating calcium-binding proteins that facilitate ion sequestration in root tissues (Poór et al., 2019). The relatively smaller decline in calcium content under SA-treated roots under stress conditions indicates that SA helps maintain ionic balance and root metabolic stability. Collectively, the results suggest that exogenous SA enhances calcium uptake, translocation, and retention under both optimal and heat stress environments. The combined application of foliar spray and root supplementation was most effective, likely due to synergistic improvement in both absorption and transport mechanisms. These findings support the hypothesis that SA confers thermo-tolerance by sustaining mineral nutrition and preserving calcium-dependent signaling essential for cellular homeostasis and stress adaptation.

Conclusion

The present study demonstrates that exogenous application of salicylic acid (SA) can significantly mitigate the adverse effects of heat stress on wheat. Heat stress negatively affects growth, physiological traits, yield components, and mineral nutrient accumulation. SA application, whether as foliar spray, soil supplementation, or in combination, improved plant performance under both optimal and stressful conditions. Under control conditions, SA treatments enhanced shoot and root growth, fresh biomass, leaf area, and chlorophyll content, while under heat stress these improvements were sufficient to partially compensate for stress-induced reductions. Yield attributes, including number of spikes, spikelets per spike, grains per spike, and 1000-grain weight, were also significantly improved with SA application, with the combined foliar and soil treatment showing the highest gains. Furthermore, SA enhanced mineral nutrition by increasing calcium, sulphate, and phosphate contents in both shoots and roots, which may contribute to improved physiological functioning and stress tolerance. Conclusively, combined foliar and soil-applied SA proved to be the most effective, demonstrating its potential as a practical agronomic strategy to enhance heat tolerance, maintain growth, and improve yield and nutrient status in wheat under high-temperature conditions. Future research should focus on optimizing SA dosage and application timing across diverse wheat genotypes and field environments to maximize its agronomic effectiveness.

Declarations

i. Ethics approval and consent to participate

Ethical approval and informed consent were not required for this study as it did not involve human participants, human data, or animals.

ii. Consent for publication

Consent for publication is not applicable.

iii. Data availability

All data generated or analyzed during this study are included in this article.

iv. Competing interests

Authors have declared that no competing interests exist.

v. Authors' contributions

S.G.B. contributed in execution of experiments; A.W. contributed in conceptualization of the study and design of experiments; A.K. contributed in development of methodology and coordination of plant growth and stress-tolerance assays; Z.A.M. wrote the initial draft of the manuscript; Z.H. contributed in statistical analysis, interpretation of results, and revising the manuscript.

vi. Funding

No funding was received for the design, data collection, analysis, interpretation, or writing of this research manuscript.

vii. Acknowledgement

Not applicable.

viii. SDGs addressed

Zero Hunger (SDG 2); Responsible Consumption and Production (SDG 12); Climate Action (SDG 13); Life on Land (SDG 15)

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