



Temporal heat stress mitigation and physiological response in *Abelmoschus esculentus* L. by foliarly supplied salicylic acid

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Abstract

Elevated global temperatures due to climate change have emerged as a significant concern for vegetable production. Heat stress drastically affects various physiological, developmental, and growth processes in horticultural crops. The temperature was enhanced gradually when the early sown plant was 50 days old (S₁), the second sown plant was 40 days old (S₂), and the third sown plant was 30 days old (S₃). Foliar applications of salicylic acid (1.5 mM) on the vegetative and physiological response of open-pollinated okra cultivar 'Sabzpari' plants subjected to heat-induced stress. The findings indicated that all sowing intervals significantly influenced plant vegetative and physiological attributes compared to control. Furthermore, S₁ plants exhibit more resilience to heat stress in comparison to S₂ and S₃ plants. In addition, the S₁ plant with foliar supplied 1.5 mM salicylic acid, exhibited a higher number of leaves, shoot length, root length, fresh weight, dry weight, chlorophyll contents, photosynthetic rate, stomatal conductance, water use efficiency, and lower transpiration rate under heat stress conditions. By applying SA, the dry biomass of the seedling increased 1.30 times as compared to the control. In S₁, dry biomass increased 1.33 times as compared to control. Notably, the treatment of 1.5 mM SA led to a significant increase of approximately 1.19 times as compared to the control, and among sowing intervals, S₁ showed 1.19 times increase. Nevertheless, the okra plants exhibited significant resilience to heat stress when salicylic acid was applied during the S₁ interval. Hence, the application of salicylic acid (1.5 mM) through foliar spraying, specifically at the S₁ interval, presents a practical strategy for mitigating the detrimental impacts of heat stress on the okra cultivar 'Sabzpari'.

Keywords: Climate change, Heat stress, Mitigate, Okra, Salicylic acid, Sowing intervals

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Introduction

Okra (*Abelmoschus esculentum* L.) is a major summer vegetable belonging to the family Malvaceae. It is an herbaceous annual plant that grows in both tropical and subtropical regions of the world. Okra, a national vegetable of Pakistan, is considered a versatile crop due to the diverse range of uses associated with its fresh roots, buds, flowers, pods, stems, and seeds (Mihretus et al., 2013). According to Okra fruits are rich in vitamins A, B, and C, minerals, iron, iodine, and viscous fiber, but low in salt, saturated fat, and cholesterol. Fe, Zn, Mn, and Ni were also found (Biswas et al., 2016). Nutritionally, okra is a rich source of essential nutrients. The utilization of okra seeds for the treatment of genitourinary problems, acid burn, and prolonged dysentery has been documented. The utilization of the seeds of this plant encompasses both their consumption in their fresh state as well as their preservation through the process of drying. Okra is a rich source of iodine, which treats goiter (a condition characterized by the enlargement of the thyroid gland that is primarily caused by iodine deficiency) (Ochatt et al., 2013). The nutritional composition of okra includes protein, carbohydrates, and vitamin C (Dilruba et al., 2009). Okra plant production in various countries is commercialized, including India, Japan, Turkey, Iran, West

Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Myanmar, Malaysia, Thailand, Brazil, Ethiopia, Cyprus, and South America (Benjawan et al., 2007). According to the FAO, the world's total cultivated area was 2.47 million hectares and production was 10.82 million tonnes in 2021. In Pakistan, it is cultivated in the Kharif season, mostly in plain areas. In 2021, it was cultivated on an area of 25,802 hectares and production was 279,987 tonnes (MNFSR, 2021).

Most vegetables are affected by severe stress situations during the summer season (Abdelrahman et al., 2022). World temperature is increasing day by day because of global warming. Global warming poses a significant challenge to the agricultural sector and the overall stability of food production. This issue is of crucial consequence to scientists and farmers worldwide (Zahra et al., 2023). Plants require a favorable environment for their proper growth and development. Any fluctuation in the environment can change the plant's life cycle and retard its growth. There are different biotics (i.e., insects, pests, rodents, etc.) and abiotic (i.e., soil, water, temperature, moisture, wind, etc.) factors that affect plant growth and health. Worldwide, the primary reason for crop failure is abiotic stress, which also decreases average yield by 50% in significant plants. The rise in temperature above a threshold level, for instance, is

adequate to cause stable injury to plant expansion and growth. Generally, a sudden increase of 10-15°C above the optimum surrounding temperature might result in heat stress (HS). Plants are affected by heat stress during the vegetative and reproductive phases affected by heat stress. The optimum temperatures for tomatoes, brinjal, chilies, and okra are 15-30 °C, 13-21 °C, 20-25 °C and 29-32 °C, respectively. Fluctuations from this optimum range retard plant growth and cause a reduction in yield (Supran et al., 2023). The elevated temperature hinders plants' growth, development, physiological, and biochemical processes (Hassan et al., 2021).

Heat stress refers to the exposure of plants to high temperatures beyond their optimal range, leading to physiological and metabolic disruptions. This phenomenon has been recognized as a major abiotic stressor affecting agricultural production worldwide. During the vegetative stage, heat stress can impede plant growth and development. High temperatures can disrupt photosynthesis, the process by which plants convert sunlight into energy, leading to reduced carbon assimilation and limited biomass accumulation. Additionally, heat stress can induce oxidative stress, causing cellular damage and impairing the overall health of the plant. These adverse effects on vegetative growth can result in stunted plants with reduced leaf area and diminished capacity for nutrient uptake. The reproductive stage of vegetables is particularly vulnerable to heat stress, as it directly influences the formation and development of flowers (Shaffique et al., 2022). Germination of seeds is the first step of plant development; extreme heat stress disrupts the plant's germination. Heat stress harms seed germination, but the extent of damage is highly dependent on crop species (Johkan et al., 2011). Heat stress reduces the sprouts in the seed by disturbing the activities of several enzymes, which lead to the breakdown of starch and induce the synthesis of abscisic acid (ABA) (Essamine et al., 2010). In tropical regions, summer temperature exceeds the threshold level of vegetables (Ali et al., 2019). Through long-term evolutionary phenological and morphological modifications and short-term mitigation or acclimatization procedures such as changing leaf orientation, transpiration cooling, or lipid membrane composition alteration, plants show diverse mechanisms for survival under elevated temperatures. Various techniques can be adapted to mitigate HS, i.e., introducing resistant cultivars, using protected horticulture, adapting various management practices, and using chemicals. whereas the use of resistance cultivars requires breeding that is time-consuming, labor-intensive, and resource-consuming, whereas protected horticulture also demands a high initial cost. So, in the present scenario, the use of chemicals emerges as a prominent option because it is cost-effective and presents a quick solution. In chemicals, the use of naturally occurring compounds provides beneficial effects with no harmful chemical residues. The relationship between the naturally occurring compounds proline and salicylic acid is well known (Sharif et al., 2018).

The implications of climate change, encompassing amplified average global temperatures and anomalous meteorological occurrences such as frequent and intense heat waves, are materializing as a global ecological apprehension due to their ramifications on botanical flora and agricultural yield. This comprehensive analysis

compiles the intracellular procedures within plants as a reaction to thermal strain-spanning the initial detection of elevated temperatures, the ensuing sequential molecular sequences connected to the activation of thermal shock elements, and their chief objectives (thermal shock proteins)-with a focal point on the categorization and tasks of thermal shock proteins. Vegetative produce encompasses a plethora of indispensable vitamins, minerals, antioxidants, and fibers, imparting crucial health advantages to humans. The inimical consequences of thermal strain on vegetal development can be mitigated through the cultivation of vegetable cultivars exhibiting heightened thermotolerance, utilizing a gamut of genetic techniques. To actualize this objective, a robust comprehension of the molecular and/or cellular mechanisms underpinning diverse retorts of vegetables to elevated temperatures is imperative. Consequently, endeavors to pinpoint genes responsive to thermal strain, encompassing those encoding thermal shock elements and thermal shock proteins, their operative functions in vegetable crops, and their applicability to the production of heat-resilient vegetables, are comprehensively deliberated (Kang et al., 2022). The HS are significant abiotic stresses that decrease crop productivity and global food security, especially given the current and increasing impacts of climate change and the rise in frequency and intensity of stress factors. HS negatively affects crop yield, which causes lower income for farmers (Stone, 2023).

Salicylic acid, a phenolic compound, is a growth promoter produced in the ovaries of multicellular and unicellular organisms (Li et al., 2015), and is involved in various metabolic processes in plants that help resist heat. The application of salicylic acid has been found to augment the functioning of superoxide, proline, and phenolic enzymes in various horticultural crops. This enhancement has proven to be crucial in the development of resistance against heat stress (Cingoz & Gurel, 2016). The salicylic acid content of cucumber seedlings increased under high-temperature stress. Salicylic acid activates heat stress proteins to reduce heat stress (Sedaghat et al., 2017). The foliar application of salicylic acid influences many plant functions, i.e., cell growth (Nazar et al., 2017). The foliar application of salicylic acid has also been shown to increase the level of endogenous salicylic acid in plants, providing protection against many types of abiotic stresses (Hayat et al., 2010).

The impact of climate change and its associated unpredictability on the performance of agriculture, particularly horticulture crops of both annual and perennial nature, has become a matter of considerable concern. The occurrence of a short growing season is anticipated to yield a decline in the production of fruits and vegetables. The adverse impact on growth and development is expected to be significant, primarily due to the occurrence of terminal heat stress and the subsequent decrease in water availability. The horticultural production systems have been subjected to additional constraints due to the heightened uncertainties and risks arising from climate change and its unpredictable nature. The potential correlation between climate change and the escalation of prices for fruit and vegetable harvests has garnered significant attention in recent years. This phenomenon has prompted researchers to investigate possible linkages between these two variables. Currently,

there is a lack of available information regarding the impact of salicylic acid on the alleviation of heat stress in okra cultivar 'Sabzpari' sown at varying intervals. Hence, the purpose of this study is to use foliar application of salicylic acid to observe resistance to heat stress in okra cultivar 'Sabzpari', at varying sowing intervals.

Materials and Methods

Plant material and growing conditions

The research was carried out in a growth room of a vegetable stress physiology laboratory at the Institute of Horticultural Sciences, University of Agriculture Faisalabad. The growth room was fully automated to control temperature, light

(including day and night periods), and relative humidity (RH). The relative humidity was maintained at 60-65% for proper growth of the plants. Seeds of okra cultivar 'Sabzpari' were obtained from Ayyub Agriculture Research Institute, Faisalabad. The sterilized sand was filled in 6-inch plastic pots (15 cm round at the top and 8 cm high), and okra local cultivar 'Sabzpari' seeds were sown at the appropriate depth (1.3 cm). The number of seeds per pot was adjusted to about five to eight at a distance of two inches. Irrigation was applied according to the requirements of the plant and by observing sand moisture. Plants were grown at 28/22°C for 50 days. After sowing seeds, the nutrient solution (Hoagland and Arnon, 1950) was applied to plants at about half a concentration twice a week or according to their requirements. Three different sowings were done at 10-day intervals (Table 1).

Table 1 Macro and micronutrients composition of Hoagland solution applied during experiment

Reagent	Stock (g/L)	ml of stock soln. for 10L ½ conc.	ml of stock soln. for 200L ½ conc.
Macro Nutrients			
KH ₂ PO ₄	136	5	100
KNO ₃	101	25	500
Ca(NO ₃) ₂ .4H ₂ O	236	25	500
MgSO ₄ .7H ₂ O	246	10	200
Micro Nutrients			
H ₃ BO ₃	2.86	5	100
MnCl ₂ .4H ₂ O	1.81	5	100
ZnSO ₄ .7H ₂ O	0.22	5	100
CuSO ₄ .5H ₂ O	0.08	5	100
H ₂ MoO ₄ .H ₂ O	0.02	5	100
Fe-EDTA	37.33	5	100

Heat stress conditions

Heat stress began when the first sown plant was 50 days old, the second sown plant was 40 days old, and the third sown plant was 30 days old. The temperature was increased gradually by 2°C each day or night until the desired temperature (45/32°C, day, or night) was attained to avoid sudden damage to plants. A stock solution of salicylic acid was prepared by adding sodium bicarbonate in salicylic acid and in water then heated and stirred until it dissolved in water uniformly. Foliar application of salicylic acid at 1.5 mM with control was done during this stress period. One week after stress, plants were harvested, and data was taken.

Number of leaves, shoot length and root length

Three plants were randomly selected from each replication, and the total number of leaves was noted. The average was noted and evaluated statistically. Three plants were randomly chosen from each replication, and shoot length was measured with the aid of a measuring rod. The mean value was taken and statistically evaluated. Plants were gently uprooted, and roots were washed. In each replicate, three plants were chosen randomly, and root length was measured with the assistance of a scale. The value of the average was taken.

Chlorophyll contents (SPAD value)

The chlorophyll meter (CCM-200plus BioScientific USA) was used to measure chlorophyll contents (SPAD units). Fully grown young leaves were selected, and data were taken from 9 a.m. to 11 a.m. Randomly, three leaves were selected from each replication, and the average was taken.

Transpiration rate (mmol m⁻² s⁻¹), stomatal conductance (mmol m⁻² s⁻¹), photosynthetic rate (μ mol m⁻² s⁻¹), water use efficiency

Fully matured leaves of various plants were randomly selected and gaseous related/physiological attributes e.g., transpiration rate, stomatal conductance (mmol m⁻² s⁻¹), and photosynthetic rate were calculated with the help of an IRGA (Infrared Gas Analyzer) ((LCi-SD; ADC Bioscientific Ltd, Hoddesdon, UK). The growth room was automated with turn off-on lighting. Lights had to have an intensity of 12000 lux for proper photosynthesis of plants. The leaves were selected from each replication, data was collected, and an average was taken. The data was taken from 9 a.m. to 11 a.m. when the lights were fully on in the growth room. To find plants maintaining water balance inside them water use efficiency (WUE) (%) was measured by the ratio of photosynthetic rate and transpiration rate.

$$\text{Water use efficiency (\%)} = \frac{\text{Photosynthetic rate}}{\text{transpiration rate}}$$

Statistical analysis

The study was conducted using a completely randomized design (CRD). There were 4 replications in each treatment. The means of the different treatments and sowing intervals were compared using LSD tests at a significance level of 5%.

Results

Number of leaves, shoot length, and root length

During heat stress, okra plants showed a linear decrease in the number of leaves, shoot length, and root length. However, foliar application of salicylic acid (SA) on

different sowing intervals of okra exhibited a significant effect on the number of leaves, shoot length, and root length (Fig. 1A, 1B, and 1C). Application of 1.5 mM SA treatment showed about a 1.17 times higher number of leaves as compared to the control. Similarly, among sowing intervals, S₁ exhibited a 1.26 times higher number of leaves than the control (Fig. 1A). SA significantly influenced the shoot length throughout the heat stress period. In S₁, 1.5 mM SA-treated plants showed 1.16 times the maximum shoot length than the control. In S₂, the maximum shoot length was observed in SA treatment (1.25 times higher). Moreover, among treatments 1.5 mM SA showed S₃ the highest shoot length (1.37 times higher) in S₃ as compared to control (Fig. 1B). The results showed a non-significant effect on the root length of okra under heat stress conditions. Among sowing intervals, in S₁, maximum root length was recorded in SA-treated plants (1.14 times higher) compared to control plants (Fig. 1C).

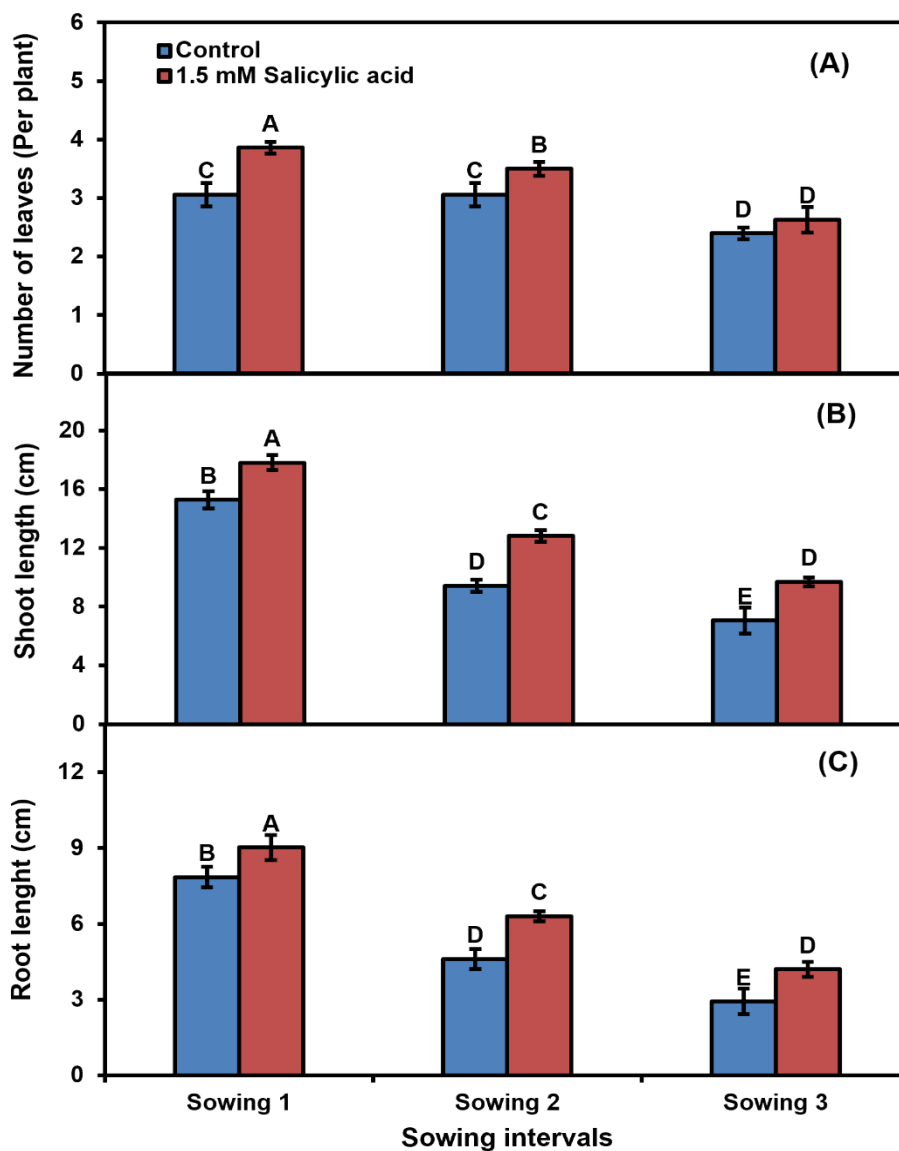


Fig. 1 Influence of foliar-supplied salicylic acid at varying sowing intervals on the number of leaves (A), shoot length (B), and root length (C) of okra cultivar Sabzpari under heat stress. Error bars with same lettering show non-significant difference while different lettering on error bars have significant difference in treatments.

Fresh weight and dry weight

Heat stress steadily influenced the growth activities in plants and decreased seedling weight and chlorophyll contents, but the seedlings treated with SA showed significant outcomes (Fig. 2A, 2B, and 2C). 1.17 times increase in seedling fresh weight was observed following the application of 1.5 mM SA in comparison to control; similarly, in S_1 fresh weight was increased 1.13 times. (Fig. 2A). Throughout the heat stress period, okra plants experienced a gradual decline in seedling dry weight. By applying SA, the dry weight of the seedling increased 1.30 times as compared to the control. In S_1 , dry weight increased 1.33 times as compared to control (Fig. 2B).

Chlorophyll contents, photosynthetic rate and transpiration rate

Notably, the treatment of 1.5 mM SA led to a significant increase of approximately 1.19 times chlorophyll content (SPAD units) as compared to the control, and among sowing intervals, S_1 showed 1.19 times increase (Fig. 2C). The physiological activities of plants are also provoked by high-temperature stress during their growth (Fig. 3A and 3B). A gradual decrease in photosynthetic rate was observed in plants under heat-stress conditions. Foliar application of SA increased the photosynthetic rate of affected plants by 1.34 times as compared to the control. Similarly, S_1 exhibited 1.29 times increase (Fig. 3A). 1.33 times decrease in transpiration rate was observed following the application of 1.5 mM SA in comparison to the control. among sowing intervals, S_1 showed 1.24 times decrease in transpiration rate (Fig. 3B).

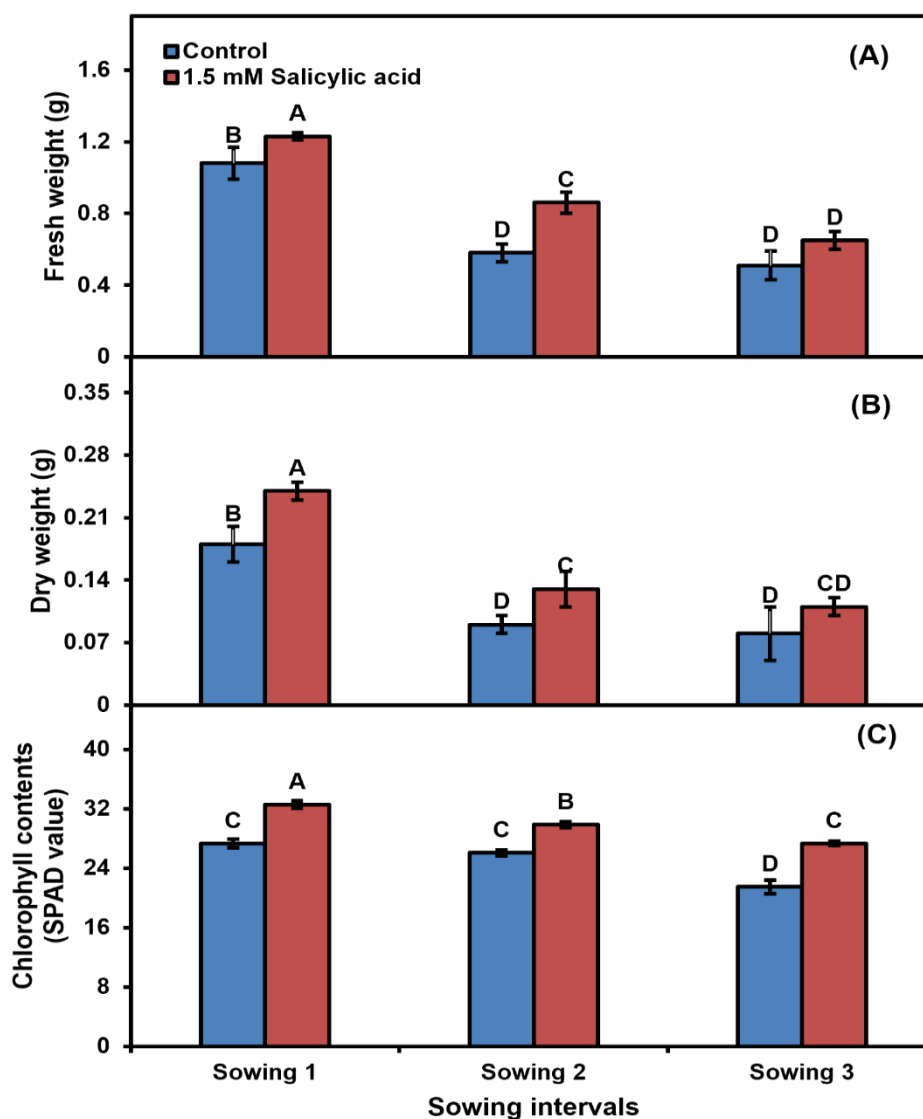


Fig. 2 Influence of foliar-supplied salicylic acid at varying sowing intervals on the fresh weight (A), dry weight (B), and chlorophyll contents (C) of okra cultivar Sabzpari under heat stress. Error bars with same lettering show non-significant difference while different lettering on error bars have significant difference in treatments.

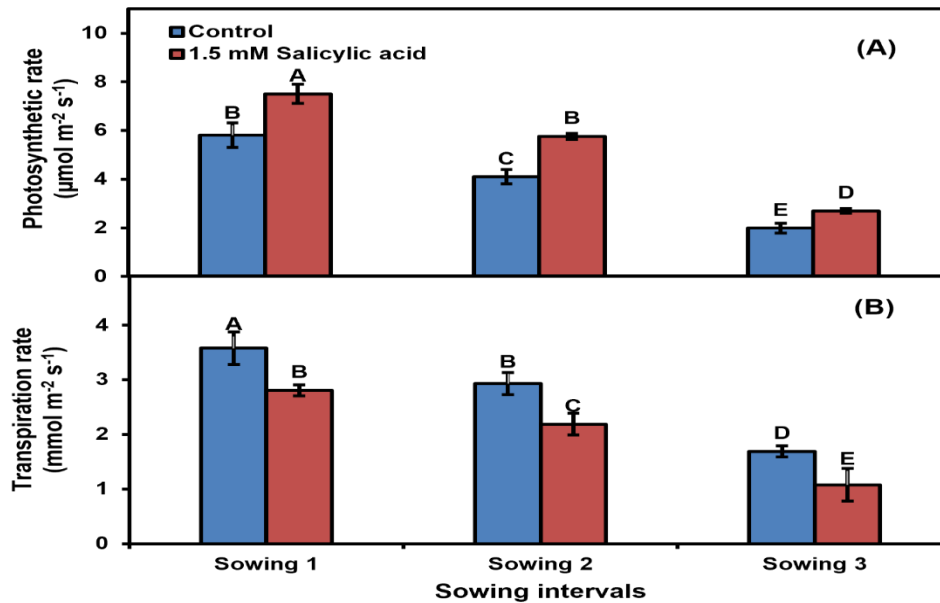


Fig. 3 Influence of foliar-supplied salicylic acid at varying sowing intervals on the photosynthetic rate (A) and transpiration rate (B) of okra cultivar Sabzpari under heat stress. Error bars with same lettering show non-significant difference while different lettering on error bars have significant difference in treatments.

Stomatal conductance and water use efficiency

Stomatal conductance and water use efficiency substantially decreased during heat stress duration in all treatments and sowing intervals. However, SA-treated plants in all sowing intervals exhibited higher stomatal conductance and water use efficiency as compared to control plants (Fig. 4A and 4B). The effects of SA and different sowing intervals on

okra were observed to have significant outcomes. In S₁, maximum stomatal conductance was recorded about 1.20 times higher in SA-treated plants than in control (Fig. 4A). The results showed that the application of SA significantly influenced water use efficiency. In S₁, 1.60 times higher water use efficiency was recorded in the SA treatment in comparison to the control (Fig. 4B).

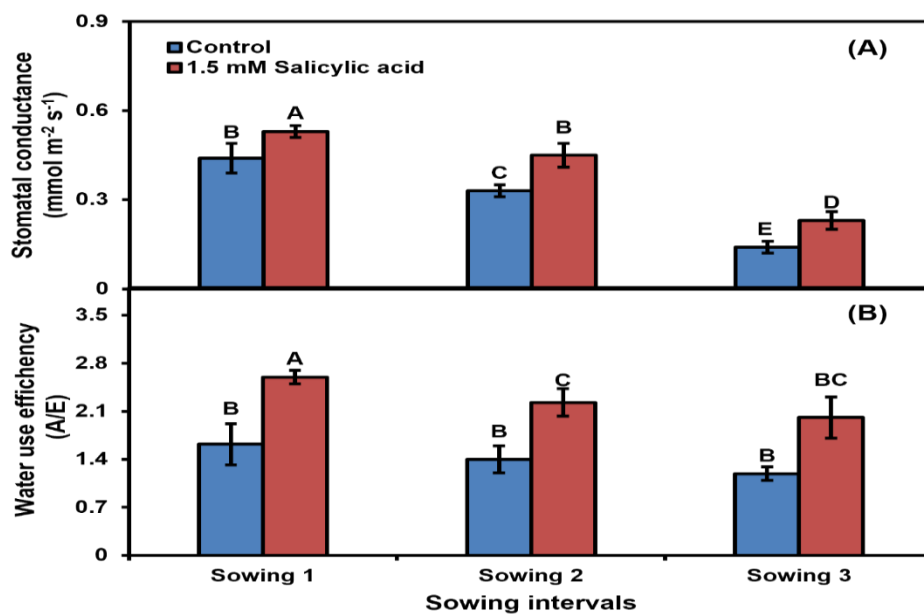


Fig. 4 Influence of foliar-supplied salicylic acid at varying sowing intervals on the stomatal conductance (A) and water use efficiency (B) of okra cultivar Sabzpari under heat stress. Error bars with same lettering show non-significant difference while different lettering on error bars have significant difference in treatments.

Discussion

The results of this study indicate that mature plants (S_1) exhibit significant responses to heat stress in comparison to their younger counterparts (S_2 and S_3). Based on the findings, it can be concluded that the application of SA showed a substantial effect on the growth parameters of the *Abelmoschus esculentus* plant during the duration of heat stress. According to a previous investigation conducted by Rai et al. (2020), it was observed that the application of SA resulted in an improvement in growth regulation. The application of SA displayed a positive influence on the elongation of shoots. The root length of the plant was observed to be influenced by the foliar application of SA. Furthermore, the application of SA accelerated the initiation of adventitious roots and a significant increase in root length in red amaranth plants (Khandaker et al., 2011). The impact of SA on thermally stressed Indian mustard plants was examined. Salicylic acid considerably boosted plant growth compared to control. Hayat et al. (2009) found that salicylic acid improved growth metrics in Indian mustard plants.

The results indicate that the SA significantly impacted the fresh weight of seedlings. In the context of rice genotypes, it has been observed that the application of salicylic acid (SA) significantly impacts various physiological parameters. Specifically, when rice plants are exposed to heat stress conditions, the presence of SA leads to a notable increase in the concentration of fresh and dry weight. Additionally, SA treatment also promotes the accumulation of both organic and inorganic solutes in rice plants under heat-stress conditions. These findings suggest that SA plays a crucial role in enhancing the tolerance of rice genotypes to heat stress, thereby positively influencing their growth and overall physiological performance. According to a study conducted by Akasha et al. in 2019, the application of salicylic acid has been found to enhance the mineral contents and activity of near-infrared (NIR) and nitrate reductase (NR) enzymes in response to heat stress. The current findings are consistent with a study by Rafique et al. (2011), who reported that under stress conditions, the application of SA led to an increase in the fresh weight of pumpkin seedlings. These combined results provide compelling evidence that SA can effectively enhance plants' growth and stress tolerance, leading to increased fresh weight and overall vigor in seedlings. SA improved plant growth and increased dry mass production. Additionally, Anitha and Das (2011) observed that under abiotic stress conditions, the application of SA led to increased root and shoot length, as well as higher fresh and dry weights in rice plants. SA plays a significant role in promoting seedling growth and dry weight accumulation, particularly under stress conditions. The foliar application of SA had a positive impact on the chlorophyll contents of plants and application led to an increase in chlorophyll levels.

The application of SA resulted in a significant increase in the photosynthetic rate of plants and this effect was observed in all sowing intervals. SA has the potential to enhance photosynthesis and improve overall plant performance, especially under challenging environmental conditions. SA treatment positively influenced photosynthesis, transpiration, and stomatal conductance, leading to the overall improvement in plant growth. In the context of plants experiencing heat stress, it has been

observed that the application of salicylic acid has resulted in notable enhancements in CO_2 assimilation and photosynthetic activity, as well as an augmentation in mineral absorption (Daneshmand et al., 2010). Salicylic acids have been observed to enhance the cellular anti-oxidation capacity and provide protection to the photosynthetic apparatus of plants. Additionally, it has been observed that this phenomenon leads to an augmentation in the process of protein synthesis. Furthermore, SA was found to enhance the photosynthetic activity of spring wheat when applied in the rooting media (Arfan et al., 2007). The foliar application of SA produced significant effects on the transpiration rate of plants. Hussain et al. (2021) reported that the transpiration rate was reduced, and photosynthesis was improved in okara when an exogenous application with proline.

The metabolic processes (stomatal conductance) in plants are adversely affected by heat stress, primarily due to the impairment of cellular water absorption mechanisms. This impairment leads to the obstruction of water-absorbing cells, ultimately resulting in the demise of roots and excessive leakage of water through the leaves. The phenomenon of high-temperature stress has been frequently observed to coincide with a reduction in water availability in field settings. In a study conducted by Morales et al. (2003), it was observed that tomato plants subjected to high-stress (HS) conditions exhibited alterations in leaf water relationships and root hydraulic conductivity. Heat stress significantly affects stomatal conductance, and these changes specifically impact plant water loss (Zhou et al., 2017), foliar application of SA also improved relationships between plant water, osmotic leaf potential, and strain of leaf turgor (Shaheen et al., 2017).

Conclusion

The study findings indicated that heat stress had a significant impact on various growth-related and physiological parameters. The application of SA as a foliar treatment has been observed to confer heat stress tolerance in okra plants. Plants 50 days after exposure to heat stress exhibit greater resilience to heat stress in comparison to plants after 40 days and 30 days, which indicates the impact of elevated temperature stress on small plants was significantly detrimental, whereas larger plants exhibited relatively superior responses in the face of heat stress conditions. The foliar application of SA (1.5 mM) resulted in notable enhancements in vegetative and physiological growth parameters when plants were exposed to heat-stress conditions.

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