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Managing Heat Stress in Tomato (*Lycopersicon esculentum* L.) Plants through Proline Foliar Application

Saeed Ur Rahman[1,a,*], Akbar Ali[2,b], Altaf Husssain[3,c], Sadia Nazeer[4,d], Mughees Ul Hassan[5,e], Waryam Abbas[6,f]

[1]*College of Agriculture, Department of Horticulture, University of Sargodha, Pakistan*
[2]*Department of Plant Breeding and Genetics, University of Agriculture, Peshawar, Pakistan*
[3]*College of Horticulture, Northwest A & F University, Yangling 712100, China*
[4]*Department Botany, Government College University Faisalabad, Pakistan*
[5]*Department of Horticulture, The University of Haripur, Pakistan*
[6]*Department of Agriculture, Karakoram International University Gilgit, Pakistan*
[*]*Corresponding author*

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ABSTRACT

The tomato is a significant vegetable worldwide in terms of consumption, nutrition, and extensive use in processed foods. During plant growth and development, amino acids especially exogenous application of proline (Pro), plays a crucial role in enhancing stress tolerance under various abiotic stresses. Among these stressors, temperature is considered as a critical and alarming factor affecting plant growth and development and even often a substantial drop in crop productivity results from a significant increase in temperature. The present investigations was conducted at the Horticulture Lab, College of Agriculture, University of Sargodha, during 2021-22 to examine the role of foliar application of 'Proline' under heat stress in tomato plants. Tomato seedlings with true leaves were exposed to high temperatures (25°C [control], 40°C, and 45°C) with exogenous 'Pro' sprays at concentrations of 0, 0.5, 1 and 1.5 $mMmL^{-1}$). Various growth attributes were studied, including morphological traits i-e number of leaves, leaf area cm^2, shoot fresh weight (mg), shoot dry weight (mg), root fresh weight (mg), and root dry weight (mg). Along this physiological parameters like photosynthetic rate (μ $mol/m^{-2}s^{-1}$), chlorophyll contents (spad), stomatal conductance (μ mols m^{-2} s^{-1}), transpiration rate (μ mol/m^{-2} s^{-1}), were also studied. The findings indicated that foliar application of 'Pro' at 1.5 mML^{-1} under heat stress at 40°C and 45°C was particularly beneficial in improving growth attributes such as the number of leaves (12.2), leaf area (8.3 cm^2), shoot length (10.39 cm), shoot fresh weight (1.88 mg), shoot dry weight (0.28 mg), and root dry weight (0.20 mg), thereby mitigating the adverse effects of heat stress in tomato plants. The variation between control and Proline treated heat-stressed plants suggests that proline may play a role in alleviating heat stress in tomato plants.

[a] saeedhorticulturist@gmail.com https://orcid.org/0009-0008-4810-0859
[b] akbaragrian@gmail.com https://orcid.org/0009-0008-6310-5666
[c] altaf@nwafu.edu.cn https://orcid.org/0009-0001-5774-9886
[d] sadianazeer2299@gmail.com https://orcid.org/0009-0006-0544-8527
[e] mughees955@gmail.com https://orcid.org/0009-0006-9556-7203
[f] waryamabbas14@gmail.com https://orcid.org/0009-0006-4747-0335

Introduction

Tomato (*Lycopersicon esculentum* L.) is globally recognized as a significant vegetable both commercially and as a food item. A member of the Solanaceae family, the tomato originates from Peru and Mexico (Ali et al., 2020). Globally, and even in Pakistan, tomatoes can grow in a variety of soil types and climates. In Pakistan, tomatoes are cultivated on 55,258 hectares yielding an average of 10.15 tons per hectare and an annual production of 561,293 tons (FAOSTAT, 2022). Tomato production is significantly affected by three main constraints: heat stress, drought, and salinity. In response to high temperatures, most plants produce a range of heat shock proteins to some extent (Meena et al., 2018). Among them, chitin-binding proteins (CBP) family member CaChiVI2 plays a crucial role in eliminating the impact of adverse environmental conditions, such as cold and salt stress (Ali et al. 2020).

Heat stress is a phenomenon where increased temperature leads to morpho-physiological and metabolic changes in plants, potentially limiting growth and potentially reducing economic yield (Sher et al., 2022). Heat stress has adverse effect on morpho-physiological and biochemical attributes of tomato plants. Air temperatures higher than 35°C inhibited fruit development, plant growth and diminished flowering (Haque et al., 2021). High temperature stress impacts plant development, including germination, expansion, and reproduction, particularly in early growth phases of tomatoes and vegetables. It disrupts chloroplasts' photosynthetic machinery, affecting carbon metabolism and chemical processes (Alsamir et al., 2020). (Ali et al., 2020) testified that high temperature reduces seed germination because it disrupts the task of enzymes necessary for the digestion of starch and accumulation of abscisic acid, as well as hampers protein synthesis. One of the severe and troubling threats to the environment that restricts plant development and production is heat stress.

Proline is a notable osmolyte and non-essential amino acid that is crucial to plants for fundamental metabolism. It helps maintain cellular turgidity, lessen oxidative harm, and ease the stress on living membranes and intracellular structure (Zulfiqar et al., 2023). Proline has been found to be applied exogenously under various abiotic stressors like water deficiency, salt toxicity, and high temperature. Numerous studies indicated that as proline accumulated, plants exhibit enhanced tolerance to stress (Sadeghipour et al., 2020). According to studies, fruiting becomes more difficult when high temperatures stress the flow of photosynthates to the reproductive structures (Hassan et al., 2021). Additional effects of heat stress include sunburn, denaturation of proteins, early flowering of the plant, twisting, increased membrane fluidity, and stunted plant growth development. (Hussain et al., 2021) found that the exogenous application of 2.5 mM proline resulted in elevated levels of proline, glycine betaine, free amino acids, and chlorophyll in the leaves of stressed plants. Higher concentrations of antioxidant enzymes (SOD, CAT, and POX) were found in heat-tolerant genotypes of okra when subjected to high temperatures, compared to heat-sensitive genotypes. (Priya et al., 2019) and (Ullah et al., 2012) studied the effects of exogenous proline treatment on mungbean (*Vigna radiata* L.) plants during flowering stage under high temperature environment and found that proline application and management improved stigma and pollen fertility, as well as increased internal proline in both reproductive and vegetative parts. It also reduced photosynthetic damage and improved leaf hydration status, which significantly affected mung bean yield (Ullah et al., 2012). The investigations of (Orsini et al., 2018) exhibited that effects of foliar application (5 µM) under high temperature and saline conditions on lettuce and found improved growth and yield traits. Additionally, applying proline topically to different crops under varying stress scenarios strengthened plant tolerance and growth (Hosseinifard et al., 2022).

Based on the previous findings and problems associated with tomato plants, the significance of the study were: to judge the role of proline in moderating stress due to extreme temperature in tomato plants, to control the biological and morphological characteristics that enable tomatoes tolerate high temperatures and figure out the optimal proline concentration that provides the optimum resilience towards thermal stress.

Materials and Methods

This experiment was conducted at Horticulture Lab, College of Agriculture, University of Sargodha, Pakistan during 2021-22 aimed to alleviate the effect of high temperature in tomatoes *(Lycopersicon esculentum* L.) by applying proline through foliar spray. Tomato seeds (cv. Roma) were obtained from the Ayub Agricultural Research Institute (AARI) Faisalabad.

Growth Conditions

The planned study was done to reveal the foliar application of proline at the seedling stage in tomatoes under temperature stress. The seeds were cleaned with a 5% sodium hypochlorite solution. Three seeds of tomatoes were sown in white plastic pots (10 × 4 cm) filled with fine sandy soil (1:1) as a medium of growth. The growth process was carried out at 25°C ambient temperature. Seeds were irrigated daily with tap water until germination was completed. The pots were closed at one end with a small hole to improve drainage. After complete germination, thinning was practiced to maintain one vigorous plant in each pot. The plants were permitted to grow under controlled conditions from germination until the emergence of true leaves. After true leaf emergence, the plants were exposed to high-temperature strain. The temperature was incrementally raised by 2°C each day to elude any abrupt shock while waiting for the targeted high temperature level (25, 40, and 45°C) was attained. Proline was applied at different levels (0, 0.5, 1, and 1.5 mM ml^{-1} via spray respectively).

Growth Attributes

In the present research, the data were taken on the following parameters at their proper time and methods i-e number of leaves, leaf area (cm^2), shoot fresh weight (mg), shoot dry weight (mg), root fresh weight (mg), root dry weight (mg), and physiological parameter; photosynthetic rate the rate of CO_2 assimilation (µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) measured under steady-state conditions, chlorophyll contents (spad) measured using a SPAD meter to assess chlorophyll content as an indicator of leaf health, stomatal conductance (µmolm^{-2} s^{-1} Measured in mol $H_2O \cdot m^{-2} \cdot s^{-1}$) and on transpiration rate (µmolem^{-2} s^{-1}),) the rate of water vapor loss (mol $H_2O \cdot m^{-2} \cdot s^{-1}$).

Statistical Analysis

A completely randomized design (CRD) which incorporates two factorial configurations and three replications was used to set up the experiment. The data for all parameters were examined by the analysis of variance (ANOVA) technique. The least significant differences test was implemented to evaluate the treatment means at the 0.05% probability level (Steel et al., 1980).

Results

From the current assessment, it was determined that all parameters were affected considerably under high temperature strain. Application of proline not only help to alleviate the heat stress, but it also helped to increase the highest number of leaves (12.2) recorded at 25°C + 1.5 mML^{-1} proline level, while the minimum number of leaves (8.4) was produced at 45°C + 1.5 mML^{-1} proline in tomato plants (Table 1). The interactions of foliar applied proline under heat stress for the number of leaves was found non-significant which is mentioned in (Table 3).

The leaf area of tomato plants under heat stress showed a significant decline. Foliar application of proline illustrates a positive relationship and increases the leaf area (8.3 cm^2) at 25°C + 1.5 mM L^{-1} pro. Among several foliar proline levels, 1.5 mM L^{-1} was found to be more rewarding to enhance leaf area (7.1 cm^2) at 40°C + 1.5 mM L^{-1} pro (Table 1).

Table1. Number of leaves, leaf area (cm^2), shoot length (cm), shoot fresh weight (mg), shoot dry weight (mg), root fresh weight (mg) and root dry weight (mg) of tomato as affected by temperature, and exogenous application of proline at 0.5, 1.0, and 1.5 $MmolL^{-1}$.

T	Proline sprays	No. of leaves	Leaf area (cm^2)	shoot fresh weight (mg	shoot dry weight (mg)	root fresh weight (mg)	root dry weight (mg)
25°C	Control	8.2	6.1	1.43	0.22	0.5	0.13
	0.5	10.4	6.4	1.75	0.24	0.57	0.14
	1	11.3	7.4	1.82	0.26	0.58	0.15
	1.5	12.2	8.3	1.88	0.28	0.59	0.16
40°C	Control	7.2	6.4	1.58	0.18	0.47	0.11
	0.5	8.4	6.9	1.62	0.17	0.52	0.12
	1	9.6	6.6	1.68	0.23	0.46	0.13
	1.5	10.3	7.1	1.72	0.24	0.51	0.14
45°C	Control	5.7	5.5	1.18	0.16	0.37	0.11
	0.5	6.5	6.1	1.39	0.17	0.39	0.11
	1	7.6	6.4	1.48	0.19	0.42	0.1
	1.5	8.4	6.7	1.58	0.22	0.47	0.12

Y: Temperature

Table 2. Physiological parameters: Photosynthetic rate (µmolm-2 s-1[1]), chlorophyll contents (spad), stomatal conductance (µmol m-2s-1)] and transpiration rate (µ mol/$m^{-2}s^{-1}$), of tomato as affected by temperature, and exogenous application of proline.

T	Proline sprays $Mmml^{-1}$	Photosynthetic rate (µ$molm^{-2}$ s^{-1})	Chlorophyll contents (spad),	Stomatal conductance (µmol $m^{-2}s^{-1}$	Transpiration rate (µ $molm^{-2}s^{-1}$
25°C	Control	2.49	10.53	0.019	2.13
	0.5	3.09	11.42	0.021	2.78
	1	3.23	12.45	0.024	2.13
	1.5	3.52	12.87	0.028	2.56
40°C	Control	2.32	9.03	0.015	1.85
	0.5	2.91	10.45	0.017	2.11
	1	2.97	11.78	0.019	2.22
	1.5	3.23	12.27	0.020	2.38
45°C	Control	1.02	6.54	0.014	1.36
	0.5	1.68	7.82	0.012	1.38
	1	2.26	8.58	0.013	1.61
	1.5	3.06	9.25	0.014	1.96

Y: Temperature; LSD test was non-significant NS, significant *, and highly significant** at 1 and 5%, probability level respectively.

Table 3. ANOVA about no of leaves, leaf area, Shoot fresh weight, Shoot dry weight, Root fresh weight and Root dry weight.

Treatments\Traits	No of leaves	leaf area	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
Temp.	1883.614*	7.46**	4.27*	159.32*	6.53*	6.98**
Proline	1249.824*	14.66*	5.54*	13.51*	16.50**	4.42*
Temp x Pro	616.702^{NS}	0.33**	6.86**	0.22*	0.12 NS	2.78*

LSD test with the significance level of P < 0.05; Marked * as Significant, ** Highly Significant, and NS for Non-Significant respectively

The interaction between foliar applied proline and high temperature stress in tomato plants showed a progressive response for shoot fresh weight (Table 3).

Maximum shoot fresh weight (1.88 mg) was observed at 25°C + 1.5 mM L^{-1} pro followed by (1.72 mg) at 40°C + 1.5 mM L^{-1} pro (Table 1). Non-significant trends were observed among the interactions of foliar applied proline under heat stress for root fresh weight. Statistically significant results were observed for shoot dry weight. The maximum shoot dry weight (0.28 mg) was noted at 25°C + 1.5 mM L^{-1} pro, while the lowest shoot dry weight (0.22 mg) was noted at 45°C + 1.5 mM L^{-1} pro. Moreover, improvement in root dry weight was recorded at its maximum (0.25 mg) at 25°C + 1.5 mM L^{-1} pro), trailed by 0.12 at 40°C + 1.5 mM L^{-1} pro (Table 3).

Among various concentrations of proline and different temperature strains the highest upturn in root fresh weight (0.59 mg) was observed for treatment (25°C +1.5 mM L^{-1} pro) while intermediate response regarding root fresh weight (0.51 mg) was recorded for treatment 40°C +1.5 mM L^{-1} pro. The lowest root fresh weight (0.47) was perceived by treatment 45°C +1.5 mM L^{-1} pro. The interaction among different temperature strain and exogenous applied proline were found non-significant for root fresh weight (Table 3).

The supreme root dry weight (0.25 mg) was recorded in treatment (25°C + 15 mM L^{-1} pro) while lowest root dry weight (0.12 mg) was observed for the treatment (45°C +1.5 mM L^{-1} pro).

Table 4. ANOVA for Photosynthetic rate, Chlorophyll Content, Transpiration rate and Stomatal conductance.

Treatments\Traits	Photosynthetic rate	Chlorophyll Content	Transpiration rate	Stomatal conductance
Temp.	4.78*	3.58*	13.65**	3.68*
Proline	3.28*	6.50**	3.62*	3.44*
Temp x Pro	1.34^{NS}	0.120^{NS}	0.46^{NS}	2.91*

Marked * as Significant, ** Highly Significant, and NS for Non-Significant respectively LSD test with the significance level of P < 0.05

However, intermediate response (0.11 mg) was observed in treatment (40°C + 1.5 mM L^{-1} pro). Results concerning interaction among different temperature strains and exogenous applied proline for this parameter were found significant (Table 3). The maximum photosynthesis (3.52 µmolm^{-2} s^{-1}) was noted in (25°C + 1.5 mM pro) followed by (3.23 µmolm^{-2} s^{-1}) in treatment (40°C +1.5 mM L^{-1} pro), while treatment (45°C + 1.5 mM L^{-1} pro) revealed minimum rate of photosynthesis (3.06 µmolm-2 s-1^{1}) in tomato plants. Moreover, the interaction of high temperature strain and foliar application of proline showed non-significant variations for photosynthetic rate (Table 4).

The highest chlorophyll contents (12.87 spad) was observed in (25°C 1.5 mM L^{-1}pro) followed by (12.27 spad) in treatment (40°C + 1.5 mM L^{-1} pro), while treatment (45°C +1.5 mM pro) showed lowest chlorophyll contents (9.25 spad) in tomato plants. On the other hand, the cumulative influence of heat stress and foliar applied proline was noted non-significant for this character.

The highest stomatal conductance (0.028 µmol $m^{-2}s^{-1}$) was noted for treatment (25°C + 1.5 mM L^{-1} pro) followed by (0.020 µmol m^{-2} s^{-1}) in treatment (40°C +1.5 mM L^{-1} pro), while treatment (45°C + 1.5 mM pro) indicated least amount of stomatal conductance (0.014 µmol $m^{-2}s^{-1}$). The investigations about interaction between foliar application of proline and high temperature stress were found significant for stomatal conductance.

The maximum transpiration rate (2.56 µ mol/$m^{-2}s^{-1}$) was noted in treatment (25°C with 1.5 mM L^{-1} pro) followed by (2.38 µ mol/$m^{-2}s^{-1}$) in treatment (40°C +1.5 mM pro), while treatment (45°C +1.5 mM L^{-1} pro) exhibited lowest transpiration rate (1.96 µ mol/$m^{-2}s^{-1}$). Results concerning interaction between foliar application of proline and high temperature stress were found non-significant for this trait

Discussion

Recently, crop production faces substantial challenges such as ups and downs in climate, rising temperatures, irregular and abrupt rainfall, and harsh meteorological environments, leading to reduced revenues (Clay and King, 2019; Ullah et al., 2014). Agricultural experts have considered heat stress as the primary factor significantly affecting the yield potential of crop species (Ali et al., 2020). The current study observed the performance of tomato seedling under heat stress condition, with exogenous application of proline. The results revealed that heat stress significantly affected all tested parameters such as; total number of leaves per plant, leaf area, fresh shoot mass, dried shoot mass, fresh root weight as well as physiological characteristics such as photosynthetic rate, transpiration rate, chlorophyll and stomatal conductance in tomato plants (Wang et al., 2020). In many countries worldwide, including Pakistan, heat stress severely restricts sustainable crop production, and high temperatures have been found to damage crops and cause significant losses (Fahad et al., 2019). Increase in normal temperature can cause pretty recognizable deviations, involving scorching of foliage and stems, and other issues like sunscald on leaves, twigs, and stems, premature leaf senescence, fruits drop, and a decline in yield (Santos et al., 2022).

In many countries worldwide, including Pakistan, heat stress severely restricts sustainable crop production, and high temperatures have been found to damage crops and cause significant losses

High temperature stress can be alleviated by several strategies, including thermotolerance, genetic approaches, and utilising osmolytes protectants (Oyebamiji et al., 2023). Some research works have supported that external proline supplementation on plants enhanced their growth and productivity during abiotic stress like salinity, high temperature and drought (Santos et al.,2022). Numerous researchers demonstrated that introducing exogenous proline can decline such negative impacts on tomato plants.

Some growth parameters were improved in chillies by applying (5 and 10 mM L^{-1}) proline which also assisted in heat stress-tolerance up to some extent under high temperature stress of 40°C for thirty days (Akram et al., 2021). The outcomes issued by (Kahlaoui et al., 2013) on tomato plants indicated that 10 mg L^{-1} Proline induced growth more efficiently in both tomato cultivars; Rio Grande and Heinz-2274. (Posmyk et al., 2007) scrutinized that external proline utilisation has upgraded growth and catalysed germination of *Vigna radiata* L. seeds when treated with proline. Hare et al., (2003) described that exogenously proline spraying enhances the seed germination of *Arabidopsis thaliana*. According to the conclusions obtained by (Fedina et al., 1993) the execution of proline foliage spray boosted plant growth as well as productivity of peas plants under chilling stress. Under different stress regimes, plant growth was improved by exogenous use of proline in several crops (Ashraf & Foolad, 2007). Hence, selecting tomatoes as a versatile crop, an experiment was steered to improve tomato plants growth and heat tolerance by applying proline as a foliar spray under high temperature stress conditions, aiming to identify the optimal concentration for optimal response.

Phenological Parameters

Number of Leaves Plant^{-1}

The stress caused by heat has an impact on a plant's leaf quantity in tomato seedlings. The results revealed deviations between the various concentrations of proline application on tomato plants. Statistically significant results concerning leaves plant^{-1} mentioned in (Table 1) revealed that the highest number of leaves (12.2) was documented from treatment (25°C + 1.5 mM L^{-1} pro), an intermediate response regarding the number of leaves (10.3) was recorded from treatment (40°C + 1.5 mM L^{-1}

pro), however, the lowest number of leaves (8.4) was observed by treatment (45°C with 1.5 mM pro). Hence, non-significant interaction between heat stress and the exogenous application of proline on tomatoes was noted (Table 3). Tomato seedlings under heat stress indicated progressive reaction to externally driven proline operations and an increase in the quantity of leaves. Our results were with same channel with the findings of (Kahlaoui et al., 2018), who had reported that under abiotic stress (Salinity) stressed tomato plants that were not treated with proline had fewer leaves than those that had received proline externally at 10 mgL^{-1} concentration and subsequently increased the amount of leaves in tomatoes.

Leaf area (cm^2)

Results regarding leaf area in (Table 1) displayed that under heat stress leaf area was significantly affected. With the various exogenous applications of proline, variation was found among leaf areas. The maximum increment in leaf area (8.3 cm^2) was recorded in treatment (25°C with 1.5 mM L^{-1} pro), followed by 7.1 cm^2 in (treatment 40°C with 1.5 mM L^{-1} pro). Treatment (45°C with 1.5 mM L^{-1} pro) indicated a minimum increase (6.7 cm^2) in leaf area. Hence, interaction between high temperature and the application of proline as a foliar spray on tomatoes for this parameter revealed highly significant. Heat stress initiated sizable damages like reduction and the burning of leaves (Santos et al., 2022). Our outcomes are in agreement with the former conclusions of (Gupta et al., 2022), who documented that high temperature decreased leaf area and reduced photosynthetic activity.

Shoot fresh weight

The results concerning shoot fresh weight in (Table 1) indicated that rise in temperature have adversely affected shoot fresh weight. A significant relationship in temperature stress with various doses of proline applied on tomato seedling was noted. Maximum enhancement in shoot fresh weight (1.88 mg) was observed for treatment (25°C with 1.5 mM L^{-1} pro) and trailed by 1.72 mg for treatment (40°C with 1.5 mM L^{-1} pro). Treatment (45°C with 1.5 mM L^{-1} pro) showed the lowest increase (1.58 mg) in shoot fresh weight. Here, the interaction between foliar-applied proline and high temperature stress on tomatoes showed a highly significant response for shoot fresh weight. A similar limiting effect of heat stress on this character of plants has been observed, and it was determined that heat stress severely declines the normal shoot growth (Bonsu et al., 2022). Our findings correspond with (Khan et al., 2015) who concluded that high temperature had reduced the shoot fresh weight of okra plants, but exogenous use of proline had expressed improvement in shoot weight.

Shoot dry weight (mg)

Significant results pertaining in (Table 1) showed that shoot dry weight in tomato were highly affected by high temperature stress. Variations among various concentrations of proline application were noted in the shoot dry weight of tomato plants. The highest increase in shoot dry weight (0.28 mg) was found from treatment (25°C with 1.5 mM L^{-1} pro), while an intermediate response regarding shoot dry weight (0.24 mg) was recorded from treatment (40°C with 1.5 mM L^{-1} pro). The lowest shoot dry weight (0.22) was perceived by treatment (45°C with 1.5 mM pro). In addition, the interaction between foliar-applied proline and high temperature stress on tomatoes for this trait showed a positive response. Related conclusions were presented by (Guo et al., 2022), who observed the decrease in shoot dry weight under high temperature stress in tomato plants but spray of GA_3 has bring improvement in shoot dry weight.

Root fresh weight (mg)

Significant results concerning (Table 1) revealed that temperature strains have a harmful influence on root fresh weight of tomato seedlings. All the treatment was significantly different when proline was sprayed on the tomato. Among various concentrations of proline, the highest upturn in root fresh weight (0.59 mg) was observed in treatment (25°C with 1.5 mM L^{-1} pro), while an intermediate response regarding root fresh weight (0.51 mg) was recorded in treatment (40°C with 1.5 mM L^{-1} pro). The lowest root fresh weight (0.47) was perceived by treatment (45°C with 1.5 mM L^{-1} pro). Afterward, the interaction among different temperature strains and exogenous applied proline was found to be non-significant for root fresh weight. High temperatures harshly decline the root fresh weight in many vegetables. This decline in root fresh weight may be due to the inadequate stock of metabolites in undeveloped emergent tissues because metabolite production is considerably agitated by heat stress, whichever is due to a limited supply of water uptake or the detrimental effect of Na Cl^+. Our findings favour the results found by (Giri et al., 2017) who identified that high temperature decreased root weight and production of tomato.

Root dry weight (mg)

Statistical assessment from (Table 1) concluded that root dry weight in tomatoes was reduced under heat stress. With different levels of proline applied indicated highly significant results. The maximal root dry weight (0.25 mg) was noted at 25°C with 1.5 mM L^{-1} pro, while the lowest root dry weight (0.12 mg) was observed at 45°C with 1.5 mM L^{-1} pro. However, an intermediate response (0.11 mg) was observed in treatment (40°C with 1.5 mM L^{-1} pro). Results concerning the interaction among different temperature strains and exogenous applied proline for this character were found to be significant. Our observation confirmed the results obtained by (Alsamir et al., 2017) that Dry matter content was considerably provoked by high-temperature strain, and heat stress encouraged alterations in the physiology of tomato plants or might modify the configuration of growth.

Physiological Attributes

Photosynthetic rate

Statistically significant results in (Table 2) revealed that under heat stress photosynthetic rate was decreased. Maximum photosynthesis (3.52 µmolm^{-2}s^{-1}) was noted in treatment (25°C with 1.5 mM L^{-1} pro), followed by (3.23 µmolm ^{2}s^{-1} $^{-}$) at treatment (40°C with 1.5 mM L^{-1} pro), while treatment (45°C with 1.5 mM L^{-1} pro) revealed the minimum rate of photosynthesis (3.06 µmolm^{-2}s^{-1}) in tomato plants. The interaction of high-temperature strain and foliar application of proline showed non-significant variations in photosynthetic rate. Our results hence approve the conclusions of (Inayat et al., 2024), that proline application in radish has improved photosynthetic activities by maintaining cell turgor, stabilizing membranes under abiotic salt stresses.

Chlorophyll contents

Statistically significant analysis from (Table 2) argued that under heat stress chlorophyll contents were vastly affected in tomato plants. The highest chlorophyll contents (12.87 spad) were observed by treatment (25°C + 1.5 mM L^{-1} pro), followed by 12.27 spad at treatment (40°C + 1.5 mM L^{-1} pro), while treatment (45°C + 1.5 mM L^{-1} pro) showed the lowest chlorophyll contents (9.25 spad) in tomato plants. Moreover, the cumulative influence of heat stress and foliar-applied proline was noted as non-significant for this parameter. Chlorophyll contents were reduced when plants were subjected to an extraordinary temperature strain. These outcomes align with the observations of several past researchers (Jahan et al., 2019 and Zhou et al., 2019) who explored that the drastic rise in temperature may lead to the decline in chlorophyll contents in plants that hamper plant growth. However, (Inayat et al., 2024) demonstrated that under salinity in radish chlorophyll contents showed positive relationship when proline was applied.

Stomatal Conductance

The findings assessed in (Table 2) indicated that stomatal conductance was boosted under the influence of heat stress. The stomatal conductance was substantially affected by the proline concentrations.

Stomatal conductance decreased with the use of proline on tomato plants. The highest stomatal conductance (0.028 µmol m-2s-1) was noted from treatment (25°C + 1.5 mM L^{-1} pro), followed by (0.020 µmol m-2s-1) treatment (40°C + 1.5 mM L^{-1} pro), while treatment (45°C + 1.5 mM L^{-1} pro) indicated the least amount of stomatal conductance (0.014 µmol m-2s-1). The investigations about the interaction between foliar application of proline and high temperature stress were found significant for stomatal conductance. Stomatal conductance was increased under heat stress which directly alters plant water relations and photosynthesis (Urban et al., 2017). Our observations are in agreement with the results of (Gadallah et al., 1999) who published that in Vicia faba, the upper and lower stomata reacted in different ways with several concentrations of exogenously applied proline, showing that the stomata on abaxial surfaces were more resistive than those on axial surfaces when treated with proline. It is thus evident that the control of stomatal behaviour can be influenced by the application of proline during stress.

Transpiration rate

The outcomes that were displayed in (Table 2) sponsored that transpiration rate was significantly affected under the influence of heat stress. A decline was recorded in the status of transpiration rate in response to high temperature strain. The maximum transpiration rate (2.56 µ $mol/m^{-2}s^{-1}$) was noted at treatment (25°C + 1.5 mM L^{-1} pro), followed by (2.38 µ $mol/m^{-2}s^{-1}$) in treatment (40°C + 1.5 mM L^{-1} pro), while treatment (45°C + 1.5 mM pro) exhibited the lowest transpiration rate (1.96 µ $mol/m^{-2}s^{-1}$). The interaction between foliar application of proline and high temperature stress were found to be non-significant for this trait. However, among various concentrations of exogenous applied proline, a 1.5 mM L^{-1} pro level was found to be best under heat stress. Our results are matching with (Rajametov et al., 2021) who investigated that under heat stress high proline content combined with increased transpiration rate maintain a constant level of photosynthetic rate which allows rapid recovery of heat damage to heat-tolerant cultivars.

Conclusion

Tomatoes cultivation is severely hindered by heat stress therefore; it is need of the day to recognise stress-tolerant characteristics of tomatoes that can thrive in high-temperature conditions. Exogenously application of proline has a positive role in alleviating the waves of heat stress in tomato seedlings. The results of this study suggested that foliar spraying on tomato plants with proline could mitigate the negative impacts of heat stress and promote both the plant's phenological and physiological responses. The recommended concentration of proline (1.5 mM L^{-1}) may help to reach certain goals of thermo-tolerance, since foliar applications of proline in this experiment amended different aspects of plant growth and other growth attributes like no of leaves, shoots fresh weight, chlorophyll contents etc. Under heat stress (40°C and 45°C), and an exogenous foliar application strategy of 1.5 mM L^{-1} proline could be advantageous for improving the plant physiological changes during the early growth stage of tomato plants.

Declarations

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Conflicts of Interest

The authors declare no conflict of interest.

Authors Contribution Statement

Performed the experiments: S.U.R., Analysed the data: S.U.R. & A.A., Contributed materials/ analysis/ tools: A.H., S.N. Wrote the paper: S.U.R., A.A., & S.N., Revision M.U.S., & W.A.

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