

SALICYLIC ACID ASSOCIATED MODULATION OF PHYSIOLOGICAL ATTRIBUTES OF HEAT TOLERANT AND HEAT SENSITIVE TOMATO GENOTYPES RESULTS IN HIGHER YIELD

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Abstract

Heat stress is an important factor curtailing yield in summer crops, particularly in semi-arid and plains having a sub-tropical climate of the globe. It not only lowers the yield but also results in a squeezed growing season because of suppressed reproductive growth and hastened maturity. An experiment was designed under field conditions to study the physiological and biochemical changes in various tomato genotypes owing to the heat stress. Tomato seedlings were raised in nursery on 15 February, 01 March and 15 March. One-month old seedlings were transplanted in the field on 15 March, 01 April and 15 April respectively. Furthermore, salicylic acid (SA) mediated modulation of the processes was also studied in relation to yield. The results revealed that SA enhanced photosynthesis, intrinsic water-use efficiency, and chlorophyll contents. Whereas, the transpiration rate was non-significant. Furthermore, significantly lowered electrolyte leakage, sub-stomatal conductance to CO₂ and water, and the temperature of the leaf surface were observed. These significant variations resulted in increased trusses per plant, fruits per truss, fruits per plant, the average weight of fruit, and ultimately per plant yield. It was concluded that under field conditions salicylic acid increased the heat tolerance in tomato plants by altering the physiological processes. A more vibrant response was noted in heat-tolerant genotypes as compared to heat-sensitive ones. It was further confirmed that SA enhanced heat resistance to a certain level of stress (44°C) and became lessened under extremely high temperature ($\geq 47^\circ\text{C}$) conditions.

Key words: Vegetables; Drought stress; High temperature; Photosynthesis; Fruit yield.

Introduction

Crops are significantly affected by abiotic stresses restraining yield (Araus *et al.*, 2002, Lalarukh *et al.*, 2022). It is known that major abiotic stresses are heat, drought, and salt stress. These stresses lessen plant growth and eventually affect harvest (McCue *et al.*, 1990; Hussain *et al.*, 2021). Besides all these, heat stress appears to be imposing thoughtful losses in crops like a tomato by lessening growth span, as plant undergoes wilting; deficiency of fruit set, and enforced maturity from May onward in the Province of Punjab. Central Punjab (the center of this study) has desiccated and scorching summer with a semi-arid climate. The normal temperature of the zone is above 40°C with temperature crests above 45°C (Syed *et al.*, 2021). This scenario is to worsen more in the future under changing climate (Huddleston, 2012; Syed *et al.*, 2021).

Photosynthetic food forming process in plants is one of the most heat-sensitive procedures and can entirely be terminated before any other signs of stress becomes evident (Abdelmageed, 2009) Heat tolerant genotypes have a higher CO₂ assimilation rate rather than heat-sensitive under heat stress environment and are ascribable to their effective photosynthetic apparatus. Reduced stomatal conductance under heat stress is evident from the literature and it might be due to lack of diffusion of CO₂ in chloroplast because of partial stomatal resistance. Higher fruit set concerning

higher photosynthetic rate is evident that under heat stress conditions rate of photosynthesis can be used as a signal of heat tolerance in tomatoes. Respiration rate is reduced due to heat stress resultantly there is higher transpiration. Higher transpiration rate causes buds, flowers, and fruits ultimately lesser yield. Vegetative and reproductive growth and gaseous movement due to genetic diversity in genome provide us with useful information about cultivar potential for higher yield under high-temperature environments (Hussain *et al.*, 2021).

There is a key role of Salicylic acid in several plant physiological and biochemical processes, helpful for maintaining growth and yield (Arberg, 1981). Foliar application of salicylic acid in wheat increased chlorophyll contents (Hayat *et al.*, 2005), chlorophyll contents increment is direct indication of increased photosynthesis in plants as reported in the literature (Ghai *et al.*, 2002; Fariduddin *et al.*, 2003), in case of *Brassica juncea* and *B. napus*. In the case of soybean and corn, foliar application improved the transpiration, increased area of the leaf, and accelerated the carbon assimilation (Khan *et al.*, 2003). Exogenously applied salicylic acid proved to be beneficial in wheat by increasing yield through increased plant height, leaf numbers, area of leaves, the diameter of the stem, and dry mass of the plant as a whole (Hussein *et al.*, 2007). Reduction in plant metabolism result in lesser growth and yield (Ramagopal, 1987) and increased by the

foliar application of salicylic acid (Shakirova, 2007). Another study proved increased root system in *Tagetes erecta* (Sandoval-Yapiz, 2004) and soybean (Gutierrez-Coronada *et al.*, 1998) when there was a foliar application of Salicylic acid. A vital attribute for yield i.e. flowering was enhanced in *Sinningia* species when there was a spray of salicylic acid (Martin-Mex *et al.*, 2005). Fruit set was also increased in Papaya by exogenous application of salicylic acid (Martin-Mex *et al.*, 2005). Results showing increased yield by SA application were reviewed in tomato and cucumber (Larque-Saavedra & Martin-Mex, 2007).

The present series of experiments instigated with the screening of 191 genotypes of tomato for their thermal tolerance potential (Study-I), then they were grouped into tolerant and sensitive ones (Shaheen *et al.*, 2016). Salicylic acid was then optimized (Study-II) to elucidate the adverse effects of heat stress on tomato (Shaheen *et al.*, 2019). This experiment presented here was planned to probe the positive effect of salicylic acid in the modulation of varying degrees of temperature stress on selected tomato lines under field settings. Physio-biochemical and yield-related parameters were recorded to get the final conclusion. Genotypes used in this study can be exploited by the breeder to improve heat tolerance in tomato. Further Salicylic acid solution @ 1.5mM can be used by the farmers to cope with the deleterious effects of heat stress in tomato.

Material and Methods

Seed sowing, transplanting, and meteorological details:

Two heat tolerant (L00090 and L00091) and two heat-sensitive (CLN1462A and CLN1466E) were screened out from a set of 191 genotypes of tomatoes imported from The World Vegetable Center Taiwan were planted in field at Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Punjab, Pakistan (Shaheen *et al.*, 2016). Seeds were sown (Mid February, 01 March and Mid March 2019) in pots containing sterilized peat moss (50% organic matter). So it is growing substrate represented by peat moss 1 kg in each pot as growth media in three splits (Mid February, 01 March and Mid March 2019) to obtain the 04 weeks old seedlings for transplanting under field settings as per treatments. Hoagland's nutrition recipe was used for a nutritional purpose. Seedlings were grown in a growth chamber with a controlled environment (28°C day and 22°C night temperature). Three different times were decided for field transplantation based on the climatic data of the experimental site (Vegetable Research Area, Agriculture University, Faisalabad, Pakistan (31°26'02" N 73°04'36" E) with elevation from sea level 186 m (Fig. 1). Seedlings of four weeks were transplanted in the field in accord with the treatment plan i.e., T₁ Control (after 30 days: optimum sowing time Mid march), T₂ (at 45 days seedling age: late 01 April) and T₃ (at 60 days seedling age: very late Mid April), to examine the effect of foliar applied salicylic acid in selected tomato genotypes in field conditions to reduce the adverse effects of heat stress. Salicylic acid solution @ 1.5mM (Study-II, Glaxosmithkline, Karachi Pakistan) was exogenously applied two weeks after transplanting as optimized in previous study (Shaheen *et al.*, 2019). In addition, the total 480 pots were prepared with three different planting times and four cultivars with or

without salicylic acid application by split-plot experimental design analyzing, the two factors genotype and salicylic acid. Each treatment contained 20 replicates.

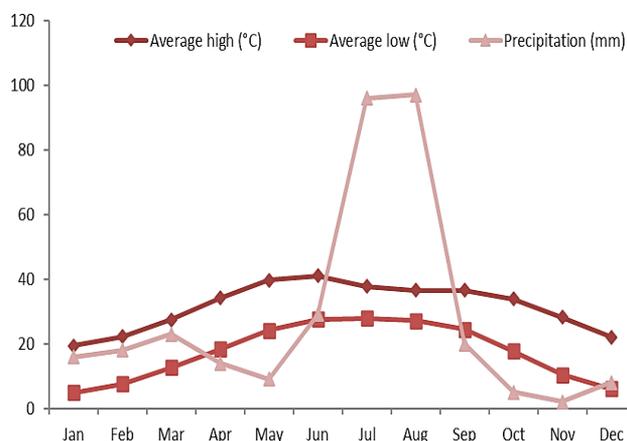


Fig. 1. Climate data of the study area during 2014 (Source: Meteorology Cell, Agriculture University, Faisalabad, Pakistan).

Physiological traits: Infra-Red Gas Analyzer (IRGA) (LCi-SD, ADC Bio-scientific, UK) was used for the recording of physiological traits. Physiological attributes like photosynthesis ($\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), sub-stomatal CO_2 ($\text{mmol CO}_2 \text{ mol}^{-1}$) and stomatal conductance to water ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were recorded. For this purposefully developed three young and healthy leaves plant^{-1} were placed in the chamber of mobile apparatus i.e. Infra-Red Gas Analyzer (IRGA). All the data related to above mentioned physiological parameters was recorded at day time from 10.00 to 12.00 a.m. at air's molar flow per unit area of leaf $403.3 \text{ mmol m}^{-2} \text{ s}^{-1}$, 99.9 kPa atmospheric pressure, 6.0 to 8.9 bar was water vapor pressure in chamber, $1711 \mu\text{mol m}^{-2} \text{ s}^{-1}$ was PAR at surface of leaf, 22.4 to 27.9°C was ambient temperature and CO_2 's ambient concentration was $352 \mu\text{mol mol}^{-1}$ (Zekri, 1991; Moya, 2003). Ratio of photosynthetic rate (Pn) and transpiration rate (E) is defined as water use efficiency (WUE) and measured as:

$$\text{Water use of efficiency (WUE)} = \frac{\text{Photosynthetic rate (A)}}{\text{Transpiration rate (E)}}$$

Biochemical attributes: Chlorophyll meter (CCM-200plus, Bio-Scientific, USA) was used to record the chlorophyll contents (SPAD value). For this purpose leaf at full development stage (same leaf number and development stage) was taken at the base of petiole. Leaves were washed with tap water and then with deionized water. Two samples per treatment were taken from each replication. These samples were contained in tubes having 25 ml deionized water. Tubes were sealed with capes to lessen the evaporation and placed overnight at room temperature. In each tube the solution's conductivity was measured. Then samples in tubes were autoclaved and left to cool down for few hours. After autoclave the solution's conductivity was measured. Ratio of both solution's conductivity before and after autoclave was measured as electrolyte leakage (%). It was supposed that solution's conductivity after autoclave shows complete (100%) electrolyte leakage.

Yield related attributes: For the recording of yield related parameters we selected plants from each replication and computed yield plant⁻¹ on the basis of recorded data. Following yield related attributes were recorded; trusses plant⁻¹, fruits truss⁻¹, fruits plant⁻¹, average weight of fruit.

Statistical analysis

The experiment was planned under randomized complete block design in factorial arrangements with three factors, consisting of two tomato genotypes, 3 times of transplanting of tomato seedlings and Salicylic acid application in 4 blocks as replication. Analysis of data was performed in Statistics 8.1 for windows and Tukey's test at $p \leq 0.05$ was used to analyzed treatment means for significant differences among them. Heat map has been designed by using Microsoft excel.

Results

Effect of SA and transplanting time on transpiration rate and stomatal conductance of water for tomato genotypes: The results showed that there was a non-significant ($p > 0.05$) effect of SA foliar application on transpirational rate (Table 1). There was a significant reduction in stomata's conductance to water due to SA application (Table 1). Lowest value recorded for stomata's conductances in L00090, under T₁ and T₂. CLN166E showed highest conductance to water through stomatas under T₃ with SA application. In treatment 3 sensitive lines did not survive without SA application.

Effect of SA and transplanting time on photosynthesis and water use efficiency of tomato genotypes: Photosynthesis was significantly ($p < 0.01$) increased in tomato lines due to SA application (Fig. 2). Highest photosynthesis recorded in in L00091 sown under T₁ and T₂ and L00090 was at par with L00091 in T₃. There was an efficient response by both L00090 and L00091 to SA application.

The results showed significant increase in WUE due to foliar application of SA in tomato lines (Fig. 2). The highest value for WUE recorded in L00090 in treatment 1 and WUE was at par in L00091 in the same treatment. CLN1462A showed lowest value for WUE in T₃. It was observed that no plant could survive in sensitive genotypes without SA application in T₃.

Effect of SA and transplanting time on sub-stomatal CO₂ (gs) and electrolyte leakage (EL) of tomato genotypes: Sub-stomatal CO₂ significantly ($p < 0.01$) lowered due to SA application in tomato (Fig. 2). Under treatment T₂ L00090 showed low sub-stomatal CO₂ that was equal to sub-stomatal CO₂ in L00091 in treatment 1. There was a decrease in sub-stomatal CO₂ in CLN1462A and CLN1666E genotypes. In treatment 3 highest CLN1462A and CLN1666E were recorded in CLN1466E. EL were significantly lowered with SA application (Fig. 2). Lowest EL at T₁ was recorded in L00090, while CLN1466E showed highest value of EL under T₃. Regardless of transplanting date L00090 and L00091 showed similar values for EL.

Effect of SA and transplanting time on chlorophyll content and number of trusses per plant in tomato genotypes: In chlorophyll contents significant ($p < 0.01$) increase was recorded due to SA application in tomato lines (Table 2). Regardless of sowing date highest value for chlorophyll was recorded in L00090 which was at par with L00091. Regarding chlorophyll contents CLN1462A and CLN1466E fell at 3rd position except 3rd transplanting time. Under treatment 1 highest value for chlorophyll was observed in L00091, L00090 and CLN1462A. Lowest value was recorded in CLN1466 in T₃. Significant increase in trusses plant⁻¹ was observed due to SA application in tomato lines (Table 2). Great increase in L00090 was recorded for trusses plant⁻¹ without considering planting date followed by L00091. Lower number of trusses plant⁻¹ than heat tolerant ones regardless of sowing dates. Under T₁ highest number of trusses plant⁻¹ was recorded in L00091, whereas CLN1462A produced lowest number of trusses plant⁻¹ in treatment 3.

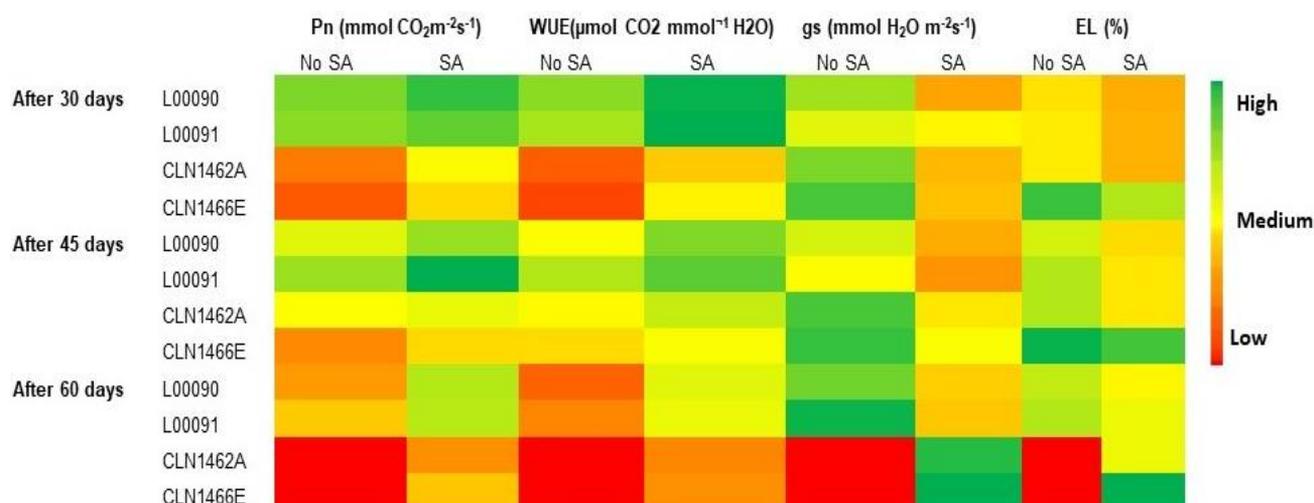


Fig. 2. Effect of SA and transplanting time after 30, 45 and 60 days on photosynthetic rate (Pn mmol CO₂ m⁻² s⁻¹), water use efficiency (WUE μmol CO₂ mmol⁻¹ H₂O), stomatal conductance (gs mmol H₂O m⁻² s⁻¹) and Electrolyte leakage (EL %) of tomato genotypes.

Table 1. Effect of SA and transplanting time on transpiration rate and stomata's conductance to water in tomato genotypes.

SA x Genotypes	Transpiration rate (mmol m ⁻² s ⁻¹)			Stomatal conductance to water (mmol m ⁻² s ⁻¹)				
	T1 (After 30 days)	T2 (After 45 days)	T3 (After 60 days)	Means	T1 (After 30 days)	T2 (After 45 days)	T3 (After 60 days)	Means
No SA × L00090	3.2 ± 0.10dg	3.4 ± 0.17af	4.5 ± 0.13a	3.7 ± 0.19	0.3 ± 0.009ae	0.3 ± 0.023bf	0.3 ± 0.017a-d	0.3 ± 0.010
No SA × L00091	3.4 ± 0.20af	3.4 ± 0.22bg	4.4 ± 0.15abc	3.86 ± 0.17	0.25 ± 0.008bg	0.2 ± 0.011ch	0.3 ± 0.015a	0.3 ± 0.015
No SA × CLN1462A	3.9 ± 0.43ae	4.3 ± 0.12ad	0 ± 0.00i	2.8 ± 0.61	0.29 ± 0.017ae	0.3 ± 0.009abc	0 ± 0.000k	0.2 ± 0.044
No SA × CLN1666E	3.8 ± 0.40ae	4.5 ± 0.12ab	0 ± 0.00i	2.8 ± 0.61	0.3 ± 0.006abc	0.3 ± 0.016ab	0 ± 0.000k	0.2 ± 0.046
SA × L00090	2.2 ± 0.21gh	2.8 ± 0.18eh	3.6 ± 0.20ae	2.9 ± 0.20	0.1 ± 0.013ij	0.2 ± 0.013hij	0.25 ± 0.006fj	0.2 ± 0.008
SA × L00091	2.4 ± 0.13fgh	3.3 ± 0.42cg	3.8 ± 0.15ae	3.2 ± 0.22	0.2 ± 0.024di	0.1 ± 0.022j	0.2 ± 0.014fj	0.2 ± 0.016
SA × CLN1462A	2.1 ± 0.17h	3.7 ± 0.10ae	4.5 ± 0.10a	3.4 ± 0.31	0.2 ± 0.028gj	0.2 ± 0.013ej	0.3 ± 0.022ab	0.2 ± 0.023
SA × CLN1666E	3.4 ± 0.10af	4.1 ± 0.23ad	4.3 ± 0.24abc	3.9 ± 0.16	0.2 ± 0.017fj	0.2 ± 0.008ch	0.3 ± 0.011a	0.2 ± 0.022
Mean	3.1 ± 0.14	3.7 ± 0.11	3.2 ± 0.34		0.2 ± 0.011	0.2 ± 0.012	0.2 ± 0.025	

Table 2. Effect of SA and transplanting time on Chlorophyll content and number of trusses per plant in tomato genotypes.

SA x Genotypes	Chlorophyll content (SPAD value)			Number of trusses per plant			
	T1 (After 30 days)	T2 (After 45 days)	T3 (After 60 days)	Means	T1 (After 30 days)	T2 (After 45 days)	T3 (After 60 days)
34.6 ± 2.68bc	27.2 ± 2.86c	19.1 ± 1.20b	26.9 ± 2.28	13.5 ± 0.65be	8.7 ± 0.63fgh	10.7 ± 0.85cf	11.0 ± 0.70
31.1 ± 2.44c	23.2 ± 2.46cd	14.9 ± 1.17c	23.1 ± 2.28	13.7 ± 0.85be	6.2 ± 0.48ghi	9.5 ± 0.65efg	9.8 ± 0.99
26.9 ± 1.98cd	19.5 ± 1.66e	0.00 ± 0.00e	15.5 ± 3.51	15.0 ± 1.47bc	4.5 ± 0.65hi	0.0 ± 0.00j	6.5 ± 1.96
25.1 ± 2.17cd	17.7 ± 2.42ef	0.00 ± 0.00e	14.3 ± 3.32	10.2 ± 0.48dg	7.2 ± 0.25fi	0.0 ± 0.00j	5.8 ± 1.31
50.9 ± 3.82a	37.5 ± 1.81a	25.6 ± 1.10a	38.0 ± 3.39	15.7 ± 0.85ab	17.5 ± 1.32ab	15.7 ± 0.48ab	16.3 ± 0.56
50.7 ± 6.21a	34.4 ± 2.68ab	24.7 ± 1.76a	36.6 ± 3.86	19.7 ± 0.75a	11.2 ± 0.85cf	13.7 ± 0.85be	14.9 ± 1.16
50.4 ± 3.90a	31.2 ± 2.82b	12.3 ± 1.50c	31.3 ± 4.93	14.2 ± 1.03bcd	7.7 ± 0.95fi	3.5 ± 0.65ij	8.5 ± 1.41
38.35 ± 1.18b	27.9 ± 1.25c	6.1 ± 0.74d	24.1 ± 4.09	13.2 ± 1.03be	9.5 ± 0.65efg	4.7 ± 0.85hi	9.2 ± 1.14
38.5 ± 2.11	27.3 ± 1.38	12.8 ± 1.74	14.4 ± 0.54	14.4 ± 0.54	9.0 ± 0.71	7.2 ± 1.04	

Table 3. Effect of SA and transplanting time on the number of fruits per truss and number of fruits per plant in tomato genotypes.

SA x Genotypes	Number of fruits per truss			Number of fruits per plant			
	T1	T2	T3	Means	T1	T2	T3
No SA×L00090	4.7 ± 0.48abc	6.0 ± 0.41a	5.7 ± 0.63ab	5.5 ± 0.31	64.2 ± 7.49cd	53.0 ± 6.28b	61.7 ± 7.94b
No SA×L00091	4.7 ± 0.48abc	5.5 ± 0.29abc	4.7 ± 0.25abc	5.0 ± 0.21	66.2 ± 10.51c	34.5 ± 3.52de	45.5 ± 4.94c
No SA×CLN1462A	4.2 ± 0.48abc	4.0 ± 0.41abc	0.0 ± 0.00d	2.7 ± 0.62	64.2 ± 9.51cd	37.0 ± 15.5d	0.0 ± 0.00e
No SA×CLN1666E	4.5 ± 0.65abc	4.2 ± 0.48abc	0.0 ± 0.00d	2.9 ± 0.67	46.0 ± 7.11e	30.7 ± 3.33de	0.0 ± 0.00e
SA × L00090	5.5 ± 0.65abc	6.0 ± 0.41a	5.7 ± 0.85ab	5.7 ± 0.35	80.0 ± 7.70ab	94.5 ± 2.87a	81.2 ± 5.82a
SA × L00091	5.5 ± 0.29abc	4.5 ± 0.65abc	4.5 ± 0.65abc	4.8 ± 0.32	86.0 ± 4.06a	49.5 ± 5.81bc	63.2 ± 12.5b
SA × CLN1462A	5.2 ± 0.48abc	5.5 ± 0.65abc	3.2 ± 0.48bc	4.7 ± 0.41	74.2 ± 7.47b	44.7 ± 8.77bc	14.0 ± 2.48d
SA × CLN1666E	4.7 ± 0.25abc	4.0 ± 0.41abc	3.0 ± 0.41c	3.9 ± 0.29	62.5 ± 4.33cd	38.7 ± 6.39d	13.7 ± 2.46d
Mean	4.9 ± 0.17	4.9 ± 0.21	3.4 ± 0.42		67.9 ± 3.13	47.8 ± 4.15	34.9 ± 5.67

Table 4. Effect of SA and transplanting time on fruit weight and yield of tomato genotypes.

SA x Genotypes	Average fruit weight (grams)			Yield per plant (grams)			
	T1	T2	T3	Means	T1	T2	T3
No SA×L00090	56.8 ± 4.39bcd	71.9 ± 1.64a	56.0 ± 3.97bcd	61.6 ± 2.88	3733.0 ± 670.59b	3828.4 ± 501.76b	3466.8 ± 534.7b
No SA×L00091	40.6 ± 1.36efg	49.3 ± 1.36def	48.0 ± 2.87def	45.9 ± 1.56	2692.1 ± 428.91bcd	1691.4 ± 158.52cd	2224.7 ± 357.9c
No SA×CLN1462A	18.9 ± 2.22ij	16.5 ± 0.82ij	0.0 ± 0.00k	11.8 ± 2.63	1195.2 ± 218.99e	603.6 ± 244.77fg	0.0 ± 0.000f
No SA×CLN1666E	15.0 ± 1.28ij	25.6 ± 1.60hij	0.0 ± 0.00k	13.5 ± 3.22	710.5 ± 163.62g	774.3 ± 065.73ef	0.0 ± 0.000f
SA × L00090	64.7 ± 4.21abc	68.1 ± 2.90ab	56.6 ± 7.54bcd	63.1 ± 3.11	5263.3 ± 817.08a	6430.4 ± 276.85a	4626.2 ± 744.9a
SA × L00091	34.8 ± 2.01fgh	43.1 ± 1.59dg	50.2 ± 3.64cde	42.7 ± 2.33	3003.3 ± 278.38bc	2121.5 ± 210.48c	3200.8 ± 688.9bc
SA × CLN1462A	18.6 ± 2.04ij	20.1 ± 0.95hij	20.6 ± 1.39hij	19.8 ± 0.83	1410.6 ± 277.06ef	898.9 ± 187.04ef	287.4 ± 56.74de
SA × CLN1666E	12.4 ± 2.20jk	29.5 ± 1.67ghi	22.1 ± 1.23hij	21.3 ± 2.30	781.1 ± 144.45g	1166.5 ± 232.48e	311.7 ± 73.73d
Mean	32.7 ± 3.46	40.5 ± 3.61	31.7 ± 4.18		2348.6 ± 304.29	2189.4 ± 346.68	1764.7 ± 337.43

Effect of SA and transplanting time on number of fruits per truss and number of fruits per plant:

The results showed a significant ($p < 0.01$) increase in fruit number truss⁻² by SA foliar application under heat stress condition (Table 3). Less number of fruit truss⁻¹ was recorded in L00090 followed by L00091 ignoring sowing date. Less number of fruit truss⁻¹ was recorded in sensitive genotypes compared with tolerant but at par for each other ignoring the sowing times. Under treatment T₂ and T₃ higher number of fruits truss⁻¹ were recorded in L00090 whereas it was minimum in CLN1466E under T₃. SA application showed an increase of fruits plant⁻¹ (Table 3). Maximum number of fruits plant⁻¹ was recorded in L00090 followed by L00091 irrespective of the sowing date. A minimum number of fruits plant⁻¹ was found in sensitive lines compared with tolerant ones. Under T₂ highest fruit number plant⁻¹ was recorded in L00090 whereas it was lowest in CLN1466E and CLN1462A under T₃.

Effect of SA and transplanting time on fruit weight and yield of tomato genotypes:

Average fruit weight significantly ($p < 0.01$) increased by SA application without considering the sowing date (Table 4). The highest fruit weight was recorded in L00090 at all treatments whereas the lowest fruit weight was observed in CLN1462A that was at par with CLN1466E. It was also found that L00091 produced less fruit weight under T₁ and T₂. Yield plant⁻¹ in tomato lines was significantly increased by SA application grown under stress conditions (Table 4). Maximum yield plant⁻¹ was recorded in L00090 followed by L00091, irrespective of sowing time. At T₃ yield was less in CLN1462A and CLN 1466E under stress condition.

Discussion

The current investigation showed a non-significant change for the rate of transpiration and reduction in stomatal conductance, which supports by a previous study wherein transpiration rate was lessened in Common beans and Asiatic dayflower was witnessed owing to SA exogenous application and this reduction in the transpiration rate was ascribed to the fact that closure of stomata is due to the salicylic acid application (Bharath *et al.*, 2021; Larque-Saavedra, 1979). Whereas contrary to our findings, a previous finding showed an increment in transpiration rate and stomatal conductance in response to exogenous application of SA in corn and soybean (Khan *et al.*, 2003). However, contrary to these results, findings of the present study confirmed with some past reports that SA foliar application encourages tolerance against abiotic and biotic stresses (Delany *et al.*, 1994; Senaratna *et al.*, 2000). A previous investigation on tomatoes under heat stress described a positive decrease in photosynthesis and further confirmed that reduction in photosynthesis was more prompt in heat-sensitive genotypes rather tolerant ones. Also, a 50% reduction in CO₂ assimilation occurred in sensitive genotypes (Camejo *et al.*, 2005; Nazir *et al.*, 2017). The results of the current investigation confirm a

previous report wherein it was informed that the SA application enhanced the photosynthesis in tomato grown under heat stress conditions (Ananieva *et al.*, 2002; Stevens *et al.*, 2006). Under drought conditions, SA provides protection to the photosynthetic system (Rajasekaran *et al.*, 1999; Singh *et al.*, 2003). Salicylic acid-mediated enhancement of intrinsic water use efficiency witnessed in the present study supports a previous finding on soybean and water use efficiency was increased due to foliar-applied SA (Kumar *et al.*, 2000). They also reported enhanced internal CO₂ concentration, which was also contrarily reduced in the present investigation.

In the present study, the SA application by foliar method lessened the electrolyte leakage in tomatoes under heat stress. Another study reported a 44% reduction in electrolyte leakage in SA treated crops as compared to the non-treated ones (Stevens *et al.*, 2006). Increased chlorophyll owing to salicylic acid application confirms some previous report that temperature stress lessened the contents of chlorophyll as observed in tomato genotypes and increased contents of chlorophyll in wheat seedlings grown from seeds pretreated with SA (Hayat *et al.*, 2005; Camejo *et al.*, 2005).

Results showed that by foliar application of SA there was an increase in trusses per plant, fruits per truss under heat stress conditions. Similarly, in flowering Spirodela and yield in cucumber, increased due to foliar application of aspirin combined with SA (Larque-Saavedra and Martin-Mex, 2007; Khurrana and Maheswari, 1980).

Thus, it may be concluded that salicylic acid acts as an endogenous regulator that potentially affects the growth and productivity of plants. The present study showed that salicylic application significantly enhanced the number of fruits per plant, average fruit weight, and ultimately increased per plant yield. Furthermore, a positive correlation between photosynthetic rate and fruit set has been reported earlier (Abdel mageed & Gruda 2009]. So, the photosynthetic rate can be used as an indicator to estimate the heat tolerance potential of a genotype. The present study also confirms some previous findings that exposure to a high temperature above optimum reduced the fruit fresh weight in tomato and reverse can be attained by exogenous SA application as witnessed in the present investigation (Sato *et al.*, 2000; Firon *et al.*, 2006).

Conclusions

It can be concluded from the results that salicylic acid increased the heat tolerance in tomato plants under field conditions. Tolerant Genotypes (L00090 and L00091) responded more efficiently to SA application as compared to the sensitive ones (CLN1462A and CLN1466E) in many of the attributes studied. It was further noted that SA induced heat resistance to a certain level of stress (44°C) and became less effective under extreme conditions of high temperature (above 47°C). Further farmers can use SA@1.5mM to extend their production period in high temperature prevailing areas.

References

- Abdelmageed, A.H.A. and N. Gruda. 2009. Performance of different tomato genotypes in the arid tropics of Sudan during the summer season. ii. generative development. *J. Agri. Rural Dev. Tropics Subtropics.*, 110: 147-154.
- Abdelmageed, A.H.A., N. Gruda and M.M.A. El-Balla. 2009. Performance of different tomato genotypes in the arid tropics of Sudan during the summer season. *J. Agri. Rural. Dev. Tropics Subtropics.*, 110: 137-145.
- Ananieva, E.A., Alexieva and L.P. Popova. 2002. Treatment with salicylic acid decreases the effects of paraquat on photosynthesis. *J. Plant Physiol.*, 159: 685-693.
- Araus, J.L., G.A. Slafer, M.P. Reynolds and C. Royo. 2002. Plant breeding and drought in C₃ cereals: what should we breed for? *Annals of Botany*, 89(7): 925-940.
- Arberg, B. 1981. Plant growth regulators. Monosubstituted benzoic acid. *Swed Agric Res.*, 11: 93-105.
- Bharath P, Gahir S, Raghavendra AS. 2021. Abscisic Acid-Induced Stomatal Closure. An Important Component of Plant Defense Against Abiotic and Biotic Stress. *Front. Plant Sci.*, 12: 615114. doi: 10.3389/fpls.2021.615114
- Camejo, D., Rodriguez, P., Morales, M.A., Dell'Amico, J.M., Torrecillas and J.J. Alaron. 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J. Plant Physiol.*, 162: 281-289.
- Delany, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward and J. Ryals. 1994. A central role of salicylic acid in plant disease resistance. *Sci.*, 266: 1247-1250.
- FAO. 1998. Grassland and Pasture Crops: Country Pasture and Forage Profiles, Pakistan.
- Fariduddin, Q., Hayat and A. Ahmad. 2003. Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in Brassica juncea. *Photosynthetica*, 41: 281-284.
- Firon, N., R. Shaked, M.M. Peet, D.M. Pharr, E. Zamski, K. Rosenfeld, Althan and E. Pressman. 2006. Pollen grains of heat tolerant tomato cultivars retain higher concentration under heat stress conditions. *Sci. Hort.*, 109: 212-217.
- Ghai, N., R.C. Setia and N. Setia. 2002. Effects of paclobutrazol and salicylic acid on chlorophyll content, hill activity and yield components in *Brassica napus* L. (cv. GSL-1). *Phytomorphology*, 52: 83-87.
- Gutierrez-Coronado, M., C.L. Trejo and A. Larque-Saavedra. 1998. Effects of salicylic acid on the growth of roots and shoots in soybean. *Plant Physiol. Biochem.*, 36: 563-565.
- Hanna, H.Y. and T.P. Hernandez. 1982. Response of six tomato genotypes under summer and spring weather conditions in Louisiana. *Hort Sci.*, 17: 758-759.
- Hayat, S., Q. Fariduddin, Ali and A. Ahmad. 2005. Effect of salicylic acid on growth and enzyme activities of wheat seedlings. *Acta Agron Hung.*, 53: 433-437.
- HKO. Hong Kong Observatory data. <http://www.meteo.fr/test/gratuit/wwis/047/c00891.htm> 2012.
- Huddleston, N. 2012. Evidences, Impacts and Choices. National Research Council, National Academy of Sciences, USA. *Climate Change.*, pp. 3-4.
- Hussain, R., C.M. Ayyub, M.R. Shaheen, S. Rashid, M. Nafees, S. Ali, M. Butt, M. Ali, A. Maqsood, S. Fiaz, S. Ahmar, T. Mahmood and F.M. Poblete. 2021. Regulation of osmotic balance and increased antioxidant activities under heat stress in *Abelmoschus esculentus* L. triggered by exogenous proline application. *Agronomy*, 11: 685:1-16. <https://doi.org/10.3390/agronomy11040685>
- Hussain, T., I.A Khan, M.A. Malik and Z. Ali. 2006. Breeding Potential for high temperature tolerance in corn (*Zea mays* L.). *Pak. J. Bot.*, 38: 1185-1195.
- Hussein, M.M., Balbaa, L.K and M.S. Gaballah. 2007. Salicylic acid and salinity effects on growth of maize plants. *Res. J. Agric. Biol. Sci.*, 3(4): 321-328.
- Khan, W., B. Prithviraj and D.L. Smith. 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. *J. Plant Physiol.*, 160: 485-492.
- Khurana, J.P. and S.C. Maheshwari. 1980. Some effects of salicylic acid on growth and flowering in *Spirodela polyrrhiza* SP20. *Plant Cell Physiol.*, 21: 923-927.
- Kumar, P., Lakshmi and V.P. Mani. 2000. Interactive effects of salicylic acid and phytohormones on photosynthesis and grain yield of soybean (*Glycine max* L. Merrill). *Physiol. Mol. Biol. Plants*, 6: 179-186.
- Lalarukh, I., S.A. Al-Dhumri, L.K.T. Al-Ani, R. Hussain, K.A. Al-Mutairi, N. Mansoor, S.F. Amjad, M.H.H. Abbas, A.A. Abdelhafez, P. Pocza, K.R. Meena and T.M. Galal. 2022. A combined use of rhizobacteria and moringa leaf extract mitigates the adverse effects of drought stress in wheat (*Triticum aestivum* L.). *Front. Microbiol.*, 13: 813415. doi: 10.3389/fmicb.2022.813415.
- Larque-Saavedra, A. 1979. Stomatal closure in response to acetylsalicylic acid treatment. *Z. Physiol.*, 93(4): 371-375.
- Larque-Saavedra, A. and F. Martin-Mex. 2007. Effects of salicylic acid on the bioproductivity of the plants. In: (Eds.): Hayat, S. and A. Ahmad. Salicylic Acid, *A Plant Hormone. Springer Publishers, Dordrecht, Netherlands.*
- Martin-Mex, R., E. Villanueva-Couoh, Herrera-Campos and A. Larque-Saavedra. 2005. Positive effect of salicylates on the flowering of African violet. *Sci. Hort.*, 103: 499-502.
- McCue, K.F. and A.D. Hanson. 1990. Drought and salt tolerance: toward understanding and application. *Trends Biotechnol.*, 8: 358-362.
- Moya, L.J., A. Gomez-Cadenas, E. Primo-Millo and M. Talon. 2003. Chloride absorption in salt-sensitive Carrizo citrange and salt-tolerant *Cleopatra mandarin* citrus rootstocks is linked to water use. *J. Exp. Bot.*, 54: 825-833.
- Nazir, A., M.R. Shaheen, C.M. Ayyub, R. Hussain, N. Sarwer, M. Imran, M. Aurangzaib, M. Nawaz, M.F.A. Khan, Jawad and M. Iqbal. 2017. Exploring the better genetic options from indigenous material to cultivate tomato under high temperature regime. *J. Appl. Bot. & Food Qual.*, 90: 339-345. DOI:10.5073/JABFQ.2017.090.042.
- Rajasekaran, L.R. and T.J. Blake. 1999. New plant growth regulators protect photosynthesis and enhance growth under drought of jack pine seedlings. *J. Plant Growth Regul.*, 18: 175-181.
- Ramagopal, S. 1987. Salinity stress induced tissue specific proteins in barley seedlings. *Plant Physiol.*, 84: 324-331.
- Sandoval-Yapiz, M.R. 2004. Reguladores de crecimiento XXIII: efecto del acido salicilico en la biomasa del cempazuchitl (*Tagetes erecta*). *Tesis de Licenciatura. Instituto Tecnológico Agropecuario, Conkal, Yucatan, Mexico.*
- Sato, S., M.M. Peet and J.F. Thomas. 2000. Physiological factors limit fruit set of tomato (*Lycopersicon esculentum* Mill.) under chronic, mild heat stress. *Plant Cell Environ.*, 23: 719-726.
- Senaratna, T., D. Touchell, Bunn and K. Dixon. 2000. Acetyl salicylic acid (aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regul.*, 30: 157-161.
- Shaheen, M.R., Ayyub, C.M., Amjad and E.A. Waraich. 2016. Morpho-physiological evaluation of tomato genotypes under high temperature stress conditions. *J. Sci. Food Agric.*, 96: 2698-2704.

- Shaheen, M.R., C.M. Ayyub, R. Hussain, A. Manan, Z. Mustafa, M. Sarwar, M. Ahsan, N. Sarwar and M. Imran. 2019. Salicylic acid improved the heat tolerance by enhancing growth, gas exchange attributes and chlorophyll contents of tomato. *Acta Hortic.*, 1257(23): 161-168.
- Shakirova, F.M. 2007. Role of hormonal system in the manifestation of growth promoting and anti-stress action of salicylic acid. In: (Eds.): Hayat, S. and A. Ahmad. Salicylic Acid, A Plant Hormone. *Springer, Dordrecht, Netherlands*.
- Singh, B. and K. Usha. 2003. Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul.*, 39: 137-141.
- Singh, R.P., Prasad, P.V.V., Sunita, K., Giri and K.R. Reddy. 2007. Influence of high temperature and breeding for heat tolerance in cotton. *Adv. Agron.*, 93: 313-385.
- Stevens, J., Senaratna and K. Sivasithamparam. 2006. Salicylic acid induces salinity tolerance in tomato (*Lycopersicon esculentum* cv. Roma): Associated changes in gas exchange, water relations and membrane stabilization. *Plant Growth Regul.*, 49: 77-83.
- Syed, A., X. Liu, M. Moniruzzaman, I. Rousta, W. Syed, J. Zhang and H. Olafsson. 2021. Assessment of climate variability among seasonal trends using in situ measurements: A case study of Punjab, Pakistan. *Atmosphere*, 12, 939. <https://doi.org/10.3390/atmos12080939>
- Zekri, M. 1991. Effects of NaCl on growth and physiology of sour orange and Cleopatra mandarin seedlings. *Sci. Hortic.*, 47: 305-315.

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