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Thermo-Tolerance in Tomato: Acetyl Salicylic Acid Affects Growth and Yield of Tomato (*Solanum Lycopersicum* L.) Under the Agro-Climatic Condition of Islamabad, Pakistan

Atifullah Shinwari,[1] Imran Ahmad,[1] Izharullah Khan,[1] Haseeb Khattak,[1] Ahmad Samir Azimi[2]

[1]University of Agriculture, Department of Horticulture, Peshawar, Pakistan

[2]Researches Organization for Development, Kabul, Afghanistan

Correspondence: Atifullah Shinwari, University of Agriculture, Department of Horticulture, Peshawar, Pakistan, Email atifshinwari@gmail.com

Received: July 09, 2018 | **Published:** October 25, 2018

Abstract

Exogenously applied various levels of acetyl salicylic acid (ASA) including ASA^0 (Control), ASA^1 (0.30mM), ASA^2 (0.60mM), ASA^3 (0.90mM), ASA^4 (1.20mM), ASA^5 (1.50mM) were investigated for induction of thermo-tolerance in tomato seedlings, transplanted in different growth conditions i.e. open field (average 32˚C) and plastic tunnel (average 45˚C/4h daily) in Islamabad, during summer 2016. The experiment was laid out in randomized complete block design (RCBD) with split plot arrangement having twelve treatments, and three replications. Examining different growth conditions, maximum number of days to flowering (18.05), plant height (65.97 cm), stem diameter (1.69 cm), number of leaves plant^{-1} (35.37), number of flowers plant^{-1} (88.61), number of fruits plant^{-1} (39.05), total yield (28.48 tons ha^{-1}), chlorophyll content (58.78 µg.g^{-1}), and leaf relative water content (74.25%) was recorded in the open field. While investigating acetyl salicylic acid levels, ASA4 (1.20 mM) treatment showed significant results and minimized the heat stress with maximum number of leaves recorded (36.28), flowers (105.53), total yield (30.97 tons ha^{-1}), chlorophyll content (62.24 µg.g^{-1}), and leaf relative water content (74%) as compared to heat stress (control). Predominantly, ASA_4 (1.20 mM) produced better results with respect to growth and yield in both growth conditions by enhancing heat tolerance in tomato seedlings

Keywords: acetyl salicylic acid, tomato, tolerance, yield, temperature, growth

Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the family Solanaceae. It is native to the Andean regions of South America. Tomato production fluctuates, that is varied seasonally as well as annually; in 2013-14 the production of tomato was decreased up to 0.65% due to fluctuated temperatures and heavy rainfall and floods while the production also fluctuates in different seasons such as Rabi (October-March) and Kharif (July - October) crop.[1] During the summer months and monsoon season the production of tomato is less. However, with the use of right production techniques and policies, the production can be increased to an adequate level.

Tomato crop is playing a significant role in the food security and safety as it is rich with minerals, vitamins, essential amino acids, sugars and dietary fibers; hence, it contributes to the well-balanced diet, health and food needs. The reason to its role in food security is also evident from its wide range of use such as fresh consumption of tomato in salads, cooked in sauces and fish or meat dishes.[2]

High temperature during early summer not only causes reduction in crop growth and fruit setting but also causes forced maturity.[3] The optimum temperature range for tomato growth is 22-32ºC. Several environmental stresses influence the growth and production of tomato. Higher ambient temperature in summer season is one of the serious problems in tomato growing areas that generate adverse effect on production of tomato; hence, resulting in reduced fruit set and ultimately declined tomato yields.[4] Both the vegetative and reproductive phases and yield of several crops is inhibited by the elevated temperature stress.[5,6] Generally, each 1ºC increase in the average temperature during the growing season may decline the crop yield up to 17%.[7]

Today worldwide, it is imperative to adopt certain new strategies to extend tomato production and improve the yield volumes in increasing temperatures.[8] The yield of tomato crop in Pakistan is far low as compared to advanced countries. The plant growth regulators are known to improve the source sink relationship and stimulate the translocation of photo-assimilates, as a result it helps in better retention of flowers and fruits.[9]

It has been reported that acetyl salicylic acid (ASA) stimulate tolerance against several abiotic stresses including heat stress. It was

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discovered that plants grown from seeds imbibed in aqueous solutions of salicylic acid or acetyl salicylic acid (0.1-0.5 mM) displayed enhanced tolerance and considerable (100%) plant survival against heat, drought stresses and chilling injury.[10] Therefore, in light of above facts the study was aimed to investigate and identify the impact and suitable doses of acetyl salicylic acid for induction of heat tolerance in tomato and to study effect acetyl salicylic acid (ASA) application on growth and yield of tomato.

Materials and methods

Area of study, growth condition, experimental design, and treatments

As a main factor (growth conditions), tomato seedlings (cultivar: Rio Grande) were transplanted in open field (average 32˚C) and plastic tunnel (average 45˚C/4 h recorded daily) at the Directorate of Vegetable, NARC (National agricultural research centre) Islamabad, Pakistan during summer 2016 and was laid out in Randomized complete block design (RCBD) having 2 factors with split plot arrangement. Soil characteristics were analyzed by collecting soil sample from 0-15cm, 15-30cm and 30-60 cm. Soil was coarse loamy, deep and well drained with moderately calcareous nature and low in organic matter. Each treatment was replicated three times. Treatments were designed as ASA_0 (control, only distill water), ASA_1 (0.30mM), ASA_2 (0.60mM), ASA_3 (0.90mM), ASA_4 (1.20mM) and ASA_5 (1.50mM). To recognize the treatments as the only cause agents for variation control treatments were taken in open field as well as inside the plastic tunnel. Temperature recorded in the open field was also above the optimum temperature requiring for tomato growth.

Implementation production of tomato (Nursery raising and transplantation)

The tomato cv. Rio Grande seeds were sown in FYM + garden soil and irrigated through sprinkler regularly. Four weeks later the seedlings were at 3 true leaf stages, and they were transplanted on 2nd April, 2016 into normal open field and under plastic tunnel conditions. Row to row and plant to plant distance was 75 cm and 50 cm respectively.

Heat treatment

For heat treatment the plastic tunnel was built, and the plants were grown under the heat inducing plastic tunnel. Similarly, plants were grown in open field as well, for comparison between the two treatments.

Acetyl salicylic acid (ASA) application

Different levels of acetyl salicylic acid (Sigma-Aldrich) were prepared in distilled water with a minimal quantity of ethanol as a solvent. Six levels of acetyl salicylic acid were applied as foliar spray on tomato plants at 15 days interval.

Temperature and relative humidity

Both temperature and relative humidity were measured on regular basis in the open field condition as well as under the plastic tunnel condition. Temperature was recorded using Thermometer while Hygrometer was used for measuring the relative humidity percentage. The temperature was maintained by the closing and opening of the door of the plastic tunnel. The doors were closed daily for 4 h (10.00 am to 2.00 pm) to increase the temperature (maximum temperature recorded was 60˚C) inside the tunnel. Average temperature 32˚C/45˚C (Open field/ Plastic tunnel) and relative humidity 58%/47.2% (Open field/ Plastic tunnel) were recorded during the whole growing season.

Parameters measured

Chlorophyll content

The chlorophyll content of the leaf was measured with the help of SPAD meter or chlorophyll meter (Spad-502, Konica Minolta camera co. Ltd, Osaka, Japan). The spad was calibrated for measurement, to record the spad units. Five randomly selected plants were used for chlorophyll content measurement in each sub plot and averages were calculated. The leaf was inserted from the leaf tip to reach the middle of the leaf between the leaf margins and midrib. The data was taken from fully expanded leaf at the fruit initiation stage.[11,12]

Leaf relative water content (LRWC)

Leaf relative water content (LRWC) was calculated based on the methods of Gupta et al. (1996).[13] Leaf relative water content or relative turgidity is a very convenient parameter to measure the water status of the plant in relation to the cellular hydration under different stresses. Leaves were collected and its fresh weight was recorded. After weighting, the leaves were dipped and remained inside the distill water for 24 hours. After 24 hours the leaves were fully turgid, again the weight was recorded. This step was followed by drying the leaves in oven at 7˚C for 72 hours, until it reached the constant weight level. The following formula was used to calculate the relative water content (RWC) of tomato leaves;

$$RWC\ \% = (F.W-D.W)/(T.W-D.W)\times 100$$

Where;

RWC % = Relative water content percentage

F.W = Fresh weight

D.W = Dry weight

T.W = Turgid weight

Other parameters include

Number of days to flowering

Plant height (cm)

Number of leaves $plant^{-1}$

Number of fruits $plant^{-1}$

Total yield tons ha^{-1}

Number of flowers $plant^{-1}$

Statistical analysis

Data pertaining studied attributes of tomato plants were statistically analyzed using Randomized Complete Block Design with split-plot arrangement. Analysis of variances were computed by applying statistical computer software (Statistix 8.1) as described by Jandel Scientific (1991)[14] whereas, LSD (least significant difference) test was used to equate treatment means at $P\leq 0.05$ significance level.

Results and discussion

Number of days to flowering

The mean data pertaining number of days flowering is mentioned in Table 02, while its analysis of variance is presented in Table 01. Statistical analysis of mean data showed that different ASA treatments and growth conditions (open field and plastic tunnel) were have significant differences, while interaction of both the treatments were

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found non-significant ($p \leq 0.05$).Under mean values for ASA treatments (Table 02) revealed that maximum numbers of days to flowering (18.00 days)were recorded in ASA0 (control), while minimum numbers of days to flowering (13.50 days) was noted in ASA5 (1.50 mM). Analyzed data for growth conditions showed that more days to flowering (18.05 days) was observed in open field, however less days to flowering (14.00 days) was counted in plastic tunnel. Acetyl salicylic acid is tend to effect the flower induction positively, it has a direct role in plant growth, flower induction, thermogenesis and ion uptake.[15] In non-stressed plants salicylic acid (SA) regulates flowering time as because plants deficit in SA are late flowering.[16]

Table 1 ANOVA for the influence of ASA application on various attributes of tomato grown under different growth conditions

Characters	Growth condition (G)	ASA (A)	Interaction G × A
No of days to flowering	*	**	Ns
Plant height	*	**	Ns
No of leaves plant^{-1}	*	**	Ns
No of flowers plant^{-1}	*	**	**
No of Fruit plant^{-1}	*	**	Ns
Total yield (tons ha^{-1})	*	**	Ns
Chlorophyll content (µg.g^{-1})	*	**	Ns
Leaf relative water content (%)	*	**	Ns

* Ns, *, ** stands for non-significant, significant and highly significant at $p \leq 0.05$ level of significance respectively

Plant height (cm)

Mean data regarding plant height (cm) of tomato is given in Table 02, while its Analysis of variance (ANOVA) is displayed in Table 01. Analyzed data for plant height (cm) showed significant difference in ASA treatments and growth conditions however, their interaction was found non-significant ($P \leq 0.05$). Among mean value for ASA treatments (Table 02) shows that highest plant height (65.29 cm) was observed in ASA5 (1.50 mM) while lowest plant height (58.63 cm) was recorded in ASA0 (control). Observing mean values for growth conditions tallest plants (65.97 cm) was observed in open field, while shortest plants (58.48 cm) were found in plastic tunnel. Lowest Salicylic acid is also a prerequisite for the synthesis of auxin[17]which is produced in the meristematic tissue of the plant that is responsible for plant growth, therefore the increase in the plant height recorded is due to the ASA application and its effect on auxin. Plant height may also increase due to the change in the hormones responsible for plant height, and ASA is believed to be involved in changing the hormonal status of plant.[18] Similarly, plant height is boosted when ASA concentration is increasing from 0.25, 0.75 up to 1.25 mM.[19]

Number of leaves

Mean data regarding number of leaves is displayed in Table 02, while its analysis of variance (ANOVA) is given in Table 01.From ANOVA table 01, it is obviously evident that ASA treatments and growth conditions shows a significant variation, however, their interaction was observed non-significant ($P \leq 0.05$). In mean values of number of leaves for ASA treatments (Table 02) more number of leaves (36.28 leaves) were counted in ASA4 (1.20mM) while less number of leaves(28.65 leaves) were noted in ASA0 (control). Among mean values of number of leaves for growth conditions maximum leaves (35.37 leaves) were recorded in open field while minimum leaves (30.61 leaves) were noted in plastic tunnel. The production of secondary metabolites during stress by utilizing the plant carbohydrates is reverted by the exogenous application of ASA, which inhibits the deleterious effects of high temperature, thereby maintaining normal metabolism and nutrient production. ASA also stops the leaves abscission by reversing the effect of ABA and ethylene[20]; hence, it improves the plant response to ethylene by promoting proline metabolism and photosynthesis. Similarly, the higher number of leaves and leaflets per leaf were produced by the application of (1.25 mM) ASA.[19]

Number of fruits plant^{-1}:

Mean data regarding number of fruits plant^{-1} is specified in Table 02, while its analysis of variance (ANOVA) in Table 01. ANOVA table 01 showed significant differences among ASA treatments and growth conditions, however, no significant ($P \leq 0.05$) difference was found among their interaction. Mean values concerning number of fruits plant^{-1} (Table 02) of ASA treatments expressed that more number of fruits plant^{-1}(36.75 fruits) were recorded in ASA_4 (1.20mM), while less number of fruits plant^{-1}(26.08 fruits) was found in ASA_0 (control). Among the mean values for growth conditions (Table 02) deliberated that maximum number of fruits plant^{-1} (39.05 fruits) was recorded in open field, while minimum number of fruits plant^{-1} (22.87 fruits) were observed in plastic tunnel. Flowering is directly related to the yield and fruits productivity.[21] Similar results are in strong conformity with current findings which reports that SA is generally effective on photosynthetic pigments, yield, quality and quantity of tomato fruit.[22]

Total yield tons ha^{-1}

Mean data regarding total yield tons hac^{-1} is mentioned in Table 03, while its analysis of variance (ANOVA) is mentioned in Table 01.The ANOVA table indicated significant differences among ASA treatments and growth conditions. However, their interaction was found non-significant. Mean values for total yield tons ha^{-1} (Table 03) displayed that highest total yield (30.97 tons ha^{-1}) was observed in ASA^4 (0.120 mM), while lowest total yield (20.61tons ha^{-1}) was recorded in ASA0 (Control). Table (03) also indicated significant variation for total yield tons ha^{-1} among different growth conditions, where in maximum total yield (28.48 tons ha^{-1}) was observed in open field while minimum total yield (21.76 tons ha^{-1}) was noted in plastic tunnel. Due to positive effect of ASA_4 (0.120 mM) on the flowering and number of fruits plant^{-1}, the fruit fresh weight and number of fruits in each plant increases, which ultimately increase the total yield tons hac^{-1}.[20] Another reason for increase in total yield tons ha^{-1} is increase in number of leaves plant^{-1} hence, promoting the proline metabolism and photosynthesis.

Chlorophyll content

Data pertaining mean of chlorophyll contents is displayed in Table 03, while its analysis of variance (ANOVA) is presented in Table 01. The analyzed variations among means in Table (01) indicated that chlorophyll contents was affected significantly by both ASA treatments and growth conditions however, their interaction was recorded non-significant at ($p \leq 0.05$) level of significance. Mean values (Table 03) for chlorophyll contents of ASA treatments stated that the highest chlorophyll content (62.24µg.g^{-1}) was recorded in ASA_4 (1.20mM) while lowest chlorophyll content (42.66µg.g^{-1}) was observed in ASA_0 (control). Among the mean values of growth conditions maximum chlorophyll contents (58.78µg.g^{-1}) were found in open field while minimum chlorophyll contents (45.63µg.g^{-1}) were observed in plastic tunnel. Chlorophyll contents are the basic constituents for

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photosynthesis and are the pigment protein-complexes. Initially in the plants under plastic tunnel, the heat stress produced inhibitory effects on chlorophyll content, while in the open field grown tomato plants higher chlorophyll content was recorded. With the application of ASA the negative effects of heat stress were minimized to certain extent. The production of reactive oxygen species (ROS) increases during the heat stress, which mainly cause chlorophyll breakdown, loss of photosynthetic and membrane integrity.[23] Thus, the treatment of ASA blocked the inhibitory effect of ROS and consequently increased and maintained the chlorophyll content, photosynthetic activity and membrane structure. Higher doses in comparison with the lower doses were more prominent to cause retardative effects on the ROS; therefore, it enhances the process of photosynthesis while the lower doses were not enough to retard ROS.

Table 2 Days to flowering, Plant height (cm), No. of leaves plant^{-1} and No. of fruits plant^{-1} of tomato as affected by ASA under different growth conditions

Characters	Days to flowering	Plant height (cm)	No. of leaves plant^{-1}	No. of fruits plant^{-1}
Treatments	**Acetyl salicylic acid (A)**			
ASA_0 (Control)	18.00 a	58.63 e	28.65 c	26.08 d
ASA_1 (0.30 mM)	17.16 ab	60.93 d	30.86 bc	26.90 d
ASA_2 (0.60 mM)	16.33 b	61.13 d	33.61 ab	30.77 c
ASA_3 (0.90 mM)	16.16 b	63.00 c	33.72 ab	33.00 b
ASA_4 (0.120 mM)	15.00 c	64.38 b	36.28 a	36.75 a
ASA_5 (0.150 mM)	13.50 d	65.29 a	34.84 a	32.25 b
LSD at 5%	1.08	0.89	3.27	0.96
	Growth conditions (G)			
Open field	18.05 a	65.97 a	35.37 a	39.05 a
Plastic tunnel	14.00 b	58.48 b	30.61 b	22.87 b
LSD at 5%	1.26	1.24	4.34	3.33
	Interaction			
A×G	NS	NS	NS	NS
LSD at 5%	NS	NS	NS	NS

*Mean values followed by different letters are significantly different ($p \leq 0.05$) according to LSD test. NS: Non-significant.

Table 3 Total yield (tons ha^{-1}), Chlorophyll (µg.g^{-1}) content and Leaf relative water content (%) of tomato as affected by ASA under different growth conditions

Characters	Total yield (tons ha^{-1})	Chlorophyll content (µg.g^{-1})	Leaf relative water content (%)
Treatments	**Acetyl salicylic acid (A)**		
ASA_0 (Control)	20.61 d	42.66 d	49.90 e
ASA_1 (0.30 mM)	22.73 d	49.33 c	60.33 d
ASA_2 (0.60 mM)	24.30 c	52.29 bc	65.83 c
ASA_3 (0.90 mM)	26.18 b	56.13 b	69.89 b
ASA_4 (0.120 mM)	30.97 a	62.24 a	74.00 a
ASA_5 (0.150 mM)	25.94 b	50.60 bc	70.38 b
LSD at 5%	0.95	5.93	2.80
	Growth conditions (G)		
Open field	28.48 a	58.78 a	74.25 a
Plastic tunnel	21.76 b	45.63 b	55.85 b
LSD at 5%	6.04	9.67	6.53
	Interaction		
A×G	NS	NS	NS
LSD at 5%	NS	NS	NS

*Mean values followed by different letters are significantly different ($p \leq 0.05$) according to LSD test. NS: Non-significant.

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Leaf relative water content

Mean data concerning leaf relative water content (%) is indicated in Table 03, while its analysis of variance (ANOVA) is indicated in Table 01. Analysis of variance of ANOVA table shows significant difference in ASA treatments and growth conditions while its interaction is found non-significant. Mean values for leaf relative water content (%) (Table 03) of ASA treatments depicted that maximum leaf relative water content (74.00 %) was recorded in ASA_4 (1.20mM), in contrast lowest leaf relative water content (49.90 %) was found in ASA_0 (control). However, the mean values of different growth conditions show that more relative water contents (74.25 %) is found in open field, while low relative water content (58.85 %) was noted in plastic tunnel. Leaf relative water content (LRWC) is a very convenient measure of the water status of the plant in relation. The two doses ASA_4 and ASA_5 (application with 1.20 and 1.50mM acetyl salicylic acid) had 20-24 % increase in RWC. It is well known that heat stress reduces the relative water content of the plant due to increase in the process of transpiration. On the other hand, salicylic acid is involved in the closure of stomata during stress condition; it has a role in the function of guard cells and therefore it reduces the transpiration. The transpiration decreasing effect of SA on leaves and epidermis of wheat also exists and determined.[24] Similarly, the enhanced photosynthetic activity of ASA tends to increase the production of sap in the leaf lamella which improves the maintenance and retention of leaf relative water contents in the leaf as well as initiate better growth.[25]

Number of flowers plant^{-1}

Analysis of variance is displayed in AOVA Table 01, which clearly revealed that there is a significant variation among ASA treatments, growth conditions and their interaction. Mean values of interaction in Figure I shows that maximum number of flowers plant^{-1} (117.46 flowers) were recorded with the application of ASA_4 (1.20mM) in open field growth while the minimum number of flowers (63.07) were found in ASA_0 (control) in the growth condition under plastic tunnel. ASA is playing a vital role in the induction of flowers and thermogenesis, as because it helps in carbohydrates assimilation and partitioning it towards the reproductive organs; thereby increases pollen production, viability, dehiscence, and stigma elongation. SA is playing a key role in physiological process like flower induction, photosynthesis and protein synthesis.[26] Correspondingly,[22] application of exogenous SA on tomato resulted in a wide range of responses including number of flowers plant^{-1}.

Conclusion and recommendation

It has been determined that acetyl salicylic acid had produced significant effect on tomato growth and yield. In contrast to the plants grown under plastic tunnel, a positive attribute was noted against all parameters by tomato plants in the open field.

ASA_4 (1.20 mM acetyl salicylic acid) attributed significantly in mitigating the negative effects of heat stress on tomato and delivered high yield that was exacerbated by improved vegetative growth, reproductive growth as well as the chlorophyll content and leaf relative water content, closely followed by ASA_5 (application with 1.50 mM acetyl salicylic acid) with maximum plant height (cm) and minimum number of days to flowering.

It is recommended to use ASA_4 (1.20 mM acetyl salicylic acid) for inducing heat tolerance in tomato crops. It is further suggested to investigate the ASA_5 (application with 1.50 mM acetyl salicylic acid) and high levels of ASA on tomato seedlings for identification of the impact of these concentrations on tomato crop.

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