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Salicylic acid upsurges the heat tolerance of tomato by improving plant water relations and antioxidants activity

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Abstract

Tomato is an economically important but heat prone crop. This study was designed with the view of expanding its growing period and tomato genotypes were grown in the field on three transplanting dates (optimum, late and very late) in order to study the effect of prevailing high temperature and sprayed with salicylic acid to check its effect in mitigation of heat induced damages. The results revealed that heat stress significantly reduced the crop productivity and foliarly applied salicylic acid played substantial role in combating the adversities of high temperature. Improved plant water relations, leaf osmotic potential was measured maximum 0.6 mp4 during the early phase while leaf turgur pressure was found maximum 0.8 mp4 during the middle phase. catalase, peroxidase were recorded 1.8 and 2.0 u/mg respectively during the early and middle of growing while superoxidases were found 20 u/mg in plants sprayed with salicylic acid. The study further reviled that salicylic acid induced tolerance becomes less pronounced under prolonged high level of stress. The findings of this study will not only help in getting economical yield under high temperature conditions but will also play its role in ensuring food security under global warming scenario.

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Introduction

The majority of scientists working on the climate agree to one point that the human interventions like burning of fossil fuel and emission of chlorofluorocarbons (CFCs) gasses are changing the climate to the level that it is not far off when these changes will be a serious threat to human survival in many cases by jeopardizing the food production. Earth's mean surface temperature has risen by more than 1.4°F (0.8°C) in last 100 years, out of which major change took place in last 35 years. An upsurge of 1.4°F may not look of great significance if we think in terms of a seasonal or daily fluctuation, but it is quite noteworthy when we think it as a permanent increment averaged across the planet. While doing so, we should also keep it in our minds that a diminution of only 9°F (5°C) in global average temperatures is the projected difference between today's climate and the ice age (Huddleston 2012).Heat stress is the most important factor in crop production that adversely affects the crop productivity from seed germination till harvesting i.e. the whole phenology of the plant. Susceptibility to high temperature stress varies with genotype and for every one degree rise in temperature above the ambient temperature, yield losses may range up to 10-15% (Upadhyaya *et al.*, 2011). Peng *et al.* (2004) showed a yield reduction in rice by 10% for every 1°C upsurge in temperature and a similar heat stress's outcome on tomato crop yield has been reported earlier (Hanson *et al.* 2002). High temperature stress results in yield loss, creating a great difference in actual yield and potential yield. Drastic effects of heat stress on vegetable crops have been explored and are described by various scientists (Gruda 2005; Hazra *et al.* 2007; Abdelmageed & Gruda, 2009; Shaheen *et al.,* 2015).

Central Punjab (the focus of this study) has semi-arid climate with a dry and hot summer. The average temperature of the areais above 40°C with temperature peaks above 45°C (FAO 1998; HKO 2012). Availability and affordability of vegetables are the two major issues the general consumer confronts in the country (Ali 2000). In the summer season, damage in vegetable crops due to heat greatly surpasses the losses that occur due to other issues such as diseases, insect/pest pressure and conventional management practices. Cultivation under controlled conditions is quite unpractical in developing countries like Pakistan because of small land holdings, limited resources and high energy prices. Unfortunately, very little work has been done so far on evaluation and enhancement of heat tolerance potential in vegetables in Pakistan. There is thus an urgent need to explore the options of introducing heat resistant germplasm along with combating the meaner by applying stress imparting chemicals to the tomato crop. To impact farming families and the general consumer, the present work was designed to increase vegetable production in Pakistan where more than 70% of the population is linked directly or indirectly to agriculture.

The popular but heat prone vegetable, tomato was selected with the aim of expanding it growing season in the plains of Punjab, Pakistan. It is grown at the start of summer season when they produce proficiently where as production is markedly reduced in the peak summer. In Pakistan, tomato is being cultivated on an area of 52.3 thousand hectares with 6.7 thousand hectares in Punjab while the total production is 529.9 thousand tons with 87.9 thousand tons produced in Punjab (Anonymous 2012). Tomato crop requires moderate day and night temperature of 28°C and 22°C, respectively, to express its production potential. The limitations imposed either by low or high temperature in the months of February and June, respectively, restricts the growth period to 3 months only (March, April and May) in plains of Punjab, Pakistan. This is very short period to contribute to good yield. This is the main factor responsible for low yield in Punjab plains as compared to the crop grown at high altitudes where the temperature is mild during summer and crop flourishes for six months (April to September). This can also be estimated by the fact that the average yield per hectare in Pakistan is 10.1 tons, which is quite low as compared to 33.6 tons per hectare in modern agriculture systems of the world (Iqbal *et al.* 2014).

One of the reasons for this low yield is high temperature. With the rise in temperature accompanied by global warming the yield gap may further widen at the two sites because of further reduction in growth period at plains where the crop is mostly concentrated. Furthermore, the sudden rise in temperature in June enhances abrupt maturity of tomato fruit leading to glut in market and consequently lower income to the growers. It is thus evident that devising a strategy to mitigate the effects of high temperature in tomato is the need of the time to improve the disappointing yield.

There are many potential chemical, which when applied exogenously, induced stress tolerance in crop plants. Salicylic acid (SA) is one of them. Exogenous SA application regulates antioxidant enzyme activities along-with enhanced plant tolerance to abiotic stresses (Gunes *et al.*, 2007; Eraslan *et al.*, 2007). Eraslan *et al.* (2007) did research on carrot grown under salt stress and later on sprayed with salicylic acid as stress alleviating chemical. Improved stimulation of antioxidant enzymes was observed in tomato plants applied with salicylic acid (Szepesi *et al.*, 2005).

The aim of this research was to expand the growing period of tomato by inducing heat tolerance through exogenous application of salicylic acid under filed settings.

Material and Methods

Four tomato genotypes i.e. two tolerant (L00090 and L00091) and two sensitive (CLN1462A and CLN1466E), from a lot of 191 tomato genotypes imported from AVRDC-The World Vegetable Center Taiwan China and screened for their heat tolerance potential under controlled environmental conditions (Shaheen *et al.*, 2015), were selected. The seeds were sown in pots containing sand as growth media on 15 February, 01 March and 15 March. Hoagland's Solution was employed as nutrient medium. Plants were kept in growth room under controlled conditions (28/22°C day and night temperature). Four weeks after emergence plants were transferred to the field according to the treatments i.e. optimum (15 March), late (01 April) and very late (15 April), in order to study the effect of heat stress on tomato under late sown conditions. Salicylic acid @ 1.5mM (optimized in preliminary experiments) was foliarly applied two weeks after transplanting. Experiment was designed following Randomized Completely Block Design (RCBD) with factorial arrangements. Experiment was replicated four times and there were five plants per replication. When the crop reached at four weeks of age, the data regarding following parameters were recorded:

Leaf water potential (Ψw)

At the end of experiment, a razor was used to cut the fully expanded leaves and was placed in the gasket of pressure chamber (Model 615, USA) to compute leaf water potential (Ψw). The data were computed in the morning before 12.00 am (10.00 am to 12.00 am).

Leaf osmotic potential (Ψs)

The same leaf used for water potential was frozen at -20 °C and were kept in Eppendorf tubes for a period of seven days. Then the frozen leaf material was thawed and cell sap extracted with the help of a steel rod. The sap so extracted was directly used for determination of osmotic potential using vapor pressure osmometer. The 10 µL of extracted sap was placed on osmometer (Wescor Model-5500) with the help of plastic syringe and Ψs measurement was taken.

Leaf turgor potential (Ψp)

Turgor potential (Ψp) signifies the difference between Ψw and Ψs, therefore Ψp was calculated, following the below mentioned equation:

$$\Psi p = \Psi w - \Psi s$$

Super oxide dismutase (SOD) (U mg^{-1} protein)

The activity of SOD was analyzed according to the protocol of Giannopolitis and Ries (1977) by calculating its potential to hinder the photo-reduction of nitrobluetetrazolium (NBT). The reaction solution (3 mL) contained 50 mM NBT, 1.3 mM riboflavin, 13 mM methionine, 75 mM EDTA, 50 mM phosphate buffer (pH 7.8) and 20-50 mL of enzyme extract. The test tubes having the reaction solution were irradiated under light (15 fluorescent lamps) at 78 mmol m^{-2} s^{-1} for 15 min.

The absorbance of the irradiated solution was noted at 560 nm by using a Spectrophotometer (Hitachi-650, Japan). One unit of SOD activity was explained as the amount of enzyme that restrained 50% of NBT photo decline.

Catalase and peroxidase

Catalase (CAT) and peroxidase (POD) activities were measured by the procedure of Chance & Maehly (1955) with some alteration. The CAT reaction solution (3 mL) comprised of 50 mM phosphate buffer (pH 7.0), 5.9 mM H_2O_2 and 0.1 mL of enzyme extract. Changes in absorbance of the reaction solution were recorded after every 20s at 240 nm. One unit CAT activity was specified as an absorbance change of 0.01 units per min. The POD reaction solution (3 mL) comprised of 50 mM phosphate buffer (pH 5.0), 20 mM guaiacol, 40 mM H_2O_2 and 0.1 mL of enzyme extract. Variations in absorbance of the reaction solution at 470 nm were calculated after every 20 seconds. One unit POD activity was assigned as an absorbance change of 0.01 units per min. The activity of each enzyme was expressed on the basis of protein content.

Ascorbate peroxidase (APX)

The enzyme activity of APX was estimated by the method of Nakano & Asada (1981). The reaction mixture comprised of sodium phosphate buffer (50 mmol L^{-1} pH = 7), EDTA (0.2 mmol L-1), ascorbic acid (0.5 mmol L^{-1}), BSA (50 mg) and enzyme extract. The hydrogen peroxide (H_2O_2) at concentration of 0.1 mmol L^{-1} was added in reaction mixture, which initiated the reaction.

The absorbance was taken at 290 nm, two minutes after reaction initiation. The difference in absorbance was divided by the ascorbate molar extinction coefficient (2.8 $mmol^{-1}$ cm^{-1}) and the enzyme activity represented as mol of H_2O_2 min^{-1} mg^{-1} protein, taking into consideration that 1.0 mol of ascorbate is required for the reduction of 1.0 mol of H_2O_2 (McKersie and Leshem, 1994).

Results

Effect of salicylic acid on leaf water potential, leaf osmotic potential and leaf turgor potential

The results revealed that salicylic acid application significantly (P<0.01) enhanced the leaf water potential of tomato genotypes.L00090 (heat tolerant genotype) gave the higher leaf water potenial and was at par with L00091 (heat tolerant genotype) regardless of transplanting time. Both L00090 and L00091 (heat tolerant genotypes) responded more efficiently to salicylic acid application. CLN1462A and CLN1666E (heat sensitive genotypes) also exhibited an incresed leaf water potentail, which was more pronounced under extreme conditions of heat (transplanting 3, when the tempeature exceeded 47.5°C), where none of the plant in both genotypes survived without salicylic acid application. The results for leaf water potentail (Fig. 1A).

A significant (P<0.01) effect of salicylic acid application in the enhancement of leaf osmotic potential lwas noted(Fig. 1B).L00090 (heat tolerant genotype) gave the higher leaf osmotic potentialand was at par with L00091 (heat tolerant genotype) regardless of transplanting time. Both L00090 and L00091 responded more efficiently to salicylic acid application. CLN1462A and CLN1666E (heat sensitive genotypes) also exhibited an incresed leaf osmotic potential, which was more pronounced under extreme conditions of heat (transplanting 3), where none of the plant in both genotypes survived without salicylic acid application. The highest leaf osmotic potential was observed in L00090 in transplanting 1, while the lowest was observed in CLN1462A in transplanting 3. The results for effects of salicylic acid application on leaf turgor potential **(**Fig. 1C) of tomato genotypes were non-significant (P>0.05).

Effect of salicylic acid on peroxidase and Superoxide dismutase activity

Salicylic acid application significantly (P<0.01) enhanced the peroxidase activity in the tomato genotypes (Fig. 2A). L00090 (tolerant genotype) showed the higher peroxidase activity, followed by L00091 (tolerant genotype) regardless of the transplanting time.

The heat sensitive genotypes (CLN1462A and CLN1466E) gave relatively lower peroxidase activity and were at par to each other, regardless to transplanting time.

The results for superoxide dismutase revealed that salicylic acid application significantly (P<0.01) improved its activity (Fig. 2B). L00090 (heat tolerant genotype) showed the highest superoxide dismutase activity followed by L00091 (heat tolerant genotype) regardless of transplanting time.

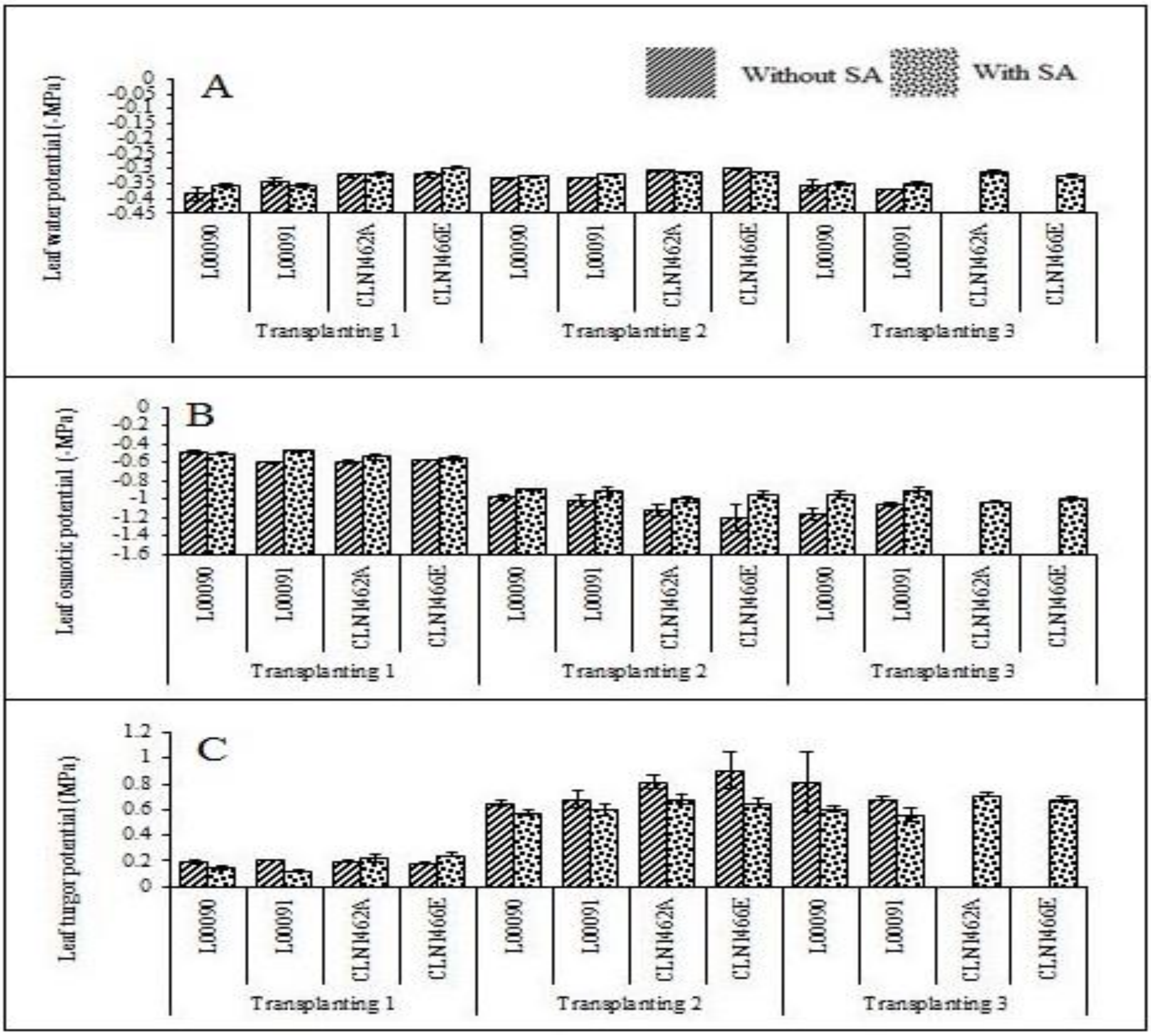

Fig. 1. Effect of salicylic acid on leaf water potential (A), leaf osmotic potential (B) and leaf turgor potential (C) of heat tolerant and heat sensitive tomato genotypes.

The sensitive genotypes CLN1462A and CLN1466E stood second and third in superoxide dismutase activity regardless of transplanting time, respectively. None of the plants of sensitive genotypes survived without salicylic acid application in transplanting 3. The highest superoxide dismutase activity was noted in L00090 in Transplanting 2, while superoxide dismutase activity was lowest in CLN1466E in Transplanting 3.

Effect of salicylic acid on Ascorbate peroxidase and Catalase activity

The results for ascorbate peroxidase activity indicated that exogenous application of salicylic acid significantly (P<0.01) enhanced its activity (Fig. 3A). L00090 (heat tolerant genotype) showed the greatest ascorbate peroxidase activity and was at par with L00091, regardless of transplanting time.

CLN1466E (heat sensitive genotype) stood second in ascorbate peroxidase activity and was at par with CLN1462A (heat sensitive genotype), regardless of transplanting time. The highest ascorbate peroxidase activity was exhibited by L00090 in transplanting 2, while the lowest was ascorbate peroxidase activity was exhibited by CLN1462A in transplanting 1.

Salicylic acid application significantly (P<0.01) enhanced the catalase activity in tomato genotypes under stressed environment (Fig. 3B). The highest catalase activity was observed in L00091 in transplanting 2, while the lowest was observed in CLN1466E.Regardless of transplanting time, L00090 (heat tolerant genotype) showed the highest catalase activity, L00091 (heat tolerant genotype) stood second in catalase activity, followed by CLN1462A (heat sensitive genotype), while CLN1466E (heat sensitive genotype) showed the lowest catalase activity.

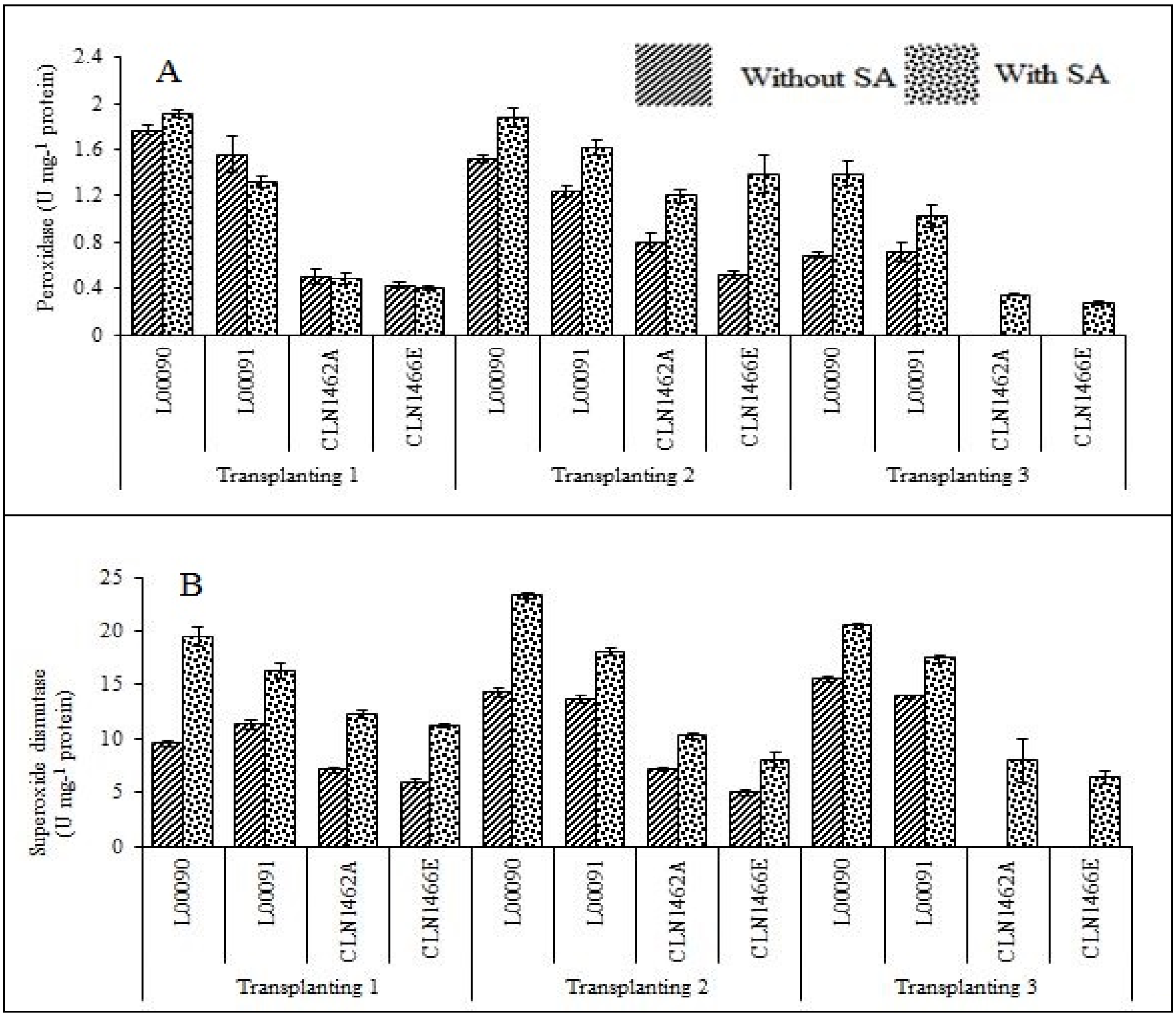

Fig. 2. Effect of salicylic acid on peroxidase activity (A) and Superoxide dismutase (B) of heat tolerant and heat sensitive tomato genotypes.

Discussion

Stress induced reduction in tissue water contents in many crops (Shangguan *et al.,* 2000; Saneoka *et al.,* 2004; Garcia *et al.,* 2007). The magnitude of such changes relies upon interactions with other abiotic factors with which the plants are interacting (White *et al.* 1996). Calcagno *et al.* (2011) reported a significant decrease in leaf water potential in stressed tomato plants as compared to non-stressed plants. The results for this study indicated that the foliar application of salicylic acid significantly increased the leaf water potential.

Szepesi *et al.,* (2005) worked on effects of exogenously applied salicylic acid on stressed tomato plants and reported an increase in the water potential by salicylic acid application under stressed environment. One of the essential factors in plant water relations under stress conditions is the acquisition of high water content (Arndt *et al.* 2001) and the capacity to do so determine its tolerance potential.

Leaf osmotic potential increased with increase in the magnitude of stress level and this reduction was more pronounced in sensitive genotypes as compared to the tolerant ones (Manaa *et al.,* 2013). According to a study by Calcagno *et al.* (2011) it was observed that leaf osmotic potential of stressed plants becomes quite low as compared to the non-stressed plants. Significance of stress induced water loss from plants has also been reported by Kav *et al.,* (2004) and Noreen *et al.* (2010).

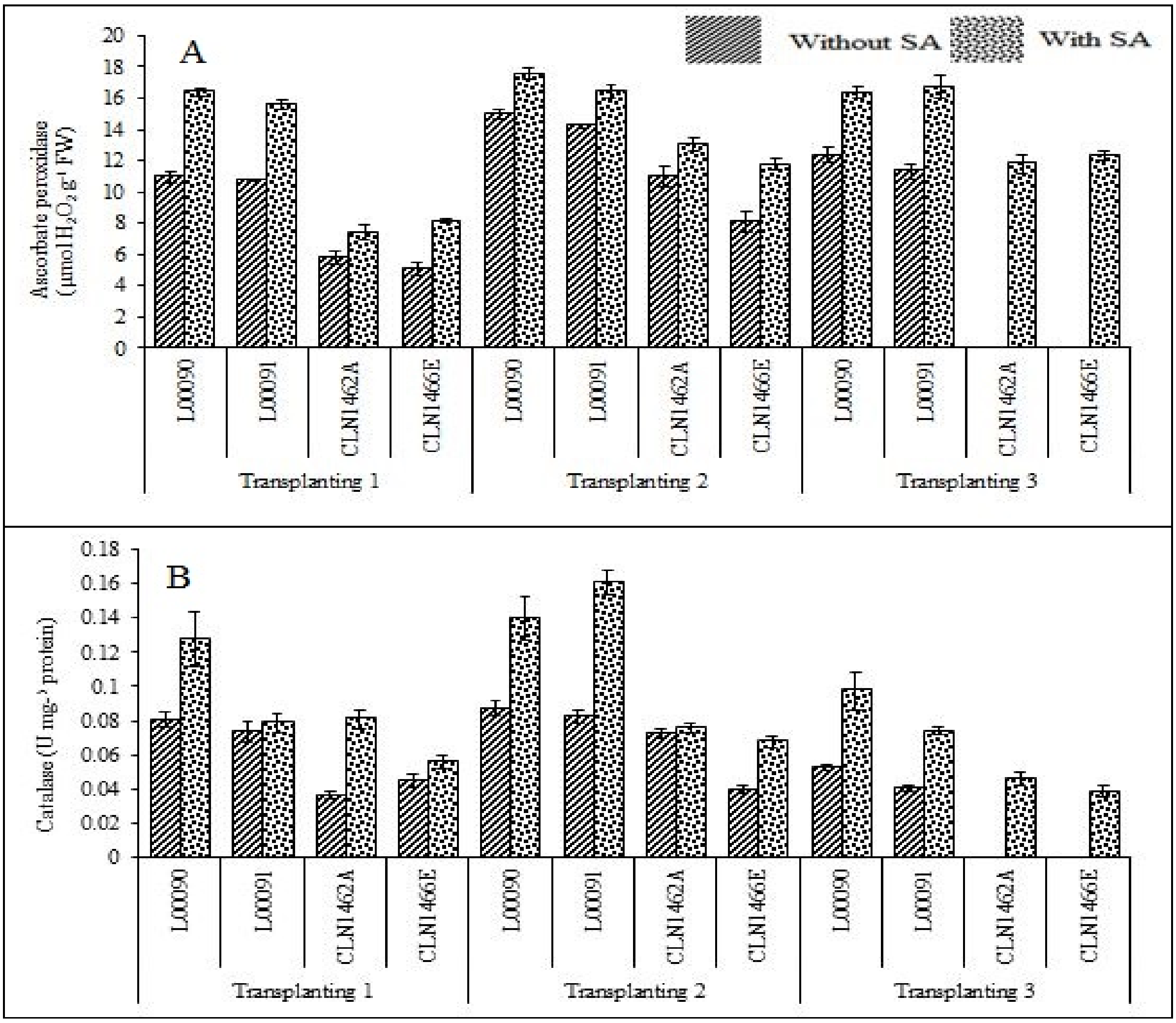

Fig. 3. Effect of salicylic acid on Ascorbate peroxidase (A) and Catalase (B) of heat tolerant and heat sensitive tomato genotypes.

The study also revealed that salicylic acid application significantly enhanced the leaf osmotic potential of plants applied with salicylic acid as compared to control with no SA application. Szepesi *et al.,* (2005) worked on salt stress in tomato and stated that under stress conditions the osmotic potential of tomato decreased and the application of salicylic acid moderated the negative effects of stress.

The maintenance of turgor potential depends on the degree of osmotic and elastic modification as well as on the interaction between the two and apoplastic water fraction (Maury *et al.* 2000).

Szepesi *et al.,* (2005) worked on effects of exogenously applied salicylic acid on stressed tomato plants and reported a decrease in turgor potential in salicylic acid treated plants but this study indicated that change in leaf tugor potential was statistically non-significant.

Under stress conditions, reactive oxygen species (ROS) i.e. superoxide radicals, hydroxyl radicals and hydrogen peroxide etc. are produced in the plants and induces oxidative stress (Panda *et al.,* 2003a, b). This upsurge in reactive oxygen species level in plants causes oxidative damage to bio-molecules (lipids, nucleic acids and proteins) and alters the redox homeostasis (Gille and Singler 1995). Reduction in oxidative damage to plants during stress is one of the major mechanisms of tolerance against stress (Kraus and Fletcher, 1994) and such magnitude of defense was credited to improved antioxidant activity (Dat *et al.,* 1988; Senaratna *et al.,* 1988). Szepesi *et al.,* (2005) reported an enhanced peroxidase (POD) activity and ultimate upsurge in tolerance against stress by exogenous application of salicylic acid.
Szepesi *et al.* (2005) reported an enhanced superoxide dismutase (SOD) activity by exogenous application of salicylic acid. The results of this study indicated significantly enhanced ascorbate peroxidase (APX) activity induced by exogenous application of salicylic acid. Szepesi *et al.* (2005) reported an improved ascorbate peroxidase activity by exogenous application of salicylic acid at the rate of 10^{-4} M. Improved antioxidant activity by foliarly applied salicylic acid under stressed environment has also been reported by various scientists (Srivastava and Dwivedi, 2000; Kang *et al.,* 2003). These antioxidant enzymes must have vital role in enhancing photosynthetic apparatus by hunting reactive oxygen species produced under stress.

Salicylic acid application significantly enhanced the catalase activity in tomato and *B. juncea* genotypes under salt stress environment (Hayat *et al.* 2008; Yusuf *et al.,* 2008).Panda and Patra (2007) used salicylic acid as priming agent to treat rice seeds under cadmium stress and reported an enhanced antioxidant activity resulted in salicylic acid treatment.

The results of this study are contrary to the findings of Choudhury and Panda (2004) who reported a decline in antioxidant activity in rice when pre-sowing treatment with salicylic acid was done. However, there are very few reports on reduced antioxidants activity by salicylic acid application. The increased antioxidants activity is a clear indication of plants ability to withstand the adverse growing conditions as it makes the plant to combat the adversities of unfavorable environment.

Conclusion

The present study indicated that spraying salicylic acid improved the plants' tolerance against heat stress, wherein the tolerant lines (L00090 and L00091) performed better as compared to the sensitive ones (CLN1462A and CLN1466E). Furthermore, salicylic acid application was proved to be beneficial in jettisoning the production gap between the stressed plants and those that were grown under optimum environmental conditions.

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