



## Effects of salicylic acid on the antioxidant enzymes activity in leaves of tomato under high temperature stress

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### Abstract

The experiment was conducted to determine the effect of salicylic acid (SA) on the antioxidant enzymes activity in tomato as a randomized complete block design with three replications. The thermo tolerant genotype namely WIR-13708 was subjected to high temperature (32°C + 45°C) stress and analyzed for antioxidative enzymes i.e., peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD) under controlled, stressed and treated conditions. Temperature above than 32 °C in tomato, cause an oxidative damage due to accumulation of reactive oxygen species (ROS). These antioxidants act as a major defense against oxidative damage caused by ROS. The exogenous applications of salicylic acid (SA) improved the activities of these enzymes. Results showed that treatment with 0.5mM salicylic acid both in the presence and in absence of stresses significantly improved the values for the above mentioned parameters.

**Keywords:** salicylic acid, tomato, antioxidative enzyme, peroxidase, catalase

### 1. Introduction

Tomato (*Lycopersicon esculentum* L.) is a member of the Solanaceae family and one of the most important vegetables in the world. It has a particular importance as being a raw material of agricultural industry besides it is used as a fresh vegetable. Tomato is a rich source of lycopene, vitamins, and minerals. Lycopene is responsible for the characteristic deep red color of ripe tomato fruits and tomato products (Helyes *et al.*, 2009) [19]. Lycopene is a key intermediate in the biosynthesis of many important carotenoids, such as beta-carotene and xanthophylls and may help in counteract the harmful effects of substances called “free radicals” and different types of cancer (De Stefani *et al.*, 2000) [10]. The current changing environmental scenario, both low and high temperatures have become a potential abiotic stress-threat to crop plants. Elevated temperature stress leads to inhibition in plant growth both in vegetative and relatively delicate reproductive developments and yield of several crops (Peet & Willits, 1998; Hussain *et al.*, 2006, Singh *et al.*, 2007) [37, 47]. Extreme temperatures have always been a serious threat to agriculture including tomato production owing to its heat and cold sensitivity. The optimum temperature range for growing tomato is 20-26/15-20°C day/night. The prevalence of high ambient temperatures in a significant proportion of the tomato growing areas of the world is one of the most crucial problems in tomato production. To devise some adaptable strategies to extend the tomato production spans and improve the yield volumes in rising temperatures is imperative. Besides genetic uplift, induction of thermo tolerance in existing high-yielding cultivars by foliar application or pre-sowing seed treatment with low concentrations of growth hormones can be a fruitful effort (Wahid *et al.*, 2007) [45]. Plants hormones modify the growth and development of plants by inducing changes in cellular, physiological, and morphological processes (Browse,

2009) [8]. Salicylic acid (SA) is a naturally occurring plant hormone, involved in the regulation of important plant physiological processes such as photosynthesis, nitrogen metabolism, proline (Pro) metabolism, production of glycinebetaine (GB), plant-water relations and antioxidant defense system under stress conditions and thereby provides protection in plants against abiotic stresses (Khan *et al.*, 2010, 2012a, b, c, 2013b, 2014; Nazar *et al.*, 2011; Miura and Tada, 2014) [30, 26, 27, 28, 29, 35, 34]. Previous studies have indicated that SA treatment influences a wide variety of plant processes including plant growth and yield (Singh *et al.* 2017; Javaheri *et al.* 2012) [42], induction of antioxidant enzymes (Ghasemzadeh and Jaafar 2013), and regulation of some photosynthetic reactions (Arfan *et al.* 2007; Li *et al.* 2014) [2]. Its role in plant tolerance to abiotic stresses such as ozone, heat, heavy metals and osmotic stress (El-Tayeb 2005; Szepesi *et al.*, 2005; Wang *et al.* 2010; Liu *et al.* 2011; Kadioglu *et al.* 2011) [12, 46, 32, 24] has been reported by several authors. Reactive oxygen species (ROS) are formed as natural byproduct of normal metabolism and play important roles in signaling (Bailey-Serres and Mittler 2006) [5]. However, excessive amounts of the ROS can cause membrane lipid peroxidation (Gunes *et al.* 2007) [18], damage to chloroplasts, inhibition of photochemical reactions, and decrease in photosynthesis (Li *et al.* 2014). Plants containing high activities of antioxidant enzymes have shown considerable resistance to oxidative damage caused by ROS (Khan *et al.* 2007; Gapinska *et al.* 2008; Frary *et al.*, 2010) [4, 14]. Important enzymes that scavenge ROS include superoxide dismutase, peroxidase and catalase (Noctor and Foyor, 1998; Ijaz, 2012) [36] and non-enzymatic metabolites like ascorbic acid (Athar *et al.*, 2008), salicylic acid (Gautam and Singh, 2009) [15], and low concentration of H<sub>2</sub>O<sub>2</sub> (Wahid *et al.*, 2007) [45] that quench these oxygen radicals and protect membranes from

injurious effects of ROS (Foyer and Noctor, 2003) [13]. The plants treated with salicylic acid increased the activity of these enzymes such as catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, glutathione reductase etc (Manochehrifar, 2010) [33]. Studies show that SA reduces the oxidative damage by maintaining superoxide dismutase activity for the removal of O<sub>2</sub> (Rao *et al.*, 1997) [39]. This experiment was designed to investigate the effect of exogenous application of SA on the antioxidant enzymes activity in the leaves of tomato plants.

## 2. Materials and Methods

Seeds of thermo tolerant (WIR-13708) genotype of tomato plants was surface sterilized with 0.01% HgCl<sub>2</sub> for 10 minutes washed thoroughly with distilled water again surface sterilized with 0.1% cetramide solution followed by another thorough washing with glass distilled water. Homogenized surface sterilized seeds were presoaked in different treatments as control in distilled water and 0.5mM concentrations of SA. For high temperature, seeds were sown in third week of February, 2015 on nursery bed and transplanted in the third week of March, 2015 in Botanical garden of Udai Pratap Autonomous College, Varanasi. The experiment was carried out in complete randomized block design with three replicates. The genotype was represented by 30 plants in each replication and supplied with 20ml of 50% of Hoagland's nutrient solution at alternate days (Hoagland and Arnon, 1950) [20]. Another experiment was conducted under control environment in which tomato genotype was grown in growth chamber at 32/26°C under 12/12 hours day/night cycle. The growing period from transplanting to harvesting was April to June. The optimum temperature and high temperature regimes were 19°C-32°C and 26°C-44°C, respectively. The last leaf of the tomato genotype was selected randomly and immediately frozen in liquid nitrogen for subsequent analysis.

### Extraction of Antioxidants

To extract antioxidant enzymes, 0.5 g of leaves were homogenized in 5ml of 50 mM phosphate buffer (pH 0.7%) containing 1% insoluble polyvinylpyrrolidone (lower MW=40,000). The homogenate was centrifuged at 15,000 rpm for 10 min. The extraction was carried out at 4°C. The supernatant was carefully decanted and used as the crude enzyme preparation for determining the activity of superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT) activity.

### Catalase (CAT)

The activity of CAT (EC 1.11.1.6) was assayed as described by (Aebi, 1983). The reaction mixture contained 100 mM potassium phosphate buffer (pH 6.5), enzyme extract equivalent to 20 µg protein and 10 mM H<sub>2</sub>O<sub>2</sub>. The decrease in absorbance at 240 nm due to the consumption of H<sub>2</sub>O<sub>2</sub> (extinction coefficient 40 mM<sup>-1</sup> cm<sup>-1</sup>) was recorded for 2 min.

### Peroxidase (POX)

Activities of POX were determined by Rao *et al.* (1996) [38]. For the activity of POX (EC 1.11.1.7), 3 mL of the reaction mixture contained 100 mM potassium phosphate buffer, pH

6.5, 16 mM guaiacol, 10 µl of 10% H<sub>2</sub>O<sub>2</sub> and enzyme extract equivalent to 10 µg protein from leaf and 1.5 µg protein from root tissue. The increase in absorbance at 470 nm (extinction coefficient 26.6 mM<sup>-1</sup> cm<sup>-1</sup>) was measured for 5 min.

### Superoxide dismutase (SOD)

Activities of superoxide dismutase were measured using the method of Das *et al.*, 2000. Pipetted out 1.4 ml aliquot of the reaction mixture in a test tube. Then 80 µl of 100 µM riboflavin was added followed by a preincubation at 37°C for 5 min and then tubes were exposed for 10 min to 200 W Philips fluorescent lamps. The control tube contained equal amount of buffer instead of sample. The sample and their respective controls were run together. At the end of the exposure time, 1.0 ml of Greiss reagent was added to each tube and the absorbance of the color formed was measured at 543 nm.

## 3. Results and Discussions

The results showed that the effect of salicylic acid on the antioxidant enzymes activity was significant (table 1). The highest enzyme activity observed for peroxidase (20.03) at the 0.5 mM SA application (table 2) followed by SOD (16.63). Salicylic acid has been shown to work in plants through different pathways. It enhances activity of the antioxidant system by inducing antioxidative enzymes or altering expression of their genes (Kang *et al.* 2013; Janda and Ruelland 2014) [25, 22]. In addition, it is now becoming clear that SA interacts both negatively and positively with other major signaling pathways including those regulated by jasmonic acid and ethylene (Singh and Gautam 2013; Janda and Ruelland 2014) [42, 22]. Heat stress also induces oxidative damage in plants caused by the generation and accumulation of superoxides (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radicals (OH), which are commonly known as reactive oxygen species (ROS) (Breusegem *et al.*, 2001). Although, the daily average temperature for optimal growth conditions of tomato is 27 to 32°C. High temperatures reduce the vegetative and reproductive growth in tomato. Changes in ambient temperature occur within hours, unlike drought and salinity stresses. Therefore, plants need to suppress and respond to the adverse effects of heat in a very short time. Investigations have shown SA as a strong and potential tool in reducing or alleviating the adverse effects of high temperature in plant. Exogenous application of SA can regulate the activities of intracellular antioxidant enzymes such as SOD, POX and increase plant tolerance to environmental stresses (Senaratna *et al.*, 2000) [40]. SA affected the processes related to seed quality including protein biosynthesis, seed primary metabolism, antioxidant enzyme production and transport of seed storage proteins which were increased like seed. Super oxide dismutase, peroxidase and catalase are antioxidant enzymes. Multiple antioxidant enzymes are involved in the scavenging of ROS. Super oxide dismutase is the first line of oxidase enzymes (Sedghi, 2010), cellular defense against free radicals and converts superoxide radicals into hydrogen peroxide. Catalase converts hydrogen peroxide to water and oxygen (El-Beltagi *et al.*, 2011) [11]. Peroxidase belongs to oxido-reductase enzymes and contributes in oxidation-reduction reactions. Oxygen is one of the precursors

for oxidase enzymes (Sedghi *et al.*, 2010).

**Table 1:** Analysis of variance for the effect of salicylic acid spraying on the antioxidant enzymes of Tomato leaves.

Mean of Squares				
S.O.V	DF	CAT	POX	SOD
BLOCK	2	0.5677 <sup>NS</sup>	0.0144 <sup>NS</sup>	0.2177 <sup>NS</sup>
SA	2	39.2744**	45.3077**	35.9744**
ERROR	4	0.0194	0.4327	0.1194

\*and\*\* indicating the significant differences at 5 and 1 percent probability level. DF: Degree of freedom; SA: Salicylic acid; SOD: Super Oxide Dismutase; POX: Peroxidase; CAT Catalase

**Table 2:** Comparison of means for the effects of salicylic acid on anti-oxidant enzymes activity in Tomato leaves

Mean of Squares			
Treatment	CAT	POX	SOD
Control	5.8667 <sup>c</sup>	12.3333 <sup>c</sup>	9.7333 <sup>c</sup>
HT	9.6667 <sup>b</sup>	15.2667 <sup>b</sup>	13.7000 <sup>b</sup>
HT + 0.5 m MSA	13.1000 <sup>a</sup>	20.0333 <sup>a</sup>	16.6333 <sup>a</sup>

In each column means with the same letter are not different significantly at 5% probability level. HT; High Temperature.

#### 4. Conclusions

The evaluation of the antioxidant- enzymes (catalase, peroxidase and superoxide dismutase activities) measured in leaves of tomato (*Lycopersicon esculentum* L.) seedlings showed different values of specific activity under heat stress. In the present study, our results indicate that the exogenous application of salicylic acid (SA) increased the activity of antioxidative enzymes. It is concluded that seed priming with 0.5 mM SA can induce the tolerance or resistance mechanisms facing environmental conditions and prepares the plant for avoiding oxidative damage.

#### 5. References

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