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The Effect of Different Concentrations of Salicylic Acid on Protective Enzyme Activities of Pepper (*Capsicum annuum* L.) Plants

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Abstract: The objective of this study was to investigate the effects of different concentration (0, 0.1, 0.7, 1.5, 3, 6 and 9 mM) of SA on antioxidant enzymes in *Capsicum annuum* L. plants. Enzyme activities of peroxidase, polyphenol oxidase, ascorbate peroxidase, catalase and glutathione reductase were measured. The plants were grown in pots vermiculite. Before applying the salicylic acid treatments, plants filled with were irrigated with based nutrient solution (Hoagland solution) for 5 weeks. After 5 weeks, plants were sprayed with different concentrations (0, 0.1, 1.5, 3, 6 and 9 mM) of salicylic acid. Data were analyzed using SPSS software and means were compared by Duncan test. In each experiment 4 replicats were used. Concentrations of 1.5, 3, 6 and 9 mM of SA caused significant increase in polyphenol oxidase and peroxidase enzyme activities in treated leaves. Concentrations of 0.7, 1.5 ad 3 mM of SA decreased in (catalase, ascorbate peroxidase and glutathione reductase) activities, but concentrations of 6 and 9 Mm of SA increased enzyme activities. Different concentrations of salicylic acid had different effects on enzyme activities in *Capsicum annuum* L.

Key words: Salicylic acid, antioxidant enzymes, *Capsicum annuum* L.

INTRODUCTION

SA is a major phenylpropanoid compound that influences plant resistance to pathogens and probably to other stress factors (Sharma *et al*., 1996; Durner *et al*., 1997; Surplus *et al*., 1 998). It is well documented that many phenolic compounds play a role in the regulation of different physiological processes, including plant growth and development, ion uptake and photosynthesis (Lynn and Chang, 1990).

Salicylic acid (SA) and related compounds have been reported to induce significant effects on various biological aspects in plants. These compounds influence in a variable manner; inhibiting certain processes and enhancing others (Raskin, 1992). Elevation of H_2O_2 levels can stimulate SA accumulation (Chamnongpol *et al*., 1998). There also reports which indicates SA can increase H_2O_2 in plants (Rao *et al*., 1997; Shirasu *et al*., 1997; Dat *et al*., 1998).

Plants have evolved protective mechanisms to keep these deleterious reactions to a minimum either by antioxidative enzymatic defence includes peroxidase, polyphenol oxidase, ascorbate peroxidase, catalase and glutathione reductase or non- enzymatic.

There is also evidence that SA can alter the antioxidant capacity in plants (Chen *et al*., 1997; Fodor *et al*., 1997; Rao *et al*., 1997). Therefore, in this research changes in the antioxidant enzymes by different concentrations of salicylic acid were studied.

MATERIALS AND METHODS

This study was done by the International Center of Science, High Technology and Environmental Sciences. Kerman, Iran in date 1 June 2006.

Plants were grown in the greenhouse at 25/20°C (day/night), with a 16 h light and 8 h dark photoperiod for 35 days. After 5 weeks, plants were treated with different

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concentrations of (0, 0.1, 0.7, 1.5, 3, 6 and 9 mM) of salicylic acid for three weeks. Four replicates were used for each treatment.

Measurements of antioxidant enzymes activity: Catalase activity was assayed by measuring the rate of disappearance of H_2O_2 following the procedure of Dhindsa *et al.* (1981). 0.5 g of leaf sample was homogenised in 5 mL of 50 mM potassium phosphate buffer pH = 7 and 1% PVP. Homogenised samples were centrifuged at 4°C for 10 min at 15000 g. An aliquot of 1 mL of the supernatant of the enzyme extract was added to the reaction mixture containing 1 mL of 1.5 M H_2O_2 and 3 mL of 50 mM potassium phosphate buffer pH = 7. Decrease in H_2O_2 is followed as decline at 240 nm during 30 sec.

Glutathione reductase activity was assayed in 2 mL of 100 mM TRIS-HCl buffer (pH = 7.2) containing 0.2 mM NADPH, 5 mM glutathione disulphide (GSSG) and 100 μL of plant extract (Anderson, 1996). The change in absorbance at 340 nm was recorded at 25°C in a spectrophotometer. Enzyme activity was based on the oxidation rate of NADPH using an extinction coefficient of 6.2 mM^{-1} cm^{-1}.

Peroxidase and polyphenol oxidase activities were determined by methods described by Kara and Mishra (1976).

APX activity was measured by the method of Nakano and Asada (1981). Enzymes activity was expressed as units/mg protein.

Protein concentration in enzyme extracts was determined according to Bradford (1976).

Statistics: The data were analysed by using analysis of variance (ANOVA). Data are expressed as the mean±SD. Statistical analyses were performed using a one-way analysis of variance (ANOVA), followed by Duncan test. Differences of $p<0.05$ were considered to be statistically significant.

RESULT

Capsicum annuum L. plants which were grown either under control or SA treatment, showed different enzymatic activities. Concentrations of 1.5, 3, 6 and 9 mM of SA caused significant increase in polyphenol oxidase and peroxidase activities in treated leaves (Fig. 1 and 2).

Concentrations of 0.7, 1.5 ad 3 mM SA decreased enzyme activities (catalase, ascorbate peroxidase and glutathione reductase), but concentrations 6 and 9 mM of SA increased enzyme activities (Fig. 3-5).

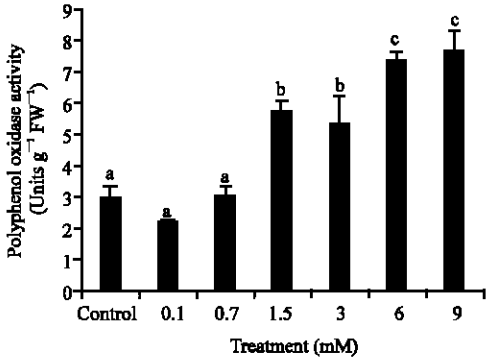

Fig. 1: Effect of salicylic acid treatment on polyphenol oxidase activity, values are the means of four replicates and bars indicates SEM, different signs show significant difference at $p<0.05$ according to Duncans test

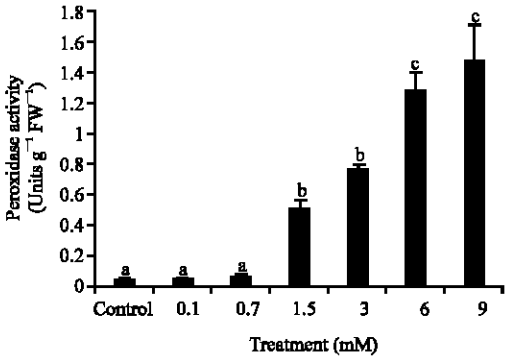

Fig. 2: Effect of salicylic acid treatment on peroxidase activity, values are the means of four replicates and bars indicates SEM, different signs show significant difference at $p<0.05$ according to Duncans test

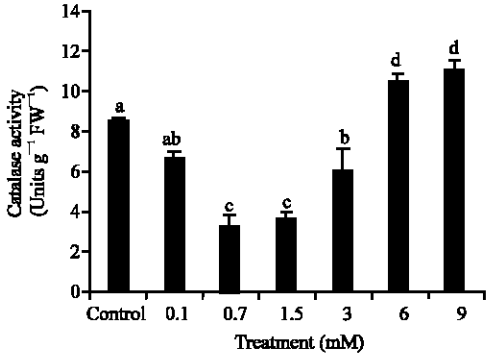

Fig. 3: Effect of salicylic acid treatment on catalase activity, values are the means of four replicates and bars indicates SEM, different signs show significant difference at $p<0.05$ according to Duncans test

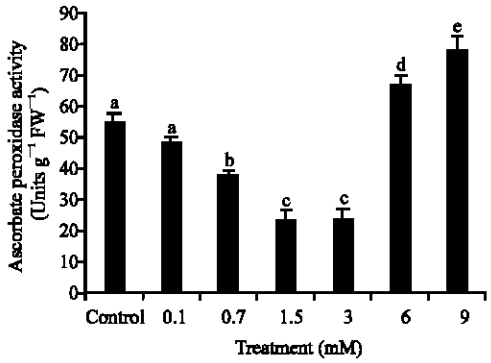

Fig. 4: Effect of salicylic acid treatment on ascorbate peroxidase activity, values are the means of four replicates and bars indicates SEM, different signs show significant difference at $p<0.05$ according to Duncans test

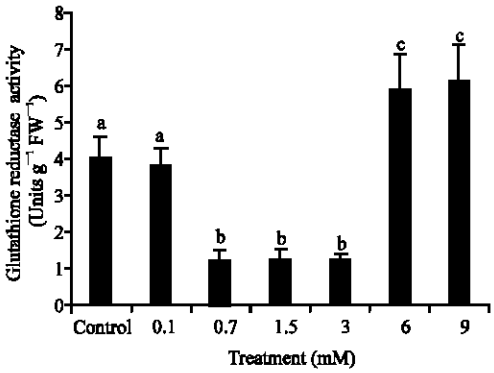

Fig. 5: Effect of salicylic acid treatment on glutathione reductase activity, values are the means of four replicates and bars indicates SEM, different signs show significant difference at $p<0.05$ according to Duncans test

DISCUSSION

There is also evidence that SA can alter the antioxidant capacity in plants (Chen *et al*., 1997; Fodor *et al*., 1997; Rao *et al*., 1997). Therefore, in this research changes in the antioxidant enzymes by different concentrations of salicylic acid were studied.

Oxidative stress in plants is buffered by the activation of antioxidant defences, including antioxidant enzymes such as peroxidase, polyphenol oxidase, catalase, ascorbate peroxidase and glutathione reductase. Oxidative stress is characterized by the synthesis of hydrogen peroxide, which is normally reduced and detoxified by CAT activity in the peroxisomes and by ascorbate peroxidase in the cytosol, mitochondria and chloroplasts (Foyer *et al*., 1997; Asada, 1999).

SA treatment increased the polyphenol oxidase and peroxidase activity in the leaves, but the activity of catalase, ascorbate peroxidase and glutathione reductase decreased when compared with the control in the leaves of pepper plants at 0.7, 1.5 and 3 mM SA, while at 6 and 9 mM their activity enhanced (Fig. 1-5).

Earlier investigations have shown the role of SA in modulating plant responses to a wide range of oxidative stresses (Shirasu *et al*., 1997). Salicylic acid inhibited a substantial portion of the catalase activity in several plant species (Raskin, 1992; Sanchez-Casas and Klessig, 1994).

In this research activity of enzymes such as peroxidase, polyphenol oxidase, ascorbate peroxidase, catalase and glutathione reductase enhanced in high concentrations of SA (6 and 9 mM) which may indicate that these enzymes might serve as acclimatization mechanisms to scavenge the toxic free radicals of oxygen produced under stressful conditions. Activity of may provide protection against high concentrations which is stressful. We concluded that, different concentrations of salicylic acid had different effects on enzyme activities in *Capsicum annuum* L.

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