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Full Length Research Paper

Changes in specific activity of ascorbate peroxidase during seed development of pea (*Pisum sativum* L.) treated with salicylic acid

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A field split-split plot experiment in randomized complete block design was conducted during years 2003-04 and 2004-05 to evaluate the effects of salicylic acid (SA) at concentrations of 0, 10^{-4} and 10^{-5} M on four pea varieties (Meteor, Climax, Greenfeast and Rondo). Four phenological growth stages were selected of seed and fruit development (BBCH 73, BBCH 77, BBCH 83 and BBCH 88) for enzyme assay. Aqueous SA was applied by three different modes: Seed treatment, seed treatment plus foliar spray and foliar spray. Maximum ascorbate peroxidase (APX) activity was exhibited by the variety Rondo as compared to other three varieties during both years of study. At BBCH 77, all the varieties showed maximum specific activities of APX which gradually decreased in BBCH 83 and BBCH 88 phenological growth stages. The specific activities of APX were recorded as maximum in the pea seeds treated with SA concentration 10^{-4} M as compared to 10^{-5} M and 0 M during both years of study. In comparison of modes of application of SA, it was observed that maximum specific activity of APX was in plants which were given seed treatment plus foliar spray (STFS) as compared to only seed treatment (ST) or foliar spray (FS).

Key words: Salicylic acid, BBCH, pea, ascorbate peroxidase, foliar application, *Pisum sativum.*

INTRODUCTION

Ascorbate peroxidase is a hydrogen peroxide scavenging enzyme that is specific to plants and algae and is indispensable to protect chloroplasts and other cell constituents from damage by hydrogen peroxide and hydroxyl radicals produced from it (Rizhsky et al., 2002). Ascorbate peroxidase has been found in the angio-sperms so far surveyed including leaves of pea (Mittler et al., 1991), nodules of legumes (Dalton et al., 1987), soybean axis (Puntarulo et al., 1988) and endosperms of caster bean (Klapheck et al., 1990). In plants, phenolic compounds like salicylic acid play an essential role in the regulation of different physiological processes, including plant growth and development, ion uptake and photosynthesis (Lynn and Chang, 1990). Salicylic acid (SA) stimulated flowering in *Lemna* and *Impatiens* and inhibited the biosynthesis of phytohormone ethylene, stomatal closure and root uptake (Raskin et al., 1990; Raskin, 1992). Among the morphogenetic processes affected by SA, are flowering and tuberization, for example, it hastens flower initiation in *Phaseolus* (Lagoa and Pereira, 1991) and induces tuberization in potato (Koda et al., 1992).

Exogenous treatment with SA before paraquat application increased the activities of the antioxidative enzymes in both chloroplasts (superoxide dismutase activity) and the other compartments of the cell (guaiacol peroxidase and catalase activity) in leaves of barley plants (Ananieva et al., 2004). SA and methyl jasmonate provided protection of photosynthesis against paraquat stress and diminished the oxidative damage caused by paraquat in barley seedlings (Popova et al., 2003). Foliar spray of SA counteracted the NaCl deleterious effects on maize and enhanced the salt tolerance in terms of

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Abbreviations: SA, Salicylic acid; **APX,** ascorbate peroxidase; **STFS,** seed treatment plus foliar spray; **ST,** seed treatment; **FS,** foliar spray; **EDTA,** ethylenediaminetetraacetic acid; **BSA,** bovine serum albumin; **ROS,** reactive oxygen species.

improving plant growth by activating the photosynthetic process (Khodary, 2004). SA greatly potentiates the effects of salt and osmotic stresses by enhancing reactive oxygen species generation during photosynthesis and germination of *Arabidopsis* (Borsani et al., 2001).

High temperature increased total endogenous SA rapidly, whereas, SA treatment and heat acclimation induced comparable sequence of changes in ascorbate and glutathione pools and antioxidant enzymes (Dat et al., 1998a, b). In barley seedlings, SA treatment completely suppressed the cadmium induced up-regulation of the antioxidant enzyme activities and plants showed an increase level of tolerance toward high cadmium concentrations (Metwally et al., 2003).

The aim of the present study was to evaluate the effects of exogenously applied salicylic acid on the specific activities of ascorbate peroxidase enzyme at four different phonological stages of seed development in pea (*Pisum sativum* L.).

MATERIALS AND METHODS

Growth conditions and treatments

The experiment was planned in a split-split plot experiment in randomized complete block design (RCBD) with three replicates. The main plots were assigned to pea cultivars (meteor, climax, green feast and rondo), with SA concentrations (0, 10^{-4} and 10^{-5} M in water) as subplots and modes of application of SA (seed treatment; seed treatment plus foliar spray; foliar spray only) as sub-sub-plots. The experiment was carried out during the growing seasons of 2003-04 and 2004-05. For seed treatment, seeds were soaked in the solutions of 0, 10^{-4} and 10^{-5} M concentrations of salicylic acid for six hours (Benavides-Mendoza et al., 2002). The plants were sprayed at phenological growth stage BBCH 60 (first flower open sporadically within the population) with aqueous solutions of SA concentrations 0, 10^{-4} and 10^{-5} M SA in the early morning when the plants had their 3rd leaf completely expanded. The sprays in all cases were carried out with a manual pump (Gutierrez-Coronado et al., 1998). The pods were sampled at four different phenological growth stages, that is, BBCH 73 (30% of pods reached average maximum length), BBCH 77 (70% of pods reached average maximum length), BBCH 83 (30% of pods ripe, dry and hard) and BBCH 88 (80% of pods ripe, dry and hard) (Feller et al., 1995).

Ascorbate peroxidase extraction and assay

One gram seeds were homogenized at 4°C with pestle and mortar in 4 ml of grinding medium composed of 50 mM Tris-HCl, pH 7.2, 0.3 M mannitol, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1% bovine serum albumin (BSA) and 0.05% cysteine. The homogenate was centrifuged at 20,000 g in SIGMA 2-16KC refrigerated centrifuge for 20 min. The supernatant was used to determine the APX activity according to the procedure used by Arrigoni et al. (1992). The reaction mixture composed of 50 µM ascorbate, 9 µM hydrogen peroxide and 50 mM potassium phosphate buffer, pH 6.5. The hydrogen peroxide dependant oxidation of ascorbate was followed by means of the linear decrease in absorbance at 265 nm. Enzyme activity was expressed in µmol of ascorbate oxidized per minute per mg protein (Esaka et al., 1992).

Protein content was determined according to the method of Bradford (1976) with BSA as a standard. Data from the two years combined were subjected to analysis of variance using computer package MSTATC. The Duncan's multiple range tests was used to separate the means.

RESULTS AND DISCUSSION

All four varieties exhibited maximum specific activity of ascorbate peroxidase at phonological growth stage, BBCH 77 (Figure 1). The variety Rondo revealed maximum APX activity at all four phenological growth stages as compared to other varieties, while the minimum specific activity of APX was recorded for the variety Greenfeast. Generally, the specific activity of all the varieties increased from phenological growth stage BBCH 73 to BBCH 77 and then declined towards the BBCH 83 and BBCH 87.

The specific activity of APX at all four phonological growth stages (BBCH73, BBCH 77, BBCH 83 and BBCH 87) of fruit development was influenced by SA concentrations (Figure 2). The maximum activity of APX was measured at BBCH 77 with all the three SA concentrations, while the lowest values for APX activity was recorded at the phonological growth stage, BBCH 87. The specific activity of APX was the highest when treated with SA 10^{-4} M as compared to SA 10^{-5} M and control. The modes of applications of salicylic acid also influenced the specific activity of ascorbate peroxidase at all four phenological growth stages during the study (Figure 3). The maximum specific activity of APX was noted for the pea plants treated with salicylic acid twice (seed treatment plus foliar spray) at all four growth phenological stages, while the lowest specific activity was recorded for the pea plants whose seeds were treated with salicylic acid.

Abiotic stresses stimulate formation of reactive oxygen species (ROS) in plant tissues. Among these, H_2O_2 is produced mainly in the chloroplasts and mitochondria of stressed cells and is the source of important cell damage (Foyer et al., 1994; Dat et al., 2000). Protection against ROS involves water-soluble and lipophilic antioxidants, as well as the ascorbate-glutathione cycle. In ascorbate-glutathione cycle, ROS catalyses the first reaction between H_2O_2 and ascorbate giving rise to mono-hydroascorbate and H_2O. APX is responsible for H_2O_2 detoxication in plant tissues (Chaudiere and Ferrari-Iliou, 1999) and is considered s a key antioxidant enzyme in plants (Orvar and Ellis, 1997). Marked changes in ascorbate metabolism occur during the development of seeds. In the early stage of seed development in *Vicia faba*, highest ascorbic acid content and a very high APX activity was observed (Tommasi et al., 1998). During the desiccation phase, ascorbate gradually decreases and finally completely disappears. With the loss of water, the APX activity also progressively decreases and becomes undetectable in the dry seed (Arrigoni et al., 1992). During the present study, seeds of the variety Rondo exhibited maximum APX activity as compared to other varieties. All the varieties showed maximum APX activities at BBCH 77 stage and

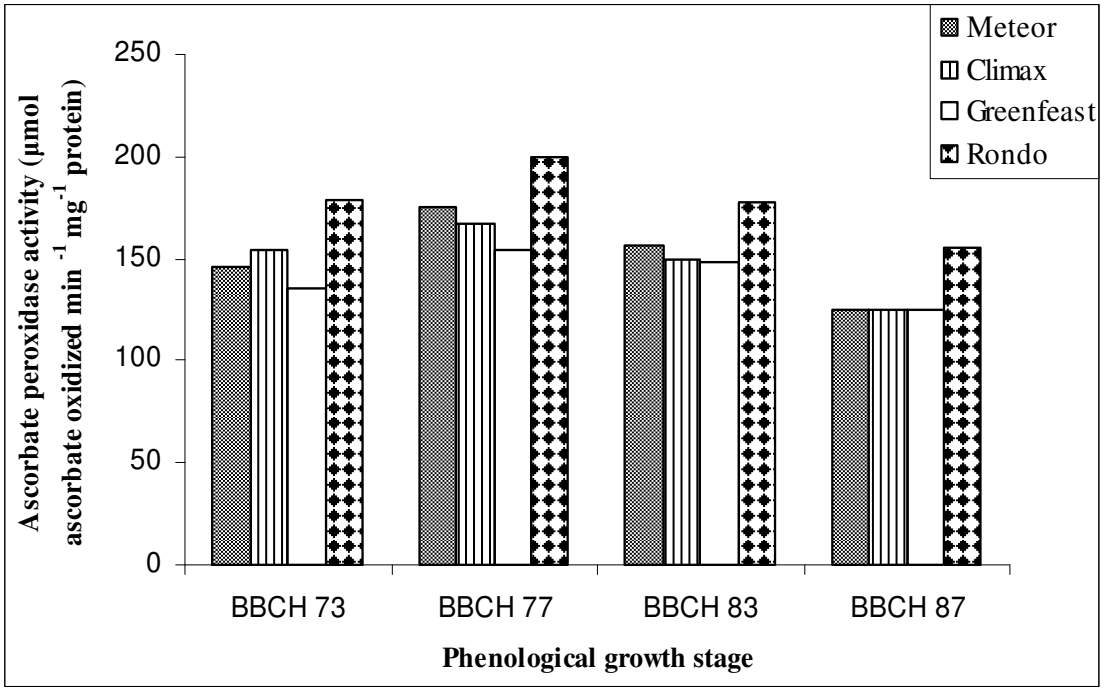

Figure 1. Effect of SA on the specific activity of ascorbate peroxidase at four phenological growth stages of pea (*P. sativum* L.) varieties during the year 2003-04 and 2004-05.

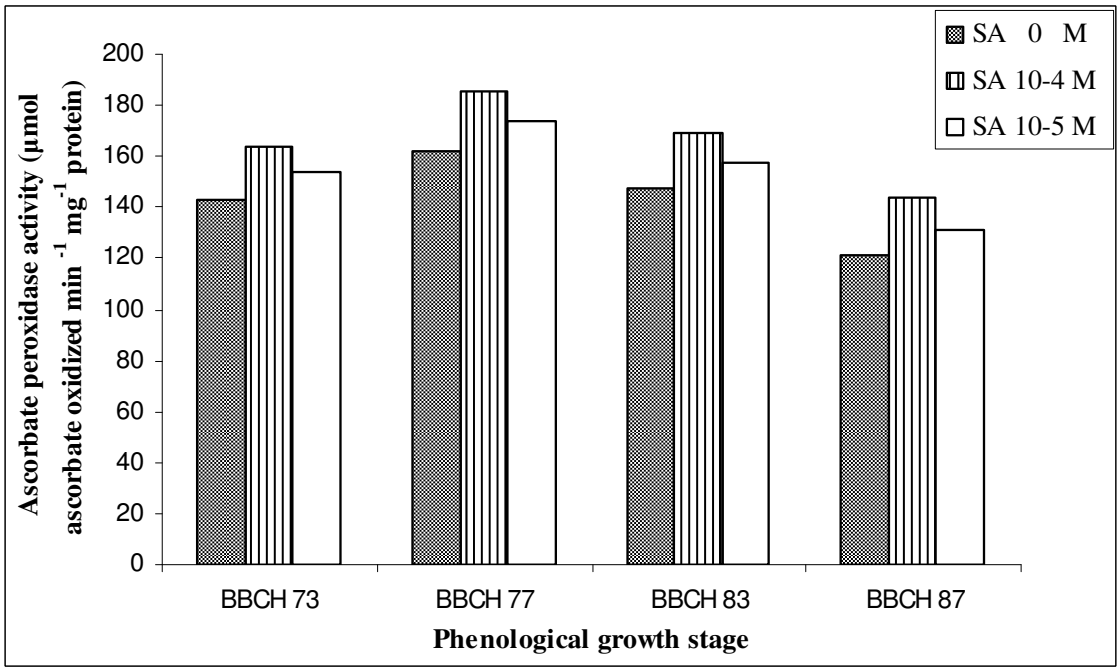

Figure 2. Effect of different SA concentrations on the specific activity of ascorbate peroxidase at four phenological growth stages of pea (*P. sativum* L.) during the year 2003-04 and 2004-05.

gradually decreased in phenological growth stages (BBCH 83 and BBCH 88). The pattern of APX specific activity shown by the varieties is in agreement with earlier workers (Arrigoni et al., 1992). The highest APX activities were recorded for SA concentration 10^{-4} M as compared to 0 M and SA concentration 10^{-5} M during both years of

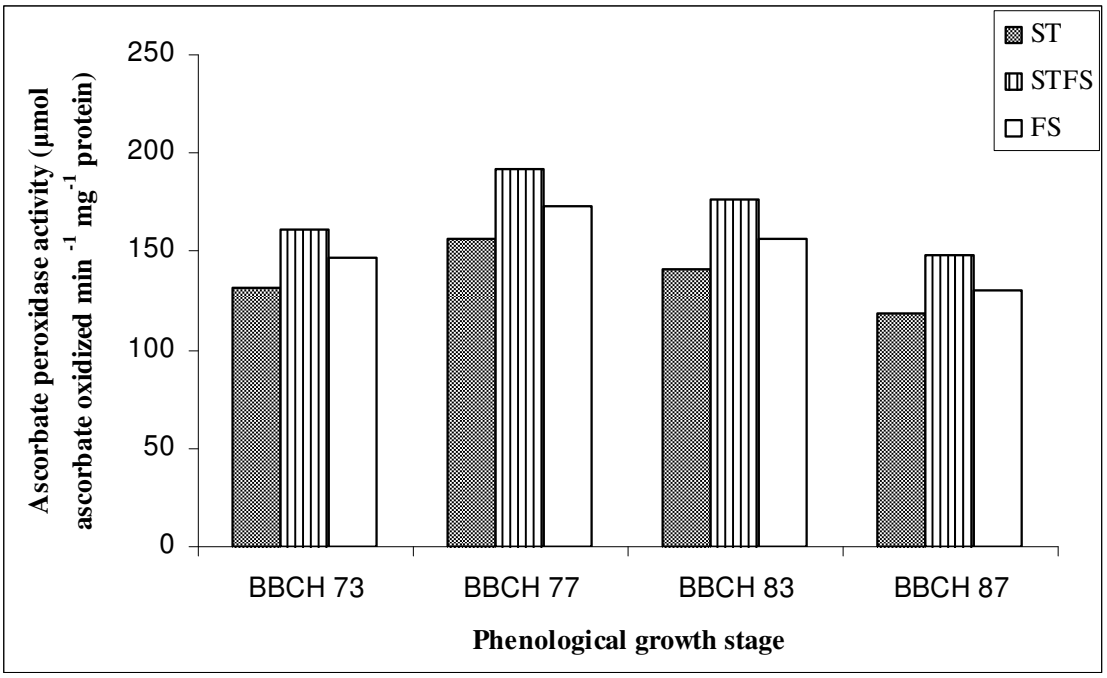

Figure 3. Effect of different modes of application of SA on the specific activity of ascorbate peroxidase at four phenological growth stages of pea (*P. sativum* L.) during the year 2003-04 and 2004-05.

study. Our findings showed that STFS had maximum specific activities of APX as compared to ST or FS. The increase in specific activities of APX induced by SA has already been revealed by earlier workers (Dat et al., 1998a, b). Higher content of ascorbate in green as compared with non green tissues of plants indicate a high requirement of ascorbate for the scavenging of hydrogen peroxide in chloroplasts.

During the present study, at BBCH 77, the highest APX activity can be correlated to maximum ascorbate content, while at phenological stages BBCH 83 and BBCH 88, the linear decrease in specific APX activities may be due to low ascorbate and water content of the seeds. There are certain reports that SA inhibits the APX activities (Conrath et al., 1995). However, from the results of the present study, it was revealed that SA is not an inhibitor of APX which was reported by other workers (Kvaratskhelia et al., 1997; Chakraborty and Tongden, 2005).

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