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RESEARCH ARTICLE

CHANGES INDUCED BY FOLIAR APPLICATION OF SALICYLATE ON ENZYME ACTIVITIES IN VIGNA MUNGO (L.) HEPPER

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ABSTRACT

Salicylic acid (SA) is a growth regulator that promotes growth of plants under stress and non stress conditions. The present study was conducted to determine the effect of foliar applications of salicylic acid (SA) on enzyme activity of *Vigna mungo* (L.) Hepper. SA was applied at five different concentrations (50ppm, 100ppm, 150ppm, 200ppm 250ppm) during the growth period of 15 days old *Vigna* seedling. Activities of antioxidant enzymes such as catalase (CAT), nitrate reductase activity (NRA), peroxidase (POD) polyphenoloxidase (PPO) and superoxide dismutase (SOD) were determined in the fresh leaves obtained from 15days old seedlings. Foliar applied SA caused a significant increase in leaf SOD and POD activity. However, leaf CAT activity was decreased with all SA treated plants compare to control plants.

INTRODUCTION

Salicylic acid (SA) is a ubiquitous phenolic compound occurring in plants in very low amounts and has been reported to regulate the physiological processes in plants such as nutrient uptake, stomatal closure, inhibition of ethylene biosynthesis, chlorophyll synthesis, protein synthesis, photosynthesis and transpiration (Khan *et al.,* 2003, Raskin 1992, Shakirova *et al.,* 2003). The expression of pathogenesis related protein genes has been regulated by SA, suggesting its key role as a signal molecule by providing resistance against pathogen attack (Raskin 1992). As an elicitor, SA regulates the PAL enzyme activity, which as a biosynthetic enzyme, catalyzes biosynthetic reactions for forming defensive compounds (Sgarbi *et al*., 2003; Solecka and Kacperska, 2003; Zhao *et al*., 2005) and SA regulates the protective enzymes such as SOD and POD, which together increase a plant's tolerance to environmental stresses (Mutlu *et al*., 2009; Shi and Zhu, 2008; Shim *et al*., 2003; Thulke and Conrath, 1998). Exogenous SA can regulate the activities of intracellular antioxidant enzymes such as SOD, POX and increases plants tolerance to environmental stresses (Senaratna *et al.,* 2000, Sakhabutdinova *et al.,* 2004). *Vigna mungo*, also known as black gram or urdbean, is a leguminous crop grown in south East Asia.

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It belongs to family fabaceae and subfamily faboideae. It originated in India and has been in cultivation since ancient times. The seeds contain about 20-25% of proteins and 40-47% of starch. It plays a very vital role in overcoming the protein caloric malnutrition especially in the developing country like India, where majority of population is vegetarian. It also has a superior mineral profile which makes it nutritionally more balanced. Moreover, its extracts have immuno stimulatory activity Solanki *et al*. (2010). The objectives of the present research was to improve our understanding of the effect of the various concentrations of SA applied to *Vigna mungo* plants as foliar spray on the catalase (CAT), nitrate reductase activity (NRA), peroxidase **(**POX**)** polyphenoloxidase (PPO) and superoxide dismutase (SOD) and its assimilation black in gram and to screen for the most effective concentration of SA.

MATERIAL AND METHODS

Plant material

Healthy and uniform seeds of *Vigna mungo* (L.) Hepper variety were purchased from Agricultural Research Station, Kovilpatti and surface sterilized with 0.1% $HgCl_2$ for a minute and washed repeatedly in distilled water. Healthy seeds were selected and sown in pots containing mixture of red soil, black soil, and sand mixed in the ratio of 2:2:1. The seeds were allowed to germinate in dark for 48 h. The percentage of seed

germination was nearly 80%. Soon after emergence, the seedlings were shifted to daylight conditions. After (15 days) growth of seedlings, the seedlings were sprayed with different concentrations of SA (50ppm, 100ppm, 150ppm, 200ppm, 250ppm) using an atomic sprayer. The seedlings were sprayed with solutions until dropping. Each plant required about 10ml of spray solution. Salicylic acid (SA-2-hydroxybenzoic acid) was obtained from Sigma Chemical Co. (St. Louis, U.S.A), SA was initially dissolved in 100µl of dimethyl sulfoxide and concentrations of 5 x 10^{-6}M to 100 x 10^{-6}M (pH 6.5) were made up with distilled water containing 0.02% Tween-20 (Polyoxyethylene sorbitan monolaurate). Plants sprayed with 0.02% Tween-20 served as the control. The plants were arranged in a completely randomized design with three replicates (Khan *et al*., 2003).

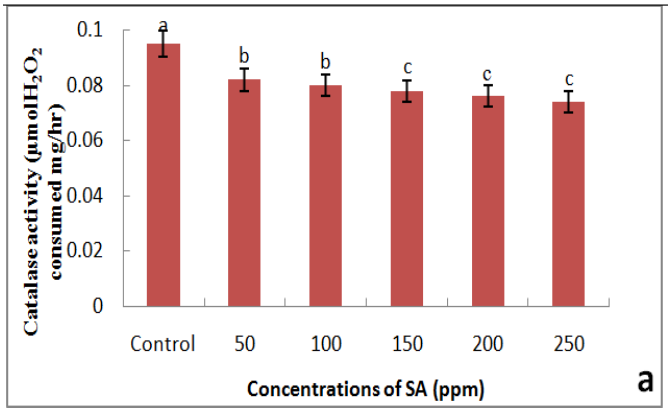
Estimation of enzyme activity

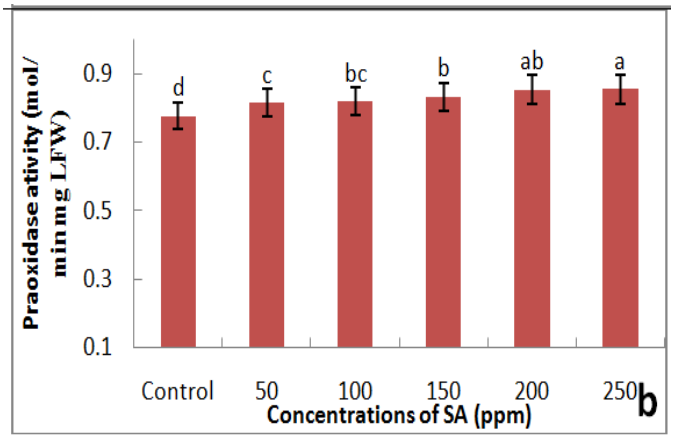
In vivo Nitrate Reductase (NR) activity was assayed according to Jaworski (1971) method Absorbance was measured at 540nm. The nitrate was estimated with the help of nitrite standard curve. To assay catalase activity, 3ml, phosphate buffer was added to 1ml of H_2O_2 and 1ml of enzyme extract (Kar and Mishra, 1976). The reaction mixture was incubated at 25°C for 1 minute. The reaction was terminated by the addition of 1ml of H_2So_4. The reaction mixture was titrated against 0.01N $KMN0_4$. The end point was the persistence of pink colour at least for 15 seconds. The catalase activity was expressed in micro moles H_2o_2 catalyzed per unit time per mg protein. To assay peroxidase activity, the enzyme extract was added to pyrogallol which gets oxidized to coloured derivative in the presence of hydrogen peroxide (1% V). The amount of purpurogallin formed during the reaction was assayed spectrophotometrically Addy and Good man (1972). Polyphenol oxidase activity was analysed by colorimatric method (Mukherje *et al*., 1975). To 2ml of enzyme extract 3ml of 0.1m phosphate buffer (pH 6.0) was added and mixed thoroughly by inverting the cuvette and placed in calorimeter. Super oxide Dimutase (SOD) activity was analyzed by Bowler *et al*. (1992) method. The absorbance was measured at 560nm.

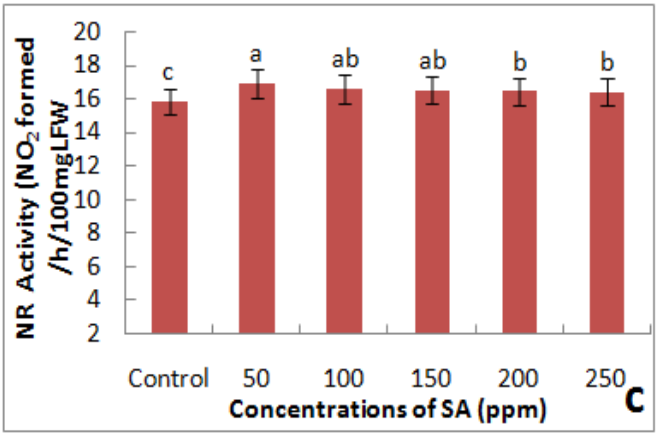
Statistical analysis: The experiments were performed in a randomized order. Datas were expressed as means of three replicates with standard error. Statistical assays were carried out by one-way ANOVA using Tukey's test to evaluate whether the means were significantly different, taking $P < 0.05$ as significant.

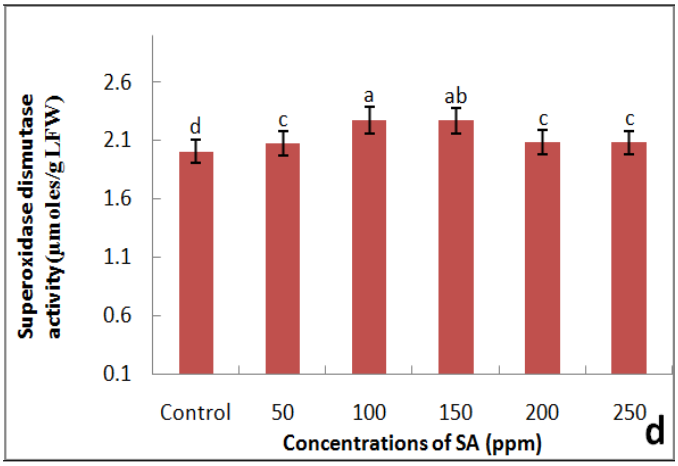
RESULTS AND DISCUSSION

Catalase, one of the antioxidant scavenging enzyme analysed in the present study was found to be decreased with all concentration of salicylic acid treated at 15 days old *Vigna* seedlings compared to control (Fig1-a).The catalase activity was found to be increased in control plant. There is an evidence that SA will decrease the CAT activity (Dat *et al*., 2000; Shi *et al*., 2006; Shi and Zhu, 2008; Shim *et al*., 2003), but in Kentucky bluegrass (He *et al*., 2005) and in wheat (Agarwal *et al*., 2005), SA actually increased the CAT activity. The greatest responses were obtained in plants sprayed with 200ppm and 250ppm of SA, with significant increases observed in POD (9% and 10%).









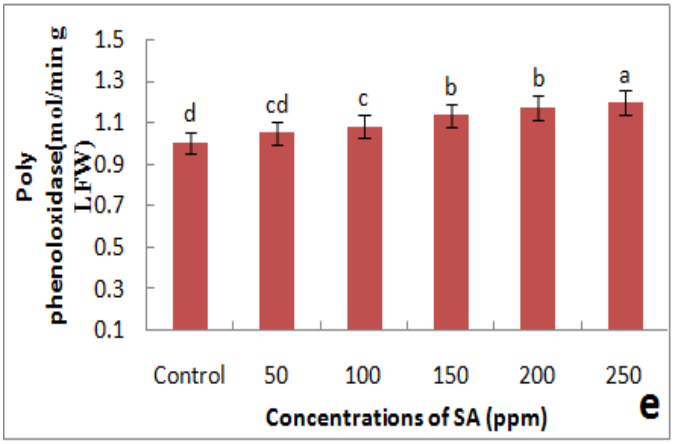


Fig. 1-(a,b,c,d,e,). Effect of Salicylate spray on enzyme activity in intact seedlings of *Vigna mungo* (L.) Hepper. The concentrations of SA were 50,100,150,200,250ppm. Each seedlings received 10ml / plant of SA spray given during the early hours of the day after 15 days old seedlings. The measurements were made 15 days old seedlings. Each value represent the mean of three independent measurements (Mean±SE, n=3). Bars carrying different letters are significantly different at $p< 0.05$

Peroxidase activity was increased with different concentrations of SA treated 15 days old *Vigna* seedlings compared to control (Fig1-b). Both the catalase and peroxidase act as the scavenger of superoxidase radicals. The treatment of millet seeds with salicylic acid caused the increase of peroxidase activity at all stages of experiment. The increase of peroxidase activity under the influence of salicylic acid is shown earlier too with the example of other cereals – barley (Ananieva, Popova, 2002) and wheat (Kolupaev *et al*., 2010). Salicylic acid can also exert the various (both activating and inhibiting) influence on the activity of peroxidase (Ruffer *et al*., 1995; Ananieva, Popova, 2002; Kolupaev *et al*., 2010), Application of 50ppm (7%) SA was significantly superior to other concentrations of *Vigna* seedlings, although lower nitrate reductase activity was observed in control plants (Fig 1-c). Our results corroborate the findings of many other related studies. In particular, they are consistent with those of Fariduddin *et al.* (2003) found that low SA concentrations increased nitrite reductase activity in *Brassica juncea*, whereas higher concentrations were inhibitory. It can therefore be assumed that SA concentration plays an important role in regulating nitrate reductase activity, with lower concentrations enhancing nitrate reductase protein and higher concentrations decreasing it by affecting the above processes. Increases in nitrate concentration and in turn nitrate reductase activity due to exogenous SA treatment under normal growth conditions have been reported previously and strongly support our observed results Hayat *et al*. (2005). Nitrate reductase activity results was found to increase with decreased in SA concentrations (Fig1-c).

SOD of 15 days old seedlings was found to be increased with the concentration of hormone. The SOD of the seedlings increased to 100ppm (13%), 150ppm (12%), were observed (Fig 1-d). The SOD is maximum around 100 and 150ppm concentrations. After that results was compared to control respectively (Fig 1-d). SOD is a major scavenger of superoxide radicals and the enzymatic action results in the formation of H_2O_2 that is then converted to H_2O and O_2 by POD and CAT (Alscher *et al*., 2002; Takahashi and Asada, 1983). Salicylic acid can increase SOD and POD activities with the resulting increase of AOS scavenging to protect plants from being injured (Mutlu *et al*., 2009; Shi and Zhu, 2008).

Regarding polyphenoloxidase, a consistent increase at 200ppm (17%), 250ppm (19%) of SA on 15 days old seedlings were observed (Fig 1-e). The polyphenoloxidase was maximum around 200, 250ppm concentrations. The minimum results were around 50 and 100ppm concentrations, the results were compared to the respective controls. (Fig. 1-e). The effects of SA on apoplastic enzyme activity in wheat plant leaves, was found to increase with SA treatment, while apoplastic catalase enzyme activity decreased, apoplastic peroxidase and polyphenol oxidase activities increased (Tasgin *et al*., 2003).

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