

# Effect Of NaCl Salt Stress On Antioxidant Enzymes Of Isabgol (Plantago Ovata Forsk.) Genotypes

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**Abstract:** Activity of antioxidant enzymes such as superoxide dismutase, catalase and peroxidase in leaves of isabgol (Plantago ovata Forsk.) genotypes viz. GI-2, HI-96, PB-80 and HI-5 were studied under salt stress at different EC levels viz. control (without salt), 5 and 10 dSm<sup>-1</sup> of nutrient supplemented NaCl salt solutions in sand filled polythene bags. Salt stress caused significant increase in the activity of superoxide dismutase, catalase and peroxidase. Maximum increase in activity of superoxide dismutase and catalase enzymes was found in the genotype GI-2 and minimum increase in the genotype PB-80. Peroxidase activity was highest in the genotype HI-96 and lowest in the genotype PB-80 under salt stress indicating genotype GI-2 and HI-96 having more capacity of scavenging reactive oxygen species produced due to salt stress and were relatively salt tolerant while genotype PB-80 was salt sensitive among the genotypes studied.

**Key words:** Plantago ovata, Superoxide dismutase, Catalase, Peroxidase, NaCl.

## Introduction

Salt stress is one of the major environmental stresses affecting crop productivity worldwide. Like other environmental stresses, it causes oxidative stress as a result of water deficit. The latter, in excess triggers generation of reactive oxygen species, which are thought to have deleterious effects on the integrity of biological membranes (Tappel, 1973; Kappus, 1985; Chaoui, et al., 1997; Del Rio et al., 2002) and thus, results either in the death of the plant or reduction in yield. The plant cells display non-enzymatic and enzymatic antioxidant system to mitigate the oxidative damages caused by reactive oxygen species (Apel and Hirt, 2004). The antioxidant enzymes include superoxide dismutase, ascorbate peroxidase, catalase, phenol peroxidase and other enzymes of the ascorbate-glutathion cycle. The non-enzymatic antioxidants include ascorbate, glutathione, carotenoids etc. Isabgol (Plantago ovata Forsk.) of the family Plantaginaceae is an important medicinal annual herb that is being grown in India in Gujrat, Madhya Pradesh, Rajasthan and in some parts of Haryana as a rabi crop. Isabgol seeds and husk are mild laxative, emollient and demulcent. Seeds have cooling effect and are used in inflammatory and bilious derangement of digestive organ. The decoction is useful in chronic diarrhoea and cough. The husk that constitutes about 25-30 % of the seed has great commercial value due to its medicinal properties and is used to cure the inflammation of mucous membrane of gastro-intestinal and gastro-urinary tracts, amoebic and bacillary dysentery and diarrhoea, duodenal ulcers, gonorrhoea and piles. Isabgol has been reported to tolerate soil salinity by several workers.

Effect of drought and salinity on growth, development and yield of isabgol HI-5 has been investigated by Surekha (1997) and Varshney and Surekha (2001). Vandana (2003) screened five isabgol genotypes viz. HI-5, HI-34, HI-96, GI-2 and PB-80 for salt tolerance. Among these genotypes GI-2 and HI-96 were found salt tolerant while PB-80 and HI-5 salt sensitive on the basis of growth, development and yield parameters. Abbreviations: EC, electrical conductivity; DAS, days after sowing; EDTA, ethylene diamine tetra acetic acid, PVP, polyvinyl pyrrolidone; NBT, nitroblue tetrazolium; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; APX, ammonium peroxidase.

## Material and method

Four isabgol (Plantago ovata Forsk.) genotypes viz. GI-2, HI-96, PB-80 and HI-5 were studied under screen house conditions. All the genotypes were grown in the dune sand filled polythene bags with control (without salt), 5 and 10 dSm<sup>-1</sup> EC level of NaCl salt solution along with nutrients. Sampling was done at vegetative stage (58 DAS).

## Enzyme extraction

Extraction conditions were standardized with respect to molarities and pH of buffer to achieve maximum extraction of enzyme in leaves. All the steps of extraction were carried out at 0-4°C. Leaves (1g fresh weight) from control and treated plants were excised. The leaves were washed with distilled water, dried with filter paper and macerated in a chilled pestle and mortar in presence of 2.5 ml of cold extraction buffer (potassium phosphate) containing 0.1 mM ethylene diamine tetra acetic acid (EDTA), 1% (w/v) polyvinyl pyrrolidone (PVP), 0.5% triton x-100 and 20% glycerol. pH was adjusted to 7.8. The homogenate was centrifuged at 10,000 x g for 15 min at 4°C. The supernatant was carefully decanted and used as the crude enzyme extract.

## Superoxide dismutase estimation

The activity of superoxide dismutase was assayed by measuring by its ability to inhibit the photochemical reduction of NBT (nitroblue tetrazolium) according to Beauchamp and Fridovich (1971). The reaction mixture contained 0, 20, 40, 60 and 80 µl of enzyme extraction in

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separate sets and to these added 0.25 ml of each of methionine, NBT and EDTA and the total volume of 3 ml was made with buffer in each set. Then 0.25 ml of riboflavin was added to each set in the last. The tubes were shaken and placed 30 cm away from light source (4 x 40 W fluorescent lamps). The reaction was allowed to run for 20 min and the reaction was stopped by switching off the light. The tubes were immediately covered with a black cloth. The absorbance was recorded at 560 nm. A non irradiated reaction mixture, which did not develop colour served as control. However, in the presence of superoxide dismutase the reaction was inhibited and the amount of inhibition was used to quantify the enzyme. Log  $A_{560}$  was plotted as a function of volume of enzyme extract used in the reaction mixture. From the resultant graph, volume of enzyme extract corresponding to 50% inhibition of the photochemical reaction was obtained and considered as one enzyme unit.

### Peroxidase estimation

A part of the extract prepared and used for SOD was taken for the estimation of peroxidase. POD activity was estimated using a modification of the method of Chance and Maehly (1955). The assay mixture contained 2.75 ml of 0.1 M phosphate buffer (pH7.0), 100  $\mu$ l of 0.1 mM guaiacol, 100  $\mu$ l of 0.1 mM  $H_2O_2$  and 50  $\mu$ l cell free extract. Reaction was started with addition of  $H_2O_2$  and increase in absorbance at 470 nm was recorded for 2 min. The activity was calculated using the extinction coefficient value of 22.6  $mM^{-1}cm^{-1}$  for guaiacol. One unit of enzyme activity was defined as amount of enzyme required for oxidation of one  $\mu$ mol of  $H_2O_2$ /minute in the assay conditions.

### Catalase estimation

A part of the extract prepared and used for SOD was taken for the estimation of catalase. The CAT activity was estimated according to the modification of the procedure described by Aebi (1984). The reaction mixture of CAT contained 2.5 ml of 0.1M phosphate buffer (pH 7.0), 100  $\mu$ l of 10 mM  $H_2O_2$  and 50  $\mu$ l of cell free extract. Reaction was initiated with the addition of  $H_2O_2$  and enzyme activity was determined by following the degradation of  $H_2O_2$  with the help of spectrophotometer at 240 nm for 2 min. The enzyme activity was calculated using the extinction coefficient value of 39.4  $mM^{-1} cm^{-1}$  for  $H_2O_2$ . One unit of enzyme activity was defined as amount of enzyme required for oxidation of one  $\mu$ mol of  $H_2O_2$ /minute in the assay conditions.

## Results

### Superoxide dismutase activity

Superoxide dismutase activity in leaves of isabgol genotypes significantly increased with increasing EC levels of the growing medium (Fig. 1). SOD activity under salt stress was found significantly different in all the genotypes studied. The relative order of SOD activity in various genotypes under salt stress was GI-2>HI-96>HI-5>PB-80. Interaction effect of genotype verses EC level (G x EC) was significant except between genotype PB-80 and GI-2 at control. Maximum increment (120.51 % of control) in SOD activity was observed in the genotype GI-2 and

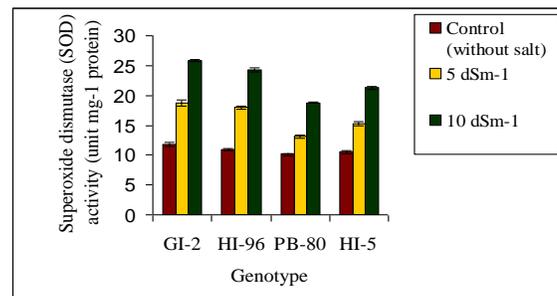
minimum increase (84.73 % of control) in the genotype PB-80 at higher (10dSm<sup>-1</sup>) EC level.

### Catalase activity

Salt stress, in general caused significant enhancement in the activity of catalase (CAT) enzyme in leaves (Fig. 2). All the isabgol genotypes responded differently with respect to CAT activity under salt stress. The relative order of CAT activity in various genotypes under salt stress was GI-2>HI-96>HI-5>PB-80. Interaction effect of genotype verses EC level (G x EC) was significant. Highest enhancement (230.70 % of control) in CAT activity was observed in the genotype GI-2 and lowest (184.43 % of control) in the genotype PB-80 at higher (10 dSm<sup>-1</sup>) level of salt stress.

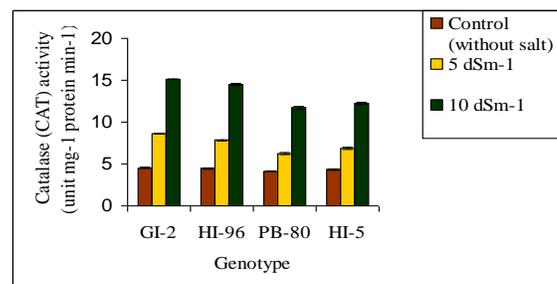
### Peroxidase activity

Activity of peroxidase enzyme in leaves of isabgol genotypes significantly increased with increasing EC levels (Fig. 3). A significant difference was observed in all the genotypes with respect to the activity of POD under salt stress. The relative order of POD activity in various genotypes under salt stress was GI-2>HI-96>HI-5>PB-80. Interaction of genotype and EC level (G x EC) with respect to POD activity was significant except at control. Maximum increase (329.30 % of control) in POD activity was detected in the genotype HI-96, while minimum enhancement (218.55% of control) was observed in the genotype PB-80 at higher (10 dSm<sup>-1</sup>) EC level.



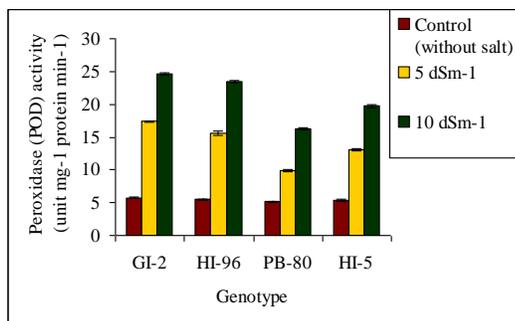
CD at 5% for G = 0.40; EC = 0.34, G x EC = 0.69; \*Bars represent mean  $\pm$  S.E.

**Fig. 1.** Effect of NaCl salt stress on superoxide dismutase (SOD) activity (unit  $mg^{-1}$  protein) of leaves of isabgol genotypes at vegetative stage



CD at 5% for G = 0.13; EC = 0.11, G x EC = 0.22; \*Bars represent mean  $\pm$  S.E.

**Fig. 2.** Effect of NaCl salt stress on catalase (CAT) activity (unit  $mg^{-1}$  protein  $min^{-1}$ ) of leaves of isabgol genotypes at vegetative stage



CD at 5% for G = 0.23; EC = 0.20, G x EC = 0.41; \*Bars represent mean  $\pm$  S.E.

**Fig. 3.** Effect of NaCl salt stress on peroxidase (POD) activity (unit mg<sup>-1</sup> protein min<sup>-1</sup>) of leaves of isabgol genotypes at vegetative stage

## Discussion

To cope with oxidative damage under extremely adverse conditions like salt stress, plants have developed an antioxidant defense system that includes the antioxidant enzymes SOD, APX, POD and CAT (Foyer and Noctor, 2005; Mittler, 2002). The level of antioxidant enzymes are higher in tolerant than in sensitive species under various environmental stresses (Bor et al., 2003; Demiral and Turkan, 2005; Turkan et al., 2005). Accordingly, we also found higher activity of SOD, CAT and POD leaves of all the isabgol genotypes under stress conditions (Fig. 1, 2 and 3). In the present investigation, highest SOD activity was observed in the genotype GI-2 and lowest in the genotype PB-80 under salt stress, which suggests that the genotype GI-2 possesses a better and genotype PB-80 least O<sub>2</sub><sup>-</sup> scavenging ability among the genotypes studied. A number of workers have also reported the increased activity of SOD under salt stress in canola (Bybordi, 2010), cowpea (Maia et al., 2010), common bean (Gama et al., 2008), pea (Ahmad et al., 2008), wheat (Kahrizi et al., 2012) and *Catharanthus roseus* (Jaleel et al., 2008). A toxic species H<sub>2</sub>O<sub>2</sub>, is byproduct of the activity of SOD, to prevent cellular damage it must be eliminated by conversion of it to H<sub>2</sub>O in subsequent reactions involving APX, POD and CAT, which regulate H<sub>2</sub>O<sub>2</sub> levels in plants. We found a significant increase in CAT and POD activity in leaves of all the isabgol genotypes with increasing salt stress. Maximum enhancement of activity of CAT enzyme was in the genotype GI-2 and POD enzyme was in the genotype HI-96 while minimum activity of both the enzymes was in the genotype PB-80 reflect increase in ROS scavenging capacity and decrease in damage to lipids of plasma membrane under stress condition was more in the genotype GI-2 and HI-96 while least in the genotype PB-80. Similar increase in CAT activity under salt stress has been reported in various plants viz., common bean (Gama et al., 2008), maize (Kholova et al., 2009), wheat (Latef, 2010), canola (Bybordi, 2010), pepper (Chookhampaeng, 2011). Kahrizi et al. (2012) however, detected a decline in CAT activity under salt stress as compared to control in wheat cultivars. Increased POD activity under salt stress has been detected in *Catharanthus roseus* plants (Jaleel et al., 2008), wheat (Latef, 2010; Kahrizi et al., 2012), canola (Bybordi, 2010) and pepper (Chookhampaeng, 2011). At biochemical level, SOD, CAT and POD are major antioxidant enzymes

associated with scavenging reactive oxygen species (ROS) and SOD is likely to be central in the defense against toxic ROS (Marschner, 1995). However, SOD detoxifies superoxide anion free radicals accompanying the formation of H<sub>2</sub>O<sub>2</sub> which is very damaging to the chloroplasts, nucleic acids and proteins and can be eliminated by CAT and POD (Marschner, 1995). An increase in the antioxidative enzymes under salt stresses could be indicative of an increased production of ROS and build up of a protective mechanism to reduce oxidative damage triggered by stress in plants. Catalase in peroxisomes break down H<sub>2</sub>O<sub>2</sub>. Peroxidase in cytosol and chloroplast can perfectly scavenge H<sub>2</sub>O<sub>2</sub>. Increase of peroxidase activity by salt treatment in plants has also been reported by Kahrizi et al. (2012).

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