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RESEARCH ARTICLE

PEROXIDASE ACTIVITY AND LIPID PEROXIDATION IN FOOD LEGUMES *Vigna mungo* (L.) HEPPER AND *Vigna aconitifolia* (JACQ.) MARECHAL SEEDLINGS UNDER SALT STRESS

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ABSTRACT

Salinity stress is a major contributor in decreasing crop productivity and threatening agricultural sustainability. In the present study the activity of lipid peroxidation and peroxidase during salt stress treatment for different periods in *Vigna mungo* and *Vigna aconitifolia* leaf tissues was done. Seedlings of *Vigna mungo* and *Vigna aconitifolia* were grown through Hydroponics system containing Hoagland solution under optimum condition of 70% humidity and temperature (28ºC). MDA contents and activity of peroxidase was determined in the leaves after 7 days of induction under salinity stress. Salt stress led to an increase in peroxidase activity, along with an enhanced accumulation of malondialdehyde (MDA) as compared to control shows variation with increased time duration and with concentration in both plants.

INTRODUCTION

Legumes are important sources of protein in arid & semi arid areas of Rajasthan. They are often exposed to adverse environmental factors. Soil salinity adversely affect plant productivity and quality (Zhu, 2001), due to excess Cl^- and Na^+ ions in plants. Seed germination in legumes is also impacted by salinity (Meena & Datta, 2014). Stress has both osmotic (cell dehydration) and toxic (ion accumulation) effects on plant cells, impairing growth, ion homeostasis, photosynthesis and nitrogen fixation among other key physiological processes (Zhu, 2001; Munns, 2002; Tejera *et al*., 2004; Bartels & Sunkar, 2005). Salt stress also causes oxidative damage, thereby affecting cellular membrane integrity, enzyme activities, and functioning of plant photosynthetic apparatus (Jithesh *et al*., 2006) and some basic plant metabolic processes such as, protein synthesis, and energy and lipid metabolism. When plant are subjected to salinity stress, reactive oxygen species (superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) are generated in response to stress condition (A. Ediga *et al*., 2013). In plant cells chloroplast, mitochondria and peroxisomes are important intracellular generators of ROS (Rich and Bonner, 1978). ROS play dual role in plant physiology. Under normal conditions, ROS are effectively scavenged by antioxidant systems, but equally they are toxic products of aerobic metabolism that accumulate within cell during abiotic stress (Huang *et al*., 2012). ROS and oxidative stress may be mediating at least some of the toxic effects of NaCl on legumes (Hernández *et al*., 1999, 2000; Jungklang *et al*., 2004).

ROS are extremely reactive, and can interact with a number of cellular macromolecules, which can induce destructive processes in plant cells when the generation of ROS is not kept under control (Mittler, 2002). ROS production overcomes the antioxidant system capacity, and oxidative stress occurs, resulting in cytotoxic protein damage, DNA damage, and lipid peroxidation (Yazici *et al*., 2007). Therefore, excessive ROS, are considered as an indicator of stress condition. Among the antioxidative defense system antioxidative enzymes play an important role in scavenging ROS through series of complex reactions (H. koca *et al*., 2007).

In our study two legume crops *Vigna mungo* and *Vigna aconitifolia* were grown hydroponically. Seeds were treated

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with Hoagland's solution and after 7 days of germination plantlets were treated with different NaCl concentration i.e. 50, 100 and 150 mM, along with control. Plantlets were harvested in interval of 24 hrs and activity level of peroxidase and lipid peroxidation was measured.

MATERIAL AND METHODS

Seeds of *Vigna mungo* (Urad Var. Uttara) and *Vigna aconitifolia* (Moth Var. RMO 40) were grown in plastic container containing Hoagland solution, in different boxes for seedling cultivation. Surface sterilized seeds were germinated in culture chamber with optimum condition (28ºC) and 70% humidity on an average. After 7 days of germination of seedling, salinity stress (i.e. 50, 100 and 150 mM NaCl) was imposed along with non salt treated plant and with the gap of four time intervals (24, 48, 96 and 120 hrs.), plantlets were harvested.

Enzyme extractions and assays: 0.3 g of leaves were frozen in liquid nitrogen and then ground in 6 mL solution containing 50 mM phosphate buffer (pH 7.0), 1% (w/v) polyvinylpolypyrrolidone, and 0.2 mM ascorbic acid (ASA). The homogenate was centrifuged at 15000 *g* for 30 min, and supernatant was collected and used for enzyme assays.

Lipid Peroxidation Assay*:* Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) produced by the thiobarbituric acid reaction (R.L. Heath and L, Packer 1968). The enzyme extract was mixed with the same volume of a 0.5% (w/v) thiobarbituric acid solution containing 20 % (w/v) tricholoroacetic acid. The mixture was heated at 95^0C for 30 min and then quickly cooled in an ice bath. The mixture was centrifuged at 3,000 *g* for 5 min and the absorbance of the supernatant measured at 532 and 600 nm. MDA concentrations were calculated by means of an extinction coefficient of 156 mM-1 cm-1: MDA (µmol/gm fresh wt.) = [(A532 - A600)/156] x 103 x dilution factor. (Du. Zhanyuan and J B William, 1992).

Peroxidase (POX) assay*:* Total peroxidase activity towards salinity stress was determined as described by H. Urbanek *et al.*, 1991, in a reaction mixture (0.4 ml) containing 100mM phosphate buffer (pH 7.0), o.1 µM EDTA, 5 Mm guaiacol, 15mM H_2O_2 and 100 µl enzyme extract .The initiation of reaction by addition of the enzyme extract and the increase of absorbance were recorded at 470 nm for every 15 second up to 5 min. The enzyme activity quantified by the amount of tetraguaiacol formed per second per mg.

Statistical analysis: All the data were analysed by the help MS Excel. The data were analyzed as the mean ± SEM of number of observations. Comparisons of means were carried out using two way ANOVA compare means between the different treatment groups. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Peroxidase activity

In the present study, in case of Moth, the activity of POX antioxidant enzyme increases on increasing NaCl concentration as compared to control. Whenever we increased the NaCl Concentration the activity of peroxidase increases significantly. At the level of 150 mM NaCl, at 72 hrs of stress imposed, POX activity is slightly more as compared to 96 hr or more. But at 120 hrs the POX activity is greatest at the level of 100 mM NaCl. In case of Urad, POX activity varies at the level of 150 mM NaCl but continuously increases at the level of 100 mM concentration of NaCl. In this POX activity is greatest at 96 hr of treatment at the level of 150 mM NaCl (Fig.1).

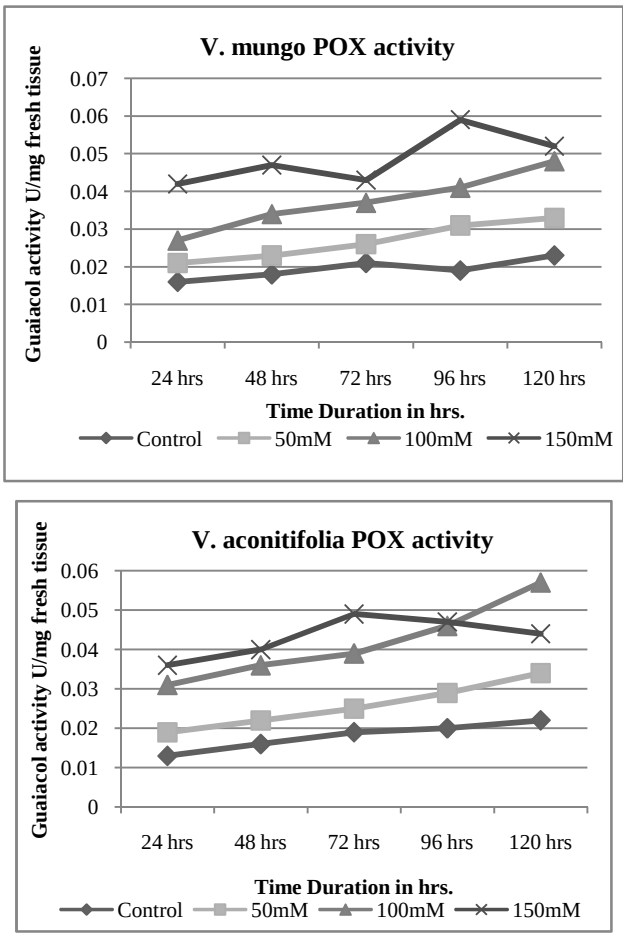

Fig. 1 Effect on Peroxidase activity by salinity stress treatment at different time duration in *Vigna mungo* and *Vigna aconitifoila* leaves. Values represent the mean.

Low basal rate and decreased POX activity seems to indicate that this enzyme does not play a crucial role in defense mechanisms against oxidative stress, or that cooperation is activated between different antioxidant enzymes to establish a proper H_2O_2 balance when POX activity is reduced by salt toxicity (Chaparzadeh *et al.*, 2004; Jaleel *et al.*, 2008a**).** Plants treated with NaCl showed increased activity of peroxidase versus the control. Similar results have been reported in *Vigna radiata* (Manivannan *et al.*, 2007). H_2O_2 is eliminated by various antioxidant enzymes in which peroxidases (**POX: EC**

1.11.1.7) (Gara *et al*., 2003) is one of them which convert H_2O_2 to water.

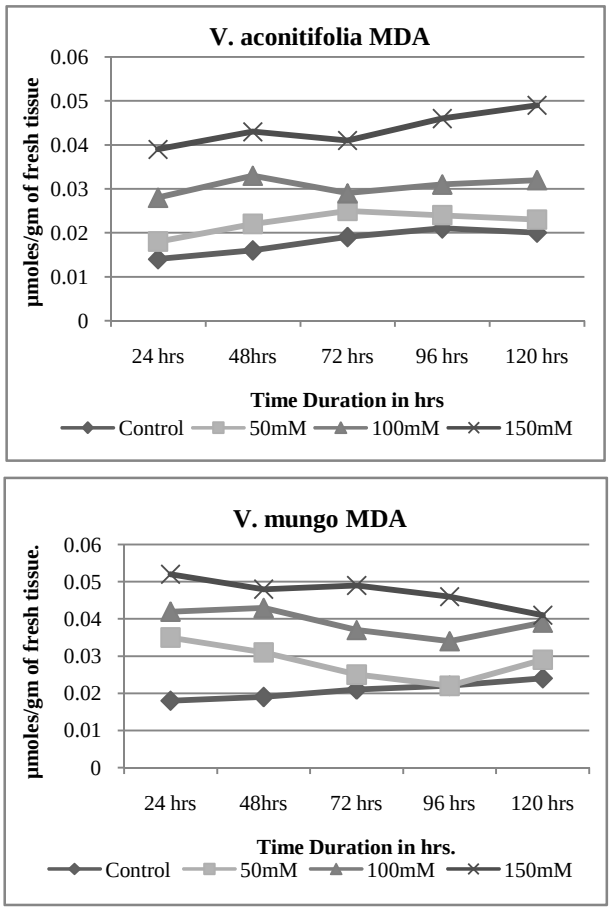

Fig.2 Lipid Peroxidation, expressed by the content of malonaldihyde (**MDA**) in leaves of *Vigna aconitifolia* and *Vigna mungo* in salt stress condition at different time duration. Values represent the mean.

Lipid Peroxidation (MDA)

In the present study, lipid peroxidation measured as MDA content is considered to be indicator of oxidative damage from stress, which is caused by ROS by reducing membrane fluidity and selectivity (Sevengor *et al.,* 2011). It has been previously demonstrated that salinity induces oxidative stress in plant tissues, and lipid peroxidation has frequently been used as an indicator of oxidative stress when plants are subjected to salinity.

The results revealed that, in case of *V. aconitifolia* the activity of lipid peroxidase significantly increased with all the treatment upto 48hrs. Little increases was found in 50mM NaCl treatment, however decline in activity of lipid peroxidation in 50mM Nacl treatment was seen after 72 hrs. At the level of 100 and 150mM NaCl treatment after 72 hrs, increase in MDA content was observed. In case of *V. mungo*, maximum activity was seen at 24hrs. of 150mM NaCl treated sample. Comparatively in *Vigna aconitifolia*, maximum activity was seen after the 120 hrs of treatment at the level of 150mM NaCl (fig. 2). In various studies it has been shown that under stress conditions, due to lipid peroxidation, MDA (malondialdehyde) accumulation take place in plants, which is an effective means of assessing oxidative stress induced membrane damage.

CONCLUSION

It is observed that salinity treatments negatively affect the growth of *V. mungo* and *V. aconitifolia*. An antioxidant enzyme enhances its activity with increasing concentration of NaCl in *Vigna mungo* and *Vigna aconitifolia*. Peroxidase scavenges the reactive oxygen species generated due to oxidative damage which occurres because of NaCl salinity. Membrane damage or leakage during salt stress is indicated by peroxidation of membrane lipids (Katsuhara *et al*., 2005). Salt stress affected both plants due to lipid peroxidation. In *V. aconitifolia* it is maximum at 120 hrs and in *V. mungo* at 24 hr of 150 mM stress condition. This indicates delayed response to salinity by *V. aconitifoila* seedling as compared to *V. mungo*. Further study to reduce the effect of salinity in both food legumes needs to be done. Various cultivars susceptible & tolerant to MDA accumulation also can be further evaluated.

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