

Salinity Induced Oxidative Damage and Antioxidant Responses in Roots of Urdbean

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Abstract

The investigation was undertaken to evaluate the effect of salinity on growth and dry weight accumulation in urdbean *Vigna mungo* (L.) Hepper cv PU-35, along with an analysis of the changes induced by salts on the antioxidant enzyme status in the roots. Low concentrations of salts were observed to be slightly promotory while excess of salinity levels inhibited root, shoot growth and reduced dry matter accumulation. The activity of antioxidant enzymes varied with the levels of salinity. A decrease in malondialdehyde (MDA) amount and stimulation of superoxide dismutase (SOD), peroxidase (PER), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) was examined in seedlings exposed to 4, 8, 12, 16 and 20 dS/m, while catalase (CAT) activity showed significant decrease above 4 dS/m. Excess of salinity induced lipid peroxidation as well as modified the activity of defense enzymes SOD, PER, CAT, PPO and PAL.

Key words : Salinity, Salts, Antioxidant enzymes, Oxidative stress, Urdbean.

Salinity is a major limitation to crop production throughout the world. It leads to metabolic alternations and reduction in growth is a cumulative effect of salinity on dry matter production through various physiological processes. However, plants, try to adapt themselves to salinity stress by accumulating several metabolites that serve as osmoticum. Environmental stresses are thought to result in the production of reactive oxygen species (ROS) in plants, causing oxidative stress. The ability of higher plants to scavenge the toxic ROS seems to be important determinant of their tolerance to environmental stresses. Oxidative damage is alleviated by a concerted task of both enzymatic and non-enzymatic antioxidant metabolisms. These mechanisms include β -carotene and enzymes including SOD, PER, CAT, PPO, PAL and lipid peroxidation MDA. There are many reports that underline the intimate relationship between enhanced antioxidant enzyme activities and increased to salinity. The present study focuses on the effect of salinity on seedling growth, biomass accumulation, examines the response of antioxidant enzymes viz., superoxide dismutase (SOD), peroxidase (PER), catalase (CAT) and also changes in enzymes such as polyphenol oxidase (PPO) and phenylalanine am-

monia lyase (PAL) in roots of urdbean. Malondialdehyde (MDA) concentration, which is an index of lipid peroxidation, was also recorded.

Methods

The research work was carried in August 2006. Urdbean *Vigna mungo* (L.) Hepper cv PU-35 seeds, procured from Genetics and Plant Breeding Department, G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand). Uniform sized seeds were surface sterilized, washed and germinated in sterilized petri dishes lined with Whatman filter paper no. 1, soaked in various levels of salinity (4, 8, 12, 16 and 20 dS/m). Distilled water soaked seeds were taken as control. The saline solutions were prepared by taking NaCl, NaHCO₃, CaCl₂ and Na₂SO₄ following procedure of Richards (1954). Twenty seeds per petri dish were used for the experiment. The petri dishes are used in triplicates. The experiment was terminated on day 4. The observations were recorded on length and dry weight of root and shoot respectively. Enzyme extraction and assays on root samples were estimated after day 4. The enzymes, namely SOD, PER, CAT, PPO and PAL were measured by standard meth-

Table 1. Effect of salinity on seedling growth and dry matter yield in *Vigna munga* (L.) Hepper, after four days of germination.

Treatment (dS/m)	Root length (cm) Mean \pm SD	Shoot length (cm) Mean \pm SD	Root dry wt (mg/seedling) Mean \pm SD	Shoot dry wt (mg/seedling) Mean \pm SD
Control	6.826 \pm 0.060008	5.689 \pm 0.02021	3.154 \pm 0.01685	9.559 \pm 0.01575
4	7.314 \pm 0.03533	6.045 \pm 0.02872	2.491 \pm 0.01549	10.016 \pm 0.009165
8	7.389 \pm 0.002557	5.973 \pm 0.0249	2.108 \pm 0.032056	9.372 \pm 0.0166
12	5.295 \pm 0.024187	5.223 \pm 2.23621	01.96 \pm 0.0167	9.275 \pm 0.015
16	3.972 \pm 0.029079	4.261 \pm 0.02166	1.20 \pm 0.0164	7.591 \pm 0.020224
20	1.336 \pm 0.02059	4.001 \pm 0.0157797	1.23 \pm 0.316165	6.884 \pm 0.0200997

ods (Dhindsa et al. 1981, Chance and Machly 1955, Kar and Mishra 1976, Yamaguchi et al. 1970, Brueske 1980), simultaneously. The MDA amount was determined using thiobarbituric acid (TBA) reaction (Heath and Packer 1968).

Results and Discussion

Salinity levels at high concentrations decreased seedling growth (length and dry matter accumulation). The deleterious effects of salinity were more pronounced in root and shoots. The seedling growth assessed in terms of root and shoot length was adversely affected by increasing levels of salinity (Table 1). In the present study, the seedlings exposed to 4dS/m showed marginal increases in length of root and shoot. The reduction in biomass accumulation

may represent the cumulative effects of damaged or inhibited physiological activities. Root elongation was adversely affected and root tip turned brown and brittle with increasing levels of salinity. The toxic effects of salinity on roots affect the entire plant as it is the main pathway for entry of saline water and other nutrients.

Excess of salinity induced lipid peroxidation and modified the activity of antioxidant defense enzymes viz., SOD, PER, CAT, PPO and PAL (Table 2). SOD catalyzes the dismutation of O_2^- to H_2O_2 and O_2 . It is a key enzyme in protecting cells from oxidative stress. SOD is an essential constituent of antioxidant defense (Boscolo et al. 2003), and is present in all aerobic organisms and in most sub-cellular compartments that generate oxidative stress. The analysis of SOD activity in the present investigation showed an

Table 2. Effect of salinity on activities of enzymes and on MDA content in roots of *Vigna munga* (L.) Hepper, after four days of germination.

Treatment (dS/m)	Peroxidase (per g fw) Mean \pm SD	Catalase (per g fw) Mean \pm SD	Superoxide dismutase (per g fw) Mean \pm SD	Phenylalanine ammonia lyase μ gt cinnamic acid (per h/g fw) Mean \pm SD	Polyphenoloxidase mmols of catechol oxide (per min/g fw) Mean \pm SD	Lipid peroxidation MDA μ mol/g fw Mean \pm SD
Control	138.361 \pm 0.511446	720.5 \pm 211.34	78.26 \pm 0.076026	82.45 \pm 0.217348	18.45 \pm 0.15013	10.41 \pm 0.013038
4	143.5 \pm 2.2472	747.1 \pm 01.45945	80.65 \pm 0.827345	131.8 \pm 0.76492	24.5 \pm 0.7937	12.87 \pm 0.01974
8	150.38 \pm 0.778048	661.3 \pm 2.8427	83.45 \pm 0.926553	147.4 \pm 0.4271	31.3 \pm 0.27203	14.48 \pm 0.012649
12	245.8 \pm 1.72047	614.2 \pm 1.30269	93.1 \pm 0.30953	173.2 \pm 0.279285	36.5 \pm 0.21213	15.84 \pm 0.01549
16	124.74 \pm 1.52197	503.9 \pm 0.943398	67.8 \pm 0.73892	182.6 \pm 0.68702	28.50 \pm 0.01183	18.69 \pm 0.013038
20	110.7 \pm 0.62769	411.3 \pm 1.689935	59.03 \pm 0.583181	162.4 \pm 3.17065	22.12 \pm 0.04483	21.84 \pm 0.01516

increase in the activity at low levels of salinity with a maximum increase 18.96% at 12 dS/m compared to the control sets. Hence, activity of SOD in the present research paper decreased by 13.3 and 24.5% on exposure to 16 and 20 dS/m simultaneously, indicating severe damage to root tissue development and growth. Reduction in SOD activity at higher levels of salinity may be attributed to an inactivation of enzyme by H_2O_2 , which is produced in different cellular compartments and also from a number of non-enzymatic and enzymatic processes in cells (Schutzendubel et al. 2002).

PER and PPO activities followed a similar trend as SOD with salinity treatments (Table 2). PER and PPO activities gradually increased with salinity treatments reaching a maximum at 12 dS/m (75.65% and 97.83% increase), and decreased thereafter. In this study the activities of PPOs showed a trend similar to that of SOD and PER, therefore, it is suggested that they may have a role in reducing oxidative stress induced by different levels of salinity. Increased or decreased PER activity has been reported in various plants (Sandalo et al. 2001). PER is thought to be stress marker enzyme and its higher induction may indicate stress exerted by high salinity, which can be correlated with amount of the accumulated salts. High level of antioxidative enzymes are a part of biochemical and physiological make up involved in salt tolerance and ameliorate the oxidative damage arising from salt stress. PPOs are responsible for oxidation of phenolic substrates to quinones and also have been reported to show increased activities to various types of stresses. In this study PPOs showed a trend similar to that of SOD and PER, hence it is suggested that they may have a role in reducing oxidative stress induced by salinity.

PAL activity increased with salinity levels reaching a maximum at 16 dS/m (121.46% increase). PAL is the entry point enzyme in the phenyl propanoid biosynthetic pathway, and increase in PAL activity in response to various types of abiotic stresses, has been reported in different species of plants (Hammerschmidt 1999).

The oxidative enzymes are known to deplete the pool of ROS from accumulating to toxic levels and thus play an important role in adaptation and survival of plants during stress. The cellular antioxidant enzymes are believed to protect the cell, to some extent,

from damages arising from salinity induced ROS production. SOD catalyses the conversion of superoxide ion to H_2O_2 , whereas CAT scavenges H_2O_2 and PER uses H_2O_2 for oxidation of various inorganic or organic substrates. Increased levels of these enzymes observed in this study, therefore, may reflect a common strategy to overcome the stress due to salts. The decreased SOD, CAT and PER activities recorded at 16 and 20 dS/m, may be due to the harmful effect of overproduction of H_2O_2 , other ROS derivatives and salinity induced inhibition of protein synthesis overrides the cellular detoxification mechanisms of plant. The reduced activities of antioxidant enzymes in the presence of high salinity levels represent an indication of severity of salts induced oxidative stress.

In present study, MDA concentration increased with increasing levels of salinity (4 to 20 dS/m) indicating, the increased presence of free radicals in the tissue and consequent increase in lipid peroxidation and oxidative stress in urdbean (Table 2). Salinity stress induced an increase in MDA has also been confirmed by (Kasturi Bai et al. 2001). Free radicals and other active derivatives of oxygen and inevitable product of natural redox reaction in various cellular compartments under different saline conditions. The free radicals mediated peroxidation of unsaturated fatty acids also referred as lipid peroxidation is a chain reaction occurring mainly in biomembranes.

Thus, salt stress modified the levels of SOD, PER, PPO, PAL and CAT, the main components of enzymatic defense system in urdbean, and the effect varied with the intensity of salinity exposure. The pattern of enzyme activities suggests that tolerance mechanism involves a system that removes free radicals and ROS, preventing their accumulation, which in turn increases the need for antioxidant/defense enzymes such as SOD, PER, PPO, CAT and PAL.

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