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Alleviation of NaCl stress by pretreatment with phytohormones in *Vigna radiata*

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Abstract

Efficiency of pretreatment as foliar spray of indole-3-acetic acid, gibberellic acid and kinetin, each ranging from 0.1 to 10.0 μM concentration, in restoring the metabolic alterations imposed by NaCl salinity was investigated in *Vigna radiata* (L.) Wilczek. Glycolate oxidase, superoxide dismutase, catalase and peroxidase activities increased under stress in leaves and roots also. Malondialdehyde content and total peroxide content also increased under stress. All the three hormones used were able to overcome to variable extents the adverse effects of stress imposed by NaCl to these parameters.

Additional key words: catalase, glycolate oxidase, malondialdehyde, peroxidase, peroxide, superoxide dismutase.

Introduction

Soil salinity is a major limitation to legume production (Abd-Alla *et al.* 1998). Salt stress enhances senescence by changing enzyme activities, reducing soluble protein content, nucleic acid content, chlorophyll content together with reduction in CO_2 fixation capacity of the leaves. Decline in CO_2 fixation rate increases NADPH/NADP ratio and under such a situation O_2 functions as an alternative electron acceptor and forms superoxide anion radical ($O_2^{\bullet-}$) and other reactive oxygen species viz. hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$). During normal cell metabolism also, $O_2^{\bullet-}$ and H_2O_2 are generated in different compartments of the cell. These active oxygen species are toxic. $^1O_2$ can react directly with polyunsaturated fatty acids, which are the main components of membranes, to form lipid peroxides (Konze and Elstner 1978). Lipid peroxidation is estimated by measuring malondialdehyde (MDA) content.

Normal cell metabolism requires detoxification of activated oxygen species. Protective mechanisms include removal of $O_2^{\bullet-}$ by glycolate oxidase and superoxide dismutase and removal of H_2O_2 by catalase or peroxidase systems. The enzyme glycolate oxidase is responsible for the formation of H_2O_2 from $O_2^{\bullet-}$ (superoxide anion radical). H_2O_2 and O_2 are also formed by the enzymatic dismutation of $O_2^{\bullet-}$ by SOD. But decrease in the activity of the enzyme can result in the formation of superoxide radical ($O_2^{\bullet-}$) and H_2O_2 which in turn can form the hydroxyl radical ($^{\bullet}OH$). Catalase plays an important role in maintaining H_2O_2 below toxic levels. SOD specially removes $O_2^{\bullet-}$ from stroma. Peroxidase is another H_2O_2 consuming enzyme which it does by the oxidation of a reducing co-substrate (variety of organic acid and inorganic compounds).

Salt-tolerant *Citrus limon* callus exhibited under salt stress the induction of a new superoxide dismutase isozyme and an increase of the peroxidase activity while the catalase activity remained unchanged (Piqueras *et al.* 1996). Treatment of *Mesembryanthemum crystallinum* for several days with NaCl enhanced activity of superoxide dismutase (Miszalski *et al.* 1998). In *Ipomoea pes-caprae*, NaCl stimulated the activities of catalase and peroxidase (Venkatesan and Chellapan 1999). In seeds of groundnut germinated in presence of NaCl and $CaCl_2$ separately or in combination peroxidase activity in cotyledons was increased (Satakopan *et al.* 1990). NaCl treatment of the chickpea plants increases peroxidase activity (Sheokand *et al.* 1995). Under NaCl salinity,

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Abbreviations: GA_3 - gibberellic acid; IAA - indole-3-acetic acid; Kin - kinetin (6-furfuryl aminopurine); MDA - malondialdehyde; SOD - superoxide dismutase.
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the lipid peroxidation as indicated by MDA formation was greater in salt-sensitive than in salt-tolerant cultivar of mulberry (Giridara Kumar *et al.* 2000).

In our experiment the main object was to determine the effect of salt stress on total peroxide and malondialdehyde content together with glycolate oxidase, superoxide dismutase, catalase and peroxidase activity in mung bean and to determine the efficiency of indole-3-acetic acid (IAA), gibberellic acid (GA_3) and kinetin (Kin) in restoring the metabolic alterations resulting from salt stress.

Materials and methods

The experiment was conducted in sandy-loam soil in the experimental garden using a salinity susceptible mung bean [*Vigna radiata* (L.) Wilczek] cultivar B-105 collected from Oil and Pulse Research Institute, Berhampore, West Bengal. Ten plants were kept in each pot and the five sets maintained were: *1*) control, *2*) NaCl stressed, *3*) IAA pretreated NaCl stressed, *4*) GA_3 pretreated NaCl stressed, *5*) Kin pretreated NaCl stressed. Plants were sprayed with IAA, GA_3, and Kin (50 cm^3 per pot) at concentrations 0.1, 1.0, and 10.0 μM mixed with Tween-20 from day 13 (emergence of first trifoliate leaf) upto day 35, once a week. Control set was sprayed with equal amount of water mixed with *Tween-20*. Then all these sets were treated with NaCl to maintain electrical conductivity (E.C.) values of the soil as 4.0 mS cm^{-1}, 8.0 mS cm^{-1} and 12.0 mS cm^{-1}. The soil used was with initial electrical conductivity 0.3 mS cm^{-1} and pH 7.6. The set receiving no NaCl was designated as control. This condition was maintained until grain filling was complete. The garden temperature was 34 ± 2 °C.

After grain filling, plants were uprooted carefully, washed thoroughly with deionized water and separated into leaves, roots and nodules. Glycolate oxidase activity was assayed according to the method of Zelitch (1953) according to the rate of reduction of 2,6-dichlorophenolindophenol measured at 620 nm on the *Hitachi U-2000* spectrophotometer (Tokyo, Japan). The assay mixture for superoxide dismutase was the same as used by Marshall and Worsfold (1978). The increase in the absorbance due to formazan formation was read at 560 nm. The increase in absorbance without the enzyme extract was taken as 100 % and the enzyme activity was calculated by determining the per cent inhibition per min. 50 % inhibition was taken as equivalent to 1 unit of superoxide dismutase activity. The lipid peroxidation was measured by the estimation of malondialdehyde content using the method of Heath and Packer (1968). Total peroxide content was measured by the ferrithiocyanate method of Thurman *et al.* (1972). The absorbance of the ferrithiocyanate complex formed was read at 480 nm and was compared with a standard curve prepared with known concentrations of H_2O_2. Catalase activity was assayed by the method of Gasper and Lacoppe (1968). Estimating the amount of $KMnO_4$ consumed total H_2O_2 was calculated. Peroxidase activity was assayed spectrophotometrically according to Chance and Maehly (1955). The absorbance at 420 nm of the reaction mixture was recorded at 0, 30, 60, 90 and 120 s. Protein content in enzyme extracts was estimated according to Lowry *et al.* (1951).

Results

Among the three different electrical conductivity (E.C) values maintained by using NaCl solution, only 4.0 mS cm^{-1} was the most suitable concentration. It produced metabolic injuries to a moderate level which could be restored to different degrees by the three hormones used. So these results have only been presented here.

Glycolate oxidase activity increased as a result of NaCl stress in leaf and root of mung bean by about 43 and 6 % over the control set. In leaf, all phytohormones considerably reduced glycolate oxidase activity (Fig. 1). In root, only GA_3 and Kin pretreatments slightly reduced the activity (Fig. 1).

Superoxide dismutase (SOD) activity also increased under stress in leaf and root over the control set. In leaf, IAA and GA_3 at all the concentrations and Kin at concentrations 1.0 μM and 0.1 μM reduced this increase (Fig. 2). In root, IAA (10.0 μM), GA_3 (0.1 μM) and Kin (0.1 μM) reduced the increase. However, 10.0 μM Kin considerably increased SOD activity (Fig. 2).

Malondialdehyde (MDA) content increased under salinity stress in leaf and root. In leaf, all phytohormones reduced increase in malondialdehyde content in stressed plants (Fig. 3). In root, only IAA and GA_3 reduced increase in MDA content as compared with the control set (Fig. 3).

Total peroxide content also increased under NaCl stress in leaf and root as compared with the control. In leaf, IAA, GA_3 and Kin at all the concentrations slightly reduced this increase (Fig. 4). But in root, all phytohormones considerably reduced total peroxide content (Fig. 4).

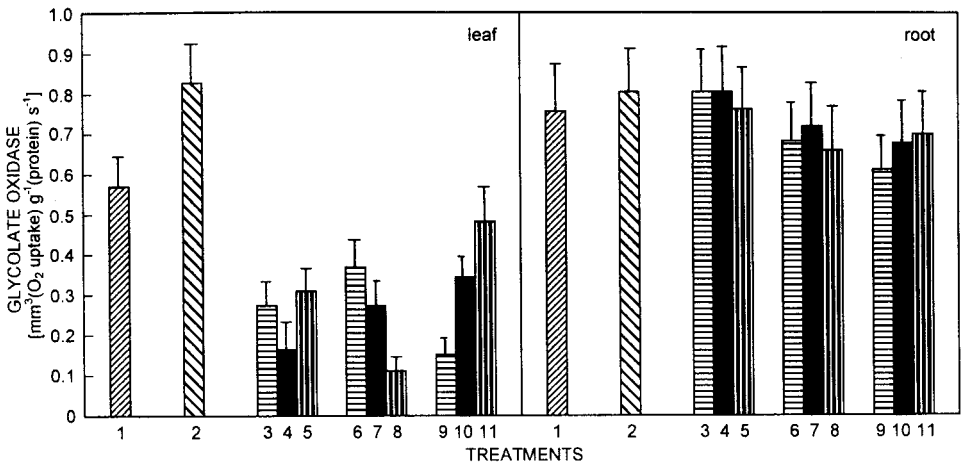

Fig. 1. Glycolate oxidase activity of control and treated mung bean leaves and roots. *Vertical bars* represent SE, $n = 3$.
1 - control set; 2 - stressed set; 3 - IAA (10.0 μM) pretreated stressed set; 4 - IAA (1.0 μM) pretreated stressed set; 5 - IAA (0.1 μM) pretreated stressed set; 6 - GA_3 (10.0 μM) pretreated stressed set; 7 - GA_3 (1.0 μM) pretreated stressed set; 8 - GA_3 (0.1 μM) pretreated stressed set; 9 - Kin (10.0 μM) pretreated stressed set; 10 - Kin (1.0 μM) pretreated stressed set; 11 - Kin (0.1 μM) pretreated stressed set.

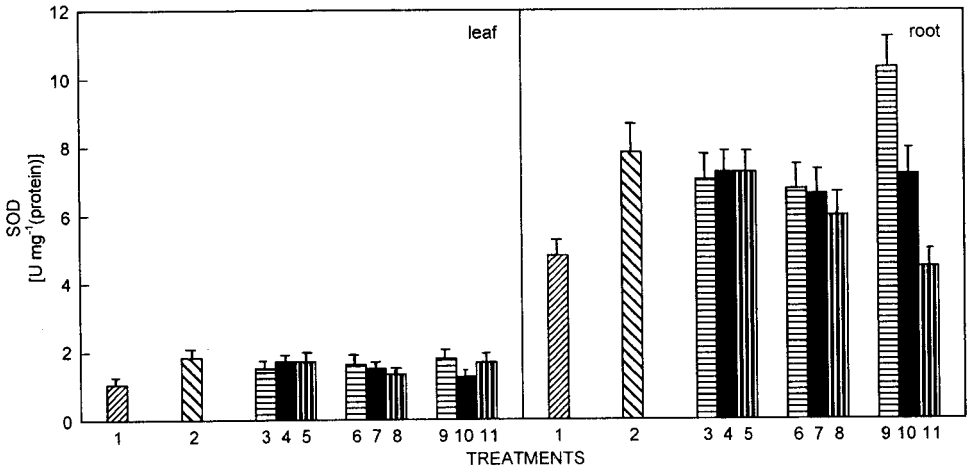

Fig. 2. Superoxide dismutase (SOD) activity (U = per cent inhibition of formazan formation per min) of control and treated mung bean leaves and roots. *Vertical bars represent* SE, $n = 3$. The same treatments (1 - 11) as in Fig. 1.

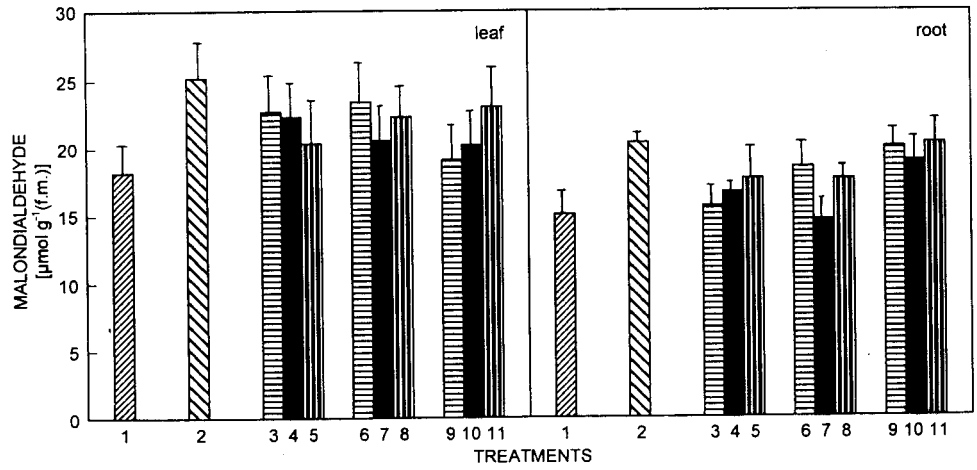

Fig. 3. Malondialdehyde (MDA) content of control and treated mung bean leaves and roots. *Vertical bars* represent SE, $n = 3$. The same treatments (1 - 11) as in Fig. 1.

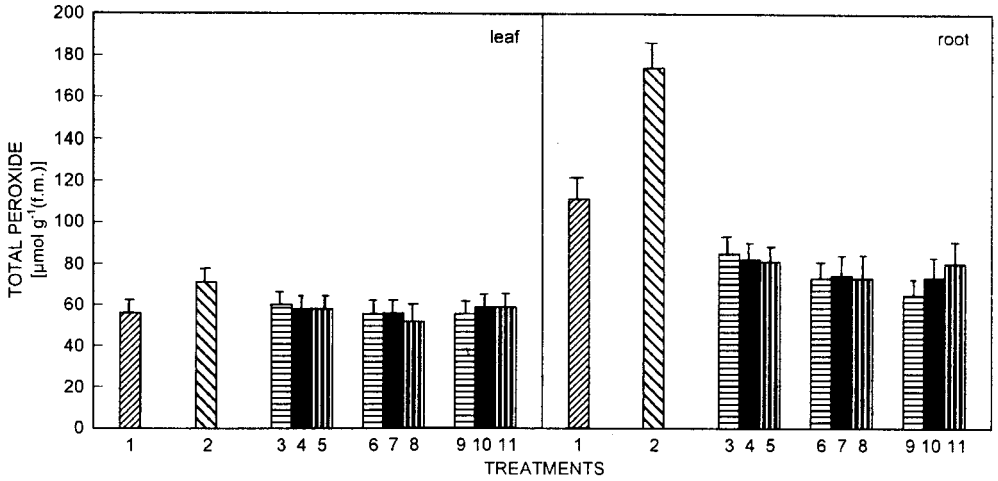

Fig. 4. Total peroxide content of control and treated mung bean leaves and roots. *Vertical bars* represent SE, $n = 3$. The same treatments (1 - 11) as in Fig. 1.

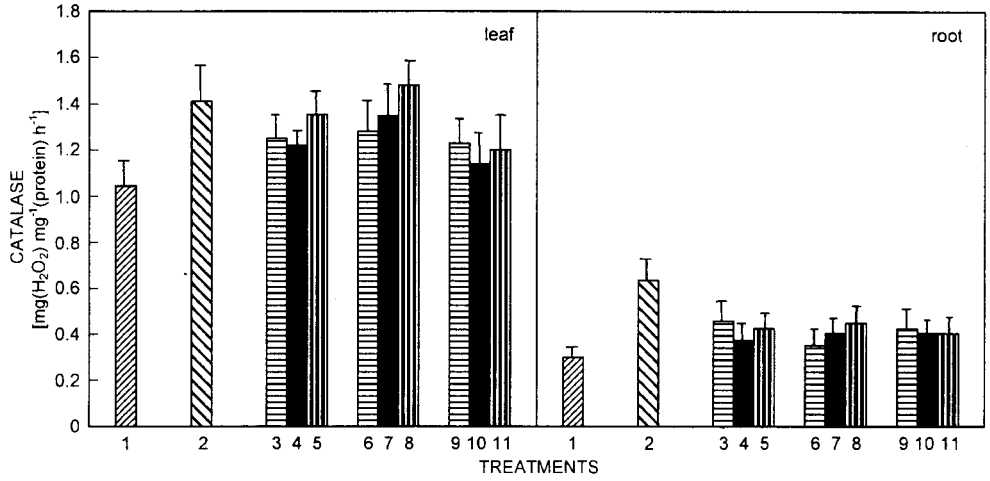

Fig. 5. Catalase activity of control and treated mung bean leaves and roots. *Vertical bars* represent SE, $n = 3$. The same treatments (1 - 11) as in Fig. 1.

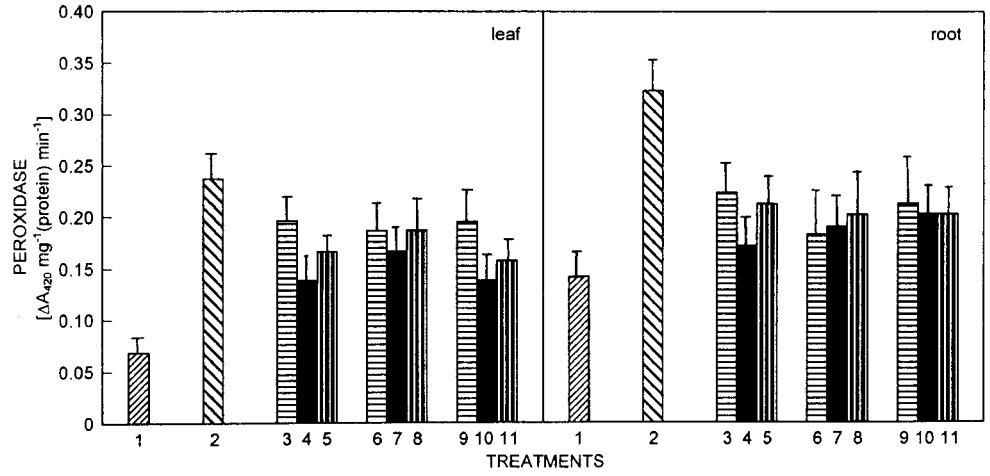

Fig. 6. Peroxidase activity of control and treated mung bean leaves and roots. *Vertical bars* represent SE, $n = 3$. The same treatments (1 - 11) as in Fig. 1.

In leaf and root of mung bean plant, catalase enzyme activity was minimum in control set and increase over the control was maximum in NaCl stressed set. In leaf, IAA and Kin at all the concentrations and GA_3 at 10.0 and 1.0 μM reduced the activity (Fig. 5). In root, IAA, GA_3 and Kin at all the concentrations considerably reduced this increase (Fig. 5).

Peroxidase enzyme activity also increased in mung bean leaf and root under stress as compared with control. But all the phytohormones used at all the three concentrations reduced this increase in both leaf and root (Fig. 6).

From all the results, it is very clear that all the three phytohormones used in the present study help to a different degree in the reversal of altered metabolism induced by salinity stress in glycophyte *Vigna radiata*.

Discussion

In the present work, total peroxide and malondialdehyde content in different plant parts of mung bean increased with simultaneous increase in glycolate oxidase, superoxide dismutase, catalase and peroxidase activities under salt stress. Similar results were also obtained in *Halimione portulacoides* and callus culture of rice (Kalir *et al.* 1984, Subhashini and Reddy 1990). With increase in peroxide content, activities of scavenger enzymes increased to protect the plant (Subhashini and Reddy 1990). Change in the activity of peroxidase induced by exposure to salinity was probably due to changes in the protein moiety of the enzyme (Kalir *et al.* 1984).

Under the present experimental condition, phytohormones pretreatment in mung bean helped to reduce the per cent increase of total peroxide (Fig. 4) and malondialdehyde content (Fig. 3), with a simultaneous decrease in the activities of scavenging enzymes as compared with the set receiving only NaCl. Since free radical-induced lipid peroxidation mediates senescence associated membrane deterioration in plant tissues, inhibition of senescence by phytohormones may be mediated by a modulation of free radical-induced lipid peroxidation (Dhindsa 1982). Phytohormones application delays senescence under stress by modulating lipid peroxidation through maintaining higher activities of superoxide dismutase, catalase and peroxidase as compared with the control.

Cytokinins retard senescence through tRNA metabolism, having effects on membrane permeability to mono- and divalent ions and localized induction of metabolic sinks (Letham 1978). Kin acts as a direct free radical scavenger or it may involve in an antioxidative mechanism linked to the prevention of purine breakdown. Free radical-induced lipid peroxidation become inhibited by Kin and GA_3 which also inhibit the activity of the enzyme lipoxygenase responsible for the oxidation of unsaturated fatty acids (Choudhuri 1988).

From the results presented here, it is pertinent to suggest the possibility of adopting different strategies for growing this crop under saline conditions. It is possible that foliar spray of the crop with phytohormones before incidence of stress would confer resistance against stress induced damage.

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