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Effect of phytohormone pretreatment on nitrogen metabolism in *Vigna radiata* under salt stress

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Abstract

Application of NaCl (electrical conductivity 4.0 mS cm^{-1}) resulted in about 52, 50 and 55 % reduction in total nitrogen contents in mung bean [*Vigna radiata* (L.) Wilczek] leaf, root and nodule, respectively. In nodule, nitrogenase activity was reduced by about 84 % under stress as compared with the control set. Glutamine synthetase activity was reduced by about 31, 16 and 23 %, glutamate oxoglutarate aminotransferase activity was reduced by 78, 57 and 42 % and glutamate dehydrogenase activity was reduced by 9, 8 and 42 % in leaf, root and nodule, respectively, under salt stress. The pretreatment with indole-3-acetic acid, gibberellic acid and kinetin, each ranging from 0.1 to 10 μM, in restoring the metabolic alterations imposed by NaCl salinity was investigated in mung bean. The three phytohormones used were able to overcome to variable extents the adverse effects of stress imposed by NaCl solution.

Additional key words: glutamate dehydrogenase, glutamate oxoglutarate aminotransferase, glutamine synthetase, mung bean, NaCl, nitrogenase.

Introduction

Salt stress generally alters a wide array of metabolic processes. Among others, salinity reduces the nitrogen content in root and stem of *Sesbania* sp. (Ramani *et al.* 1989), delays nodule initiation, decreases nodule mass and leghaemoglobin content of fresh nodules in chickpea (Ram *et al.* 1989). In pigeon pea and chickpea plants nitrogen content decreases with increasing salinity (Subbarao *et al.* 1990, Varshney *et al.* 1998). Imposition of salinity stress inhibits total nitrogen content in alfalfa (Khan *et al.* 1997).

Nitrogenase is an enzyme which is responsible for reduction of free nitrogen (N_2) to ammonia (NH_3), whereas glutamate dehydrogenase (GDH), glutamine synthetase (GS) and glutamate oxoglutarate aminotransferase (GOGAT) are ammonia assimilatory enzymes in plants. GDH directly incorporates ammonia into glutamate, GS incorporates ammonia into glutamine and GOGAT catalyses transamination from glutamine to 2-oxoglutarate resulting in the formation of 2 molecules of glutamate. In soybean, nitrogenase activity in nodules and nitrogen content in leaves and roots decrease under soil salinity. Presoaking treatment with GA_3 (0.29 mM) or IAA (1.04 mM) significantly reduces the detrimental effects of salinity on acetylene reduction activity of soybean nodules and improves the nitrogen content in leaves and roots (Abd-Alla *et al.* 1998). The decrease in N_2-fixation has been ascribed to direct effect on nitrogenase activity or an indirect effect through decrease in leghaemoglobin content (Swaraj and Bishnoi 1999).

In our experiment, attention has been given to phytohormones like IAA, GA_3 and kinetin as possible inducers of resistance to unfavourable environmental conditions. The main object was to determine the effect of salt stress on nitrogen content and nitrogenase, glutamine synthetase, glutamate oxoglutarate aminotransferase and glutamate dehydrogenase activity in mung bean and to determine the efficiency of these three phytohormones in restoring the metabolic alterations resulting from salt stress.

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Abbreviations: GA_3 - gibberellic acid; GDH - glutamate dehydrogenase; GOGAT - glutamate oxoglutarate aminotransferase; GS - glutamine synthetase; IAA - indole-3-acetic acid; Kin - kinetin (6-furfuryl aminopurine).

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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 ten pots were maintained for each set. Five sets maintained were: *1*) control, *2*) NaCl stressed, *3*) IAA pretreated NaCl stressed set, *4*) GA_3 pretreated NaCl stressed set and *5*) Kin pretreated NaCl stressed set. Sets numbered *3*, *4* and *5* were sprayed with IAA, GA_3 and Kin respectively (50 cm^3 per pot) each 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 solution to maintain electrical conductivity (E.C.) of the soil 4.0, 8.0 and 12.0 mS cm^{-1}. The soil used was with 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 where the experiment was conducted was 34 ± 2 °C.

After grain filling, the penultimate leaves, roots and nodules were collected. Nitrogen content was measured according to Kjeldahl method. Glutamine synthetase (GS) activity was estimated according to Elliott (1955), glutamate oxoglutarate aminotransferase (GOGAT) activity was assayed according to Sodek and Da Silva (1977) and glutamate dehydrogenase (GDH) activity was assayed according to Lea and Miflin (1974). Nitrogenase activity was assayed following the method of Hardy *et al.* (1968) using fresh and healthy nodules collected from 56-d-old mung bean plants.

Results

From the experimental results it is clear that among the three different concentrations of NaCl, only the lowest 4.0 mS cm^{-1} was the effective sublethal concentration. It produced metabolic injuries to a moderate level which could be restored to different degrees by the three hormones each used at three different concentrations. So the results of these four treatments [*viz.* stressed set (E.C. 4.0 mS cm^{-1}), IAA pretreated stressed set, GA_3 pretreated stressed set and Kin pretreated stressed set] have only been presented here.

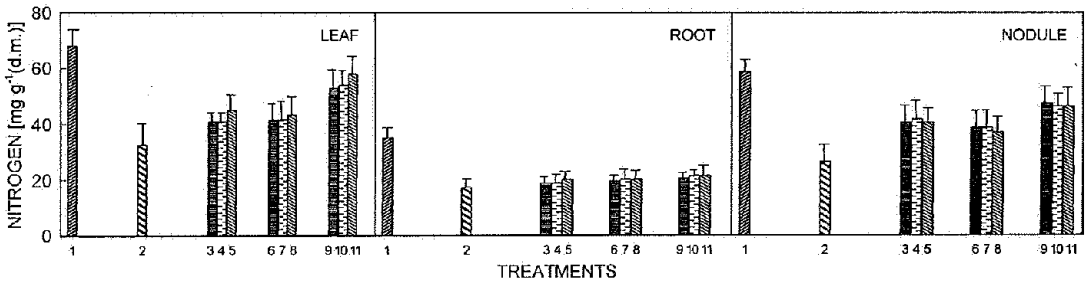

Fig. 1. Nitrogen content of control and treated mung bean leaves, roots and nodules: 1 - control; 2 - NaCl stressed set; 3 - IAA (10.0 μM) pretreated stressed set; 4 - IAA (1.0 μM) pretreated stressed set; 5 - IAA (0.1 μM) pretreated stressed set; 6 - GA_3 (10.0 μM) pretreated stressed set; 7 - GA_3 (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. *Vertical bars* represent standard error of the mean of 3 replicates ($P = 0.05$).

Total nitrogen contents of leaf, root and nodule were reduced 52, 50 and 55 %, respectively, under NaCl stress as compared with control. In leaf and root, all the three phytohormones were most effective at a concentration of 0.1 μM. IAA pretreatment ameliorate the reduction to 34 and 41 %, GA_3 to 36 and 41 %, and Kin to 15 and 39 % (Fig. 1). In nodules, all hormone concentrations, were more or less equally effective (Fig. 1).

The nitrogenase activity in nodules was reduced by about 84 % under stress as compared with the control set and IAA, GA_3 and Kin were the most effective at a concentration of 1.0 μM minimizing the reduction to 40, 20 and 28 %, respectively (Fig. 2).

Glutamine synthetase (GS) activity was reduced by about 31, 16 and 23 % under salt stress in leaf, root and nodule, respectively, as compared with the control. In case of leaf, all the three hormones at a concentratin of 0.1 μM were the most effective showing 56, 48 and 58 % increase in activity over the control set (Fig. 3). In root, 1.0 μM IAA and 0.1 μM Kin were beneficial (Fig. 3). In nodule, 1.0 μM IAA decreased the reduction from 23 to 10 %, 10.0 μM and 0.1 μM GA_3 from 23 to 5 % and

10.0 μM Kin from 23 to about 2 % (Fig. 3).

Glutamate oxoglutarate aminotransferase (GOGAT) activity was also reduced under stress as compared with control by 78, 57 and 42 % in leaf, root and nodule, respectively. In leaf, 0.1 μM IAA, 1.0 μM GA_3 and 1.0 μM Kin increased the enzyme activity by 22, 5 and 27 %, respectively, over the control set (Fig. 4). In root, IAA and GA_3 each at a concentration of 1.0 μM and 10.0 μM Kin decreased the reduction from 57 to 7, 11 and 7 %, respectively (Fig. 4). In nodule, 1.0 μM IAA, 0.1 μM GA_3 and 10.0 μM Kin increased the enzyme activity over the control set by 16, 30 and 56 %, respectively (Fig. 4).

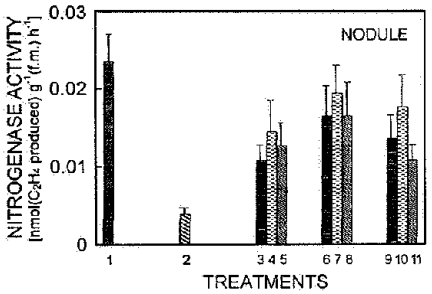

Fig. 2. Nitrogenase enzyme activity of control and treated mung bean nodules. See Fig. 1 for details.

Glutamate dehydrogenase (GDH) enzyme activity was slightly inhibited by NaCl (9, 8 and 42 % in leaf, root and nodule, respectively). In leaf, 0.1 μM IAA decreased the reduction from 9 to 4 %, 1.0 μM GA_3 from 9 to 2 %, whereas 0.1 μM Kin completely reversed the stress effect (Fig. 5). In root, IAA and kinetin each at a concentration of 0.1 μM and 1.0 μM GA_3 decreased the enzyme activity only by 3, 5 and 2 %, respectively, than the control set (Fig. 5). In nodule, IAA and Kin each at a concentration of 0.1 μM and 1.0 μM GA_3 ameliorate the reduction from 42 to 5, 16 and 5 %, respectively (Fig. 5).

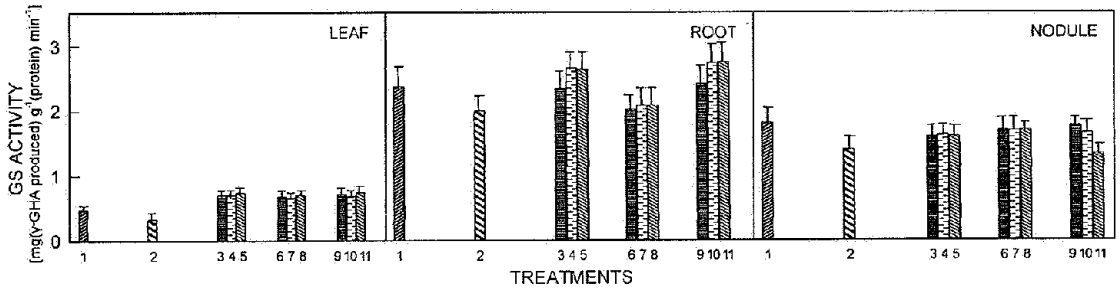

Fig. 3. Glutamine synthetase (GS) enzyme activity of control and treated mung bean leaves, roots and nodules. See Fig. 1 for details.

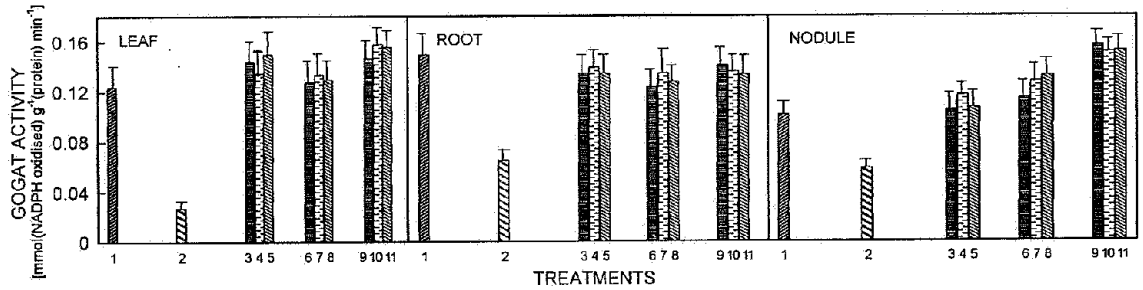

Fig. 4. Glutamate oxoglutarate aminotransferase (GOGAT) enzyme activity of control and treated mung bean leaves, roots and nodules. See Fig. 1 for details.

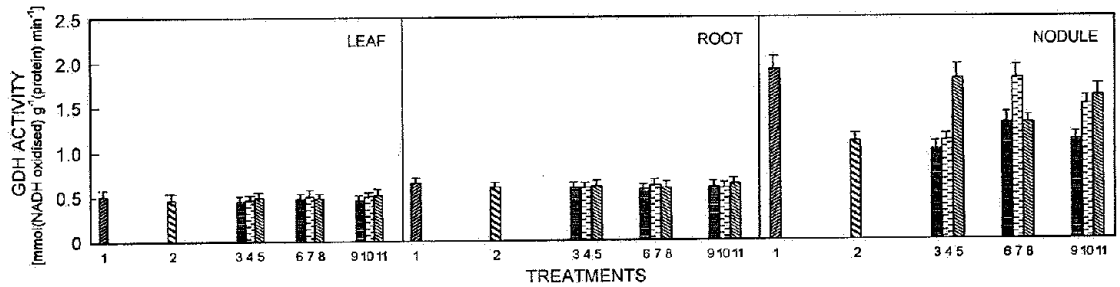

Fig. 5. Glutamate dehydrogenase (GDH) enzyme activity of control and treated mung bean leaves, roots and nodules. See Fig. 1 for details.

Discussion

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*. Salinity stress reduced nitrogen content (Fig. 1) together with decreased activities of nitrogenase (Fig. 2), GS (Fig. 3), GOGAT (Fig. 4) and GDH (Fig. 5). All the three growth regulators used at different concentrations helped to a different degree in reducing the stress effect. Similar results were also obtained in pea, chickpea, soybean, wheat, *Azolla* sp. and *Crotalaria* sp. Salinity disturbs the accumulation pattern(s) of nitrogenous fraction, leading to decrease in total and protein nitrogen (Lal and Bhardwaj 1987). It also induces hydrolysis of storage proteins by increasing protease activity as well as decreases synthesis of free amino acids (Strogonov 1962, Durgaprasad *et al.* 1996). The uptake of nitrogen and the synthesis of nitrate into organic nitrogen compounds also become prone to disruption under salinity (Lapina 1967).

Treatments with Kin, IAA and GA_3 enhance the nitrogen fixing efficiency in plants (Figs. 1 and 2). Higher efficiency may be due to the presence of functional nodules and their larger size or due to delayed senescence (Nandwal and Bharti 1982, Garg *et al.* 1992). Kin improves the rate of nitrogen fixation as well as elevates the nitrogen content. Salinity has been reported to decrease the content of endogenous cytokinins (Garg *et al.* 1995). Cytokinins decrease the levels of degradative enzymes thereby affecting the content of other hormones directly or indirectly. For instance, cytokinins inhibit the activity of indole acetic acid oxidase and other enzymes involved in IAA oxidation (Bekki *et al.* 1987). Hormone pretreatment in the present work also helps to maintain the activities of GS, GOGAT and GDH under salt stress by maintaining their active forms, a necessary factor toward an efficient catalytic activity (Figs. 3, 4 and 5).

From the results presented here, it is pertinent to suggest the possibility of adopting different strategies for growing this crop under saline conditions, and one may involve the pretreatment of the crop with phytohormones.

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