GROWTH REGULATOR MEDIATED CHANGES IN LEAF AREA AND METABOLIC ACTIVITY IN MUNGBEAN UNDER SALT STRESS CONDITION

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SUMMARY

Efficacy of treatment as foliar spray of indole-3-acetic acid, gibberellic acid and kinetin (6-furfuryl aminopurine) at 0.1 to 10 \( \mu \)M in ameliorating the harmful effect of NaCl salinity (E.C value 4 ds/m) on metabolic activity was investigated in \( Vigna \) radiata (L.) Wilczek. Application of NaCl resulted in decrease in total leaf area in mungbean. Stomatal openings on leaf surface were less prominent and trichomes were shorter in length under salinity stress in comparison with the control. Sodium and chloride contents increased in leaf, root and nodules under salinity whereas, potassium and calcium contents decreased under NaCl stress in various plant parts studied. Proline content increased under salt stress by 76, 217 and 119% in leaf, root and nodule respectively over the control set. Salt stress increased acid phosphatase enzyme activity both in leaf and root of mung bean by about 61 and 48% respectively over the control set. All the three growth regulators used in the present study were able to overcome to variable extents the adverse effects of stress imposed by NaCl solution.

Key words : Acid phosphatase, phytohormone, proline, salt stress, \( Vigna \) radiata

INTRODUCTION

Salinity of soil is a serious agroeconomical problem which leads to metabolic alterations and graded reduction in plant growth in terms of all the growth parameters. Soil salinity is a major limitation to all crops. Partially opened stomata of the salinized plants with reduced CO\(_2\) diffusion has been reported in onion, bean and cotton plants (Gale \textit{et al.} 1967). In potato, the levels of proline and sodium increase in different plant parts under salinity to maintain the osmoregulation (Abdullah \textit{et al.} 1990). Presence of excess NaCl in soil results in increasing Na\(^+\) and Cl\(^-\) contents and decrease in K\(^+\) and Ca\(^{2+}\) contents in different plant parts of avocado, grapevines, maize, \textit{Vicia faba}, horsegram, sudan grass, safflower, sweet pepper and wheat (Bernstein \textit{et al.} 1969, Bernstein 1975, Patil \textit{et al.} 1989, Sudhakar \textit{et al.} 1990, Datta 1996, Patil \textit{et al.} 1996, El-Bahr 1995, Maliwal 1997). Recent studies indicate that adaptation to salinity is closely associated with proline accumulation. Ecological studies also support the idea that proline accumulation is important in plants adaptation to dry or saline environments. Salt stress results in a significant increase in the accumulation of proline in \textit{Hordeum vulgare}, \textit{Citrus}, faba bean, tomato and mulberry (Chaudhuri \textit{et al.} 1997, Gomez-Cadenas \textit{et al.} 1998, Trinchant \textit{et al.} 1998, Aziz \textit{et al.} 1998, Kumar \textit{et al.} 2000). Phosphorus metabolism is also adversely affected in plants growing under saline stress. Acid phosphatase catalyzes a variety of biochemical reactions including carbohydrate metabolism and plays a vital role in regulating the plant cell activities through inorganic phosphorus level (Turner and Wellburn 1985). Acid phosphatase enzyme...
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activity under NaCl stress has been studied in rice and Brassica (Dubey and Sharma 1989, Mittal and Dubey 1992, Gangopadhyay et al. 1995, Gangopadhyay et al. 1997).

In our experiment, attention has been given to phytohormones like IAA, GA₃, and kinetin as possible inducers of resistance to unfavourable environmental conditions. The main object was to determine the effect of salt stress on leaf area of a single plant, leaf surface architecture, Na, K, Cl, Ca and proline contents and acid phosphatase activity in mung bean and to determine the efficiency of three classical phytohormones 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 mungbean [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) Salt stressed treated with IAA (0.1 to 10 μM), (4) Salt stressed treated with GA₃ (0.1 to 10 μM) and (5) Salt stressed treated with kinetin (0.1 to 10 μM).

IAA, GA₃ and kinetin were sprayed at the rate of 50 ml per pot each at concentrations 0.1, 1.0, and 10 μM mixed with Tween-20 from day 13 (emergence of first trifoliate leaf) up to day 35, once a week. Control set was sprayed with equal amount of water mixed with Tween-20. All these sets were treated with NaCl solution to maintain electrical conductivity values of the soil as 4, 8, and 12 dS m⁻¹. The soil used has initial electrical conductivity 0.3 dS m⁻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, average leaf area of a plant was measured from 20 randomly selected plants from each set using a leaf area planimeter. Leaf surface architecture was studied using scanning electron microscope as described by Boyde and Wood (1969) and Bradbeer et al. (1970). Mature mung bean plants were uprooted carefully, washed thoroughly with deionized water, separated into leaves, roots and nodules and oven dried for estimating sodium, potassium, chloride and calcium contents using atomic absorption spectrophotometer (AAS). The penultimate leaves and roots were collected to measure proline content (Bates et al. 1973) and acid phosphatase activity (Malik and Singh 1980).

RESULTS AND DISCUSSION

Among the three salinity levels, only 4 dSm⁻¹ was the most 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., salt stressed, IAA treated, GA₃ treated and kinetin treated have been presented here.

Compared with the control set, total leaf area of plant was reduced by about 40% under salt stress. GA₃ at a concentration of 10 μM was able to minimize the percent reduction from 40 to 10%. IAA and kinetin each at a concentration of 1.0 μM caused increase in leaf area of about 33% and 31% respectively over the control (Table 1).

Table 1. Total leaf area of control and treated 56 days old mung bean plants.

<table>
<thead>
<tr>
<th>Set</th>
<th>Leaf area (Cm²)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.11±2.31</td>
</tr>
<tr>
<td>NaCl stressed</td>
<td>13.72±2.45</td>
</tr>
<tr>
<td>IAA (10 μM) + NaCl</td>
<td>28.51±3.23</td>
</tr>
<tr>
<td>IAA (1.0 μM) + NaCl</td>
<td>30.72±2.83</td>
</tr>
<tr>
<td>IAA (0.1 μM) + NaCl</td>
<td>25.18±2.88</td>
</tr>
<tr>
<td>GA₃ (10 μM) + NaCl</td>
<td>20.85±1.44</td>
</tr>
<tr>
<td>GA₃ (1.0 μM) + NaCl</td>
<td>16.32±1.61</td>
</tr>
<tr>
<td>GA₃ (0.1 μM) + NaCl</td>
<td>14.88±1.73</td>
</tr>
<tr>
<td>Kinetin (10 μM) + NaCl</td>
<td>28.31±2.94</td>
</tr>
<tr>
<td>Kinetin (1.0 μM) + NaCl</td>
<td>30.26±2.59</td>
</tr>
<tr>
<td>Kinetin (0.1 μM) + NaCl</td>
<td>27.17±3.06</td>
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</tbody>
</table>

Reduced leaf area under salinity stress was due to inhibition of cell division and cell enlargement and loss of capacity to maintain an adequate tissue ion concentration (Zidan et al. 1990). Leaf area was mostly increased by...
IAA and kinetin. The ameliorative role of growth regulators on leaf size enlargement might be attributed to accelerated cell division and cell enlargement (Darra and Saxena 1971). The bioregulatants–induced high uptake of essential nutrients (like K⁺ and Ca²⁺) and antagonistically low uptake of Na⁺ ions might also be responsible for the enhanced leaf growth (Banuelos and Bangerth 1986).

Stomatal openings on leaf surface were less prominent and trichomes were shorter in length under salt stress in comparison with the control set as studied by scanning electron microscopy (Plate-I). All the three phytohormones viz. IAA, GA₃ and kinetin each used at three different concentrations were found to overcome the stress effects on leaf surface showing much prominent stomatal openings in addition to restoring longer trichomes (Plates 2, 3 and 4).

Plate 1. Scanning electron microscopic photographs of mung bean leaf surface showing stomata (®) and trichomes (t). A. Control (X 400), B. NaCl stressed (X 400).

Plate 2. Scanning electron microscopic photographs of mung bean leaf surface showing stomata (®) and trichomes (t) of salt stressed plants treated with A. IAA (10 μM), B. IAA (1.0 μM), C. IAA (0.1 μM).
Plate 3. Scanning electron microscopic photographs of mung bean leaf surface showing stomata (®) and trichomes (t) of salt stressed plants treated with A. GA₃ (10 μM), B. GA₃ (1.0 μM) and C. GA₃ (0.1 μM).

Plate 4. Scanning electron microscopic photographs of mung bean leaf surface showing stomata (®) and trichomes (t) of salt stressed plants treated with A. Kinetin (10 μM), B. Kinetin (1.0 μM) and C. Kinetin (0.1 μM).
In our experiment short trichomes and reduced stomatal openings on leaf surface under salt stress resulted in reduced gaseous exchange between the internal tissues and outer atmosphere as evidenced by reduced CO$_2$ uptake and release by leaves in comparison with the control set (Chakrabarti and Mukherji 1994).

Sodium content in leaf increased by 144% under NaCl stress as compared with the control set. IAA at a concentration of 1.0 $\mu$m, GA$_3$ at 0.1 $\mu$m and kinetin at all concentrations were effective in reducing the sodium uptake. In root, sodium content increased 71% under stress as compared with control. IAA at a concentration of 1.0 $\mu$m, GA$_3$ at 1.0 and 10 $\mu$m and kinetin at 0.1 $\mu$m were most effective and completely reversed the stress effect. In case of nodule, sodium content increased about 47% under NaCl stress as compared with the control set. IAA at 0.1 to 10 $\mu$m, GA$_3$ at 1.0 $\mu$m and kinetin at 0.1 to 10 $\mu$m were able to completely overcome the stress effect (Table 2). Potassium content in mung bean leaf decreased about 7% under NaCl stress as compared with the control set. IAA at 0.1 to 10 $\mu$m, GA$_3$ at 1.0 $\mu$m and kinetin at 0.1 to 10 $\mu$m completely reversed the stress effect. In root, potassium content decreased about 9% under stress and IAA and GA$_3$ at concentrations 0.1 and 1.0 $\mu$m reduced the per cent reduction from 9 to 2.5%. Kinetin at 0.1 to 10 $\mu$m completely reversed the stress effect. In nodule, potassium content decreased about 30% under stress as compared with the control set. IAA and GA$_3$ at a concentration of 0.1 $\mu$m and kinetin at concentrations of 1.0 to 10 $\mu$m were effective in reducing the per cent reduction from 30 to 16, 21 and 22 respectively (Table 2). Chloride content in mung bean leaf increased by 149% under NaCl stress over the control set. IAA at 1.0 $\mu$m, GA$_3$ and kinetin at 10 $\mu$m were the most effective in reducing the per cent increase of chloride from 33 to 76, 45 and 21 respectively. In root, chloride content increased by 28% under stress, whereas IAA at 1.0 to 10 $\mu$m, GA$_3$ at 0.1 to 1.0 $\mu$m and kinetin at 0.1 $\mu$m reduced the per cent increase from 28 to 15. In nodule, chloride content increased by 33% under stress as compared with the control set. IAA at 0.1 $\mu$m, GA$_3$ at 1.0 $\mu$m and kinetin at 10 $\mu$m reduced the per cent increase from 33 to 8, 12 and 8 respectively (Table 2). Calcium content decreased about 7% in mung bean leaf under stress as compared with the control set. IAA at 1.0 and 10 $\mu$m completely reversed the stress effect. GA$_3$ at 0.1 and 1.0 $\mu$m and kinetin at 0.1 to 10 $\mu$m were able to reduce the per cent reduction from 7 to 5 and 2 respectively. In case of root, calcium content decreased by 11% under NaCl stress and IAA at a concentration of 10 $\mu$m, GA$_3$ at 0.1 and 1.0 $\mu$m, kinetin at 1.0 and 10 $\mu$m completely reversed the stress effect. In nodule, calcium content decreased about 14% under NaCl stress as compared with the control set. All the three phytohormones used each at 0.1 and 1.0 $\mu$m completely reversed the stress effect (Table 2).

Hormone treatment helped in reduced accumulation of sodium and chloride content in different plant parts as compared with the stressed plants without hormone treatment, whereas, potassium and calcium contents were almost like control plants in hormone treated plants. Calcium has been reported to play an important role in the selective transport of potassium by protecting membrane integrity in the presence of excess sodium, thereby making the plants more salt tolerant (Clarkson and Hanson 1980). Less sodium accumulation was also reported in cassava when growth regulators were used to counter the stress effects (Indira and Ramanujam 1985). Phytohormones help in maintaining the cell membrane structure and ionic movement through it. Kinetin affects membrane permeability to mono- and divalent ions.

Proline content under salt stress increased by 76, 217 and 119% in leaf, root and nodule, respectively over the control set. In leaf, IAA, GA$_3$ and kinetin each at a concentration of 1.0 $\mu$m reduced the per cent increase from 76 to 56, 45 and 21, respectively (Fig. 1). In root, IAA at a concentration of 1.0 $\mu$m and GA$_3$ and kinetin each at a concentration of 0.1 $\mu$m reduced the per cent increase of proline from 217 to 164, 71 and 140 (Fig. 1). In nodule, all the three phytohormones were most effective at a concentration of 1.0 $\mu$m. IAA completely reversed the stress effect and GA$_3$ and kinetin reduced the per cent increase of proline from 119 to 53 and 105 respectively (Fig. 1).

Role of proline in plant is related to survival rather than to maintenance of growth (Greenway and Munns 1980). Hormone application before stress helped mung bean to overcome the stress effect to some extent by maintaining the intracellular water potential and ion balance which ultimately reduced the necessity of accumulating higher levels of proline.
Table 2. Sodium, potassium, chloride and calcium contents (mg g⁻¹ dry wt.) in control and treated mungbean plant parts at 56 days sowing.

<table>
<thead>
<tr>
<th>Set</th>
<th>Sodium</th>
<th>Potassium</th>
<th>Chloride</th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Root</td>
<td>Nodule</td>
<td>Leaf</td>
</tr>
<tr>
<td>Control</td>
<td>0.045 ± 0.010</td>
<td>0.210 ± 0.109</td>
<td>0.190 ± 0.092</td>
<td>0.320 ± 0.098</td>
</tr>
<tr>
<td>NaCl stressed</td>
<td>0.110 ± 0.057</td>
<td>0.360 ± 0.109</td>
<td>0.280 ± 0.092</td>
<td>0.296 ± 0.098</td>
</tr>
<tr>
<td>IAA (10 µM) + NaCl</td>
<td>0.070 ± 0.016</td>
<td>0.206 ± 0.132</td>
<td>0.170 ± 0.121</td>
<td>0.321 ± 0.121</td>
</tr>
<tr>
<td>IAA (1.0 µM) + NaCl</td>
<td>0.069 ± 0.017</td>
<td>0.207 ± 0.115</td>
<td>0.160 ± 0.127</td>
<td>0.310 ± 0.127</td>
</tr>
<tr>
<td>IAA (0.1 µM) + NaCl</td>
<td>0.071 ± 0.010</td>
<td>0.203 ± 0.178</td>
<td>0.168 ± 0.150</td>
<td>0.330 ± 0.150</td>
</tr>
<tr>
<td>GA₃ (10 µM) + NaCl</td>
<td>0.079 ± 0.015</td>
<td>0.207 ± 0.127</td>
<td>0.170 ± 0.127</td>
<td>0.300 ± 0.127</td>
</tr>
<tr>
<td>GA₃ (1.0 µM) + NaCl</td>
<td>0.073 ± 0.012</td>
<td>0.207 ± 0.196</td>
<td>0.180 ± 0.109</td>
<td>0.310 ± 0.109</td>
</tr>
<tr>
<td>GA₃ (0.1 µM) + NaCl</td>
<td>0.072 ± 0.015</td>
<td>0.205 ± 0.167</td>
<td>0.160 ± 0.109</td>
<td>0.320 ± 0.109</td>
</tr>
<tr>
<td>Kinetin (10 µM) + NaCl</td>
<td>0.081 ± 0.010</td>
<td>0.203 ± 0.144</td>
<td>0.180 ± 0.132</td>
<td>0.322 ± 0.132</td>
</tr>
<tr>
<td>Kinetin (1.0 µM) + NaCl</td>
<td>0.110 ± 0.057</td>
<td>0.206 ± 0.178</td>
<td>0.170 ± 0.155</td>
<td>0.310 ± 0.155</td>
</tr>
<tr>
<td>Kinetin (0.1 µM) + NaCl</td>
<td>0.081 ± 0.017</td>
<td>0.208 ± 0.150</td>
<td>0.170 ± 0.115</td>
<td>0.332 ± 0.115</td>
</tr>
</tbody>
</table>
Phosphorus deficiency in soil and plants is known to induce the activity of phosphatase to cope up the inorganic phosphorus requirement of the plants (Dubey and Sharma 1989). Phytohormone treatment in the present experiment retained the acid phosphatase activity under stress almost at control level indicating the presence of adequate Pi content within a cell. Adequate phosphorus supply improves tolerance to NaCl (Karaki and Ghazi 1997). So there was probably no need for increased enzyme activity in hormone treated stressed sets.

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 take recourse to the treatment of the crop with growth regulators.

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