PRESOWING SEED TREATMENTS WITH GLYCINEBETAINE AND MINERAL NUTRIENTS OF WHEAT (TRITICUM AESTIVUM L.) UNDER SALINE CONDITIONS

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Salinity is at present one of the most serious environmental problems influencing crop growth. It has been extensively demonstrated that salinity affects several processes in the plants. Exogenous application of glycinebetaine as seed treatment was observed on wheat under control or saline conditions. Although GB presowing effect was not prominent in both cultivars, the concentration of Na⁺ and Cl⁻ were high in MH-97 and low in S-24, suggesting S-24 could be more salt tolerant as compared to MH-97 at initial stages of growth, but MH-97 was superior to S-24 in germination percentage both under control and saline conditions.

Key words: Salt stress, ions uptake, Glycinebetaine priming

INTRODUCTION

There are a number of strategies to improve the salinity tolerance of crops (Ashraf, 1994; Flowers, 2004). One of the most important is the seed priming (seed hardening) with different chemicals including inorganic salt solutions. Seed priming is a pre-germinative treatment in which seeds are held at water potential that allows imbibition but prevents radicle emergence. The degree of improvement in salt tolerance from priming depends upon the initial quality of the seed, the species being treated and specific treatment conditions (Welbaum and Bradford, 1989).

Glycinebetaine, quaternary ammonium compounds occurring naturally in a variety of plants, animals and microorganisms (Rhodes and Hanson, 1993). It has been reported that, it stabilizes both the quaternary structure of proteins and membranes against the adverse effect of drought, high salinity, and extreme temperatures (Sakamoto and Murata, 2000). Glycinebetaine is widely believed to protect cytoplasm from Na⁺ toxicity (Nomura et al., 1998). It is hypothesized that dipole character neutralize Na⁺ and Cl⁻ during salt stress and hydrophobic methyl groups stabilize hydrophobic domains of proteins (Bohnert and Jensen, 1996; Nomura et al., 1998). In vitro studies showed that GB (200-500 mM) protected enzyme activity from Na⁺ toxicity (Match et al., 1988; Colaco et al., 1992; Murata et al., 1992). Lopez et al. (2002) investigated that glycinebetaine can be used as alternative treatment to reduce effect of salt stress on the water relation of salt sensitive plants, its application increases stomatal conductance by ameliorating significantly the effect of salinity or water relation through increase in the leaf relative water content.

Keeping in view the above-mentioned reports, it is hypothesized that GB may have a role in discriminating Na⁺ uptake. The optimization of the seed priming technique becomes very important, especially at the commercial scale. Several factors affect seed priming response: solution concentration, composition, osmotic potential, the duration and temperature, and the extent of aeration. To investigate the best priming solution at germination stage of wheat and to clarify correlation between concentration and soaking period, this study screened some priming solutions.

MATERIALS AND METHODS

The experiment was conducted in growth chamber of Botany Department at University of Agriculture, Faisalabad. The seeds of wheat cultivars were obtained from Department of Botany, UAF. There were two wheat cultivars i.e. S-24 and MH-97, four pre-soaking levels of glycinebetaine i.e. control (non-soaked), water soaked, 10 mM and 30 mM of GB and two salinity treatments i.e., control and salt stressed (150 mmol L⁻¹ of NaCl).

The experiment was laid out in a completely randomized design with four replicates. The plants were allowed to establish for fifteen days after sowing in Petri plates with half strength Hoagland’s nutrient solution. After fifteen days following parameters were recorded:

1. Germination percentage
2. Chlorophyll contents
3. Mineral nutrients

Chlorophyll Contents

The chlorophylls a, b, total and chlorophyll a/b ratio were determined according to the method of Arnon (1949). The fresh leaves were cut into 0.5 cm segments and extracted over night with 80% acetone at -10 °C. The extract was centrifuged at 14000 x g for 5 minutes and then absorbance of the supernatant was read at 663 and 645 nm using a spectrophotometer (Hitachi-220, Japan).

The chl. a and b were calculated by the following formulae.

\[ \text{Chl. } a (\text{mg g}^{-1} \text{f.w.t.)} = 12.7(\text{OD}663)-2.69(\text{OD}645)j\times V/1000 \times W \]

\[ \text{Chl. } b (\text{mg g}^{-1} \text{f.w.t.)} = 22.9(\text{OD}645)-4.68(\text{OD}663)j\times V/1000 \times W \]

Where

\[ V = \text{Volume of the extract (ml)} \]
\[ W = \text{Weight of fresh leaf tissue (g)} \]
Determination of mineral elements in plant tissues

The dried ground shoot and root material (0.1 g) was digested with sulphuric acid and hydrogen peroxide according to the method of Wolf (1982).

1. Determination of Na\(^+\), K\(^+\) and Ca\(^{2+}\)

Na\(^+\), K\(^+\) and Ca\(^{2+}\) cations were determined with a flame photometer (Jenway, PFP-7). A graded series of standards (ranging from 5 to 25 mg L\(^{-1}\)) of Na\(^+\), K\(^+\) and Ca\(^{2+}\) were prepared and standard curves were drawn. The values of Na\(^+\), K\(^+\) and Ca\(^{2+}\) from flame photometer were compared with standard curve and total quantities were computed.

2. Determination of Cl\(^-\)

Shoot and root samples of 100 mg were ground and extracted in 10 ml of distilled water, heated at 80 °C till the volume became half. Maintained the volume again 10 ml with distilled water. Cl\(^-\) content was determined with a chloride analyzer (Sherwood, 926).

RESULTS AND DISCUSSION

Glycinebetaine had significant (p ≤ 0.001) effect on germination percentage. Salinity effect and cultivars difference were also highly significant (p ≤ 0.001), indicating that the cultivars differed greatly in response to glycinebetaine. MH-97 showed greater value of germination percentage under both saline or control conditions (Table 1; Fig.1). These results are related to the findings that germination % age significantly reduced under saline conditions (Lovato et al., 1994; Iqbal et al., 1998; Junmin et al., 2000).

Under control conditions the response of both cultivars was consistent while under saline conditions, S-24 exceeded in chlorophyll \(a\) concentration. Chlorophyll \(a\) contents of seeds soaked in water or 30 mM GB were

Table 1. Mean squares from analyses of variance of data for chlorophyll pigments and mineral nutrients of wheat (\textit{Triticum aestivum} L.) when eight hours presoaked seeds with glycinebetaine were germinated for 15 days under control or saline conditions.

<table>
<thead>
<tr>
<th>Source of variations</th>
<th>Degrees of freedom</th>
<th>Germination % age</th>
<th>Chlorophyll (a)</th>
<th>Chlorophyll (b)</th>
<th>Total chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycinebetaine (GB)</td>
<td>3</td>
<td>4075.5***</td>
<td>0.011 ns</td>
<td>0.001 ns</td>
<td>0.024 ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>495.1***</td>
<td>0.014 ns</td>
<td>0.024***</td>
<td>0.007 ns</td>
</tr>
<tr>
<td>Cultivars (cvs)</td>
<td>1</td>
<td>6847.5***</td>
<td>0.81***</td>
<td>0.068***</td>
<td>1.32***</td>
</tr>
<tr>
<td>GB x S</td>
<td>3</td>
<td>112.7*</td>
<td>0.006 ns</td>
<td>0.005*</td>
<td>0.04 ns</td>
</tr>
<tr>
<td>GB x cvs</td>
<td>3</td>
<td>603.5***</td>
<td>0.027*</td>
<td>0.011***</td>
<td>0.063*</td>
</tr>
<tr>
<td>S x cvs</td>
<td>1</td>
<td>937.5***</td>
<td>0.873***</td>
<td>0.117***</td>
<td>1.73***</td>
</tr>
<tr>
<td>GB x S x cvs</td>
<td>3</td>
<td>154.8***</td>
<td>0.338**</td>
<td>0.003ns</td>
<td>0.068*</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>0.18</td>
<td>0.009</td>
<td>0.001</td>
<td>0.022</td>
</tr>
</tbody>
</table>

| Shoot Na\(^+\)       | 26.21 ns           | 6.66 ns           | 1.131 ns         |
| Root Na\(^+\)        | 15432.9***         | 158.2***          | 135.4***         |
| Shoot K\(^+\)        | 11215.8***         | 166.7***          | 5.00 ns          |
| Root K\(^+\)         | 11215.8***         | 166.7***          | 5.00 ns          |
| Shoot Ca\(^{2+}\)    | 23.14 ns           | 19.57**           | 0.481 ns         |
| Root Ca\(^{2+}\)     | 11232.9***         | 11232.9***        | 12.87*           |
| Shoot Cl\(^-\)       | 17.19 ns           | 25.22**           | 4.020 ns         |
| Root Cl\(^-\)        | 14.85              | 4.412             | 1.926            |

| Glycinebetaine (GB)  | 3                  | 86.4***           | 9.368ns          | 85.49***        | 20.51**          |
| Salinity (S)         | 1                  | 118.9***          | 4.1 ns           | 8237.8***       | 37860.4***       |
| Cultivars (cvs)      | 1                  | 140.1***          | 98.75**          | 96.34***        |
| GB x S               | 3                  | 8.516**           | 74.18***         | 52.19**         | 25.88***         |
| GB x cvs             | 3                  | 80.83***          | 8.65 ns          | 39.01**         | 70.98***         |
| S x cvs              | 1                  | 5.096 ns          | 553.4***         | 181.23***       | 250.2***         |
| GB x S x cvs         | 3                  | 14.47***          | 13.27 ns         | 24.11 ns        | 71.01***         |
| Error                | 48                 | 1.383             | 4.785            | 9.136           | 3.25             |

* = significant at 0.05, ** at 0.01 and *** at 0.001 levels respectively
ns = non-significant
high under saline conditions. GB had non-significant effect on chlorophyll b in wheat seedling. Overall, chlorophyll b concentration under control conditions were higher than there in saline conditions. GB had non significant effect on total chlorophyll. When seeds were treated by GB, the total chlorophyll was high in S-24 under saline conditions while under non-saline conditions it was high in MH-97. The highest level of total chlorophyll was at 30 mM of GB under saline condition in MH-97 (Table 1; Fig. 1). This non significant effect on chlorophyll a due to increased salinity was also reported by (Manceau et al., 2004). It has been observed that due to increased salinity chlorophyll decreased which contrasts with our results but it had no effect on chlorophyll a and b ratio (Ashrafuzzaman et al., 2000). The reduction in chlorophyll might be due to enhancement of chlorophyllase activity at higher salinity levels or due to reduction in de novo chlorophyll synthesis (Sudhakar et al., 1991).

Shoot Na$^+$ was increased significantly by salinity ($p \leq 0.001$) and glycinebetaine ($p \leq 0.05$) presoaking levels. Whereas the interaction GB x cvs was non-significant. Significant interaction of GB x S x cvs ($p \leq 0.01$) showed that both cultivars differed variably under saline conditions when treated with GB. Overall, MH-97 accumulated more Na$^+$ as compared to S-24 under all the treatments, except at 10 mM GB under saline condition. A non-significant effect of glycinebetaine on root sodium was observed. Of the two cultivars, MH-97 had greater concentration of root Na$^+$ especially under saline conditions. GB had non-significant effect on shoot and root potassium of wheat plant. Highest concentration of potassium was recorded at 30 mM concentration of GB in MH-97 while in S-24 it was higher at 10 mM presoaked treatment of GB (Table 1; Fig. 2).
Fig. 2. Shoot and root mineral nutrient concentrations of wheat (*Triticum aestivum* L.) when eight hours presoaked with glycinebetaine seeds were germinated for 15 days under control or saline conditions.

NS = non-soaked

WS = water soaked
In conclusion, NaCl increase on shoot Cl− content in both shoots and roots elsewhere in barley (Khan et al., 1999; Hussain et al., 2002) that are in accordance to our results i.e., both Na+ and Cl− in the plant parts increased while K+ decreased and Ca2+ remained unaffected but differ in that due to increase in NaCl, K+ decreased while Ca2+ unaffected. So decreased K+ concentration could be attributed to antagonistic effect of Na+ on K+ uptake (Cramer et al., 1985).

Both salinity and GB had significant effect (p ≤ 0.001) on shoot calcium in wheat seedling. Ca2+ concentration in MH-97 was high as compared to S-24. Only cultivar difference was significant (p ≤ 0.001) whereas salinity and GB levels both had non-significant effect on root Ca2+. MH-97 had greater value of calcium as compared to S-24 both in control or saline conditions. The highest value of calcium was observed in roots of MH-97 in control condition. Higher sodium chloride had no effect in S-24 which suggested that S-24 was more salt tolerant as compared to MH-97 at initial stages of growth, although MH-97 was superior to S-24 in germination percentage both under control or saline conditions.

LITERATURE CITED


