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# 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 pre-sowing 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 m*M*) protected enzyme activity from $Na^+$ toxicity (Matoh *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 m*M* and 30 m*M* 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 $^oC$. 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.

Chl. *a* (mg $g^{-1}$ f.wt.) = [12.7(OD663)-2.69(OD645)]xV/1000xW
Chl. *b* (mg $g^{-1}$ f.wt ) = [22.9(OD645)-4.68(OD663)]xV/1000xW
Where
V = Volume of the extract (ml)
W = 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 $^oC$ 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 ($p \leq 0.001$) effect on germination percentage. Salinity effect and cultivars difference were also highly significant ($p \leq 0.001$), 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 m*M* GB were

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

| Source of variations | Degrees of freedom | Germination %age | Chlorophyll *a* | Chlorophyll *b* | Total chlorophyll |
|---|---|---|---|---|---|
| Glycinebetaine (GB) | 3 | 4075.5*** | 0.011 ns | 0.001 ns | 0.024 ns |
| Salinity (S) | 1 | 495.1*** | 0.014 ns | 0.024*** | 0.007 ns |
| Cultivars (cvs) | 1 | 6847.5*** | 0.81*** | 0.068*** | 1.32*** |
| GB x S | 3 | 112.7* | 0.006 ns | 0.005* | 0.04 ns |
| GB x cvs | 3 | 603.5*** | 0.027* | 0.011*** | 0.063* |
| S x cvs | 1 | 937.5*** | 0.873*** | 0.117*** | 1.73*** |
| GB x S x cvs | 3 | 154.8*** | 0.338** | 0.003ns | 0.068* |
| Error | 48 | 0.18 | 0.009 | 0.001 | 0.022 |
| | | **Shoot $Na^+$** | **Root $Na^+$** | **Shoot $K^+$** | **Root $K^+$** |
| Glycinebetaine (GB) | 3 | 33.03* | 26.21 ns | 6.66 ns | 1.131 ns |
| Salinity (S) | 1 | 5643.7*** | 14532.9*** | 586.2*** | 135.4*** |
| Cultivars (cvs) | 1 | 10.08 ns | 11215.8*** | 166.7*** | 5.00 ns |
| GB x S | 3 | 58.5** | 35.28 ns | 9.22 ns | 2.59 ns |
| GB x cvs | 3 | 18.73 ns | 23.14 ns | 19.57** | 0.481 ns |
| S x cvs | 1 | 0.14 ns | 11123.9*** | 0.66 ns | 12.87* |
| GB x S x cvs | 3 | 65.57** | 17.19 ns | 25.22** | 4.020 ns |
| Error | 48 | 10.86 | 14.85 | 4.412 | 1.926 |
| | | **Shoot $Ca^{2+}$** | **Root $Ca^{2+}$** | **Shoot $Cl^-$** | **Root $Cl^-$** |
| Glycinebetaine (GB) | 3 | 86.64*** | 9.368ns | 85.49*** | 20.51** |
| Salinity (S) | 1 | 118.9*** | 4.1 ns | 8237.8*** | 37860.4*** |
| Cultivars (cvs) | 1 | 254.1*** | 1408.1*** | 98.75** | 65.34*** |
| 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, 0.01 and 0.001 levels respectively
ns = non-significant

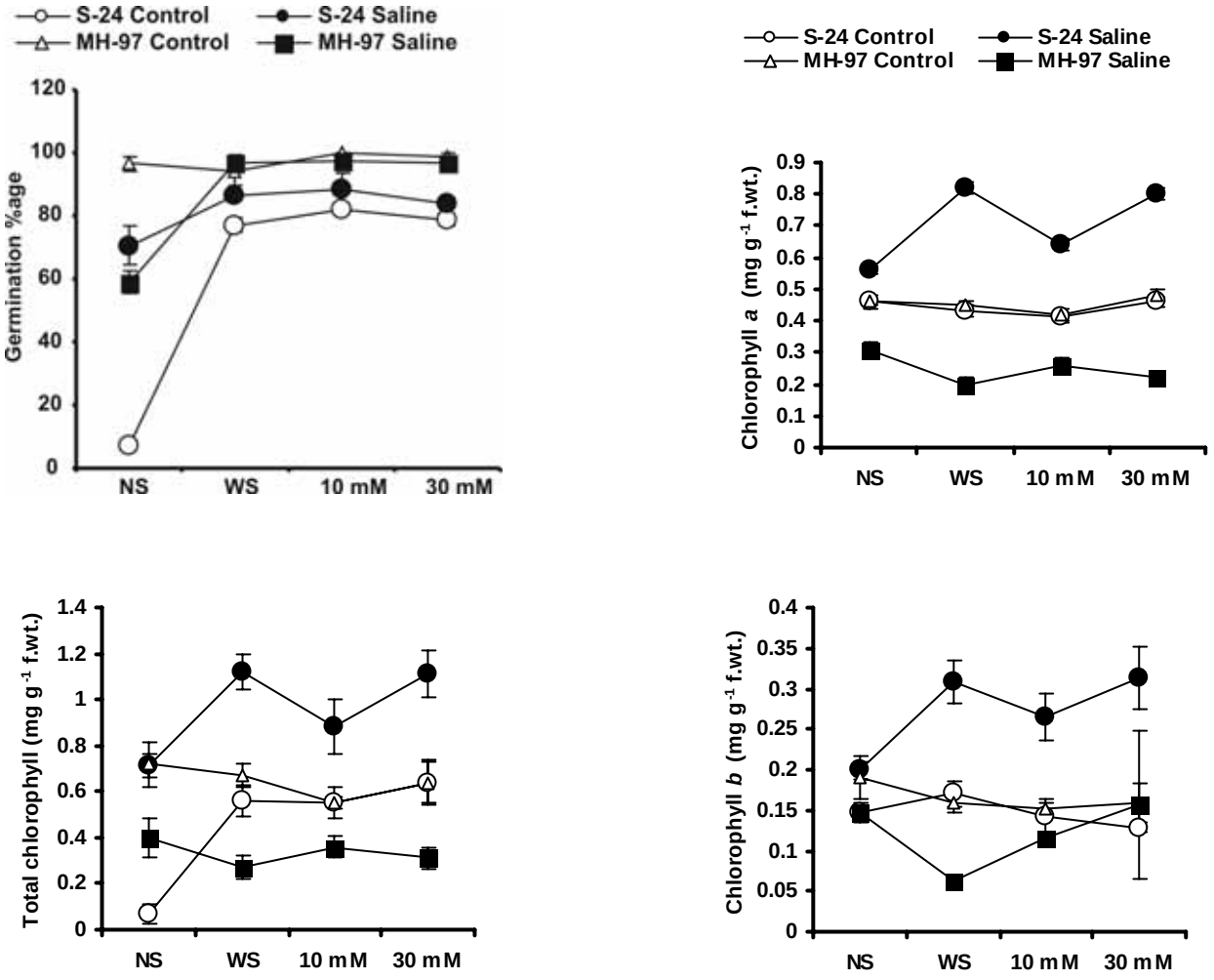


**Fig. 1. Germination percentage and chlorophyll pigments of wheat (*Triticum aestivum* L.) when eight hours presoaked with glycinebetaine seeds were germinated for 15 days under control or saline conditions.**
**NS = non-soaked WS = water soaked**

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 m*M* 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 m*M* 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 m*M* concentration of GB in MH-97 while in S-24 it was higher at 10 m*M* presoaked treatment of GB (Table 1; Fig. 2).

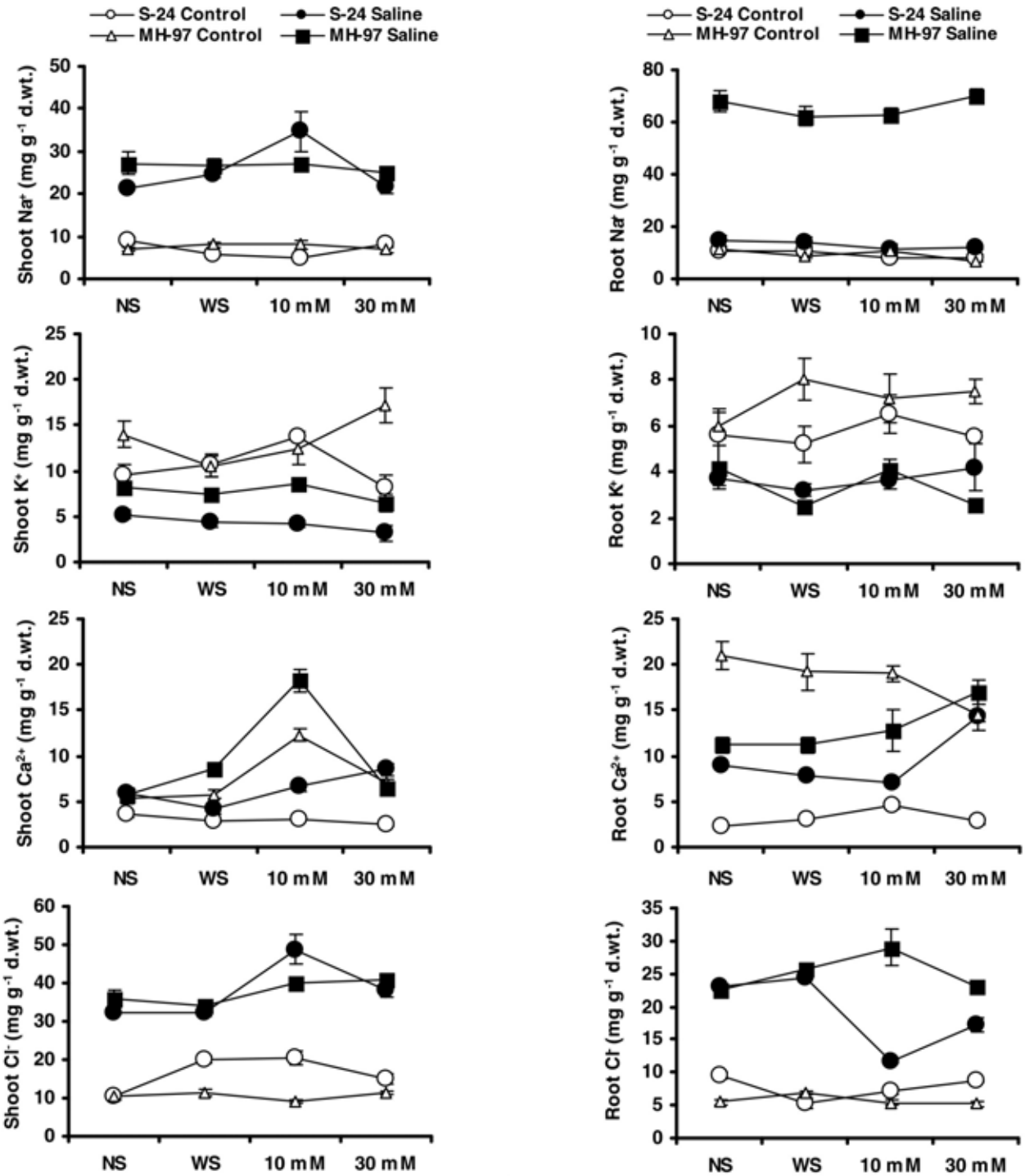


Fig. 2. Shoot and root mineral nutrient concentrations of wheat (*Triticum aestivum* L.) when eight hours presoaked with glycinebetaine seeds were germinated for 15 days under control or saline conditions.
NS = non-soaked WS = water soaked

Begum *et al.* (1992) found that NaCl stress significantly increased the accumulation of $Na^+$ and $Cl^-$ but decreased $K^+$ accumulation in germinating seeds. Increased NaCl concentration caused an increase in $Na^+$ and $Cl^-$ contents 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 $Ca^{2+}$ remained unaffected but differ in that due to increase in NaCl , $K^+$ decreased while $Ca^{2+}$ 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 ($p \leq 0.001$) on shoot calcium in wheat seedling. $Ca^{2+}$ concentration in MH-97 was high as compared to S-24. Only cultivar difference was significant ($p \leq 0.001$) whereas salinity and GB levels both had non-significant effect on root $Ca^{2+}$. 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 on shoot $Ca^{2+}$, but it increased root $Ca^{2+}$ which is in accordance to the results reported earlier (Hamada, 1996; Ashraf *et al.,* 2000). The findings of (Jafri and Ahmad, 1994) that salinity increased the uptake of $Na^+$ while the effect on $K^+$ and $Ca^{2+}$ uptake varied between cultivars and growth with decreased on increasing salinity in cotton also support our results.

Application of salinity caused significant ($p \leq 0.001$) increase on shoot $Cl^-$ under saline conditions. This increase was more prominent in MH-97 as compared to S-24. Data for root $Cl^-$ showed that effect of GB, salinity and cultivar difference were all highly significant. Root $Cl^-$ was high at control levels, while effect of GB was not enhancing in control conditions (Table 1; Fig. 2). It has been suggested that salt stress affects the various physiological processes through changes of water and ionic status in the cells (Hasegawa *et al.,* 2000). There are some reports about ionic imbalance in the cells due to excessive accumulation of $Na^+$ and $Cl^-$ and reduces uptake of other mineral nutrients, such as $K^+$, $Ca^{2+}$, and $Mn^{2+}$ (Lutts *et al.,* 1999).

In conclusion, $Na^+$ and $Cl^-$ were high in MH-97 but low 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.

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