Journal of Applied Biological Sciences 3(2): 67-72, 2009
ISSN: 1307-1130, www.nobel.gen.tr

Effect of NaCl Salinization on Germination of *Glycine max* (L.) Merr. Seeds in Supplement with or without External Proline or Cysteine

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Received: April 15, 2009
Accepted: May 30, 2009

ABSTRACT

In the present study, effect of NaCl salinization supplement with or without external proline or cysteine on germination, root growth and free proline accumulation were investigated in *Glycine max* seeds. Seeds were germinated under control (NaCl free) and saline (25-100 mM NaCl) conditions supplement with or without proline (0.5 mM) or cysteine (0.5 mM). Tested concentrations of singly NaCl decreased seed germination with respect to control. The minimum final germination percentages were recorded for singly NaCl, NaCl plus proline and NaCl plus cysteine at 100 mM as 66.7 %, 63.3 % and 60.0 %, respectively. Germination rates (velocity index) of *G. max* seeds were decreased with increasing NaCl concentrations at all applications. While the highest rate was estimated at control as 45.9 %, the minimum rates were estimated for singly NaCl, NaCl plus proline and NaCl plus cysteine at 100 mM concentrations as 28.4 %, 28.3 % and 21.7 %, respectively. Free proline accumulation of cotyledons and roots of *G. max* seedling was increased by NaCl treatments in all tested concentration and combinations.

Key Words: *Glycine max*, germination, proline, cysteine, NaCl.

INTRODUCTION

Salinity problem mostly seen in arid and semiarid regions may also be a problem in irrigated land. It was estimated that about one-third of irrigated land has been affected by salinity problem [1]. In addition, nearly 300.000 ha irrigated land lose their productivity because of mis-irrigation every year in the world [2]. In Turkey, the areas affected by salts is about 2 million ha, but it may increase if proper management systems are not considered. In the GAP region, a vast area of land was introduced by irrigation. Farmers have not mush information about irrigation system and soil interaction. They think that much water means much yield. As a result of this, excess and mis-irrigation are common in the region which will result in salinity problem near future [3].

The effect of NaCl on germination is considered to be the result of both ionic toxicity and the osmotic effect produced by a lowered osmotic potential.

These two factors are inter-related and co-exist under saline conditions [4]. In general, seed germination is more sensitive to salinity stress than is the growth of established plants [5]. Uhvitz [6] described the main effects of salinity on seed germination in terms of delayed beginning of the process, reduced germination rate, and lowered final germination percentages. Osmotic stress under saline conditions termed as physiological drought, subjects plants to dehydration. Ionic toxicity resulting from the accumulation of specific ions such as Na and Cl, in the cytoplasm or apoplast, interferes with plant metabolic functions [7].

Proline accumulation is a common metabolic responses of higher plants to water deficits, and salinity stress, and has been the subject of numerous reviews [8-10]. The proline accumulated in response to water stress or salinity stress in plants is primarily localized in the cytosol [11]. Proline protects membranes and proteins against the adverse effects of high concentrations of inorganic ions [12,13].

Proline may also function as a protein-compatible hydrotrope [14] and as a hydroxyl radical scavenger [15]. Exogenously supplied proline is osmoprotective for bacteria, facilitating growth in highly saline environments [16]. Exogenously supplied proline can also be osmoprotective to higher plant cells [17].

Cysteine is a thiol (–SH) containing aminoacid which is found in structure of glutathione (GSH, *L*-γ-glutamyl-*L*-cysteinyl-glycine). The non-protein thiol has unique structural properties and a broad redox potential. Environmental factors alter both the size and the redox state of the glutathione pool. For example, salt stress [18], heavy metals [19] and low temperature [20] all lead to GSH accumulation.

This study was initiated to determine the effect of NaCl salinization supplement with or without proline or cysteine on the germination of *G. max* seeds, and to determine the free proline accumulation.

MATERIAL AND METHODS

Seed and treatment

For the germination test, *Glycine max* (Soybean) seeds were used. The seeds were supplied from Department of Field Crops in the University of Cukurova, Adana, Turkey. The seeds were treated with 0, 25, 50 and 100 mM NaCl (Merck) supplement with and without 0.5 mM proline (Merck) or 0.5 mM cysteine (Sigma). Four replicas of 10 seeds for each NaCl treatment, or for the control, non-treated seeds, were placed on two layers of filter paper in 90 mm petri dishes. Before experiment, the seeds were sterilized with 2% sodium hypochloride solution for 15 min, and washed with sterile water three times. The filter paper was moistened with distilled water for the controls, or with aqueous solutions of each treatment. Distilled water or application solutions were added periodically maintaining the filter paper wet during the course of the experiment. The petri dishes were incubated in a growth chamber at 25±1 °C without photoperiod. The number of germinated seeds was counted every days during 4 days from the start of the test. Seeds were considered to be germinated at the emergence of the radicle (first root). Treatments were evaluated by counting the number of germinated seeds, and measuring the length of roots.

Germination tests

Germination rates were calculated according to the modification by Khan and Ungar [21] of the Timson's germination velocity index [22]: $\sum G/t$; where G is the percentage of seeds germinated after 2 days intervals, and t is the total time of germination. Seedling vigour index (SVI) was calculated according to Abdul-Baki and Anderson [23] as SVI = Percent germination x root length. The percentages of relative seed germination (RSG) after 4 days, and relative root growth (RRG) and germination index (GI) after 4 days of exposure to NaCl solutions were calculated as follows [24]:

$$RSG\ (\%) = \frac{\text{Number of seeds germinated in NaCl solution}}{\text{Number of seeds germinated in control}} \times 100$$

$$RRG\ (\%) = \frac{\text{Mean root length in NaCl solution}}{\text{Mean root length in control}} \times 100$$

$$GI\ (\%) = \frac{RSG \times RRG}{100}$$

Free proline analysis

Determination of free proline content was done according to Bates et al. [25]. Root or cotyledon (without embryo) samples (0.5 g) from each group were homogenized in 3% (w/v) sulphosalycylic acid and homogenate filtered through filter paper. After addition of acid ninhydrin and glacial acetic acid, resulting mixture was heated at 100 °C for 1 h in water bath. Reaction was then stopped by using ice bath. The mixture was extracted with toluene, and the absorbance of fraction with toluene aspired from liquid phase was read at 520 nm. Proline concentration was determined using calibration curve and expressed as µmol proline g^{-1} F.W.

Statistical analysis

For statistical analyses we chose the analysis of variance (ANOVA) in Statistical Analysis System (SPSS 11.0 for windows). The significance of differences between mean values were determined by a multiple range test (LSD; Least Significant Difference). For this reason, alpha (α) was preferred to be 0.05, which corresponds to a confidence level of 95%.

RESULTS

Germination

The final germination percentages of the seeds are presented in Figure 1. The germination percentage of the control seeds (NaCl free) was estimated as 96.7. All the tested NaCl concentrations and NaCl with proline and cysteine combinations decreased seed germination as compared to control especially at 50 and 100 mM concentrations (p<0.05). The maximum final germination percentage of seeds were recorded for singly NaCl, NaCl plus proline and NaCl plus cysteine at 25 mM as 93.3 %, 66.7 % and 86.7 %, respectively. The minimum final germination percentages were recorded for singly NaCl, NaCl plus proline and NaCl plus cysteine at 100 mM as 66.7 %, 63.3 % and 60.0 %, respectively.

Daily germination percentage of seeds of *G. max* are given in Figure 2. Germination of the seeds are completed at 3rd days at all tested concentrations and combination. According to daily germination percentages, it is obvious that control seeds germinated more earlier than the all NaCl applied seeds.

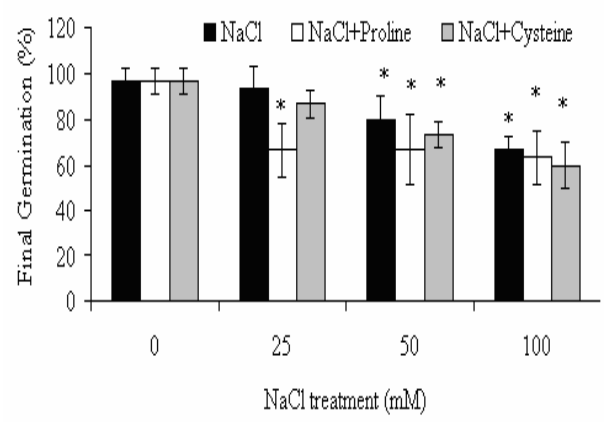

Figure 1. Effects of different NaCl concentrations supplement with and without proline or cysteine treatment on germination percentage of *G. max* seeds. Error bars represent the standard deviation of means. * $p<0.05$.

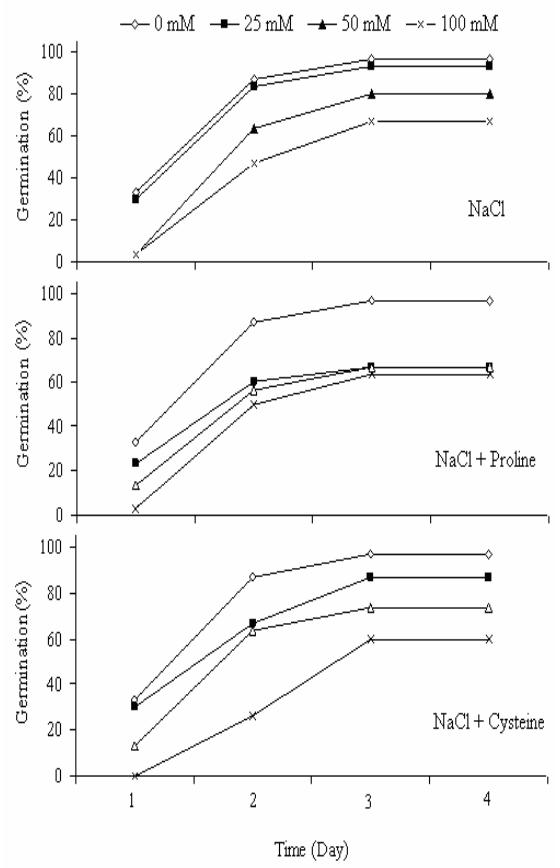

Figure 2. Germination rates of *G. max* seeds at different NaCl concentrations treated with and without proline or cysteine.

Germination rates (velocity index) of *G. max* seeds were decreased with increasing NaCl concentrations at singly NaCl and NaCl with proline or cysteine combination (Figure 3). While the highest rate was estimated at control seeds as 45.9 %, the minimum rates were estimated for singly NaCl, NaCl plus proline and NaCl plus cysteine at 100 mM concentrations as 28.4 %, 28.3 % and 21.7 %, respectively.

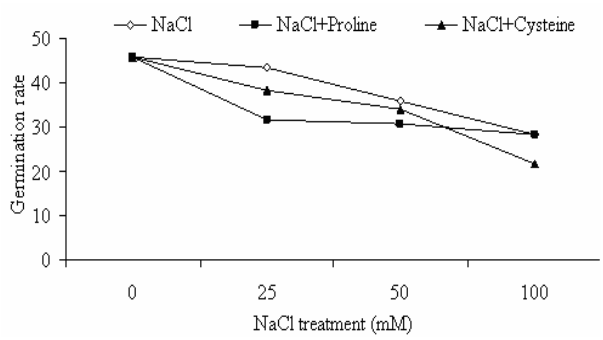

Figure 3. Germination rates of G. max seeds at different NaCl concentrations supplement with and without proline or cysteine.

Root length

In all tested NaCl concentrations and NaCl plus proline or cysteine combinations root development adversely affected with respect to control (Figure 4). Root lengths significantly decreased for singly NaCl at 50 and 100 mM, for NaCl plus proline and NaCl plus cysteine combinations at 25, 50 and 100 mM compared to control ($p<0.05$). The highest root length was measured at control as 10.2 cm. The minimum root lengths were measured for NaCl at 100 mM, NaCl plus proline at 50 mm and NaCl plus cysteine at 100 mM as 8.7, 4.4 and 3.7 cm, respectively.

RSG, RRG, GI and SVI

RSG, RRG and GI of the seeds are shown in Figure 5. According to estimation of RSG, singly NaCl concentrations inhibited seed germination. On the otherhand, supplement of exogenous 0.5 mM proline or cysteine were not alleviated NaCl effect on seed germination. The maximum inhibitions were estimated for individually NaCl and NaCl with proline and cysteine combination at 100 mM as 69.0 %, 65.5 % and 62.0 %, respectively. RRGs were decreased with increasing NaCl concentrations at all tested NaCl and NaCl with proline and cysteine combination except at 50 mM NaCl plus 0.5 mM proline combination. The maximum RRGs were estimated for NaCl, NaCl plus prolin and NaCl plus cysteine at 25 mM as 85.3 %, 50.0 % and 65.7 %, respectively. GI showed differences among all tested concentrations and combinations. Although the maximum GIs were estimated for singly NaCl and NaCl with proline and cysteine combination at 25 mM as 79.4 %, 34.5 % and 59.1 %, respectively. The minimum index were estimated for the above treatments at 100 mM as 29.1 %, 28.9 % and 21.7 %, respectively.

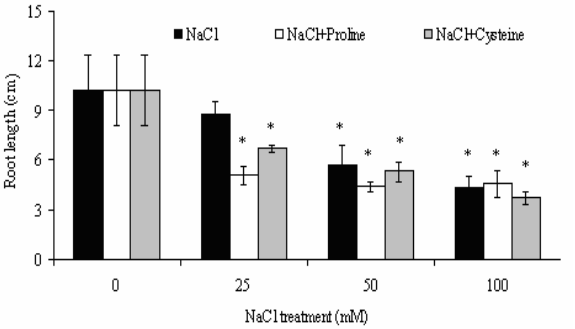

Figure 4. Root lengths of G. max at different NaCl concentrations supplement with and without proline or cysteine treatments on root development of G. max seeds. Error bars represent the standard deviation of means. * p<0.05.

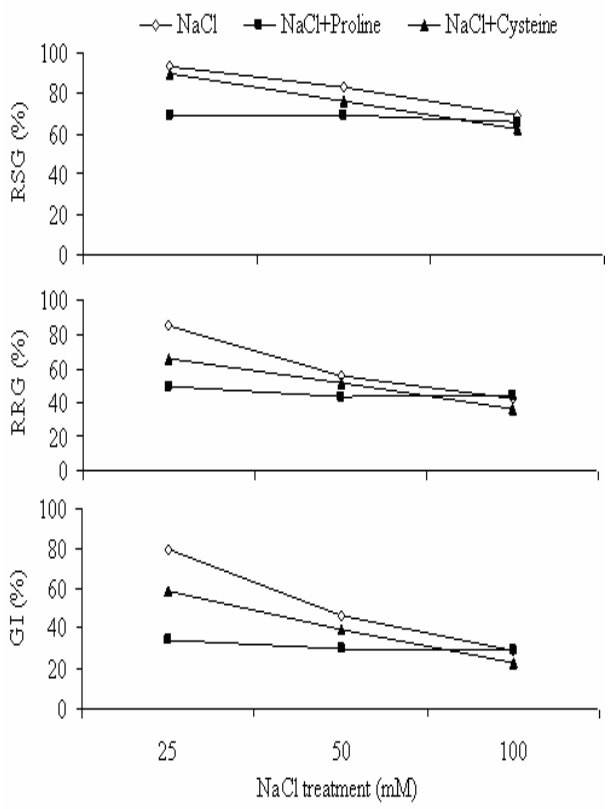

Figure 5. RSG, RRG and GI of G. max seeds at different NaCl concentrations treated with and without proline or cysteine.

In the tested NaCl concentrations and NaCl with the combinations, SVIs were sharply decreased with increasing NaCl concentrations (Figure 6). Among treatments, the highest SVI was estimated at control as 986.3. The minimum index were estimated for singly NaCl and NaCl with proline and cysteine combination at 100 mM as 284.6, 287.0 and 224.0, respectively.

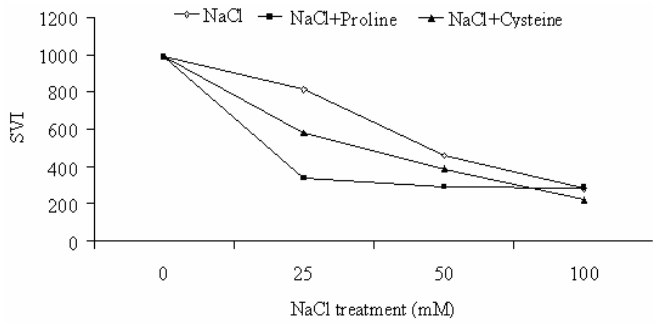

Figure 6. SVI of G. max seeds under different NaCl concentrations in the presence of proline or cysteine.

Proline accumulation

Free proline contents of *G. max* cotyledons and roots are presented in Table 1. In both parts of seedling prolin accumulation were increased by NaCl treatments in all tested concentration and combinations. Proline contents of cotyledon in controls were changed among 1.50-1.57 µmol g^{-1} FW. All tested NaCl treatments in cotyledons prolin accumulation were significantly increased with raising NaCl concentrations with respect to NaCl free treatment ($p<0.05$). The highest prolin content was measured at 50 mM NaCl plus cysteine combination as 3.97 (±0.98) µmol g^{-1} FW ($p<0.05$). Among tested treatments, the lowest proline accumulation in cotyledons was measured at 100 mM singly NaCl as 2.13 (±0.21) µmol g^{-1} FW ($p<0.05$). Comparing to the cotyledon, low proline accumulations were measured in the roots. Proline contents of the seedling roots in controls were changed among 0.44-0.47 µmol g^{-1} FW. Free prolin accumulation of root of *G. max* seedlings were higher in NaCl treatments than control. The highest prolin content in roots was measured at 50 mM NaCl plus cysteine combination as 1.37 (±0.38) µmol g^{-1} FW ($p<0.05$). Among tested treatments, the lowest proline accumulation in roots was measured at 100 mM NaCl plus 0.5 cysteine as 0.76 (±0.11) µmol g^{-1} FW ($p>0.05$).

Table 1. Free proline contents of *G. max* cotyledons and roots under different NaCl concentrations in the presence of proline or cysteine treatment.

NaCl (mM)	Free proline content (µmol g^{-1} F.W.)					
	Cotyledon			Root		
	NaCl	NaCl+Pro	NaCl+Cys	NaCl	NaCl+Pro	NaCl+Cys
0	1.53±0.21	1.50±0.26	1.57±0.15	0.44±0.14	0.47±0.04	0.47±0.08
25	2.70±0.44*	2.57±0.15*	3.73±0.76*	1.08±0.34*	1.26±0.27*	0.88±0.20*
50	2.87±0.49*	2.87±0.29*	3.97±0.98*	1.12±0.37*	1.17±0.18*	1.37±0.38*
100	2.13±0.21*	2,60±0.36*	2.43±0.25	0.91±0.09	0.82±0.26	0.76±0.11

Values are mean±SD. *, $p<0.05$.

DISCUSSION

Generally, plants are most sensitive to soil toxicity during the germination stage. Seed germination is one of the criteria usually used to screen crops or varieties for their tolerances to soil toxicity [26]. Salinity is well known to delay or reduce germination and inhibit seedling growth, however, detailed studies on the time course of events during germination are generally lacking. Salinity has been reported to inhibit first phase of germination which beings with hydration of seeds [6]. *Ougeinia dalbergioides* seeds were subjected to salinity stress up to the 0.16 M level accomplished more than 90% germination, but a higher level of salinity stress first caused a gradual and subsequently a sharp decline enabling only 10% of seeds to germinate in 0.32 M NaCl. Mean germination time increased and GI decreased, both gradually, up to the 0.16 M level of salinity and sharply beyond. Under salinity stress, a similar decrease in root length was also recorded which was significantly different at each increase of 0.08 M level of salinity stress [27]. Effects of various NaCl salinity (25, 75 and 125 mM) and proline (0.1 and 1 mM) on germination of *Zygophyllum simplex* seeds were determined by Khan and Ungar [28]. They found that rate of germination decreased with an increase in salinity. The rate of germination for each salinity level revealed that proline significantly increased the rate of germination at 0 mM NaCl, while the effect of proline was not significant at 75 or 125 mM NaCl. The germination rate (velocity) of *Z. simplex* seeds in non-saline and low salinity media with proline was substantially higher than in controls, but proline had no effect on the rate of germination in the high salinity treatments. The germinability of *G. max* seeds decreased with increasing level of salinity stress in all tested NaCl concentrations and NaCl with proline and cysteine combinations. RSG shows inhibition or stimulation of seed germination by salinity treatments. According to this, tested all singly NaCl concentrations and NaCl with proline or cysteine combinations inhibited germination of *G. max* seeds. The inhibitions were increased with raising salinity concentrations. Root lenghts were decreased with increasing NaCl concentrations at all treatments comparing to control. RRG indicates decrease or increase of root development by salinity treatments. According to this, root developments were not stimulated in NaCl with proline combination. Germination rates (velocity) were also decreased by NaCl at all treatments. Exogenous application of proline was reported to stimulate growth of cells [29] and plants and to improve metabolism under stress conditions [30]. Notwithstanding, negative effects were also reported [31,32]. We expected that exogeneous applications of proline or cysteine with NaCl stimulate seed germinability and seedling development at NaCl treatments. However, both of the applications were not alleviated seed germination as shown in Figure 1, 2, 3, 5 and 6. and root developments as shown Figure 4 and 5. Munns [33] hypothesized that plant growth is initially inhibited (phase 1) by cellular responses to the osmotic effects of external salt, i.e. by responses to the decreased availability of soil water.

In a later, second response (phase 2), growth is further inhibited by the toxic effects of excessive salt accumulation within the plant.

Free proline accumulation has been observed in response to a wide range of abiotic and biotic stresses in plants. Possibly an osmoticum, proline is considered to be one of the first metabolic responses to stress, and is perhaps a second messenger [34]. The effects of NaCl on changes in the proline level in of rice seedlings were investigated by Wanichananan et al. [35]. The proline content of rice seedlings was affected by the presence of NaCl in the growth medium. For the high level of proline, the increment of NaCl concentration from 0 to 513 mM raised the proline levels significantly, by more than a 8-fold increase. Proline contents in both cotyledons and the root of *G. max* seedlings were higher in individually NaCl concentrations and NaCl with proline or cysteine combinations than control (NaCl free). However, proline accumulation in roots and cotyledons of the seedling were not increased gradually with increasing salinity in both applications. The maximum proline content in cotyledons were determined at 50 mM NaCl plus 0.5 mM cysteine. This was 2.53-fold increase compared to the control. Between treated combinations, high prolin contents were measured at cysteine applications compared to prolin. Low proline accumulations were determined in roots with respect to cotyledons. Cotyledon is a significant part of the embryo within the seed of the plant and has biological activities more than seedling root, thus these may be probable reason.

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