EFFECT OF PRESOAKING GUAR SEEDS IN SOME PLANT VITAMINS OR PHYTOHORMONES ON GERMINATION AND SEEDLING GROWTH IN THE PRESENCE OF NaCl.
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ABSTRACT

The present experiment is planned as an attempt to minimize the harmful effects of NaCl salinity (2500, 5000 and 7500 ppm) on germination percentage (GP %) and seedlings growth (fresh and dry weight as well as seedling length) of guar, through presoaking seeds in selected phytohormones (ABA or SA) and vitamins (AsA or Thi) at 50 and 100 ppm for each. The low level of NaCl increased significantly guar seed germination percentage and seedling growth, whereas that increasing salinity levels up to 5000 ppm decreased seed germination percentage as well as seedling growth and this effect increased consistently and rapidly with increasing NaCl salinity as compared to non-salinized (control). The great reduction occurred under high salinity levels (7500 ppm NaCl). In most cases, presoaking seeds with selected material had a significant effect on seed germination as well as seedling growth under both normal and NaCl saline conditions compared to control except ABA at 100 ppm with high salinity levels. On the other hand, under normal conditions, maximum germination was achieved in seeds presoaked with AsA at both level and Thi or SA at 100 ppm. Generally, phytohormone (SA) and plant vitamin (AsA or Thi) counteracted the harmful effect of salinity on guar seedling growth against different salinity levels. These results suggested that AsA presoaking alleviate the adverse effects of NaCl presoaking guar seeds salinity on the seed germination and seedling growth of the guar followed by SA over the non-presoaking treatment.

Keywords: Guar, salinity, abscisic acid (ABA), ascorbic acid (AsA), salicylic acid (SA) and thiamine (Thi), seed germination, seedling growth.

INTRODUCTION

Salinity is one of the major and increasing problems in irrigated agriculture in Egypt. Salt effects on different morphological, physiological, and biochemical processes (Singh and Chatrath 2001). These include delays the seed germination as well as final germination percentage (Rahman et al., 2000 and Hu & Schmidhalter, 2001), ion homeostasis (Zhu, 2001) and damaged photosynthetic components and decrease in photosynthetic activity (Wang and Nii, 2000). The sensitivity of plants to salinity may depend on their developmental stage (Adams, 1990). Most plants are more sensitive to salinity during germination and seedling growth ((Fowler, 1991 and Reinhard et al., 1995). Seed germination is an important and critical development phase in the life cycle of plants (Gutterman, 1993 and Kigel, 1995), where the most sensitive period of growth occurs during germination or early seedling growth and development (Catalan et al., 1994). Presoaking seed treatments have been shown to enhance stand establishment in non-saline areas (Khan, 1992) and have potential in saline areas as well (Ashraf...
Presoaking seeds is a promising technique, being cost, and low risk. Imposition of abiotic stress during germination and early cycle of plant life results in altered level of plant hormones. The decreased salicylic acid and increased abscisic acid contents observed in salt stressed plants has led to the suggestion that salt stress induced change in endogenous hormonal levels. Exogenously seed treatment with phytohormons and plant vitamins generally stimulate (directly or indirectly). Guar or cluster bean, Cyamopsis tetragonoloba (L.) Taub, is a member of the Fabaceae (Leguminosae) family was used as a protein rich cattle feed. The endosperm of guar seed is a rich source of mucilage or gum, which forms a viscous gel in cold water, and is used as an emulsifier, thickener and stabilizer in a wide range of foods and industrial applications. Guar is a moderate sensitive plant to salt stress and salinity stress is one of the major abiotic stresses affecting. The present study is conceived with to investigate the effects of presoaking guar seeds in varying concentrations of phytohormone (ABA or SA) or plant vitamins (AsA or Thi) on guar seed germination and seedling growth under normal and NaCl saline conditions.

**MATERIALS AND METHODS**

A homogenous lot, healthy and almost uniform size, of guar seeds surface sterilized by soaking in 0.01% mercuric chloride for 3 minutes, then repeatedly washed with distilled water and divided into 9 groups. The first group of seeds was soaked (12 hours) in distilled water to serve as control (S0) and the remaining 8 groups were separately soaked for 12 hours in aqueous solutions of abscisic acid (ABA), ascorbic acid (AsA), salicylic acid (SA) and thiamine (Thi) at 50 or 100 ppm of each. Every group was divided into four sub-groups. The sub-group transferred to sterile Petri dishes (11 cm diameter) containing two layers of the filter papers (Whitman No. 1). The first sub-group was moisture with 10 ml of distilled water (control). The remainder sub-groups were salinized with 10 ml distilled water added with NaCl at 2500, 5000 and 7500 ppm for treatments (S1, S2 and S3, respectively). In order to avoid water losses, 5 ml of these solutions added Petri dishes, every 3 days. Thirty of each guar seeds were allowed to germinate at about 25±2°C in the dark. Petri dishes were tightly sealed with the impermeable colorless film in order to avoid water losses during the incubation. Thiram was added to the solutions at a concentration of 0.2% (w/v) to control the fungi infection. The number of seeds that sprouted and germinated was counter after 7 days. Seeds were considered to have germinated when shoot extended to more than 2 mm from the seeds. After 14 days final germination, seedlings were harvested and washed with water after harvest. The experiment was repeated tow times and a complete randomized block design with three replicates was followed. The following data were recorded: germination percentage (ISTA, 1999), shoot and root length (cm), was measured on ten seedlings randomly taken from each replicate, weighed, and the fresh weight per 10 seedling was calculated. In addition, shoot and root dry weight were (mg) measured using the same seedlings.
taken for the determination of fresh weight. They were oven-dried at 70°C until constant weight was reached. The obtained data were subjected to statistical analysis of variance according to Gomez and Gomez (1984) LSD value for comparison.

RESULTS AND DISCUSSION

a-Germination Percentage (GP %):

Data in Table (2) and Fig (2) indicated that the low level of NaCl increased significantly guar seed germination percentage whereas, decreased gradually with increasing NaCl levels. The great reduction occurred under high salinity levels (7500 ppm NaCl). In most cases, presoaking seeds with either selected phytohormones or plant vitamins had a significant effect on seed germination under both normal and NaCl saline conditions compared to control except ABA at 100ppm had no effect under high salinity levels. On the other hand, under normal conditions, maximum germination was achieved in seeds presoaked with AsA at 100 ppm. Furthermore, phytohormones (SA) or plant vitamins (Thi or AsA) used combined with low salinity level (2500 ppm) increased germination percentage as compared to control (untreated seeds). Under moderate NaCl saline (5000ppm), maximum germination was obtained in seeds presoaked with AsA, however lowest was achieved in seeds presoaked with ABA. On the other hand, under high saline level (7500 ppm), presoaking guar seeds with AsA at both levels and SA at 50 ppm enhanced seed germination as compared to control (untreated seeds). However, maximum reduction in percentage was recorded in seeds presoaked with ABA as compared to remaining treatments including control. Generally, both phytohormones and plant vitamin used enhanced germination percentage under moderate and high salinity levels (5000 and 7500 ppm) as compared to the untreated plants under such salinity level. Furthermore, AsA were more effective than SA in increasing germination percentage of guar. In view of these results obtained, it is obvious that salt stress caused a reduction in guar germination. These findings are agreement with Hamada & Al-Hakimi, 2001; Shalata and Neumann, 2001; El-Bassiouny and Bakheta., 2005; Khan et al, 2006 and Bassuony et al., 2008) in different plant species. The effect of salinity on germination seeds in many species is not only on lowering the percentage of germination, but also lengthening the time needed to complete germination (Ayers, 1952). In addition, some plants are sensitive to salinity at the seedling stage, because the mechanism of the tolerance to salinity is not yet fully developed, (Almodares et al., 2007).
Table (1): Effect of presoaking guar seeds in ABA, SA, AsA or Thi on germination percentage, seedling shoot and root length (cm) grown under normal and NaCl conditions.

<table>
<thead>
<tr>
<th>Salinity levels (ppm)</th>
<th>Treatment</th>
<th>Seed germination%</th>
<th>Shoot length (cm)</th>
<th>Root length (cm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>S0</td>
<td>S1</td>
<td>S2</td>
<td>S3</td>
</tr>
<tr>
<td>water</td>
<td>70</td>
<td>87</td>
<td>61</td>
<td>56</td>
</tr>
<tr>
<td>ABA (50)</td>
<td>73</td>
<td>80</td>
<td>67</td>
<td>63</td>
</tr>
<tr>
<td>ABA (100)</td>
<td>70</td>
<td>73</td>
<td>69</td>
<td>61</td>
</tr>
<tr>
<td>SA (50)</td>
<td>85</td>
<td>90</td>
<td>78</td>
<td>73</td>
</tr>
<tr>
<td>SA (100)</td>
<td>90</td>
<td>90</td>
<td>73</td>
<td>64</td>
</tr>
<tr>
<td>AsA (50)</td>
<td>90</td>
<td>93</td>
<td>80</td>
<td>73</td>
</tr>
<tr>
<td>AsA (100)</td>
<td>92</td>
<td>96</td>
<td>86</td>
<td>80</td>
</tr>
<tr>
<td>Thi (50)</td>
<td>93</td>
<td>80</td>
<td>77</td>
<td>62</td>
</tr>
<tr>
<td>Thi (100)</td>
<td>90</td>
<td>83</td>
<td>78</td>
<td>70</td>
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<tr>
<td>Mean</td>
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<td>LSD at 5%</td>
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</table>

NaCl salinity inhibits seed germination through increased osmotic potential; hence the seeds were unable to imbibe the water required for germination (Maas & Nieman, 1978) or decrease of the water movement into the seeds during imbibitions (Hadas, 1977) and/or reduced water uptake which arrested radicle emergence (Hampson and Simpson, 1990; Begum et al., 1992) and/or accumulation of Na+ and Cl ions changes in the activate a number of metabolic processes necessary for germination (Kayani et al., 1990) and/or accumulation of toxic ions, facilitate the intake of ions in
sufficient amounts to be toxic for the embryonic activities due to the influence
of the cations more than anions of acid radical used, the entry of the ions to
the seeds that might have been toxic to the embryo or the developing
seedlings (Huang and Redmann, 1995 and Almodares et al., 2007) and/or
inhibition of the uptake of several essential nutrients causing nutritional or
ionic imbalance (Al-Ansari, 2003 and Taamalli et al., 2004). Furthermore,
excess of Na+ might cause problems with membranes and enzyme inhibition
(Dodd and Donvn, 1999), disturbance in metabolism leading to increase in
phenolic compounds (Ayaz et al., 2000) which lead to decreasing both cell
division and cell elongation (Ghoulam and Fares, 2001). In addition, the
germination processes might have been stopped as a result of contact of the
seeds with high concentration of Na+ and Cl- ions (Almodares et al., 2007)
and/or by accumulation of these factors. On the other hand, data revealed that
AsA or SA or Thiamine alleviated partially the adverse effect of salt stress on
guar germination. The results proved that phytohormone (SA) and vitamin
(AsA) partially counteracted the harmful effect of NaCl salinity on guar seed
germination.

![Fig. (1): Effect of presoaking in ABA, SA, AsA or Thi on germination percentage of guar seeds grown under normal and NaCl conditions at 7 days from sowing.](image)

Concerning the inhibitor effect of ABA on seed germination in this
study. The results are in agreement with Schopfer and Plachy (1984), they
stated that ABA may interfere with seed germination by changing the water
status of the seed so that water uptake is inhibited. In addition, Baskin and
Baskin, (1998) stated that ABA seed treatments failed to improve germination
percentage and seedling growth. Moreover, Kabar (1997) found that
exogenous ABA is a potent inhibitor of seed germination and seedling
growth in many plant species. Concerning the promotive effect of AsA, many researchers revealed the positive effect of AsA on germination and
seedling growth i.e. Huan, (1988) and Ishibashi & Iwaya (2006), they
concluded that presoaking seeds with AsA increased the germination rate
and improved germination process. In addition, Shaddad et al., (1999) found
that AsA counteracted the adverse effects of salinity on seedling growth as
well as metabolic mechanisms and metabolic activities in the plants.
Regarding the effect of Thi may be due to the significance of vitamin B1 in

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cellular respiration as co-enzyme in the decarboxylation of pyruvate and α-ketoglutarate to acetyl and succinyl CO-A is well established and affects the respiratory process (Rajagopal et al., 2001). And increase hydration of seeds would help hydrolysis of food reserves in the germinating seeds and would consequently enhance the availability of respiratory substrates for energy production and nutrition. It could thus account for the observed increase in germination count. A similar promotive effects on seed germination by Ansari et al., (1990) and Samiullah et al., (1991). Most published data indicate that the salinity delayed seed germination as well as final germination percentage (Gutierrez-Boem et al., 1994; Huang and Redman, 1995; Munoz et al., 1996; Mohamed, 2002 and Zeinali et al., 2002). Concerning the interactions, pre-soaking seeds with AsA or SA is much effective in alleviating stress effects of salinity on the plants reported earlier by Ashraf and Foolad, (2005) and Ashraf et al., (2008). Moreover, Tari et al. (2002) showing that salt tolerance is induced in seedlings raised from seeds primed with salicylic acid. Also with Afzal et al., (2005) and Farooq et al., (2005). These results are also supported by Senaratna et al., (2000), they observed that the enhanced tolerance of tomato and bean seedlings sprayed with SA and ASA to drought stress. In this concern, under the influence of salt stress the osmotic potential greatly decreased and both AsA and SA pre-soaking moderated it (Szepesi et al., 2005) and might be the result of augmented activities of proteinase in endosperm and the contents of soluble sugar, protein and free amino acids (Zhang et al., 1999) and may be due to enhanced oxygen uptake and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis under saline conditions (Kathiresan et al., 1984) and may be due to increased rate of cell division in the root tips of seedlings from primed seeds (Rivas et al., 1984).

b-Seedlings Growth:

Data in Tables (2&3) revealed that increasing salinity levels up to 5000 ppm decreased seedling growth (seedling root and shoot lengths as well as fresh and dry weights) and this effect increased consistently and rapidly with increasing NaCl salinity as compared to non-salinized (control). Pre-soaking in phytohormones (ABA or SA) or plant vitamins (AsA or Thi) treatments showed a significant effect on guar seedling growth under normal and these treatments, in most cases, decreased seedling length under high NaCl saline conditions when compared with untreated plants (control). During normal conditions, minimum root length was recorded in seeds treated with 100 ppm ABA. While, maximum shoot length was attained in seeds pre-soaked with salicylic acid at 50 ppm and AsA or at 100 ppm, while ABA at both level drastically effected under high level of NaCl (7500 ppm). Generally, ABA at 100 ppm used levels decreased significantly seedling shoot length while, AsA application proved optimum values for seedling shoot and root length as well as seedling length. Moreover, SA or AsA reduce the harmful effects of salinity on the root length. In the present investigation, the stimulation effect of low NaCl salinity level (2500 ppm) on seedling length (shoot and root length) may be resulted from the beneficial effect of low concentration of chloride on many physiological processes as photosynthesis and osmoregulators. The inhibitory effects of salt stress on guar seedling
growth are in agreement with the observations of Abraham and Kiran, (2003) ; Neveen and Shawky, (2003); Silva et al., (2003) and Azooz et al., (2004). Moreover, Cicek and Cakirlar (2002) reported that salinity reduced shoot length, fresh and dry weight of maize seedlings. In addition, Shannon and Grieve (1999) indicated that salinity reduced fresh weight of some vegetables. Also, Jeannette et al. (2002) that total fresh weight of root and shoot was significantly reduced with increased salt stress.

Moreover, Hatung (2004) suggested that the bad effects of salinity on seedling shoot and root length may be due to the bad effects of salinity on meristematic cell division and elongation as well as root penetration. Also, Hawker and Walker (1978) revealed that this reduction in growth is due to reduced cell division or cell enlargement caused by salinity stress. Moreover, Greenway (1963) recorded that salinity reduced seedling length either by making osmotic cell enlargement dependent on soluble accumulation, and effect of salinity on cell size and number of cells per unit area. Yet, Tesu et al., (1980) revealed that three stages of cell growth were adverse affected particularly elongation and differentiation with increasing salinity level. Moreover, the reduced seedling growth weight under salt stress conditions.
could be attributed to the physiological drought induced by the low water potential and osmotic adjustments as a result of increased ionic concentration in their cells, which result in deformation of macromolecules by disrupting their shell or bound water (Schwarz, 1985) and/or may be due to the expenditure of energy on the synthesis of organic or inorganic solutes for osmotic adjustment rather than for growth (El-Banna, 1985).

![Graph showing effect of presoaking in ABA, SA, AsA or thi on guar seedling shoot fresh weight (mg/10 seedling) grown under normal and NaCl conditions.](image)

In addition, seedling grown in saline media accumulate high levels of salt and an osmotic adjustment is needed to keep root water potential lower than that of the external medium, energy must be expended to create such osmotic adjustment and this may lead to seedling growth reduction (Yeo, 1983) and/or might be attributed to the osmotic effect resulting from salt stress which cause disturbances in water balance and inhibited apical growth and internal hormonal imbalance (El-Desouky and Atawia, 1998; Younis et al., 2003) and/or may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings (Hajibagheri et al., 1989) and/or suppressing effects of salinity on both meristematic cell division and elongation as well as root penetration (Bernstein, 1971) and/or may be another reason for this decrease.

![Graph showing another effect of presoaking.](image)
**Fig (5) Effect of presoaking in ABA, SA, AsA or Thi on guar seedling root fresh weight (mg/10 seedling) grown under normal and NaCl conditions**

Concerning the improve of AsA or SA presoaking are in accordance with the Senaratna et al., 2000, Afzal et al., (2005) and Farooq et al., (2005). These results are also in confirmation with Tari et al., (2002) showing that salt tolerance is induced in seedlings raised from seeds primed with salicylic acid. Stress tolerance due to presoaking of seeds suggests that these molecules trigger the expression of the potential to tolerate stress rather than having any direct effect as a protecting (Senaratna et al., 2000). Also, Khodary (2004) found that SA increases the fresh and dry weights of shoot and roots of stressed maize plants. Results are also in accordance with the findings of Afzal et al., (2005) for wheat seedlings primed with SA under saline conditions. Salinity tolerance in seeds presoaked with SA might be the result of augmented activities of proteinase in endosperm and the contents of soluble sugar, protein and free amino acids under stress conditions (Zhang et al., 1999). The improving effect of AsA on seedling growth may be due to the fact that AsA is a major primary antioxidant (Nijs and Kelley, 1991), plays an important role in preserving the activity of enzymes (Padh, 1990). Furthermore, Noctor and Foyer (1998) stated that AsA is a small, water soluble antioxidant molecule which acts as a primary substrate in the cyclic pathway for enzymatic detoxification of hydrogen peroxide, it acts directly to neutralize superoxide radicals and/or regulates implicated in regulation of cell division (Smirnoff, 1996) and cell wall expansion and cell elongation (Gonzalez-Reyes et al., 1994; El-Yazal, 2007). Moreover, Smirnoff (1996) obtained that cell wall ascorbate and cell-localized ascorbate oxidase have been implicated in control of growth; high ascorbate oxidase activity is associated with expanding cells. Who pointed out that the beneficial effect of AsA on root length may be attributed to the fact that AsA is involved in the regulation of root elongation, cell vacuolation and cell expansion.

**Table (2): Effect of presoaking guar seeds in ABA, SA, AsA or Thi on seedling fresh and dry weight (mg/10 seedling) grown under normal and NaCl conditions after 14 days from sowing.**

<table>
<thead>
<tr>
<th>Salinity levels (ppm)</th>
<th>Seeding shoot fresh weight (mg/10 seedling)</th>
<th>S0</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>Mean</th>
<th>1st Exp.</th>
<th>S0</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>Mean</th>
<th>2nd Exp.</th>
<th>Mean</th>
</tr>
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<tbody>
<tr>
<td>Water (50)</td>
<td></td>
<td>352</td>
<td>353</td>
<td>323</td>
<td>233</td>
<td>214</td>
<td>282.8</td>
<td>352</td>
<td>353</td>
<td>323</td>
<td>233</td>
<td>214</td>
<td>282.8</td>
<td>325.1</td>
</tr>
<tr>
<td>ABA (100)</td>
<td></td>
<td>323</td>
<td>333</td>
<td>243</td>
<td>154</td>
<td>154</td>
<td>273.3</td>
<td>323</td>
<td>333</td>
<td>243</td>
<td>154</td>
<td>154</td>
<td>273.3</td>
<td>271.3</td>
</tr>
<tr>
<td>SA (50)</td>
<td></td>
<td>460</td>
<td>474</td>
<td>306</td>
<td>214</td>
<td>214</td>
<td>363.5</td>
<td>460</td>
<td>474</td>
<td>306</td>
<td>214</td>
<td>214</td>
<td>363.5</td>
<td>313.8</td>
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<tr>
<td>SA (100)</td>
<td></td>
<td>510</td>
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<td>333</td>
<td>213</td>
<td>213</td>
<td>392.3</td>
<td>510</td>
<td>513</td>
<td>333</td>
<td>213</td>
<td>213</td>
<td>392.3</td>
<td>381.5</td>
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<tr>
<td>AsA (50)</td>
<td></td>
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<td>516</td>
<td>302</td>
<td>216</td>
<td>216</td>
<td>386.0</td>
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<td>316</td>
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<td>223</td>
<td>401.3</td>
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<td>223</td>
<td>401.3</td>
<td>391.3</td>
</tr>
<tr>
<td>Thi (50)</td>
<td></td>
<td>480</td>
<td>523</td>
<td>273</td>
<td>182</td>
<td>182</td>
<td>364.5</td>
<td>480</td>
<td>523</td>
<td>273</td>
<td>182</td>
<td>182</td>
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<td>Mean</td>
<td></td>
<td>485.1</td>
<td>515.7</td>
<td>301</td>
<td>198.3</td>
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<td>481.7 / 415.3</td>
<td>248.8</td>
<td>181.0</td>
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<tr>
<td>LSD at 5%</td>
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</tbody>
</table>

Salinity: 12  Treatment: 18  Interaction: 30

Salinity: 23  Treatment: 17  Interaction: 37
Generally, phytohormones (SA) and plant vitamins (AsA or Thi) counteracted the harmful effect of salinity on guar seedling growth against different salinity levels. In addition, AsA or SA was more effective than other treatment to reduce the effect of salinity on seedling fresh and dry weights. Our results suggested that ASA presoaking alleviate the adverse effects of salinity on the seed germination and seedling growth of the guar followed by SA presoaking over the non-presoaking treatment. Moreover, Cicek and Cakirliar (2002) reported that salinity reduced shoot length, fresh and dry weight of maize seedlings. In addition, Shannon and Grieve (2000) indicated that Salinity reduced fresh weight of some vegetables. Similar result was observed by Jeannette et al. (2002) that total fresh weight of root and shoot was significantly reduced with increased salt stress.

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