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Salt stress alters physiological indicators in cotton (*Gossypium hirsutum* L.)

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Abstract

A pot experiment was conducted to evaluate performance of four upland cotton (Gossypium hirsutum L.) varieties, Deir-Ezzor22, Niab78, Aleppo118 and Deltapine50 grown under non-saline conditions (control) and salt stress (200 mol m^{-3} NaCl) for 7 weeks. Results showed that seedling height, root length, leaf number, leaf area, leaf chlorophyll a and b, osmotic potential, chlorophyll content index (CCI), dry biomass and root/shoot weight ratio were significantly reduced with salinity treatment. This reduction was more pronounced in Deltapine50 and Aleppo118 than Niab78 and Deir-Ezzor22. Leaf relative water content (RWC) was strongly reduced for Deltapine50 and Aleppo118, while, it was slightly increased for Niab78 and Deir-Ezzor22. In conclusion, osmotic potential, RWC, CCI, dry biomass and root/shoot weight ratio could be considered as useful indictors for salt tolerance screening among cotton varieties.

Keywords: Cotton, chlorophyll content index, relative water content, salt stress, variety

Introduction

Land surfaces submitted to salt stress are increasing day by day in arid, semiarid regions and especially in the Mediterranean area. This increase is due to a pull up of salts from the soil after irrigation and also to the use of water loaded in salt for watering. Currently, one third of all irrigated lands in the world are affected to a greater or lesser degree by salinity and the salinity problem continues to increase (Munns, 2005). Salt stress is an abiotic stress that can affect plant growth and the development at physiological and biochemical activities as- such as photosynthesis activity and chlorophyll content, *e.g.* in tomato (Hajer *et al*., 2006), *Catharanthus roseus* (Jaleel *et al*., 2008) and cotton (Basal, 2010). The effect of high salt concentration on growth and yield has been mentioned in many reports (Ahmad *et al*., 2002; Ali *et al*., 2004; Demiral *et al*., 2005; Jaleel *et al*., 2008; Basal, 2010). Salinity is one of the most serious factors for limiting crop production, especially for the sensitive ones (Manivannan *et al*., 2007). Plants show variable capacity to salinity tolerance that ranges from negligible effect to plant death. Considerable differences are found between plant species. For example, after exposure to 200 mol m^{-3} NaCl, a salt- tolerant species such as sugarbeet might have a reduction of only 20% in dry weight, whereas, a moderately tolerant species such as cotton might have a 60% reduction, and a sensitive species such as soybean might die (Munns, 2002). Cotton is one of the major fiber crops of the world which is classified as a salt tolerant crop (Ahmad *et al*., 2002). Leaf relative water content (RWC) is considered as an alternative measure of plant water status, reflecting the metabolic activity in plant tissues *e.g.* in *Atriplex nummularia* L (de Araújo *et al*., 2006), safflower (Siddiqi and Ashraf, 2008), and pea (Noreen and Ashraf, 2009a). Several researchers mentioned that fresh and dry weights might be affected under saline conditions (Munns, 2002; Hajer *et al*., 2006; Jaleel *et al*., 2008; Khatoon *et al*., 2010). Thus, this investigation is focused on some critical indicators for fast screening of salt tolerance capacity in some cultivated cotton varieties in Syria.

Materials and Methods

Seeds of Upland cotton (*G. hirsutum*. L) Niab78, Deir-Ezzor 22, Deltapine 50 and Aleppo 118 were provided by the Cotton Research Administration (Cotton Bureau) in Aleppo, Syria. The experiment was carried out at the Atomic Energy Commission of Syria-Damascus (AECS). Seeds were soaked in distilled H_2O for 24 h and then planted in pots filled with a 1/3:2/3 (v/v) mixture of perlite:peat mosse. Germination was carried out in a greenhouse at temperature of 18 ºC, 12-h photoperiod and relative humidity of 80 %. Seedlings were allowed to grow in a greenhouse under controlled conditions (temperature of 25 ºC, 12-h photoperiod and relative humidity of 80 %). Seedlings were irrigated with tap water for one week before the initiation of NaCl treatment. The seedlings were subjected to salt stress at the first real leaf stage by adding NaCl (200 mol m^{-3}) to the water. Plants were irrigated twice a week by water with or without salt. The same environmental conditions were maintained during the experiment.

The experiment (Five replicates by treatment) was carried out in the greenhouse for 7 weeks. Plants were

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harvested 7 weeks after salt application; plants were up-rooted carefully and washed properly under tap water. Plant height (PH), root length (RL) and leaf number (LN) were recorded. The plants were then separated into roots, leaves and stems. Leaf area (LA) was measured with an Area meter AM100 (ADC, Bioscientific) instrument and expressed as cm^2. The osmotic potential was measured using Micro Osmometer (OSMOMETTE) apparatus. Chlorophyll SPAD reading were determined using Minolta Chlorophyll Meter-SPAD 502 (Spectrum Technologies Inc., IL). Leaf chlorophyll SPAD measurement was used to determine the chlorophyll content index (CCI) according to Richardson *et al*. (2002). While, chlorophyll (Chl) *a* and *b* content were determined according to Arnon (1949). Plant samples were fractionated into roots, leaves and stems just before the initiation of flowering. Roots, leaves and stems dry weights (DW) were obtained after oven drying at 70 °C for 72 h. While, for relative water content measurements: two leaves from each plant were excised, and their fresh weight was scored immediately. After floating them in deionized water at 4 °C overnight, their rehydrated weight was determined. Finally, they were dried in an oven at 70 °C overnight and weighed. RWC was calculated as indicated RWC = (fresh weight - dry weight)/ (rehydrated weight - dry weight) x 100.

All statistical analyses were performed using Statview 4.5 statistical package (ABACUS, 1996) at 5% significance level ($p = 0.05$). Data were subjected to analysis of variance (ANOVA) for the determination of differences in means between tested plants of each concentration of NaCl applied. Differences between means were tested for significance by Fisher's PLSD test. Data are expressed as mean of five replicates.

Results and Discussion

Plant height and root length, leaf number and leaf area

Salt treatment exhibited deleterious effect on vegetative growth with genotypic variation among the four tested cotton varieties. Salt stress impaired both seedling height (Figure 1A) and root length (Figure 1B) of all tested varieties. This decline in seedling height (PH) was more pronounced in Aleppo 118 and Deltapine 50 compared to the other tested varieties. These observations are similar with the findings in cotton (Basal, 2010) and in Black Seeds (*Nigella sativa* L.) (Hussain *et al*., 2009). While, in the case of root length (RL), the largest reduction was below their control recorded in Deltapine 50 and Aleppo 118 by 22 and 11%, respectively. Whereas, the lowest reduction was recorded in Niab78 (7%). While, this parameter was slightly increased in Deir-Ezzor22 by 2%.

Leaf number (LN) also decreased in a similar trend (Figure 1C). It has been noted that the leaf area (LA) decreased in stressed plants compared to their respective control in all varieties tested except for Deir-Ezzor22 where there was an increase in leaf area with salinity treatment (Figure 1D). This reduction was more accentuated on Aleppo 118 and Deltapine 50 by 16 and 8%, respectively, below their control, but increased by 4 and 13% in Niab78 and Deir-Ezzor22, respectively, up to their respective control. Heigh salinity levels have led to reducing leaf area due to turgescence reduction resulting from salt stress which can cause inhibition of cell division and expansion (Manivannan *et al*., 2007). In this respect, the results are in agreement with results obtained in cotton by Qadir and Shams (1997) and Ganieva *et al.* (1998). Similar observation has also been recorded in other species (Ali *et al*., 2004; Netondo *et al*., 2004; Jaleel *et al*., 2008).

Chlorophyll content and Relative water content

Reduction in leaf SPAD readings (expressed as CCI) was observed in all tested varieties under saline conditions compared to their respective control (Figure 2). This parameter was less pronounced in Niab 78 and Deir-Ezzor 22 compared to other varieties. From the data collected after experiment regarding chlorophyll content we observed that salt stress significantly ($p<0.01$) reduced chlorophyll content by <1%, 5%, 32% and 61% in Niab 78, Deir-Ezzor 22, Deltapine 50 and Aleppo 118, respectively, below the control (Figure 2A). Consequently, Niab78 and Deir-Ezzor22 seem to be more tolerant to NaCl compared to the other varieties. The present data clearly support the general correlation between the photosynthetic capacity and leaf area which is in agreement with the hypothesis of Reich *et al*. (1999), that no species can improve photosynthetic capacity without increasing leaf area due to biophisical limitation. Chlorophyll (Chl) content can be considered as one of the few physiological parameters that can show good correlation with salinity tolerance (Ashrafuzzaman *et al*., 2000; Hakam *et al*., 2000; Ali *et al*., 2004).

A decline in Chl *a* and *b* content was also observed at 200 mol m^{-3} compared to the respective control (Figure 2B). A significant reduction ($p<0.01$) in leaf Chlorophyll *a* and *b* was observed in all accessions due to salt stress. However, the adverse effect of salt stress on the previous parameter was higher in Aleppo 118 and Deltapine 50 for both leaf Chl *a* and *b* content than in the other accessions (Figures 2C and 2D). These results were in accordance with Netondo *et al*. (2004), Hajer *et al*. (2006) and Jaleel *et al*. (2008). These results suggest that the reduction of chlorophyll content could be used for screening of plants for salinity tolerance.

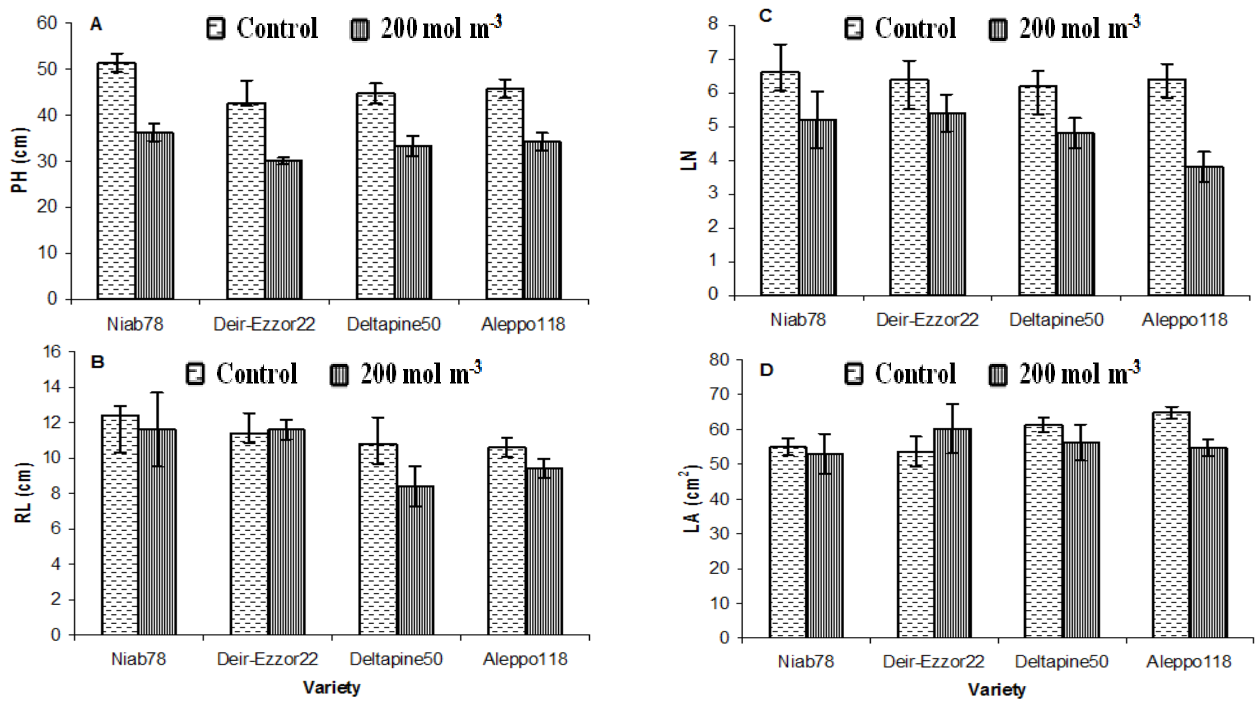

Figure 1: Plant height PH (A), root length RL (B), leaf number LN (C) and leaf area LA (D) of four cotton varieties at control and 200 mol m^{-3} NaCl

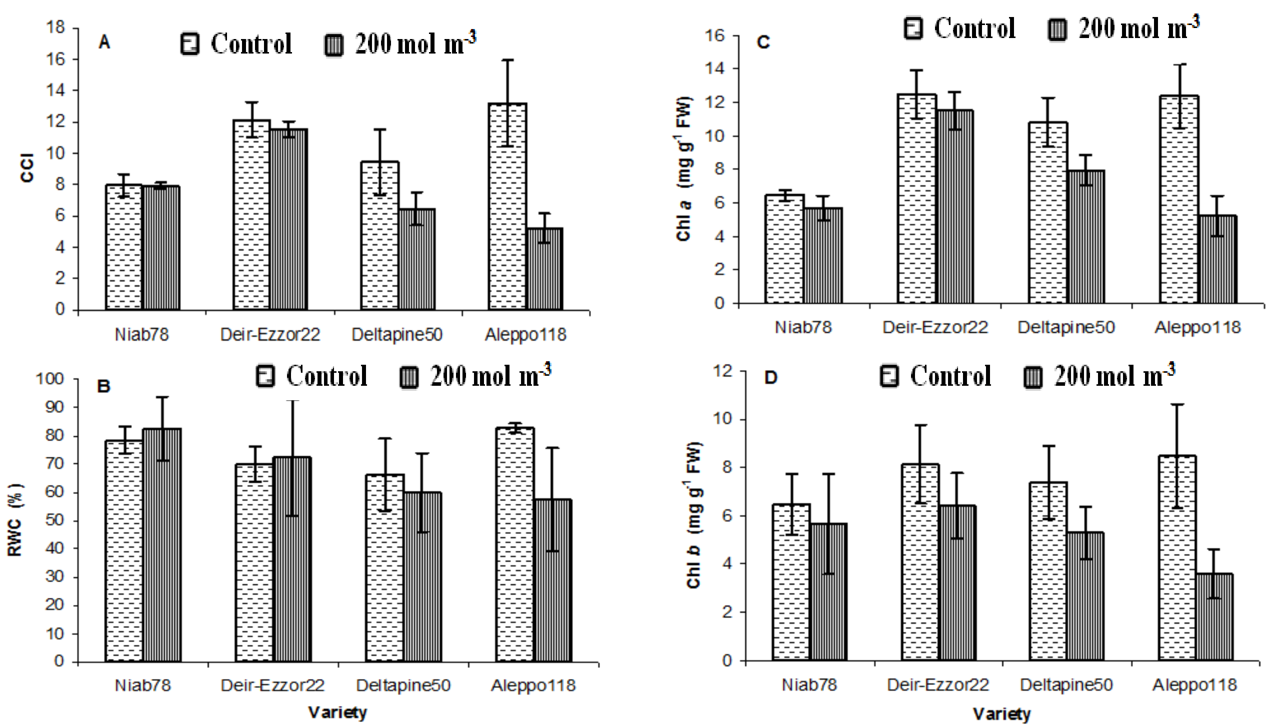

Figure 2: Chlorophyll content index CCI (A), relative water content RWC (B), leaf Chl *a* (C) and leaf Chl *b* content (D) of four cotton varieties at control and 200 mol m^{-3} NaCl

Like wise, our data showed that accessions differed significantly in this water relation parameter. Sodium chloride stress adversely affected the relative water content (RWC). It has been noticed that, the highest values of the previous parameter were recorded for Niab78 followed by Deir-Ezzor22 reflecting their salt tolerance compared to the other tested varieties (Figure 2B). Whereas, the lowest one was recorded for Deltapine50 (Figure 2B).

Extent of salt-induced effects on relative water content has been used as one of the vital water relation parameters for assessing degree of salt tolerance in plants (Siddiqi and Ashraf, 2008; Noreen and Ashraf, 2009a). Sodium chloride stress adversely affected RWC. Note that leaf dry weight was significantly reduced with NaCl application. At 200 mol m^{-3} NaCl seedlings of Niab78 and Deir-Ezzor22 were able to adjust osmotically, leading to maintenance of relative water content (RWC), in contrast to Aleppo118 and Deltapine50, RWC was 31 and 23 % lower than in the unstressed control. Whereas RWC of that organ was about 3 and 5 % (p<0.01) more than the control for Deir-Ezzor22 and Niab 78, respectively. The ability of Niab78 and Deir-Ezzor22 accessions to keep leaf RWC above the control was similar to the observation of de Araújo *et al*. (2006) in *Atriplex nummularia* L.

Osmotic potential

Our results showed that osmotic potential (ψ) was considerably decreased under saline treatment compared to their respective control for all tested varieties (Figure 3). This decrease can be attributed to the cellular water loss under salinity (Stoeva and Kaymakanova, 2008). Analysis of variance indicated that the effect of 200 mol m^{-3} NaCl level on this parameter was highly significant (p<0.01). Under 200 mol m^{-3} NaCl, this index was negatively affected and the reduction was almost about 63, 89, 96 and 152 % for Niab 78, Deir-Ezzor 22, Deltapine 50 and Aleppo 118, below the control, respectively. It is reported by Gama *et al*. (2007) that the reduced water potential could be explained by the fact that during stress carbon allocation, osmotic adjustment and accumulation of soluble sugars compete with other factors and can affect growth. Wild range of salt tolerance in cotton varieties is recorded (Ahmad *et al*., 2002).

Biomass and root/ shoot weight ratio

Reduction in dry biomass was observed in all tested varieties under saline conditions compared to the control (Figure 4). The results are in agreement with the studies supported in cotton by Khan *et al*. (2001), Ahmad *et al*. (2002), Basal (2010), Munns (2002) and Saleh (2011). This reduction was more pronounced for Deltapine50 and Aleppo118 at root, leaf and stem levels compared to Niab78 and Deir-Ezzor22 (Figure 4A, B and C, respectively). Similar observation has also been recorded in other species (Hajer *et al*., 2006; Hussain *et al*., 2009; Khatoon *et al*., 2010).

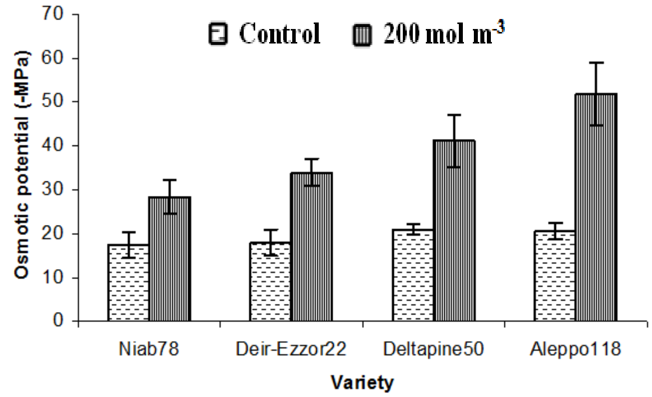

Figure 3: Leaf osmotic potential of four cotton varieties at control and 200 mol m^{-3} NaCl

In the case of root dry weight (RDW) (Figure 4A), it has been noticed that the decline in this parameter is can be arranged as the following: Deir-Ezzor 22 (10%) < Niab 78 (23%) < Aleppo 118 (53%) < Deltapine 50 (56%). These values fall within the same range of Munns (2002) who reported that the reduction in dry weight of cotton tissues reach 60%. While, for leaf dry weight (LDW) (Figure 4B), this reduction ranged from 23% (Niab78 and Deir-Ezzor22) to 39% (Aleppo118). Whereas, this decline for stem dry weight (SDW) (Figure 4C), varied between 20 (Deir-Ezzor22) and 46% (Aleppo118). Ashraf and Ahmad (2000) and Basal (2010) reported that salt-tolerant cotton varieties (*G. hirsutum* L.) had higher shoot biomass production than salt-sensitive varieties at the vegetative stage. The decline in dry weight detected due to increased salinity can be attributed to a combination of osmotic and specific ion effects of Cl^- and Na^+ (Hajer *et al*., 2006; Basal, 2010).

Previous studies carried out on cotton (Meloni *et al*., 2001) showed that shoot growth was more inhibited by NaCl than the root growth. Data regarding root/shoot weight ratio estimated for the four tested varieties showed different response towards salt application (Figure 4D) *e.g.* this ratio was reduced by 33% and 16% in Deltapine50 and Aleppo118, respectively, while it was increased by 20 and 17% in Niab78 and Deir-Ezzor22, respectively, compared to their respective control. Based upon this observation, we can suggest that Niab78 and Deir-Ezzor22 could be classified as salt-tolerant varieties, while, Deltapine50 and Aleppo118 as susceptible one. Similarly, Saleh (2011) investigation, reported that Deir-Ezzor 22 variety is differed by showing high salt tolerance relative to Aleppo 118. Thus,

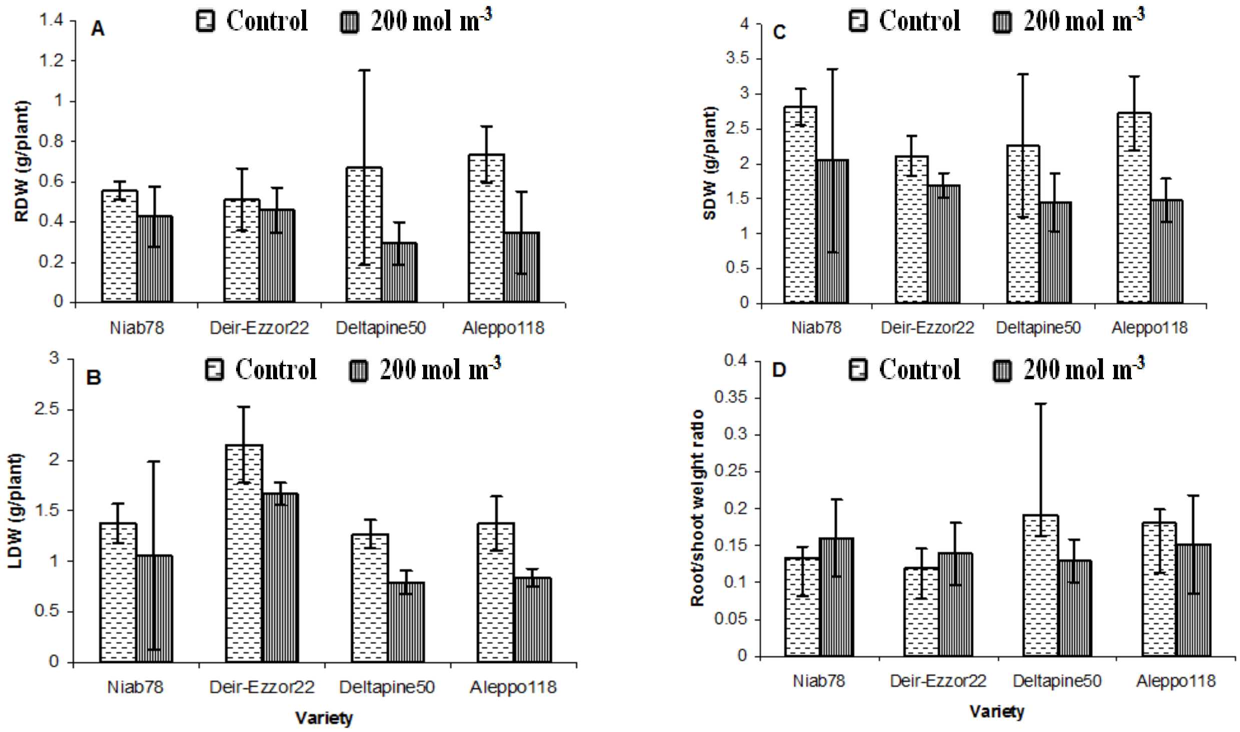

Figure 4: Root dry weight RDW (A), leaf dry weight LDW (B), stem dry weight SDW (C) and root/shoot weight ratio (D) of four cotton varieties at control and 200 mol m^{-3} NaCl

increased root/ shoot ratio appears to be an adaptation to salinity, resulting in a more efficient water and nutrient uptake under saline stress (Gorham *et al*., 1985).

Conclusion

Various physiological indices were evaluated in this study. Among these indicators, leaf area, chlorophyll SPAD, leaf Chl *a* and *b* content, relative water content, osmotic potential, biomass and root/shoot weight ratio could be considered as useful parameters for screening salt tolerance among different cotton varieties cultivated in Syria. Thereby, cotton varieties that could be considered as salt tolerant will help up in boosting plants production in salt affected regions. Further determinants such as: ion partitioning, transpiration rate and osmolytes accumulation, are needed for a better understanding of the response towards salt treatment among these tested varieties.

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