Assessing the impact of salinity stress on some morpho-physiological traits of two chickpea genotypes under hydroponic conditions

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SUMMARY

Evaluating the performance of crop species to salinity stress is considered an intricate task due to differences in performance, response and susceptibility at different phenological stages of chickpea crop. Assessment of the performance of chickpea genotypes in response to NaClinduced salinity stress at the initial vegetative phase is of great importance to have a crystal idea about the threshold level of tolerance. An experiment was carried out under hydroponic conditions to evaluate the performance of two chickpea genotypes (ELMO and ORION), in response to different salinity levels (0, 25, 50 and 75 mM NaCl) as factorial arrangement under completely randomized design with three replications. The average of shoot and root dry matter weight was significantly higher for the ELMO genotype at the control treatment (1.143, 0.4133 g respectively), while it was significantly lower in the two genotypes ORION and ELMO at the highest salinity level (0.267 and 0.2700; 0.0433 and 0.0533 g respectively). The root to shoot ratio was significantly higher in both genotypes in the control and the lowest salt level (25 mM NaCl), without significant differences among them (47.98, 43.30, 37.10 and 36.25% respectively). The relative water content and stomatal conductance were significantly higher in the ORION genotype (88.01%; 335.40 mmol m⁻² s⁻¹) compared to ELMO (84.09%; 299.10 mmol m⁻² s⁻¹), and increasing salinity level caused a proportional decline in both traits, where they were significantly lower at the highest salt level (75Mm) (77.45%; 87.50 mmol m⁻² s⁻¹). Results indicate genotypic variability in response to NaCl-induced salinity stress under hydroponic conditions and the physiological traits are more expressive and reliable as selection criteria than morphological ones.

Keywords: Abiotic stress; relative water content; root to shoot ratio; stomatal conductance; Cicer arietinum L.

INTRODUCTION

Salinity is a significant environmental threat that may result in significant crop losses, especially in arid and semi-arid regions of the world (Zulfiqar and Ashraf, 2021). Over 6% of the world's land is thought to be saline, and 20% of all irrigated land is thought to be saline, according to some credible estimates (Buttar et al., 2021). This is predicted to cause more than US\$30 billion in agricultural losses yearly. Poor management practices are the primary cause of the ongoing expansion of salt-affected soils (Munns et al., 2020a).

Chickpea (Cicer arietinum L.) is one of the earliest seed crops that humans cultivated. In terms of food legumes produced worldwide, chickpea is currently produced in the third mass lace behind field peas (Pisum sativum L.) and beans (Phaseolus spp.). Although chickpea is produced in more than 50 countries, only 22 of those countries have more than 20,000 hectares of planted land; 19 of those nations have between 10,000 and 20,000 hectares of covered land. The entire yearly production in the world is 8.4 million tons. India accounts for 65% of the world's yearly production of chickpea, followed by Pakistan (10%), Turkey (7%), Iran (3%), Myanmar (2%), Mexico (1.5%), and Australia (1.5%). Ethiopia, Iraq, Jordan, Morocco, Syria, Tanzania, Malawi, and Canada are significant producers of chickpea (FAO, 2020). Chickpea seeds are rich in protein (20–25%) and CHO (49-55%), along with vitamins and minerals, making them suitable for use in animal feed as well as human nutrition (Gaur et al., 2010). Chickpea plants have the

capacity to biologically fix nitrogen from the atmosphere, which increases soil fertility. Because of their exceptional nutritional properties, chickpeas are the foundation of a healthy diet; further research on their sensitivity to saline stress increases the possibility for producing them (Muller et al., 2011). Especially in the early stages of growth and maturation, chickpeas are highly vulnerable to salt stress (Pujol et al., 2000). Within 75 days of exposure to 40 mM NaCl, even salttolerant chickpea cultivars are killed (Samineni et al., 2011). The most sensitive chickpea genotypes, according to Flowers et al. (2019), did not grow even at 25 mM NaCl, indicating a large variance among the genotypes. The more resilient genotypes, however, were able to withstand the salt stress brought on by 100 mM NaCl. Limited crop output can arise from modifications in many physiological and metabolic processes in plants, contingent on the degree and intensity of saline stress. Plant growth is initially suppressed by soil salinity through osmotic stress; subsequently, ion toxicity takes place (Rahnama et al.,

Chickpea is fairly salt-susceptible legume crop, during vegetative growth, which is evident in an abrupt reduction in the production of dry matter (Varshney et al., 2021). Chickpea plants may experience growth losses in response to NaCl levels deemed mild for other crops (James et al., 2011). Stress symptoms, in particular have been linked to sodium toxicity alone (Rouphael et al., 2017). The majority of salinity investigations have employed NaCl as the primary source of salt. Naturally, NaCl concentrations more than 30–40 mM can inhibit the uptake of water and ions



by plants, hence restricting their growth and development (Shin et al., 2020). Early stress responses mostly include stomata closing, which is brought on by abscisic acid (Buckley, 2019). However, compared to the carbon supply of photosynthesis, the primary carbon sink, which is represented by the rapid growth of roots and young leaves, is impacted earlier and more severely (Muller et al., 2011). However, significant drops in the rate of photosynthesis and CO2 uptake should be anticipated. Redirecting assimilate mobility could also promote root growth, but at the expense of shoots (Li et al., 2018). Abiotic stress limits the ability of roots to transfer nutrients and water to shoots, which negatively impacts the functional equilibrium between roots and shoots and lowers the shoot:root ratio (Ashraf and Munns, 2022). Nevertheless, the degree to which a crop responds to salt varies among crop species and genotypes. Numerous crops respond to salt stress in a wide range of mechanisms (Annunziata et al., 2017). However, the sensitivity of different growth stages and genotypes is still poorly understood. Different genotypes of chickpeas may differ in their capacity to hold onto water. For example, in conditions of salinity stress, more salt-tolerant varieties may be able to keep the water content of their shoots higher than more sensitive ones. It is still unknown, though, why chickpeas are particularly vulnerable to salt stress and what are the stress-associates traits at each and every developmental stage (Farooq et al., 2017).

According to the available research, regardless of the experimental context, the rank order of genotypes' salt resistance is consistent, supporting the analysis of the impact of salt stress on vegetative growth in hydroponics, pots, or fields. As a result, identifying salt-tolerant chickpea genotypes would be very helpful for the development of salt-tolerant cultivars with high yielding capacity via breeding programs to effectively ensure sustainable crop production in moderately salinity-affected soils. It is also necessary to identify critical salinity limits and some morpho-physiological features of the assessed chickpea genotypes that could be used as a basis for the development of this crop in saline land. Therefore, the objective of the conducted hydroponic experimentation is to determine which genotype(s) is/are tolerant to NaCl-induced salinity stress while retaining production capacity by analyzing the effects of different levels of salinity stress on two chickpea genotypes during the early vegetative growth stage based on certain morphological and physiological traits.

MATERIALS AND METHODS

The assessment of the impact of NaCl-induced salinity stress (25, 50 and 75 mM NaCl), in addition to control was done under hydroponic conditions in a controlled environment chamber at the Department of

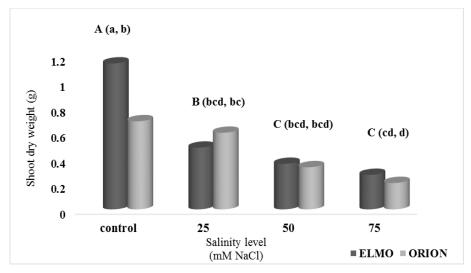
Applied Plant Biology, University of Debrecen, Hungary, where the air temperature was maintained at 24 °C /18 °C day/night temperature; 10/14 h light/dark photoperiod; 45% relative humidity and 300 µmol m⁻² s⁻¹ light intensity. Seed surfaces of the two investigated chickpea genotypes (ELMO and ORION) were sterilized with 6% v/v H₂O₂ for 20 minutes before being properly washed and rinsed with deionized water, then were grown geotropically at 24 °C between moist filter sheets. Soaked and sprouted seeds (small seedlings: five-day age) were transplanted into plastic pots (1.7 liters: 4 plants per pot) in a hydroponic environment, filled with dicot nutrient solution that consisted of the following substances (0.7 mM K₂SO₄, 0.5 mM MgSO₄, 0.1 mM KH₂PO₄, 0.1 mM KCl, 0.5 μM MnSO₄, 0.5 μM ZnSO₄, 10 µM H₃BO₃, and 0.2 µM CuSO₄ along with 2.0 mM Ca (NO₃)₂. Additionally, iron was given in the form of 10⁻⁴ M Fe-EDTA) (Marschner et al., 1990). Nutrient solution of each pot was replaced with fresh alternative every 3 days. The experiment was done as factorial arrangement (genotype × salt concentration) in completely randomized design, replications. Samples were collected from 35-day old seedlings after salinity stress treatment, for estimation of the investigated morphological (shoot and root dry weight; root/shoot dry weight ratio) and physiological (relative water content as described by Schonfeld et al., 1988 and stomatal conductance as described by Guerrier et al., 2019) traits at the end of the experiment. The tabulated obtained data were statistically analyzed using MSTAT-C program, to calculate the least significant differences (LSD) at 0.01 between genotypes, salinity levels and their interaction and the co-efficient of variation (CV%) for each and every investigated trait.

RESULTS AND DISCUSSION

Shoot dry matter weight: The statistical analysis did not exhibit significant differences in the shoot dry weight between the genotypes, while there were significant differences (p≤0.01) among the salt levels and the interaction between the genotypes and the salt levels. The shoot dry matter weight was significantly higher in the control (0.9167 g), followed with significant difference by the lower salt levels (25 mM NaCl) (0.5417 g), while it was significantly lower at both 50 and 75 mM NaCl (0.3433 and 0.2383 g respectively) without significant differences between these two higher salinity levels (Figure 1). It was demonstrated that the average shoot dry matter weight was significantly higher for ELMO genotype at the control (1.143 g), while it was significantly lower in the two genotypes ORION and ELMO at the highest salinity level (75 mM NaCl (0.267 and 0.2700 g respectively) (Figure 1).



Figure 1. Effect of NaCl-induced salinity stress on shoot dry weight (g) of two chickpea genotypes under hydroponic conditions

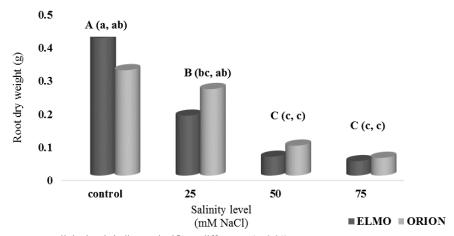


Different capital letters among salinity levels indicates significant differences ($p \le 0.01$). Different small letters at each salinity level between genotypes indicates significant differences ($p \le 0.01$).

Root dry matter weight: Results did not show significant differences in the root dry weight between the investigated genotypes, while the differences were significant ($p \le 0.01$) among salt levels and the interaction between the genotypes and the salt levels. The root dry weight was significantly higher in the control (0.3650 g), while it was significantly the least in the 75 and 50 mM NaCl salt levels without significant differences between them (0.04833 and

0.07333 g respectively). It has been found that the root dry weight was significantly higher for the two genotypes ELMO and ORION in the control, and the genotype ORION at the salt level of 25 Mm (NaCl) without significant differences among them (0.4133, 0.3167 and 0.2600 g respectively), while it was significantly the least for the rest interactions with no significant differences among them (*Figure 2*).

Figure 2. Effect of NaCl-induced salinity stress on root dry weight (g) of two chickpea genotypes under hydroponic conditions



Different capital letters among salinity levels indicates significant differences ($p \le 0.01$). Different small letters at each salinity level between genotypes indicates significant differences ($p \le 0.01$).

Root to shoot dry weight ratio: Results of the statistical analysis did not reveal significant differences in the roots to shoot ratio between the studied chickpea genotypes, while the differences were significant ($p \le 0.01$) among salt levels and the mutual interaction between the genotypes and the salt levels. It was the

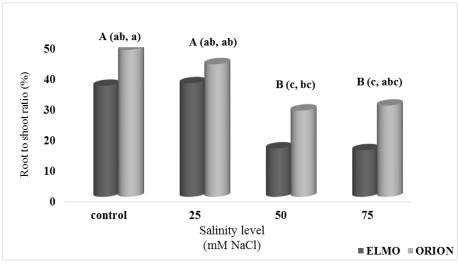
highest in the control and the 25 mM salt level, without significant differences between them (42.11 and 40.20% respectively), while it was least at 50 and 75 mM NaCl, without significant differences between them (22.51and 21.99% respectively), indicating that the growth of both shoots and roots of chickpea



genotypes is sensitive to salt stress conditions and this elucidates the complete demise of chickpea plants at the salt level of 100 mM NaCl. Taking into account the interaction between genotypes and salt levels, the ratio was significantly higher between the two genotypes ORION and ELMO in the control and the salt level of

25 mM NaCl, without significant differences among them (47.98, 43.30, 37.10 and 36.25% respectively), while it was the least for the rest of the interactions, with no significant differences between them (*Figure 3*).

Figure 3. Effect of NaCl-induced salinity stress on root to shoot dry weight ratio (%) of two chickpea genotypes under hydroponic conditions



Different capital letters among salinity levels indicates significant differences ($p \le 0.01$). Different small letters at each salinity level between genotypes indicates significant differences ($p \le 0.01$).

With salinity imposition, as plant water content decreases, the cells shrink, and the cell wall relaxes, resulting in loss of turgor, causing a reduction in leaf water potential (ψ) and in cell division and expansion (Faroog et al., 2009). If the salinity-induced osmotic stress is imposed early in the developmental stages, the inhibition of cell expansion results in a reduced leaf area, and stunted growth of both shoots and roots (Farooq et al., 2012). The decline in the shoots and roots dry weight, in the investigated genotypes of chickpea was a result of the high intensity of NaClinduced salinity stress in the nutrient solution, which lead to a remarkable decrease in the water potential (ψ) , thus reducing the amount of water available to be taken up by the plants, which adversely affects the rate of water absorption by the root system, and as a consequence the amount of absorbed water becomes insufficient to compensate the quantity of water lost through transpiration during the gas exchange process, which leads to a drop in the leaf cell pressure potential (ψ_D) , thereby inhibiting leaf expansion, because the turgor potential is considered as the physical driving force for the cell walls to elongate (Cossgrove, 1989). On the other hand, the decrease in the pressure potential (ψ_p) will also lead to a significant and proportional decline in the stomatal conductance (gs) with the increase in the concentration of soluble salts (NaCl) in the growth medium, which negatively affects the gas exchange process, and thus reducing rate of CO₂diffusion, due to a parallel increase in the stomatal resistance (rs), which negatively affects the

intercellular CO_2 concentration (Ci) in the CO_2 -fixation sites within chloroplasts, which in turn leads to a decline in the assimilation rate, and the production and accumulation of dry matter, causing a reduction in the growth rates and development of the various plant organs (shoot and root).

The genetic variation between the studied genotypes could be attributed to variation in their efficiency in maintaining high values of turgor pressure, differences in the plant cell wall extensibility (Φ) , or variation in stomatal conductance (gs) (Xiancan et al., 2018; Carminati and Javaux, 2020). In general, a decrease in elongation of leaf cells will lead to a decrease in the source size (leaf area index), which negatively affects the amount of dry matter accumulation and photo-assimilates allocated for the growth and development of the root system (Cisse et al., 2019).

Relative water content: Results showed that there were significant differences (p≤0.01) in the relative water content between the two investigated chickpea genotypes, and between the salt levels, and the interaction between them. The relative water content was significantly higher in ORION genotype (88.01%) compared to ELMO (84.09%). It was significantly the highest at the control treatment and at the 25 mM NaCl, without significant differences between them (93.17 and 89.89% respectively), while it was significantly lower at the highest salt level (75 mM) (77.45%). This trait was found to be significantly higher in both genotypes in the control and 25 mM NaCl, without



significant differences between them (95.10, 91.34, 91.25 and 88.45% respectively), while it was significantly lower in ELMO at 75 Mm NaCl (73.16%),

followed by ORION genotype at 75 mM NaCl (81.74%) (*Figure 4*).

A (ab, a) A (abc, ab) B (bc, bc) 100 C (d, c) Relative water content (%) 80 60 40 20 0 75 50 control Salinity level **■ELMO** ■ ORION (mM NaCl)

Figure 4. Effect of NaCl-induced salinity stress on relative water content (%) of two chickpea genotypes under hydroponic conditions

Different capital letters among salinity levels indicates significant differences ($p \le 0.01$). Different small letters at each salinity level between genotypes indicates significant differences ($p \le 0.01$).

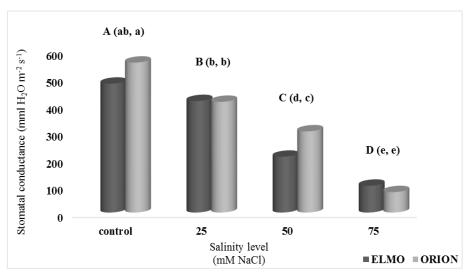
The variation in the leaf relative water content between the studied genotypes of chickpea (ELMO and ORION) is attributed to the difference in the proline content of the leaves, where the proline content was significantly higher for the ORION genotype (7.961 μmol g⁻¹) compared to the ELMO (5.760 μmol g⁻¹) (data not shown). In general, increasing the biosynthesis and accumulation of the compatible organic solutes (proline) leads to a decrease in the leaf cell water potential for both roots and shoots, which increases the difference in the water potential gradient between the growth solution and the plant roots, so the rate of water absorption by roots will increases, because it is the driving force for the movement of water from the higher water potential site (growth solution) (less negative) to the site of a low water potential (more negative), which enables plants to absorb a sufficient amount of water to compensate for the water lost through transpiration and maintain relatively high levels of water potential (Ismail and Horie, 2017). The variation in the leaf relative water content among different salt levels is due to the availability of more amount of free water molecules in the growth medium of the control treatment which is free of dissolved mineral salts (NaCl), that usually bind water molecules, reducing the number of free and absorbable water, so the presence of high concentration of dissolved salts in the growth medium (above 25 mM) leads to a remarkable decline in the water potential of the aqueous solution (more negative).

Stomatal conductance: Results revealed that the stomatal conductance (gs) was significantly ($p \le 0.01$)

higher in ORION genotype (335.40 mml m^{-2} s^{-1}) compared to ELMO (299.10 mmol H_2O m⁻² s⁻¹). Increased soluble salts (NaCl) in the growth medium caused a significant and progressive decrease in the stomatal conductance, as it was significantly higher for the control (516.30 mmol H₂O m⁻² s⁻¹), while it was significantly lower at the highest salt level (75 mM NaCl) (87.50 mmol H₂O m⁻² s⁻¹). Taking into account the interaction between the genotypes and salt levels, it has been shown that stomatal conductance was significantly higher among the two genotypes Orion and Elmo for the control without significant differences between them (555.30 and 477.30 mmol $H_2O \ m^{-2} \ s^{-1}$ respectively), while it was significantly lower at the highest salt level (75 mM) with no significant differences between them (75.67 and 99.33 mmol H₂O m⁻² s⁻¹ respectively) (Figure 5). The variation in the stomatal conductance between the genotypes and the salt levels is mainly due to the variation in the relative water and proline contents, where it was recorded that the relative water content was significantly higher in the ORION genotype (88.01%) compared to ELMO (84.09%). On the other side, proline content was significantly higher in the genotype ORION compared to ELMO (data not shown). The relative water content was also significantly higher in the control and the 25 mM salt level (93.17 and 89.89% respectively). This indicates the importance of the osmotic adjustment mechanism in maintaining high values of the relative water content, which in turn allows the stomata to remain open.



Figure 5. Effect of NaCl-induced salinity stress on stomatal conductance (mmol H_2O m⁻² s⁻¹) of two chickpea genotypes under hydroponic conditions



Different capital letters among salinity levels indicates significant differences ($p \le 0.01$). Different small letters at each salinity level between genotypes indicates significant differences ($p \le 0.01$).

CONCLUSIONS

Chickpea genotypes exhibited variation in terms of responding to NaCl-induced salinity stress. Taking into account all the studied parameters, it was observed that ORION genotype was more tolerant to salinity stress compared to ELMO. Maintaining high values of relative water content in the leaves is one of the most important physiological characteristics determining the degree of stomata opening and the level of cytoplasmic membrane integrity and stability under stressful environments. Maintaining high values of the relative water content ensures higher stomatal conductance,

guarantees the maintenance of the source size and, as a consequence, the photosynthetic capacity of the plant.

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