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Influence of Salt Stress on Seed Germination and Agro-morphological Traits in Chickpea (*Cicer arietinum* L.)

Munqez J.Y. Shtaya, Heba Al-Fares, Tawfiq Qubbaj, Hassan Abu-Qaoud, Faisal Shraim 10.18805/LR-633

ABSTRACT

Background: The experiment was conducted at the Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Palestine under growth chamber and greenhouse conditions.

Methods: To study the effect of NaCl on germination, fifty seeds in each treatment for each cultivar were germinated on a filter paper in petri dishes moistened with the different NaCl concentration levels (0.0, 50 and 100 mM). To study the effect of NaCl on plant growth and productivity, 2 seeds per variety were grown in 10 liter pots filled with peat moss-sand. Pots were irrigated with the different NaCl levels.

Result: The tested chickpea cultivars showed different tolerance levels in response to NaCl stress levels. NaCl reduced final germination percentage (FGP) and germination index (GI). The highest plant height was obtained by Einalbeda (38.63 cm) in the control group, while the lowest one was found in Arij (28.25 cm) under 100 mM NaCl. NaCl did not affect root/shoot ratio in all genotypes. Reduction in fresh weight ranged between 25.6 and 74.2% under 50 and 100 mM NaCl.

Key words: Chickpea, Germination index, NaCl, Root/shoot ratio, Salinity.

INTRODUCTION

Soil salinity negatively affects plant growth and development. Salinity is estimated to affect around one-third of all irrigated land in the world, the number is increasing (Singla and Garg, 2005; Shahid *et al.*, 2018). The middle east is considered as the world's most salt-affected area (Hossain, 2019). The elevation of soil soluble salts will lead to specific ion toxicity and ionic imbalances (Munns, 2003). Therefore, drastic reduction in crop plant growth and productivity may occur as a result of the accumulative salinity in arable land and even plant death may occur (Rout and Shaw, 2001).

Improving crop tolerance to salinity is one of the eco-friendly and sustainable measures that can be used to reduce the negative effects of salinity on plants. As a result, there has recently been an increase in interest in researching and selecting salinity-tolerant genotypes using both conventional and nontraditional selection and breeding techniques (Ceritoğlu *et al.*, 2020).

Chickpea (*Cicer arietinum* L.) is classified as one of the main legumes and in many countries, it is considered an important source of protein and is widely used as fodder crop or even as green manure (Geethanjali *et al.*, 2018). Chickpea seeds are rich in amino acids, vitamins, protein (≅ 20.6%), fat (≅ 2.2%) and carbohydrates (≅ 61.2%) (Flowers *et al.*, 2010; Ceritoğlu *et al.*, 2020; Jha *et al.*, 2021). Chickpea is classified as a highly sensitive crop to salinity (Hossain *et al.*, 2015). It is reported that salinity could decrease growth, grain yield and other yield components in chickpea (Sohrabi *et al.*, 2008; Kandil *et al.*, 2012; Pushpavalli *et al.*, 2020).

Therefore, the identification of new sources of salinity tolerant genotypes is of great realistic importance. Chickpea is native to arid areas; consequently, it may have an important level of adaptability to different environmental stresses, including salinity (Krouma, 2009). Therefore, it provides a valuable source of more resistant cultivars with minimal yield loss when grown in saline conditions and it could be a viable solution to the salinity problem (Rao *et al.*, 2002). The study was planned with an aim to compare the responses of Kabuli and Desi chickpea cultivars to salinity in terms of growth and yield.

Department of Plant Production and Protection, Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Nablus P.O. Box 707, Palestine.

Corresponding Author: Tawfiq Qubbaj, Department of Plant Production and Protection, Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Nablus P.O. Box 707, Palestine. Email: tqubbaj@najah.edu

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MATERIALS AND METHODS

Plant material and experimental design

Three chickpea varieties were used in the study, including two desi landraces (Einalbeda and Arij) and Kabuli variety (Hadas). All varieties were obtained from the Palestinian national agricultural research center. Experiments were implemented under controlled conditions in the growth chamber and under greenhouse conditions during the 2020 -2021 growing season at the Faculty of Agriculture and

Veterinary Medicine, An-Najah National University, Palestine. Both experiments were set in factorial arrangement in a complete randomized design (CRD) with four replications.

Effect of salinity on seed germination

To study the effect of NaCl on germination, fifty seeds per treatment per cultivar were used. Seeds of uniform size, were surface sterilized in 3% (v/v) of bleach (sodium hypochloride 5.25%) solution for 10 min, rinsed three times in distilled water and then were germinated on a filter paper in 12 cm square petri-dishes. The filter paper was moistened with the solution of the three different NaCl levels (0, 50 and 100 mM). The petri-dishes were kept at 22°C (day/night temperatures) and under darkness for germination. Germination was evaluated after 8 days. Seeds were considered as having germinated when the radicle protruded to a length of 2 mm and the following parameters were calculated:

1. Final germination percentage (FGP): FGP was calculated using the following equation:

$$FGP = \frac{\text{Number of germinated seed}}{\text{Total number of seed tested}} \times 100$$

2. Germination index (GI): GI was calculated according to the following equation:

$$GI = \frac{\text{\% Germination each treatment}}{\text{\% Germination in the control}} \times 100$$

Effect of salinity on seedling growth and production

Two pre-germinated seeds per variety were grown in a 10 liter pot filled with a mixture of peat moss-sand (1:2, v/v). Pots were irrigated with fresh water for two weeks and then the following levels (0, 50, 75 and 100 mM) of NaCl were applied (for the control treatment, 0 mM NaCl, fresh water was added). Pots were irrigated every second day by adding 250 ml of the corresponding NaCl concentration. Seventy days after sowing, the experiments were terminated and the following plants' parameters were collected and analyzed:

1. Plant height (cm): Measured from the soil surface to the top of the plant.
2. Root and plant fresh weight (g).
3. Root and plant dry weight (g): Dried in an electric oven for 72 h at 70°C.
4. Plant height reduction (PHR): It was calculated using the following equation:

$$PHR\ (\%) = \frac{\text{PH at control - PH at the saline condition}}{\text{PH at the saline condition}} \times 100$$

5. Plant fresh weight reduction (PFWR): It was calculated using the following equation:

$$PFWR\ (\%) = \frac{\text{Plant weight at control} - \text{Plant weight at the saline condition}}{\text{Plant weight at the saline condition}} \times 100$$

6. Root/shoot (R/S) ratio: The R/S ratio was calculated as:

$$\text{Root/shoot ratio} = \frac{\text{Roots dry weight}}{\text{Shoot dry weight}}$$

Data analysis

ANOVA analysis was conducted for the different tested parameters, followed by the Bonferroni mean separation test using MINITAB (18) software.

RESULTS AND DISCUSSION

In the present study, the tested chickpea cultivars showed different tolerance levels in response to NaCl stress levels. The inhibitory effects of salinity on morphological traits were observed on the plants at the beginning of the third week after NaCl application. However, the early negative effect of NaCl was observed during seed germination, where NaCl reduced FGP and GI.

Final germination percentage (FGP)

The final germination percentage (FGP) showed significant differences between varieties and NaCl treatments with a significant interaction between the two factors (Table 1). In comparison, all tested varieties completed the FGP in the control treatment (100% of the seeds germinated). The same germination reduction pattern was reported by Farooq *et al.* (2017) and Dadaşoğlu *et al.* (2020) on other chickpeas varieties and the 100 mM NaCl concentration found to be the critical threshold for germination in chickpea (Dadaşoğlu *et al.* 2020).

Similar results were also reported in rice (Islam and Karim, 2010) and chickpea (Özaktan *et al.*, 2018; Ceritoğlu *et al.* 2020). Although 50 and 100 mM NaCl did not significantly affect the final germination percentage and germination index of Arij (Small seed variety) (93.33 and 83.33% respectively). Einalbeda was the most affected variety where FGP was significantly reduced at 50 and 100 mM NaCl (80 and 73.33% respectively). Hadas (big seed variety) germination was not affected under 50 mM NaCl (86.67%) while FGP was significantly reduced under 100 mM NaCl (76.67%). Al-Mutawa (2003) reported that the rate and percentage of seed germination of chickpea were significantly reduced by increasing salinity levels and the magnitude of the reduction varied among genotypes Kaya *et al.* (2008) reported that small seed chickpea varieties can germinate in a shorter time than large seed varieties under saline conditions. The results showed that increasing NaCl concentration caused crucial impacts not only on germination rate but also on the agronomic traits; these findings agree with the results reported by Ceritoğlu *et al.* (2020). Flowers *et al.* (2010) stated that chickpea is a plant affected by salinity, where the most tolerant genotypes can't survive for a long time at 100 mM NaCl solution while the susceptible genotypes will die in just 25 mM NaCl solution.

Plant height

After 70 days of growth under different NaCl concentrations (50, 75 and 100 mM), the tested chickpeas varieties showed some differences in terms of growth parameters. The plant height showed significant differences between varieties and NaCl treatments with a significant interaction (Table 2). These findings agreed with the results reported by Ceritoğlu *et al*. (2020) and Yousef *et al*. (2020). The highest plant height was obtained by Einalbeda (38.63 cm) in the control group, while the lowest one was found in Arij (28.25 cm) under 100 mM NaCl. The mean plant height of cultivars ranged between 31.67 and 34.42 cm. The reduction rates in plant height depending on NaCl treatments are given in Fig 1. Plant height reduction was relatively higher under 100 mM than the lower NaCl concentrations. Welfare *et al*. (2002) reported that high salinity has negative effects on plant height. Arij showed a higher tolerance rate regarding plant height under 50 mM and 75 mM, respectively, while it was the most sensitive under 100 mM NaCl. It was reported that the growth rate was higher in tolerant chickpea genotypes compared with susceptible ones under salinity stress (Kafi *et al*. 2011).

Root/shoot ratio

To test the effect of different NaCl levels on plant growth, the shoot/root ratio was calculated (Table 2). Obtained results show that NaCl did not affect root/shoot ratio in all genotypes, indicating that roots and shoots are in the same range of sensitivity to NaCl stress. A higher root shoot length ratio indicated that the selection of stress tolerance might help improve grain yield (Mazhar *et al*. 2020; Masood *et al*. 2020).

Plant fresh and dry weight

There were significant differences in plant fresh and dry weight between varieties and NaCl treatments. Significant interaction also affected the fresh and dry weights of the plants (Table 3). These findings are consistent with those stated by Ceritoğlu *et al*. (2020). According to Welfare *et al*. (2002), Sohrabi *et al*. (2008) and Hossain *et al*. (2015), high salinity has a negative impact on shoot weight. Fig 2 showed the reduction percentage in plant fresh weight. The reduction was varied among the three varieties, Hadas was the most influential one. Reduction in fresh weight ranged between 25.6% and 74.2% under 50 and 100 mM NaCl, respectively (the average reduction was 54.3%). The least affected variety was Arij; the reduction in fresh weight ranged between 4.9% and 27.2% under 50 and 100 mM NaCl, respectively affected by NaCl. Similar results were reported in chickpea and pea (Yousef *et al*., 2020; (the average reduction was 12.9%). Einalbeda showed the lowest reduction in plant fresh

Table 1: The effect of NaCl on final germination percentage and germination index of three chickpea cultivars.

Variety	Final germination percentage (FGP)			Germination index (GI)		
	Control	50 mM	100 mM	Control	50 mM	100 mM
Einalbeda	100.00^{a}	80.00^{bc}	73.33^{c}	1.00^{a}	0.80^{bc}	0.73^{c}
Arij	100.00^{a}	93.33^{ab}	83.33^{abc}	1.00^{a}	0.93^{ab}	0.83^{abc}
Hadas	100.00^{a}	86.67^{abc}	76.67^{bc}	1.00^{b}	0.87^{abc}	0.77^{bc}

Numbers share the same letter(s) are not significantly differ (Bonferroni test, P = .05).

Table 2: The effect of different levels of NaCl on plant height (cm) and on shoot/root ratio of three chickpea cultivars.

Variety	Plant height (cm)				Shoot/root ratio			
	Control	50 mM	75 mM	100 mM	Control	50 mM	75 mM	100 mM
Einalbeda	38.63^{a}	35.50^{abc}	36.38^{ab}	36.25^{ab}	0.34^{ab}	0.27^{ab}	0.24^{b}	0.50^{ab}
Arij	31.63^{abc}	32.25^{abc}	31.63^{abc}	28.25^{c}	0.25^{b}	0.26^{ab}	0.25^{ab}	0.34^{ab}
Hadas	33.00^{abc}	31.63^{abc}	30.38^{bc}	30.50^{bc}	0.52^{ab}	0.42^{ab}	0.54^{a}	0.45^{ab}

Numbers share the same letter(s) are not significantly differ (Bonferroni test, P = .05).

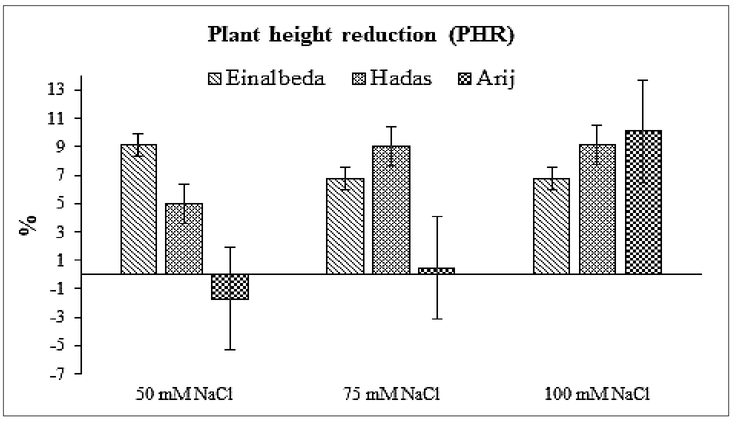

Fig 1: Plant height reduction percentage of three chickpea cultivars depending on NaCl treatments.

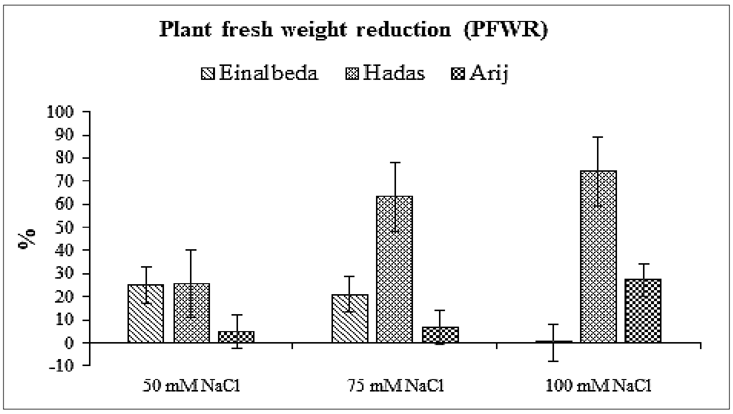

Fig 2: Plant fresh weight reduction percentage of three chickpea cultivars depending on NaCl treatments.

Table 3: The effect of different levels of NaCl on Plant fresh and dry weight of three chickpea cultivars.

Variety	Plant fresh weight (g)				Plant dry weight (g)			
	Control	50 mM	75 mM	100 mM	Control	50 mM	75 mM	100 mM
Einalbeda	22.93^{ab}	15.61^{cd}	16.15^{bcd}	19.52^{abc}	8.68^{a}	7.04^{abc}	7.45^{abc}	7.35^{abc}
Arij	15.20^{cd}	14.49^{cd}	14.24^{cd}	11.95^{d}	7.04^{abc}	6.69^{abc}	6.53^{abc}	5.65^{c}
Hadas	24.10^{a}	19.20^{abc}	14.77^{cd}	13.84^{cd}	8.29^{ab}	7.87^{ab}	6.09^{bc}	5.94^{bc}

Numbers share the same letter(s) are not significantly differ (Bonferroni test, P = .05).

Table 4: The effect of different levels of NaCl on root fresh and dry weight of three chickpea cultivars.

Variety	Root fresh weight (g)				Root dry weight (g)			
	Control	50 mM	75 mM	100 mM	Control	50 mM	75 mM	100 mM
Einalbeda	8.81^{bc}	4.27^{cd}	3.90^{d}	10.05^{ab}	0.54^{b}	0.40^{b}	0.36^{b}	0.37^{b}
Arij	3.73^{d}	3.74^{d}	3.51^{d}	4.16^{cd}	0.42^{b}	0.33^{b}	0.35^{b}	0.30^{b}
Hadas	14.77^{a}	7.92^{bcd}	7.81^{bcd}	6.82^{bcd}	0.96^{a}	0.60^{b}	0.58^{b}	0.44^{b}

Numbers share the same letter (s) are not significantly differ (Bonferroni test, P = .05).

weight under 100 mM (0.1% reduction) (Fig 2). Plant dry weight was significantly Dadaşoğlu *et al*. (2020) Einalbeda showed the highest dry weight over NaCl treatments without significant difference from the control (Table 3).

Root fresh and dry weight

The root fresh weight showed significant differences between varieties and NaCl treatments, whereas no significant difference was observed in root dry weight between the tested varieties (Table 4). This is in agreement with the results reported in chickpea (Abdiev *et al*., 2019) and forage pea (Acikbas *et al*., 2021). Despite the salinity, Hadas showed the highest root fresh and dry weights (14.77 and 0.96 g, respectively) compared to Einalbeda and Arij.

CONCLUSION

According to the present results, varieties and NaCl showed significant effects on studied traits and the interaction between varieties and NaCl treatments significantly affected the majority of the studied traits. The results showed that increasing NaCl concentration caused crucial impacts not only on germination rate but also on the agronomic traits. Plant height reduction was relatively higher under 100 mM than the lower NaCl concentrations. Obtained results show that NaCl did not affect root/shoot ratio in all genotypes, indicating that roots and shoots are in the same range of sensitivity to NaCl stress.

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