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## **Effect of Salinity on Germination and Some Agro-morphological Traits in Chickpea Seedlings**

### **Abstract**

Salinity is a global problem threatening all agriculture lands on the World. The  $700 \times 10^7$  ha land is arable on the world, of which  $150 \times 10^7$  ha can be cultivated,  $34 \times 10^7$  ha is saline and,  $56 \times 10^7$  ha is sodic. Moreover, it was reported that salt-affected areas are increasing by the day. Chickpea which is one of the most produced agricultural products overall the World is a sensitive genus to salt stress, like the other members of grain legumes. So, the aim of the study is to understand the effects of salinity on chickpea and variations in showed responses by cultivars in the early seedling stage. The 7 cultivars, including Diyar-95, Arda, Sarı-98, Yaşa-05, Hisar, Çakır and Aydın-92 and 3 NaCl doses (control, 50 mM and 100 mM) were used as a factor in the study. The experiment laid out in completely randomized design (CRD) with 6 replications. The investigated traits in the study were noteworthy affected by increasing NaCl doses. According to results, the maximum and minimum reduction rates in germination percentage, plant height, number of branches, stem diameter and fresh weight compared with the control were calculated as 8.4-39.6%, 10.5-36.7%, 15.1-43.3%, 8.4-31.0% and 12.5-42.5%, respectively. The present study indicated that cultivars exhibited a large variation in terms of responding to salinity. In addition, it was observed that Çakır and Arda cultivars were tolerant of salinity while Diyar-95 and Sarı-98 were susceptible. Consequently, the early seedling characteristics of different genotypes can be used as a substantial indicator of breeding programs.

## INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important and cultivated grain legumes overall World. The FAO (2018) stated that almost 13 million tones of chickpea were produced in 2018. The agronomical significance of chickpea is based on its high nutritious seed content that approximately contains 54-59% carbohydrate, 18.4–29.0% protein, 2.1-3.2% fiber, 4.5–6.6% lipid and 2.9-4% ash (Jukanti et al., 2012). Chickpea is also a rich source of amino acids, vitamins, phosphorus, potassium, iron, calcium and magnesium (Akibode and Maredia, 2011). In addition, protein content can be increased depending on supplementary irrigations at various growth stages (Varol et al., 2020). Due to these vulnerable traits, chickpea has a vital role in human and animal nutrients in a wide geography. However, one of the most substantial problems is salinity in chickpea cultivation. Chickpea is a very sensitive genus to salt stress like the other grain legumes (Elsheikh and Wood, 1990). The redundant exogenic salt concentration causes osmotic stress, ion toxicity and production of reactive oxygen species (ROS), and consequently be shown some morphological, physiological and biochemical responses by plants (Eyidogan

and Öz, 2007). It has been reported by many researchers that decreasing in growth stages, seed yield and yield components in chickpea plants exposed to salinity stress (Sohrabi et al., 2008; Kandil et al., 2012; Pushpavalli et al., 2020). Salinity in the soil is a major abiotic stress for all cultivated crops. The 700 x 10<sup>7</sup> ha land is arable on the world, of which 150 x 10<sup>7</sup> ha can be cultivated, 34 x 10<sup>7</sup> ha is saline and, 56 x 10<sup>7</sup> ha is sodic. Moreover, it was reported that salt-affected areas are increasing by day (Shahid et al., 2018). Almost 10 million ha of irrigated areas convert to impractical land annually due to salinization, waterlogging and sodification (Szabolcs, 1989). Most of the salt-affected areas commonly located in semi-arid and arid regions, in which low-quality groundwater is used for irrigation and the evapotranspiration rate is noteworthy higher compared with the other regions. The highest salt-affected region in the world is the Middle East, followed by Oceania and North America (Hossain, 2019). It is stated that if the salinization of non-affected lands continues at such a rate, 50% of the useful lands will be lost up to 2050 (Hasanuzzaman et al., 2014). The food requirement for human and animals have been increasing by the day. So, it is not only necessary to make provision for salinization

and also more tolerant plants should be enhanced to salt stress using breeding technologies. The aim of this study is to investigate the response of different chickpea genotypes to salinity stress and understand the difference among the genotypes is noteworthy or not.

## **MATERIAL and METHODS**

### ***Varieties of Seeds Used and Experimental Design***

The 7 chickpea cultivars were registered in Turkey were used in the present study, including Diyar-95 (DYR), Arda (AR), Sarı-98 (SR), Yaşa-05 (YA), Hisar (HSR), Çakır (ÇKR) and Aydın-92 (AY). The cultivars used in the study are the most commonly cultivated chickpea varieties in a different region of Turkey. Also, three doses of salt as NaCl (control, S1: 50 mM and S2: 100 mM) were used as a factor. The experiment was laid out in completely randomized design (CRD) with six replications.

### ***Preparations and Applications***

The present study was laid out in the climate chamber conditions of Siirt University where average temperature and humidity were between 23-25 °C and 45-55%, respectively. The potting medium was preferred to study so that soil mixture and

ecological factors could thoroughly be stabilized. The pods, 5 kg capacity, of which 19 cm depth and 24 cm diameter were used. Each pod was filled with a 4 kg dry soil mixture that consists of sand to silt ratios of 3:1. The soil in the pods was arranged as field capacity. The 250 ml of each, distilled water, 50 mM and 100 mM NaCl, were filled to regulate field capacity of control, S1 and S2 treatments, respectively. The 4 seeds were sown in each pot in the depth of 3cm and more than 1 plant was eradicated after emerging. So, one plant placed per pot during the growth process. After emerging, 100 ml solution (distilled, 50 and 100 mM NaCl) was given to per pot 3 times in 1-week intervals. The study was laid out throughout 30 days.

### ***Sampling and Data Collection***

Germination percentage (GP), plant height (PH), stem diameter (SD), number of the branch (NB) and fresh weight of plant (FW) were investigated in the study. The GP was recorded at ten days after sowing. The formulas given below were used to calculate the GP and reduction in traits compared with control plants:

$$\text{Germination percentage} = \frac{\text{Number of complete germinated seeds}}{\text{Number of sowed seeds}} \times 100$$

$$\text{Reduction in trait} = \frac{\text{Value at control} - \text{value at treatment}}{\text{Value at control}} \times 100$$

The PH was measured by a portable meter and found by calculating the distance between the soil surface and the top of the plant. The SD was measured at the base with an electronic digital caliper (Mitutoyo 500-182-30 digital caliper, Co. Ltd., Japan) (Garcia-Jimenez et al., 2018). While calculating the NB, all branches were counted. The FW was determined by cutting the stem from the base after the harvest.

### ***Statistical Analysis***

The Shapiro-Wilk test was used for the normality of data (Korkmaz et al., 2014). Data were calculated by analysis of variance in the JUMP software according to the Completely randomized design (Kalaycı, 2005). The results were grouped according to the TUKEY test (Rushing et al., 2013).

## **RESULTS**

The used cultivars and salinity levels caused significant differences in tested traits. It is known that salinity has an inhibitory effect on plant growth that can show differences among families, genus,

species and even varieties (Elsheikh and Wood, 1990). Besides, the response of various cultivars showed differences in saline conditions. In the present study, used cultivars had different tolerance levels under salinity. The inhibitory effects of salinity on morphological traits, e.g. changing leaf color, symptoms of water deficit, were observed at the end of the second week. However, the first negative impact was determined during seedling emergence that GP reduced and emerging time delayed with NaCl doses.

### ***Analysis of variance***

Analysis of variance indicated that all investigated traits were affected with increasing NaCl doses and the responses of tested cultivars exhibited different characteristics. At the early seedling stage, the reduction in plant length and weight demonstrated genetic variation in vegetative growth responses to salinity among cultivars. According to results, genotypes (G) and NaCl doses (S) showed statistically significant (0.01 or 0.05) effects on tested traits. Besides, the interaction

between cultivars and NaCl treatments (G x S) significantly influenced some traits (Table 1).

**Table 1.** Analysis of variance on some agronomic traits of cultivars under saline conditions

Source of variation	DF	GP		PH		NB		SD		FW	
		MS	F prob	MS	F prob	MS	F prob	MS	F prob	MS	F prob
Genotypes	6	10694.4	**	469.9	**	656.7	**	0.54	*	13.0	**
Salt doses	2	18581.7	**	2466.6	**	1043.1	**	1.10	**	24.5	**
G x S	12	5793.7	**	163.6	ns	55.8	ns	0.24	ns	2.6	*

(\*\*):  $p < 0.01$ , \*:  $p < 0.05$  ns: no significant difference, DF: Degree of freedom, MS: mean of square)

### Germination Percentage

The GP showed significant differences (0.01) depending on cultivars and NaCl treatments. Also, the interactions of factors statistically influenced (0.01) the GP. In the present study, the cultivars exhibited various responses to saline conditions. All cultivars had fully germination rates in non-

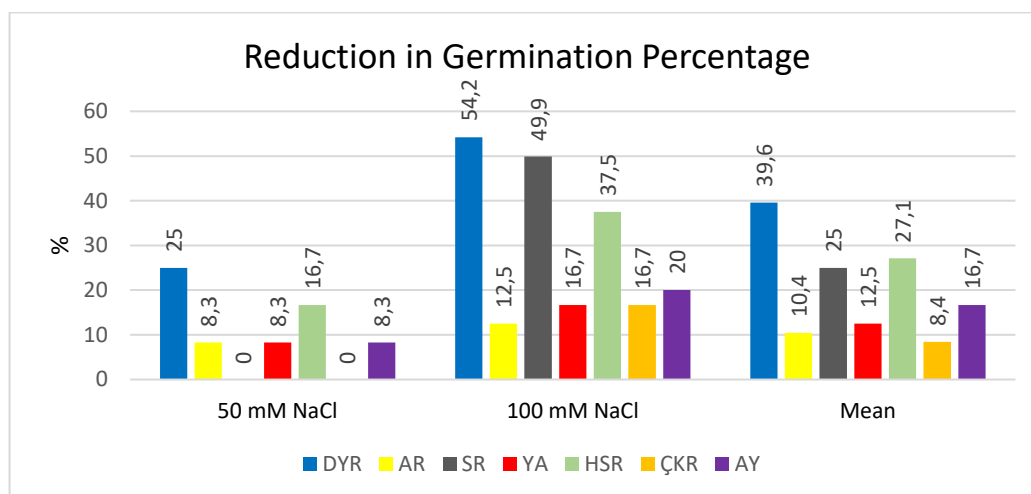
salt-applied groups except cv. SR (83.3%). However, it was determined that GP decreased with increasing NaCl treatments. Although 50 mM NaCl treatment did not affect the GP of ÇKR and SR, 100 mM caused a noteworthy loss. The mean of cultivars changed between 68.5-97.6% with increasing NaCl treatments (Table 2).

**Table 2.** Germination percentage of cultivars under NaCl stress

Genotypes	Germination Percentage (%)			
	Control	50 mM NaCl	100 mM NaCl	Mean
DYR	100.0a	75.0bc	45.8d	73.6cd
AR	100.0a	91.7ab	87.5ab	93.1ab
SR	83.3ac	83.3ac	41.7d	69.4d
YA	100.0a	91.7ab	83.3ac	91.7ab
HSR	100.0a	83.3ac	62.5cd	81.9bc
ÇKR	100.0a	100.0a	83.3ac	94.4a
AY	100.0a	91.7ab	75.0bc	88.9ab
Mean	97.6a	88.1b	68.5c	
TUKEY (G)	11.73**			
TUKEY (S)	6.07**			
TUKEY (G x S)	24.85**			

The most affected cultivar by 100 mM NaCl treatment was DYR (39.6%) while the least affected one was ÇKR (8.4%). The tolerances of other cultivars varied among

these values. The reduction rates in GP of cultivars depending on NaCl treatments were given in Figure 1.



**Figure 1.** The reduction in germination percentage of cultivars depending on NaCl treatments

### Plant Height

The PH was significantly influenced (0.01) by cultivars and NaCl treatments, however, the interactions between them did not affect it (Table 1). The highest PH was obtained by YA (32.2 cm) in the non-salt-

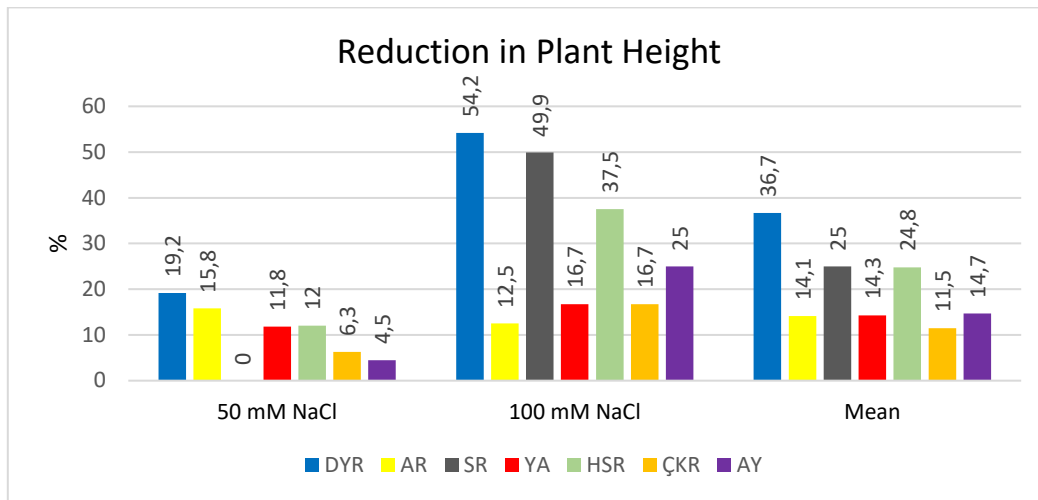
applied group while the lowest one was seen in DYR (15.5 cm) with 100 mM NaCl. The mean PH of cultivars changed by 3% and 10.5% with 50 and 100 mM NaCl treatments, respectively (Table 3).

**Table 3.** Plant height of cultivars under NaCl stress

	Plant Height (cm)			
	50 mM NaCl	100 mM NaCl	Mean	SE
DYR	29.7	24.0	15.5	23.1b
AR	33.6	28.3	21.4	27.8a
SR	26.1	27.1	18.2	23.8ab
YA	32.2	28.4	23.1	27.9a
HSR	31.6	27.8	20.0	26.4ab
ÇKR	31.8	29.8	21.0	27.5ab
AY	31.2	29.8	23.1	28.2a
Mean	30.9a	27.9b	20.4c	
TUKEY (G)	4.59**			
TUKEY (S)	2.38**			
TUKEY (G x S)	9.70			

The most affected cultivar by salt stress was DYR (36.7%) and the least affected one was ÇKR (11.5%) in the study. The

reduction rates in PH depending on NaCl treatments were given in Figure 2.



**Figure 2.** The reduction in plant height of cultivars depending on NaCl treatments

### Number of Branches

The variation among genotypes and increasing NaCl concentrations had a statistically significant (0.01) effect on the NB. The genotypes x NaCl interaction did not significantly influence the NB (Table 1). According to results, the most value of

the NB was obtained by HSR (23.3) in control while the least one determined with DYR (9.1) in 100 mM NaCl treatment. The mean NB of genotypes reduced by 18.3% and 36.7% with 50 mM and 100 mM NaCl applications, respectively (Table 4).

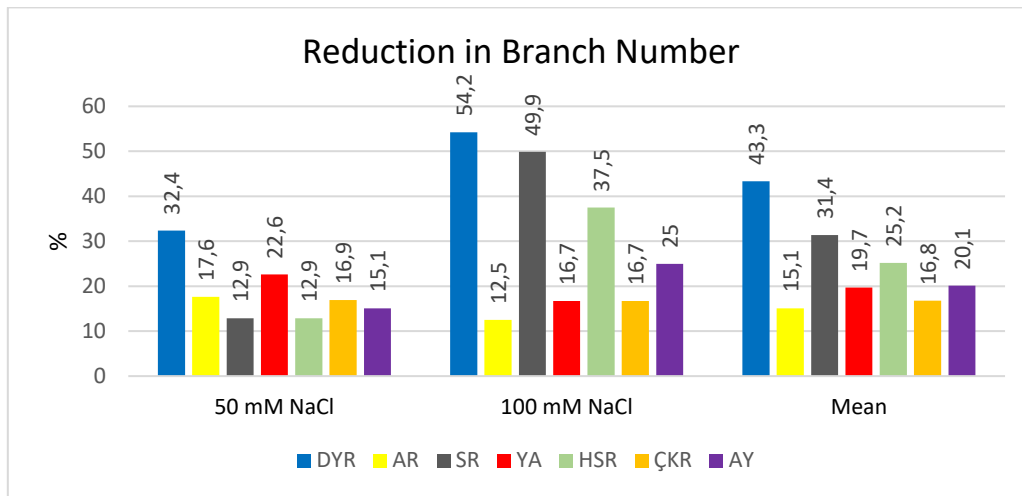
**Table 4.** Branch number of cultivars under NaCl stress

	Number of Branches (plant)			
DYR	18.5	12.5	9.1	13.3c
AR	21.0	17.3	15.2	17.9b
SR	16.3	14.2	11.2	13.9c
YA	19.0	14.7	12.0	15.2c
HSR	23.3	20.3	16.5	20.1a
ÇKR	17.2	14.3	10.2	13.9c
AY	18.5b	15.7	10.5	14.9c
Mean	19.1a	15.6b	12.1c	
TUKEY (G)	2.02**			
TUKEY (S)	1.05**			
TUKEY (G x S)	4.27			

The most and least affected genotypes by salt applications compared with control were DYR (43.3%) and AR (15.1%),

respectively. The reduction in the NB of cultivars depending on NaCl treatments was given in Figure 3.





**Figure 3.** The reduction in the number of branches of cultivars depending on NaCl treatments

### Stem Diameter

The results of the analysis of variance indicate that the SD was significantly affected by cultivars (0.05) and salt doses (0.01). The interaction of factors did not have any significant impact on the SD. As the mean of genotypes, although the YA

and HSR had the thickest (1.69 mm) SD while AR had the thinnest (1.52 mm) one. In the non-salt-applied groups, the highest SD value (1.84 mm) was determined in the AY while the lowest one (1.62 mm) was obtained by SR (Table 5).

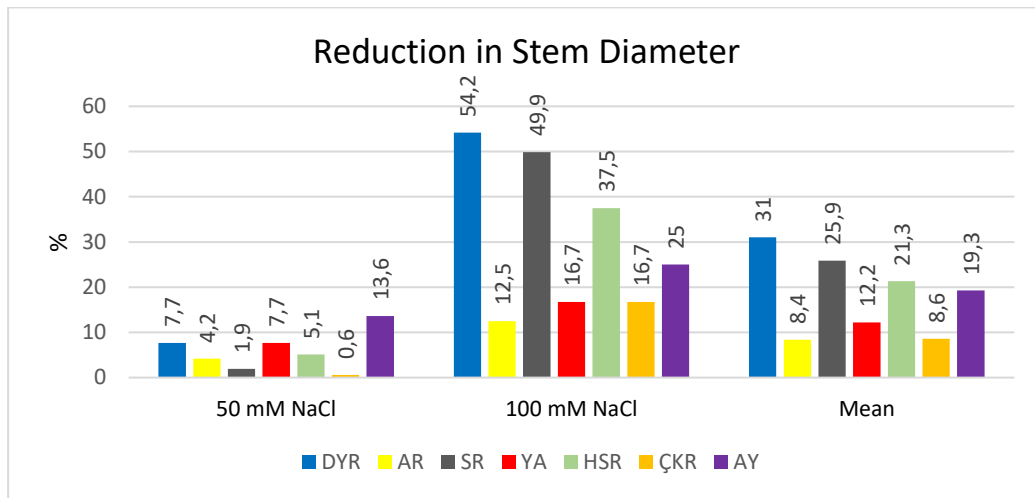
**Table 5.** Stem diameter of cultivars under NaCl stress

	Stem diameter (mm)			
	1	2	3	4
DYR	1.68	1.55	1.47	1.58ab
AR	1.65	1.58	1.33	1.52b
SR	1.62	1.59	1.41	1.54b
YA	1.82	1.68	1.58	1.69a
HSR	1.78	1.69	1.60	1.69a
ÇKR	1.69	1.68	1.60	1.65ab
AY	1.84	1.59	1.46	1.63ab
Mean	1.72a	1.62b	1.49c	
TUKEY (G)	1.89*			
TUKEY (S)	0.97**			
TUKEY (G x S)	0.40			

The results pointed out that the most affected genotype by NaCl treatment was DYR (31%) and the least affected cultivar was AR (8.4%). The SD of genotypes decreased by 5.8% and 13.4% with 50 mM

and 100 mM NaCl applications, respectively (Table 2). The reduction rates in the SD of cultivars depending on NaCl treatments were given in Figure 4.





**Figure 4.** The reduction in stem diameter of cultivars depending on NaCl treatments

### **Fresh Weight of Plants**

The genotypes and NaCl doses had a statistically significant (0.01) effect on the FW. Moreover, the interaction between cultivars and NaCl doses also influenced (0.05) the FW of salt applied plants (Table

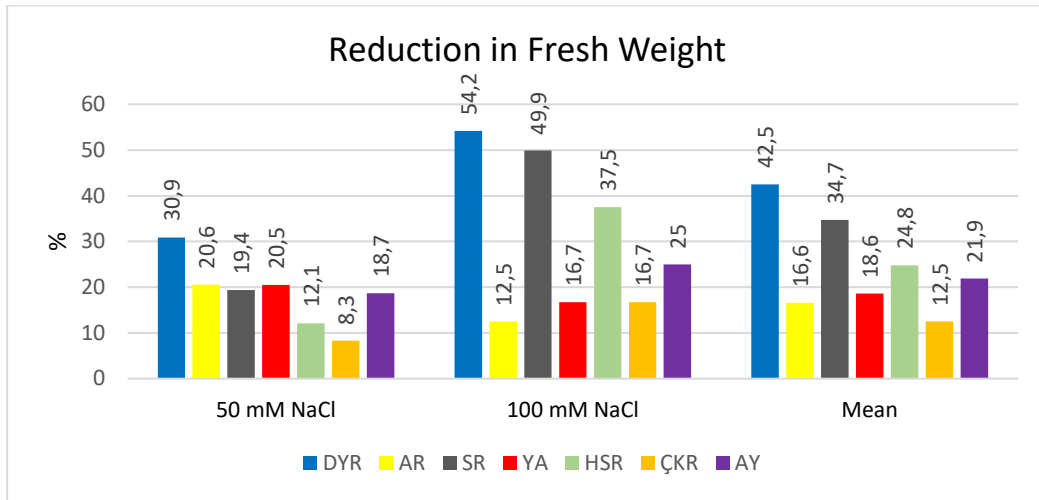
1). The HSR without NaCl application exhibited the highest performance (2.98 g) in terms of FW and the lightest FW (0.87 g) was measured by the SR and AY with 100 NaCl application (Table 6).

**Table 6.** Fresh weight of cultivars under NaCl stress

	Fresh Weight (g)			
DYR	2.46ac	1.70de	0.90f	1.68cd
AR	2.47ac	1.96be	1.74de	2.06b
SR	1.96be	1.58ef	0.87f	1.47d
YA	2.54ac	2.02be	1.46ef	2.00bc
HSR	2.98a	2.62ab	2.03be	2.54a
ÇKR	2.06be	1.89ce	1.42ef	1.79bd
AY	2.35ad	1.91be	0.87f	1.72bd
Mean	2.40a	1.95b	1.33c	
TUKEY (G)	0.34**			
TUKEY (s)	0.18**			
TUKEY (Gxs)	0.72*			

The most affected cultivar was DYR (42.5%) and the least affected one was ÇKR (12.5%) depending on NaCl applications

(Table 2). The reduction rates in the fresh weight of cultivars depending on NaCl treatments were given in Figure 5.



**Figure 5.** The reduction in plant fresh weight of cultivars depending on NaCl treatments

## DISCUSSION

Analysis of variance pointed out that the used cultivars, salinity levels and their interaction for germination, plant height, number of branches, stem diameter and fresh weight were found to be significant at 0.01 or 0.05 level (Table 1). All tested traits were adversely affected by salinity, especially with 100 mM NaCl treatment, in all cultivars whose tolerance potential exhibited differences. The high point in terms of NaCl doses was that the reduction rate of tested traits was sharp at 100 mM NaCl while change depending on cultivars. Flowers et al. (2010) stated that chickpea is a plant that is noteworthy affected by salinity, in which the most tolerant genotypes can't survive at 100 mM solution for a long time while the susceptible

genotypes die in just 25 mM solution in a hydroponic medium. Dadaşođlu et al. (2020) also indicated that the 100 mM NaCl concentration is a critical threshold for germination in chickpea. Besides, it was determined that the growth rate is higher in tolerant genotypes compared with susceptible ones under salinity stress (Kafi et al., 2011).

Salinity adversely affected both the germination percentage rapidly reduced with NaCl treatments. Moreover, the emergence time delayed with increasing salt treatments although it was not observed in the present study. Özaktañ et al. (2018) indicated that the emergence time and seedling growth are negatively affected by salinity stress and the emergence is impossible at 16 dS m<sup>-1</sup>. It is thought that the

germination process is influenced by various factors, including the toxic effect of salts, reducing water potential and preventing water uptake by seeds (Shanko et al., 2017). It was revealed that Kabuli cultivars have a higher tolerance threshold compared with Desi cultivars to salt stress (Singla and Garg, 2005; Sohrabi et al., 2008). Kaya et al. (2008) determined a relationship between germination and seed size that small seeds can germinate in a shorter time than greater seeds in high saline solution. High concentration salts in medium causes reduce protein synthesis (Colorado et al., 1995) and inhibit the germination process reducing the expression of a gene, calmodulin (Nicolas et al., 1998). Farooq et al. (2017) stated that salinity caused a more than 50% reduction in the germination of chickpea.

The results showed that increasing NaCl treatments caused vital impacts on not only germination but also agronomic traits. The primary disappointment of plant growth is thought to be caused by increases in NaCl toxicity, osmotic potential and inhibitory of water uptake. Also, different researchers stated that salinity drastically influences biological nitrogen fixation (Basu and Kumar, 2020), photosynthesis (Çiçek et al., 2018), Carbon metabolism

(Che-Othman et al., 2019), nutrient uptake (Vishnu et al., 2017) and production of reactive oxygen species (Kumari et al., 2017). Due to such physiological changes, agronomic traits, grain yield and yield components are negatively affected by salt stress. Welfare et al. (2002) stated that high salinity has negative effects on plant height, leaf size, stem diameter and shoot weight. Manchanda and Sharma (1989) reported that tolerance of chloride salinity exhibits differences depending on genotypes and increasing salt stress rigorously affected the agronomic traits in chickpea. Hossain et al. (2015) stated that salinity caused retardation in agronomic traits and germination, however salicylic and gibberellic acid treatments supported plant growth. Khan et al. (2015) reported that salinity stress caused reduce at leaf mass (52-65%), seed dry mass (60%), number of pods per plant (55%) and number of seeds per plant (44%).

## CONCLUSION

In conclusion, the harmful effects of salinity levels caused substantial damage to investigated agronomic traits in chickpea. However, tolerance to salinity stress among cultivars exhibited a large variation. This is an indicator of the importance that the salinity level can be mortal for some

cultivars while it is just detrimental for the others. So, breeding salt-tolerant genotypes can help to reduce the harmful effects of salinity in chickpea agriculture. It is estimated that some seedling traits like used in the study, can be an indicator and help to give foresight during the breeding process.

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