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Araştırma Makalesi / Research Article

Effects of Salt Stress on Seed Germination of Chickpea (*Cicer arietinum* L.) and Pea (*Pisum sativum* L.)

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ABSTRACT: Salinity is one of the most severe environmental factors limiting the productivity of agricultural crops. This study was conducted to determine the effect of salt stress on seed germination in chickpea (*Cicer arietinum* L.) and pea (*Pisum sativum* L.). For this purpose, 4 different pea (Utrillo, Serge, Jof and Bolero) and 4 different chickpea (Kanada, İnci, Azkan and Çağatay) cultivars were used. Four different salt concentrations (50, 100, 150 and 200 mM NaCl) were applied to determine and the effects on seed germination (germination percentage, germination speed, mean daily germination, peak value and germination value) were investigated. In the study, there was a significant decrease in germination parameters in both species with increasing salt level. Seed germination of pea cultivars did not occur on 150 (except Jof) and 200 mM salt stress, while chickpea cultivars didn't germinate on 200 mM (except Çağatay) salt stress. In general, it can be said the critical limit for germination in both species to be 100 mM. Jof cultivar have the highest (46%) germination percentage in 100 mM. In chickpea, the maximum germination percentage at 100 mM (63%) and 150 mM (20%) NaCl was determined in Çağatay. Our findings support that there are differences in tolerance to salt stress between different species or varieties of the same species.

Keywords: Salt stress, Germination, Chickpea, Pea

Tuz Stresinin Nohut (*Cicer arietinum* L.) ve Bezelyede (*Pisum sativum* L.) Tohum Çimlenmesi Üzerine Etkileri

ÖZ: Tuz stresi tarımsal ürünlerde üretimi sınırlandıran en şiddetli çevresel faktörlerden biridir. Bu çalışma, nohut (*Cicer arietinum* L.) ve bezelyede (*Pisum sativum* L.) tohum çimlenmesi üzerine tuz stresinin etkisini belirlemek amacıyla yapılmıştır. Çalışmada 4 farklı bezelye (Utrillo, Serge, Jof ve Bolero) ve 4 farklı nohut (Kanada, İnci, Azkan ve Çağatay) çeşidi kullanılmıştır. Çeşitlerin tuzluluğa toleranslarını belirlemek amacıyla kontrole göre 4 farklı tuz konsantrasyonu (50, 100, 150 ve 200 mM NaCl) kullanılmış ve tohum çimlenmesi üzerine olan etkileri (çimlenme oranı, çimlenme hızı, ortalama çimlenme zamanı, ortalama günlük çimlenme, pik değeri ve çimlenme değeri) incelenmiştir. Çalışmada artan tuz seviyesi ile birlikte her iki türde de çimlenme özelliklerinde önemli düşüşler görülmüştür. Bezelye çeşitlerinde 150 mM (Jof hariç) ve 200 mM tuz stresinde çimlenme olmazken, nohut çeşitlerinde 200 mM (Çağatay hariç) tuz stresinde çimlenme meydana gelmemiştir. Genel olarak değerlendirildiğinde, her iki tür içinde çimlenme için kritik sınır tuz seviyesinin 100 mM olduğu söylenebilir. Bezelyeye ait çeşitlerden Jof 100 mM'da en yüksek (%46) çimlenme oranı gösteren çeşit olmuştur. Nohutta ise Çağatay çeşidinde 100 mM (%63) ve 150 mM konsantrasyonunda en yüksek (%20) çimlenme oranı belirlenmiştir. Bulgularımız farklı türler veya aynı türe ait çeşitler arasında tuz stresine tolerans bakımından farklılıklar olduğunu desteklemektedir.

Anahtar Kelimeler: Tuz stresi, Çimlenme, Nohut, Bezelye

INTRODUCTION

Seed germination is the first critical and most sensitive stage in the life cycle of plants. Therefore, a successful germination positively affects plant growth and developmental stages. Salinity which is one of the most important environmental stress factors for crop production causes serious damages on germination and seedling development, and significantly reduces crop yield (Al-Karaki, 2001; Ghoulam and Fores 2001; Cavusoglu and Kabar, 2008; Dadasoglu and Ekinci, 2013).

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All of the leguminous plants contain annual or perennial 12.000 species, which are grown in all climatic conditions of the world except the Polar Regions and only their 200 have been cultivated. All of the edible legumes cultivated are annual. Bean (*Phaseolus vulgaris* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik., *Lens esculenta* Moench.), broad bean (*Vicia faba* L.), cowpea (*Vigna sinensis* L.) and pea (*Pisum sativum* L) are the cultivated legumes species as pulse in these species (Akcin, 1988). Legumes are very important in human and animal nutrition due to their protein content (Sehirali, 1988). In field crops, pulses are the most cultivated group after cereals in terms of sowing area and production (Sepetoglu, 2002). In the world, 66.8 million hectares of pulses are planted, and 61.2 million tons are produced. Pulses are grown at 735 thousand hectares in Turkey and the production is about 1 million tons. The most cultivated edible legumes are chickpeas, lentils, beans, broad beans and peas, while the most produced are chickpeas, lentils, beans, broad beans, peas and black beans (Anonymous, 2014).

Grain legumes are highly susceptible to salinity stress, which significantly reduces crop yields in cultivated plants (Farooq et al., 2017). Salinity is one of the important problems that reduce plant diversity and agricultural productivity in Turkey as well as in the world. Especially in arid and semi-arid climate regions, inadequate rainfall and high evaporation, insufficient drainage, wrong agricultural practices and soil properties are the leading causes of salinity and influence large areas (Anonymous, 2005). Salt stress depresses germination by inhibiting water uptake or by the effect of toxic ions such as Na^+ on embryo (Okcu et al., 2005; Farooq et al., 2015; Farooq et al., 2017). Effects of salt stress on plants depends on the plant species, the type and amount of salt applied and the duration of exposure. In saline environments, plants respond very differently depending on genotypic differences (Benidire et al., 2017; Dajic, 2006). Since most crops do not grow at high salt concentrations, salinity is considered a threat to food supply (Flowers, 2004; Kumari and Mesfin, 2015; Benidire et al., 2017). In the future, the problem of salinity is likely to be more severe due to the lack of appropriate management practices and an increasing trend towards large-scale irrigation agriculture (Hussein et al., 2017). Therefore, in order to increase food production, it seems necessary to determine the potential of plants to tolerate salinity (Athar and Ashraf, 2009).

This study was conducted to investigate the effect of salt stress (NaCl) on germination of pea and chickpea seeds. Thus, by determining the effect of salinity in the first and most important stage of plant development, cultivars that can be cultivated in problematic soils can be revealed and continuity in crop production will be ensured.

MATERIAL AND METHOD

Chickpea (Kanada, İnci, Azkan and Çağatay) and pea (Serge, Utrillo, Jof and Bolero) seeds were obtained from Bahri Dağdaş International Agricultural Research Institute (Konya, Turkey) and Department of Field Crops, Faculty of Agriculture, Uludağ University (Turkey). The study was carried out in a germination chamber under controlled conditions (25 °C±2). In the study, 4 different doses of NaCl (50, 100, 150 and 200 mM) were used. The seeds were sterilized with 5% sodium hypochlorite. The seeds were germinated in sterilized disposable petri dishes (90 mm diameter, 15 mm height) containing two Whatman No. 2 filter papers soaked with 10 ml of distilled water or 50, 100, 150 and 200 mM NaCl. Germination experiment was established with 5 different doses (0, 50, 100, 150 and 200 mM) salt application, 8 cultivars (4 peas, 4 chickpeas), 4 replications and 50 seeds in each repetition, and counts were made according to ISTA (1996) rules. Parameters such as germination percentage (GP), germination speed (GS), mean daily germination value (MDG), peak value (PV) and germination value (GV) were examined (Czabator, 1962; Ellis and Roberts, 1981; Gairola et al. (2011).

Germination percentage (GP): Germination percentage calculated by the following formula

$GP = n/\Sigma n x100$

Where, n = Number of germinated seeds

Σ n= Total number of seeds

Germination speed (GS): It is the rate of germination in terms of the total number of seeds that germinate in a time interval. Germination speed calculated by the following formula

$GS= n1/t1+ n2/t2 +……$

Where, n1, n2,… are the number of germinated seeds at times t1, t2, ….. (in days)

Mean daily germination (MDG): Mean daily germination calculated by the following formula

MDG = Total number of germinated seeds/ Total number of days

Peak Value (PV): Peak value was calculated by the following formula

PV = Highest seed germinated/ Number of days.

Germination Value (GV): Germination value was calculated by the following formula

GV = MDG x PV

Counts were made daily and above measurements were made on germination seeds. The data obtained from the study was averaged and Duncan multiple comparison test was used for statistical analysis. Statistical analysis of germination percentage was performed after Arc-sin transformation of the values.

RESULTS AND DISCUSSION

In this research, the effect of salt stress on germination properties of certain chickpea and pea cultivars was investigated. The effects of salt stress on the seed germination parameters were statistically significant in all cultivars of pea and chickpea (P <0.001).

In all the pea cultivars, it was observed that GP decreased as salt concentration increased, In terms of GP, the highest values were obtained from Jof and Serge cultivars at 0, 50 and 100 NaCl. In the control group (0 mM NaCl), 97% germination of both cultivars was obtained. GP values decreased to 46% (Jof), 30% (Serge), 12% (Bolero) and 10% (Utrillo) at 100 mM NaCl. In 150 mM salt conditions, germination occurred only in Jof cultivar with 10% GP among the pea cultivars (Figure 1).

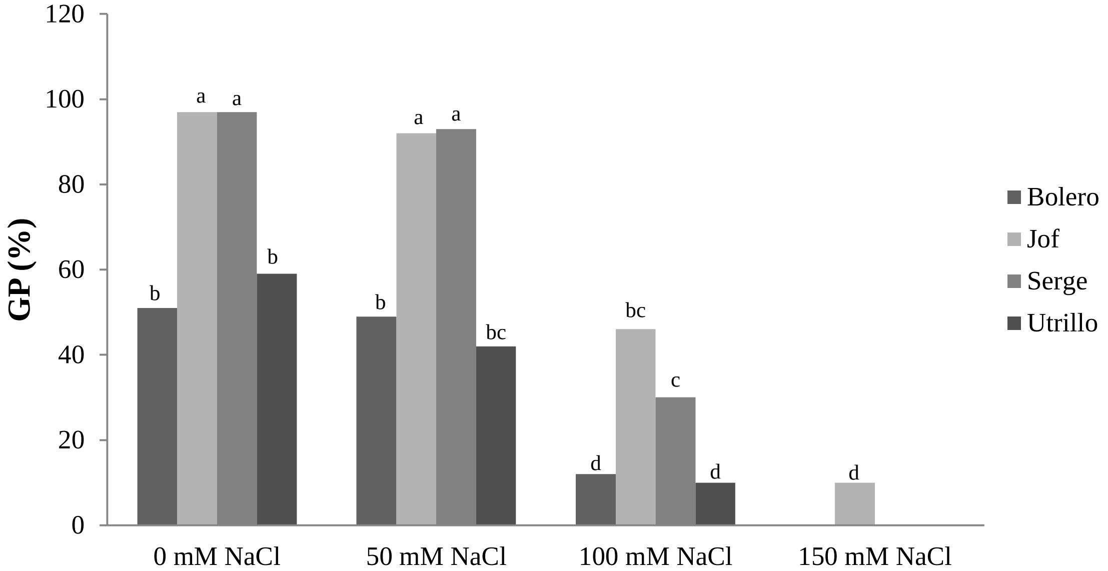

Figure 1. Effect of salt stress on GP in pea cultivars
There is no difference between the mean shown on bars in the same letter (P>0.001).

GS reduced with the increase in salt concentration in all pea cultivars. The highest GS value occurred in Serge cultivar at 0 (6.03) and 50 (4.75) mM NaCl while lowest (2.46 and 2.09) was in Bolero. Jof had the highest GS value at 100 (2.1) and 150 (0.44) mM NaCl (Figure 2).

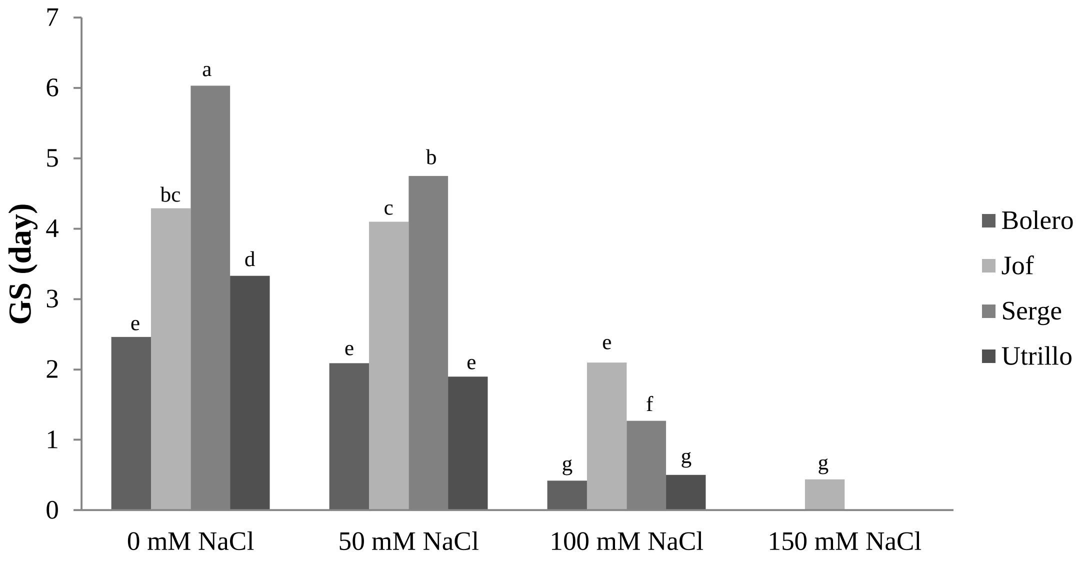

Figure 2. Effect of salt stress on GS in pea cultivars
There is no difference between the mean shown on bars in the same letter (P>0.001).

MDG of pea cultivars decreased with increasing salt concentration. Jof and Serge had higher MDG values than the other cultivars at 0 and 50 mM NaCl. The greatest value (1.44) in regard to MDG at 100 mM NaCl was obtained from the Jof cultivar. The lowest value (0.31) was obtained from Utrillo at the same dose. MDG value was 0.31 in Jof at 150 mM NaCl (Figure 3).

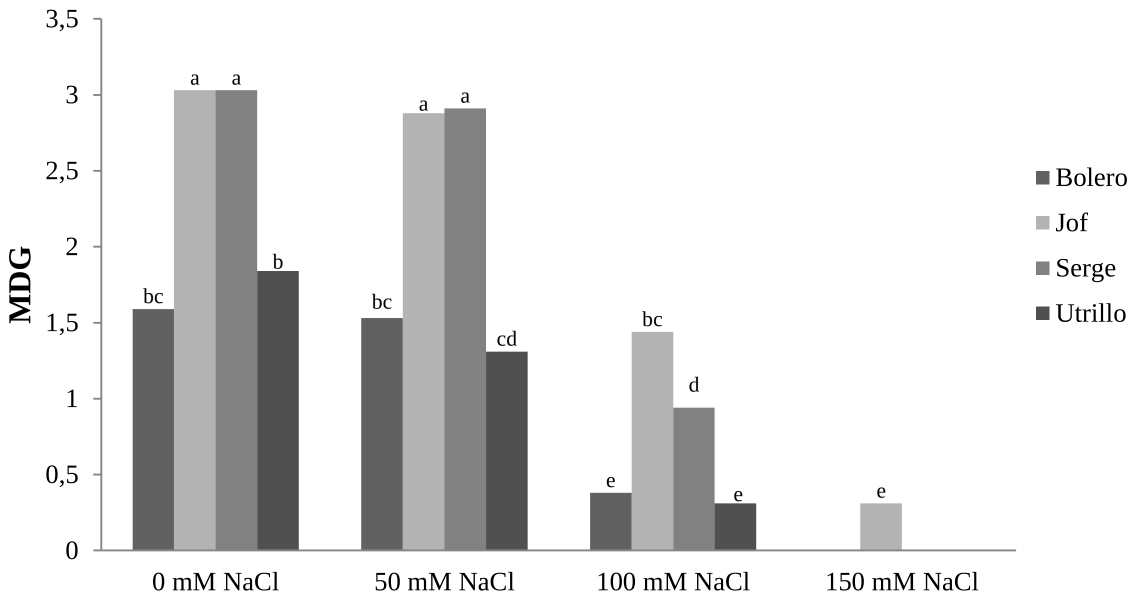

Figure 3. Effect of salt stress on MDG in pea cultivars
There is no difference between the mean shown on bars in the same letter (P>0.001).

In pea cultivars, PV was found to decrease with increasing salinity, the highest value of PV at 100 mM NaCl was 0.69 in Jof and the lowest was 0.13 in Utrillo. Jof and Serge cultivars had the highest PV values at 0 and 50 mM NaCl. At 150 mM NaCl only Jof cultivar had PV value (0.19) (Figure 4).

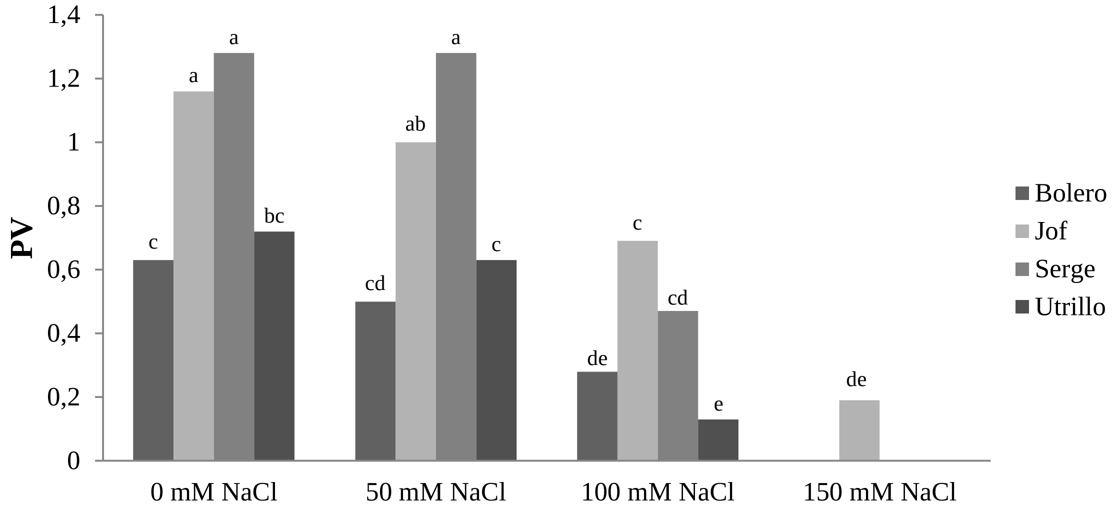

Figure 4. Effect of salt stress on PV in pea cultivars
There is no difference between the mean shown on bars in the same letter (P>0.001).

Salinity stress negatively affected GV. Serge had the greatest GV compared to other cultivars at 0 (3.9) and 50 (3.8) mM NaCl while GV was the highest in Jof at 100 (1.0) and 150 (0.08) mM (Figure 5).

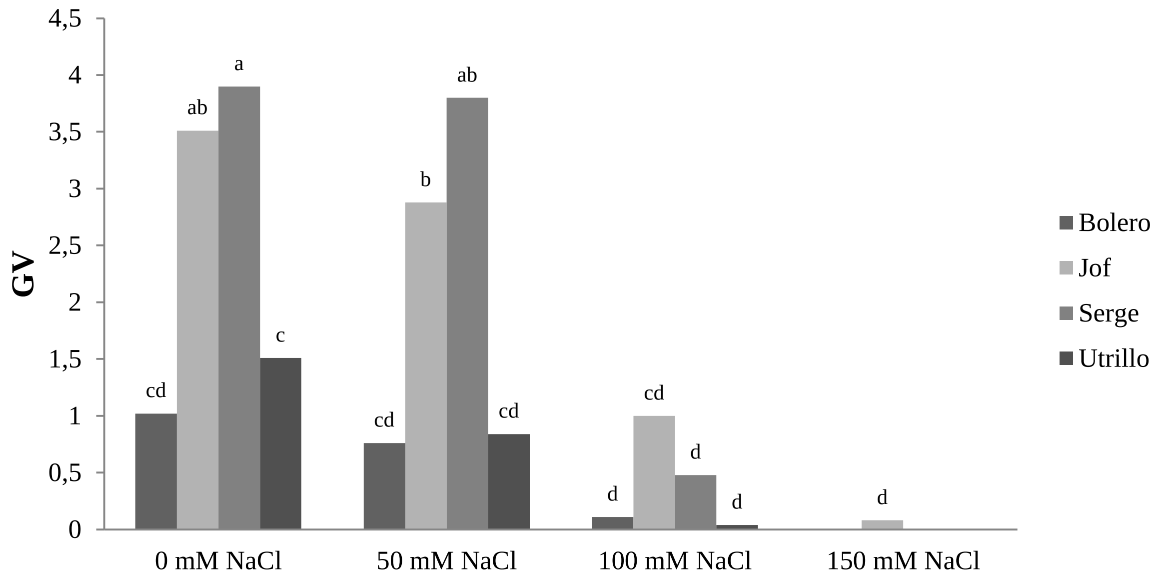

Figure 5. Effect of salt stress on GV in pea cultivars
There is no difference between the mean shown on bars in the same letter (P>0.001).

The effects of salt stress on seed germination parameters of chickpea were statistically (P <0.001) significant. GP, GS, MDG, PV and GV showed a decrease due to the increased salt level in all cultivars (Figure 6, Figure 7, Figure 8, Figure 9 and Figure 10).

Çağatay had the highest GP values at all NaCl concentrations. Çağatay was the least affected cultivar by salt stress in regard to GP, and 20% germination occurred at 150 mM salt level. Furthermore, Çağatay was the only cultivar germinating at 200 mM NaCl. İnci was the most affected cultivar from salt stress and showed the highest decrease in regard to GP (Figure 6).

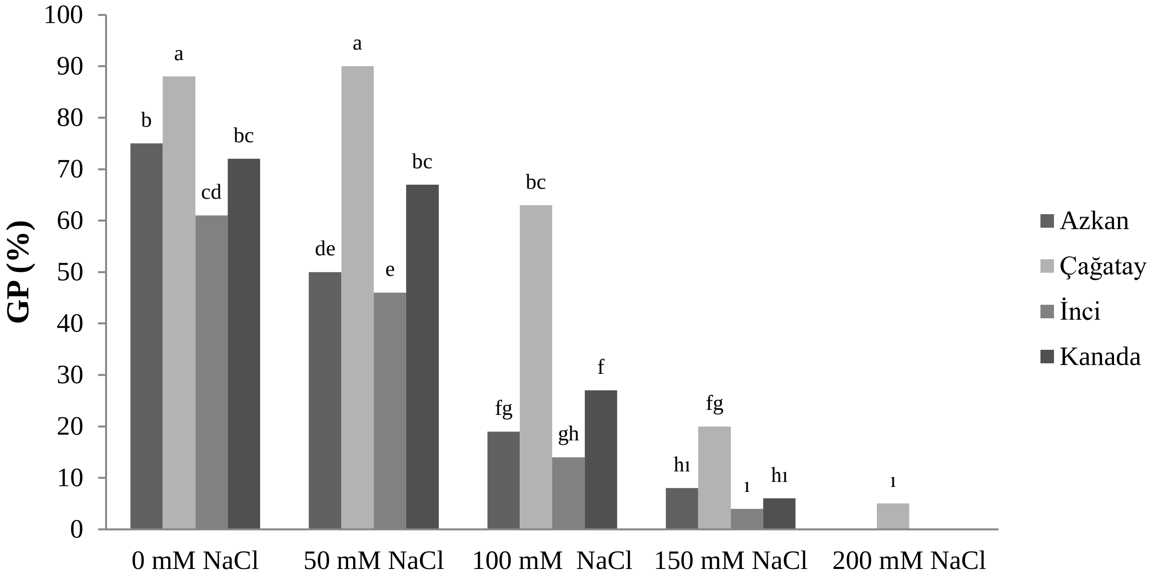

Figure 6. Effect of salt stress on GP in chickpea cultivars
There is no difference between the mean shown on bars in the same letter (P>0.001).

GS values decreased with increasing salinity concentrations. The highest GS values at 0, 50, 100, 150 and 200 mM NaCl occurred in Çağatay cultivar as 7.3, 6.17, 4.54, 1.54 and 0.26, respectively. GS value was the lowest in İnci (0.20) at 150 mM NaCl (Figure 7).

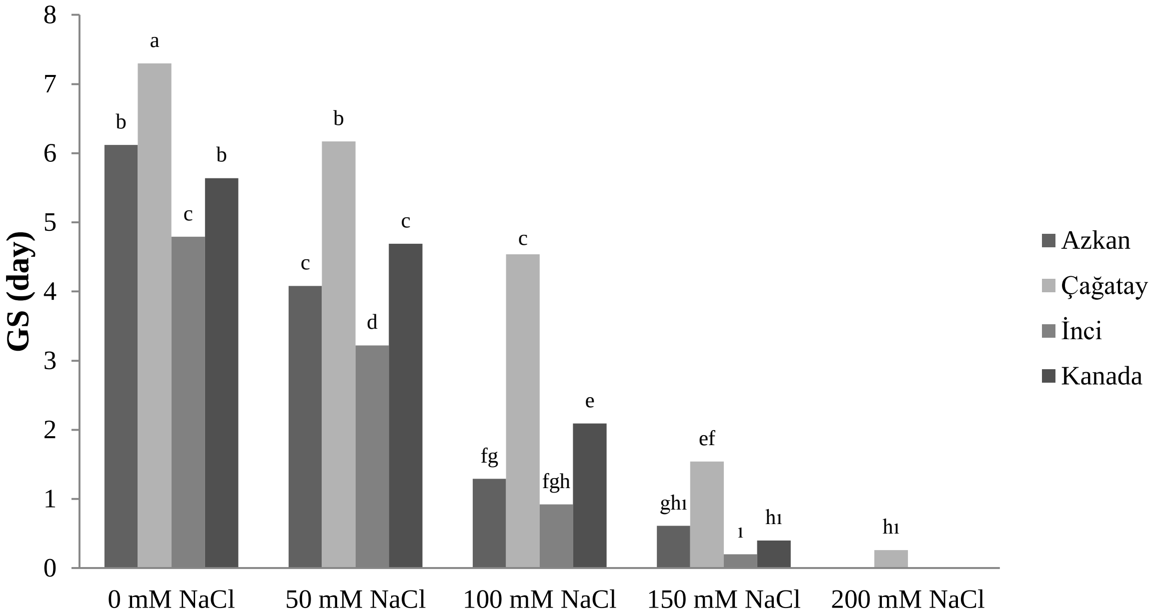

Figure 7. Effect of salt stress on GS in chickpea cultivars
There is no difference between the mean shown on bars in the same letter (P>0.001).

When the MDG values were examined, the highest value was obtained from Çağatay cultivar at 150 mM salt application (0.63) and lowest from İnci (0.13) cultivar. MDG value was 0.16 in Çağatay at 200 mM NaCl (Figure 8).

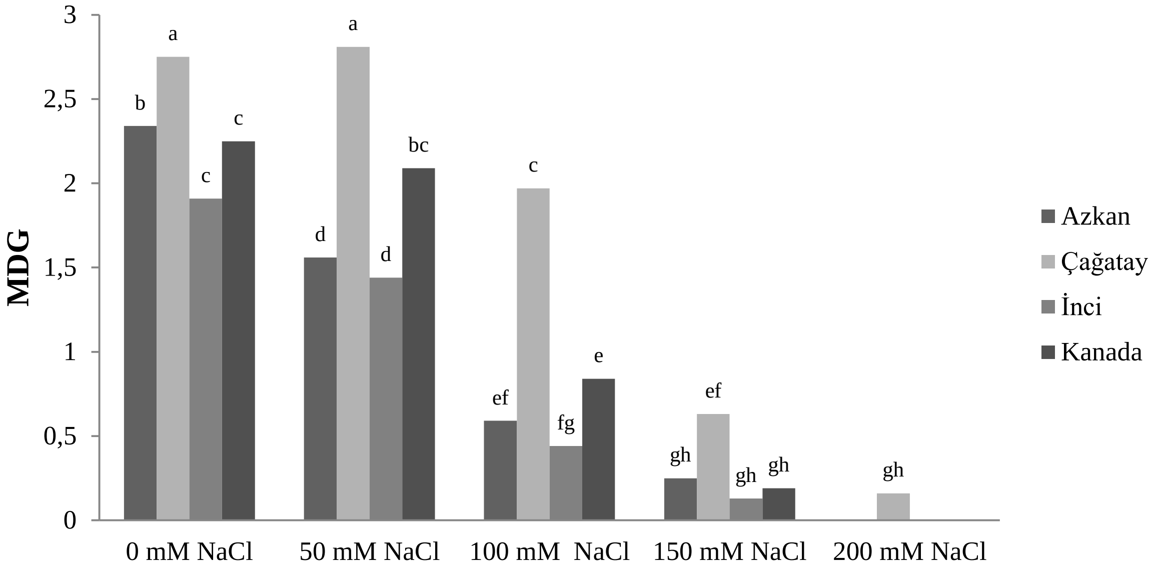

Figure 8. Effect of salt stress on MDG in chickpea cultivars
There is no difference between the mean shown on bars in the same letter (P>0.001).

The salinity conditions caused reductions on PV values of chickpea cultivars. In regard to PV, Çağatay had the highest PV values (2.38, 1.66, 1.22, 0.44 and 0.16) at 0, 50, 100, 150 and 200 mM NaCl. İnci had the lowest PV at 0 (1.38), and 50 (0.75) mM NaCl while the lowest PV were determined in Azkan and İnci 100 and 150 mM NaCl (Figure 9).

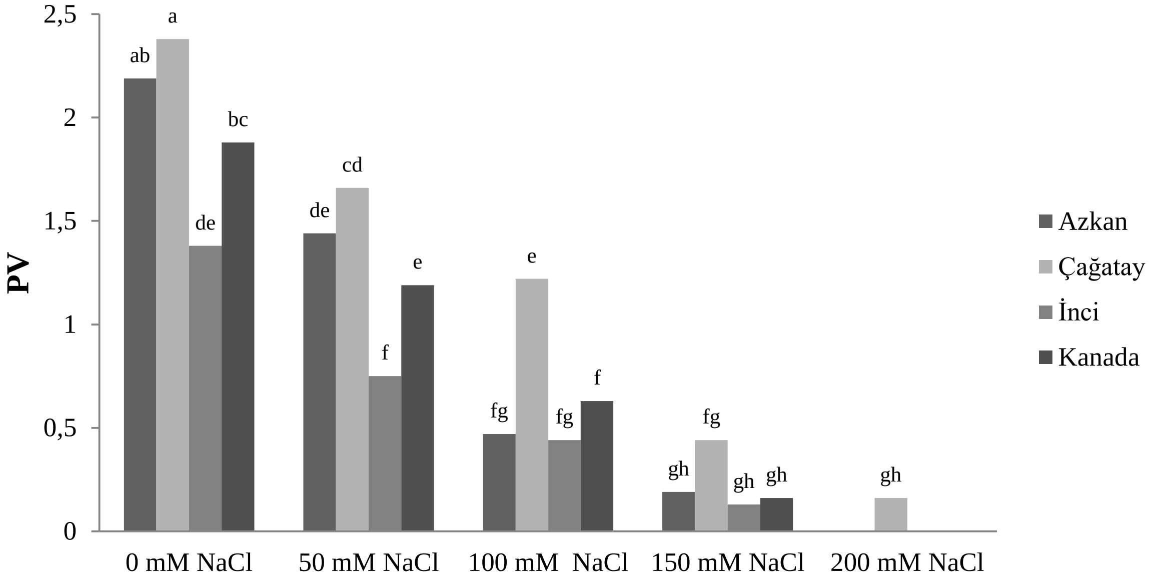

Figure 9. Effect of salt stress on PV in chickpea cultivars
There is no difference between the mean shown on bars in the same letter (P>0.001).

In chickpea, GV decreased with increasing salinity and GV was highest in Çağatay cultivar at 0 (6.61), 50 (4.68) and 100 (2.35) mM salt stress. However, 150 mM NaCl treatment did not statistically affect GV in chickpea cultivars (Figure 10).

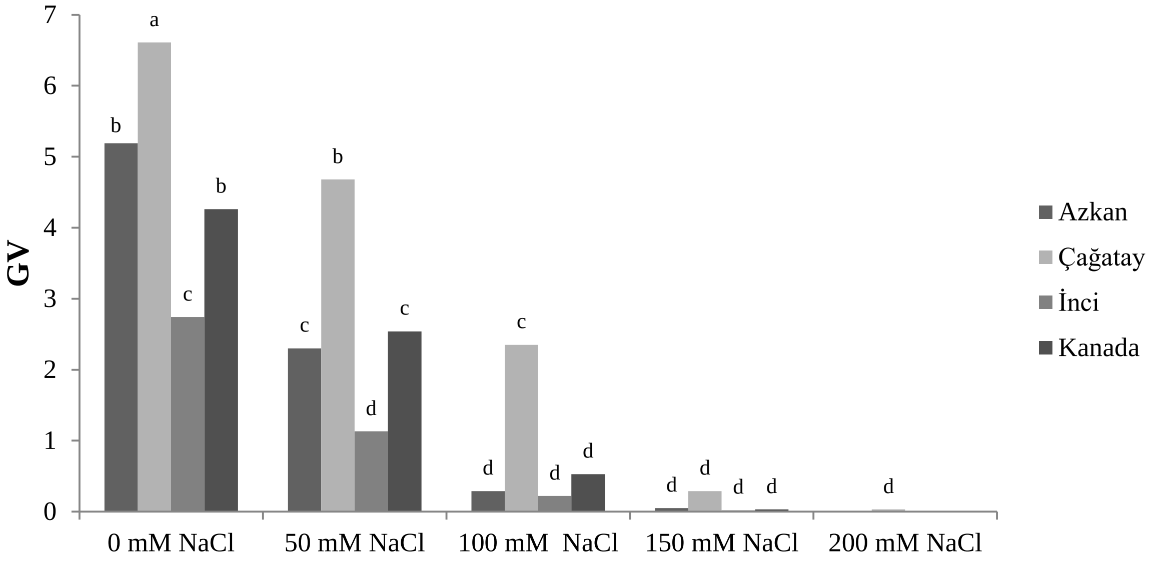

Figure 10. Effect of salt stress on GV in chickpea cultivars
There is no difference between the mean shown on bars in the same letter (P>0.001).

In the study, it was determined that the salt stress conditions had negative effects on germination of the pea and chickpea cultivars studied. Similarly, Petrović et al. (2016) stated that salinity had significant effects on seed germination of peas. Moreover, it was pointed out that increasing salinity levels caused decreases in germination parameters in faba bean (Anaya et al., 2018).

Seed germination and seedling development stage of plants is the most sensitive stage to salinity. Salt stress causes negative physiological and biochemical changes in germinated seeds. Salinity delays or prevents germination of seeds by various factors such as reducing water content, making changes in the mobilization of stored reserves and affecting the structural organization of proteins (Ibrahim, 2016). Salt stress has been known to reduce seed germination (more than 50%) by inhibiting water uptake and / or by the toxic effect of ions in the embryo in legumes (Farooq et al., 2017).

In this study, it was observed that salt stress negatively affected germination parameters in chickpea and pea. Cultivars' responses to salt stress varied more or less. Similarly, in a study conducted on rice, germination was 100% in the control group and decreased to 65% under 20 ds m^{-1} salt conditions, and Hybrid 312 was the highest germinating variety among the rice varieties (Vibhuti et al., 2015). Salinity significantly affects seed germination, seedling and plant growth. Damages in plant metabolism caused by salt stress cause plant growth and yield reductions (Dadasoglu and Ekinci, 2013).

When the results obtained from the study are examined, although the sensitivity of plants to salt stress varies, significant differences are observed in salt sensitivity even among the cultivars of the same species. Among the pea cultivars studied, Jof was the most tolerant cultivar to salt stress, and Çağatay cultivar was among chickpea cultivars during germination stage. Chickpea is a species that is sensitive to salinity, and its very sensitive genotypes can die even at 25 mM NaCl. Some genotypes have been shown to tolerate 320 mM NaCl during germination. In addition, the germination resistance of chickpea shows a wide variation in salt stress conditions and the reason of this is not known exactly (Flowers et al., 2010). In another study, salinity has been found to decrease the germination and seedling development of some pea varieties depending on the salt concentration, and salt tolerance of the cultivars varied (Noreen et al., 2007). There may be differences between the results obtained from the germination test under laboratory conditions and the results obtained from field conditions. In a study, it was stated that post-germination stage are more sensitive than germination stage in pea under salt and drought stress (Petrović et al., 2016). In addition, Noreen et al. (2007) showed that although significant variations in germination of pea varieties under salt stress conditions, an evaluation was not sufficient according to germination results in tolerance to salinity. Therefore, the results obtained from this study can be considered as a preliminary evaluation.

CONCLUSION

In this study, the germination responses of some chickpea and pea cultivars against salt stress during germination stage were investigated. In general, germination was not seen in pea cultivars after 150 mM and chickpea cultivars after 200 mM. There was a difference between cultivars in terms of salt tolerance during germination stage of both species. Based on the results obtained, it will be useful to determine the reactions to the salt stress especially during the plant development stage.

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