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Effects of Water Deficit Induced by PEG and NaCl on Chickpea (*Cicer arietinum* L.) Cultivars and Lines at Early Seedling Stages

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ABSTRACT

The effects of water deficit induced by different osmotic potential levels [0 (control), -0.4, -0.6 and -0.8 MPa] of PEG 6000 and NaCl treatments on chickpea (*Cicer arietinum* L.) cultivars and lines at germination and early growth stages by sampling on 4th and 8th days of incubation consisting of 4 days each of dark and subsequent 16 hours illumination. All of these treatments affected germination percentages of all genotypes but PEG was more effective in inhibition than NaCl at the MPa levels tested. The experimental studies showed that all of the genotypes tested could be classified as tolerant, moderately tolerant and sensitive ones. But the tolerance levels were not found to be correlated directly with MPa levels applied, as Canıtez and ILC-3279 were tolerant to PEG, but ILC-3279 was sensitive to NaCl treatment. Within this context the classification can be summarized as below: PEG tolerant (Canıtez and ILC-3279), moderately tolerant (AkN 87, FLIP 87-59C, Gökçe and Uzunlu), sensitive (AkN 290 and ER 99). NaCl tolerant (Uzunlu and FLIP 87-59C), moderately tolerant (Gökçe, Canıtez, AkN 290 and AkN 87) and sensitive (ER 99 and ILC-3279).

Key Words: *Drought, early seedling stage, germination, NaCl, PEG, salinity, water deficit*

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an ancient legume crop believed to be originated in South East Turkey, and the adjoining part of Syria [1, 2]. It is the fourth most important food legume with a total annual global production of 9.1 million M tones from 11.2 million ha [3]. Besides being an important source of human and animal food, chickpea also plays an important role in the maintenance of soil fertility, particularly in the dry, rainfed areas [4, 5]. One of the most important abiotic factors limiting plant germination and early seedling stages is water stress brought about by drought and salinity [6, 7], which are widespread problems around the world [8]. Salinity and drought affect the plants in a similar way [9]. Reduced water potential is a common consequence of both salinity and drought [10]. Water stress acts by decreasing the percentage and rate of germination [11] and seedling growth [8, 12, 13]. Since chickpea is grown mostly as a rainfed and post-rainy season crop, water stress during vegetative and/or reproductive growth stages is one of the most limiting factors for the chickpea growth [14].

Germination of seeds, one of the most critical phases of plant life, is greatly influenced by salinity [15]. Salinity is reported to decrease as well as delay germination of most of the crops. Chickpea is a salt-sensitive crop especially during germination [16]. Lower levels of salinity delayed germination whereas higher levels reduced the final percentage of seed germination [17] and vegetative plant growth is suppressed under saline conditions [18]. In addition, salinity imposes on plants other stresses such as ion toxicity, as a result of ion entry in excess of appropriate compartmentation, and nutrient imbalances, as commonly seen in the displacement of potassium by sodium. In fact, salinity damage is mainly due to altered water relations caused by high salt accumulation in the intercellular spaces [19].

Drought, like salinity, plays an important role not only in determining germination rates, but also influences seedling development [20]. Establishment of seedlings is the most critical life stage in dry environments and a lack of soil moisture is often a major reason for seedling mortality [21]. With increasing drought stress, water availability decreases, changing the percentage and velocity of germination and growth of seedlings adversely. Osmotic solutions are used to impose water stress reproducibly under in vitro conditions [7]. PEG

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widely used to induce water stress, is a non-ionic water polymer, which is not expected to penetrate into plant tissue rapidly [22]. PEG molecules with a Mr ≥ 6000 cannot penetrate the cell wall pores [23]. Because PEG does not enter the apoplast, water is withdrawn not only from the cell but also from the cell wall. Therefore, PEG solutions mimic dry soil more closely than solutions of low–Mr osmotica, which infiltrate the cell wall with solute [24].

The present investigation has been performed to evaluate chickpea (*Cicer arietinum* L.) tolerance to osmotic stress induced by polyethylene glycol (PEG) or NaCl during germination and the early seedling stages of plant development. Eight chickpea genotypes [four cultivars (Gökçe, Canıtez, ER 99 and Uzunlu) and four lines (AkN 87, AkN 290, ILC-3279 and FLIP 87-59C)] were tested.

This is the first work aimed at selecting drought and salinity tolerant chickpea genotypes in the germination and early seedling stages by using PEG or NaCl in in vitro conditions.

2. MATERIALS AND METHODS

The seeds of chickpea cultivars and lines were obtained from Ankara Central Research Institute for Field Crops for this research. The effects of drought and salt stresses induced by different osmotic potential levels [0 (control), -0.4, -0.6 and -0.8 MPa] of polyethylene glycol 6000 (PEG 6000) and NaCl treatments on germination and early seedling development of chickpea (*Cicer arietinum* L.) cultivars and lines were investigated for two sampling dates (4 and 8 days). The seeds of the cultivars (Canıtez, ER 99, Gökçe and Uzunlu) and the lines (AkN 87, AkN 290, FLIP 87-59C, ILC-3279) which were imbibed in deionized water were incubated under dark conditions for 4 days and subsequently 16 h photoperiod with 250 $\mu mol.m^{-2}s^{-1}$ light intensity for four days at 23±2 °C on humidified filter paper with an aliquot of solution of different osmotic potentials of PEG 6000 or NaCl. The wet filter papers were changed when germination of seeds was measured for the first sample day after sowing. The osmotic potentials of PEG 6000 were calculated as described by Michael and Kaufman [25] . At end of the each sampling dates, the percentage of germination (%) was determined and, the length of roots and epicotyls of genotypes were also measured (mm $seedling^{-1}$) for PEG and NaCl treatments. Determination of the tolerance of the genotypes used based on the inhibition percentage of root elongation as compared with the control groups for both of the treatments. Then genotypes were classified according to their tolerance index values.

The experiment was arranged in a completely randomized design with three replicates of eight seedlings per replications (n=24) in each sampling date. Differences among treatments and genotypes, as well as interaction between these variables, were tested using SPSS statistical programme, version 11.5. The arcsine transformation was used to stabilize variances. The data held after the transformation was acceptably normal with homogenous treatment variance. Statistical variance analysis of the data was performed using ANOVA and compared with least significant difference (LSD) at the 5% level.

3. RESULTS

3.1. Percentage of Germination

Water deficit induced by PEG and NaCl treatments affected percentage of germination of genotypes (Fig 1A and B). Maximum germination (100%) was obtained in Canıtez and AkN 87 in all levels of PEG and NaCl potentials after the first sampling date. Also, the percentages of germination of Gökçe and ER 99 were maximum in all NaCl treatments. Although it decreased only in Gökçe at -0.8 MPa PEG potential during the first incubation period, it was lower at -0.6 and -0.8 MPa PEG potentials in all samples of other cultivars (Uzunlu, ER 99) and lines (AkN 290, FLIP 87-59C, ILC-3279). However, increasing PEG potentials to -0.8 MPa caused approx. 40% reduction in FLIP 87-59C. On the other hand, percentage of germination of FLIP 87-59C and ILC-3279 lines descreased with the increasing osmotic potentials of MPa) of NaCl decreased the germination of Uzunlu. But this reduction was around 70% in that genotype at the end of the first sampling date.

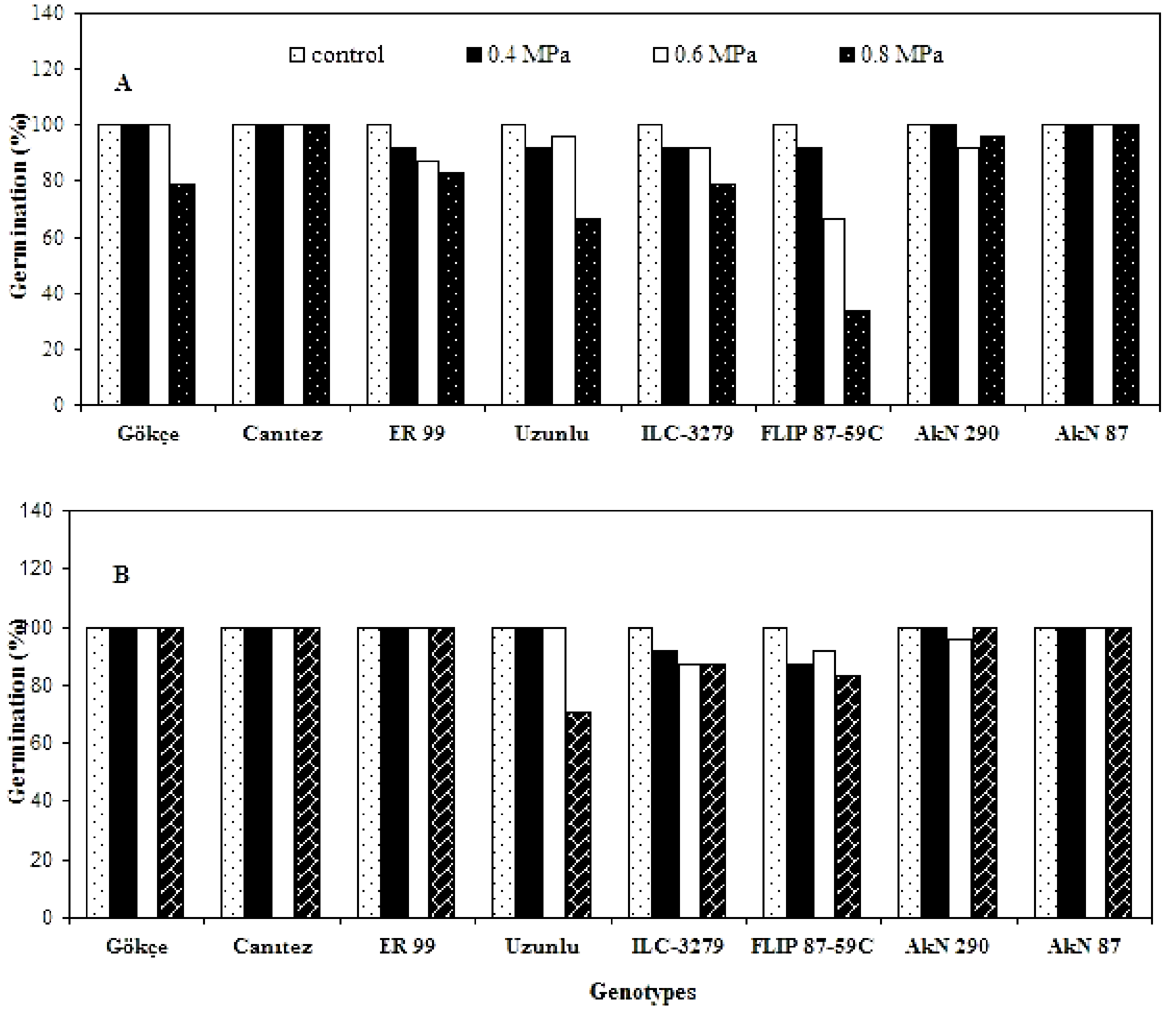

Figure1. Percentage of germination of chickpea seeds exposed to different osmotic potentials of PEG (A) and NaCl (B) at the first sampling date.

At the end of the 8th day of incubation, germination of genotypes increased slightly as compared with the 4th day of the incubation (Fig. 2A and 2B). Germination of some cultivars and lines inhibited in lower osmotic potentials of NaCl and PEG 6000 whereas delayed in higher osmotic potentials (-0.4 MPa) of NaCl and PEG 6000. Our results showed that PEG treatments had more inhibitive effects on germination of genotypes compared to NaCl treatments.

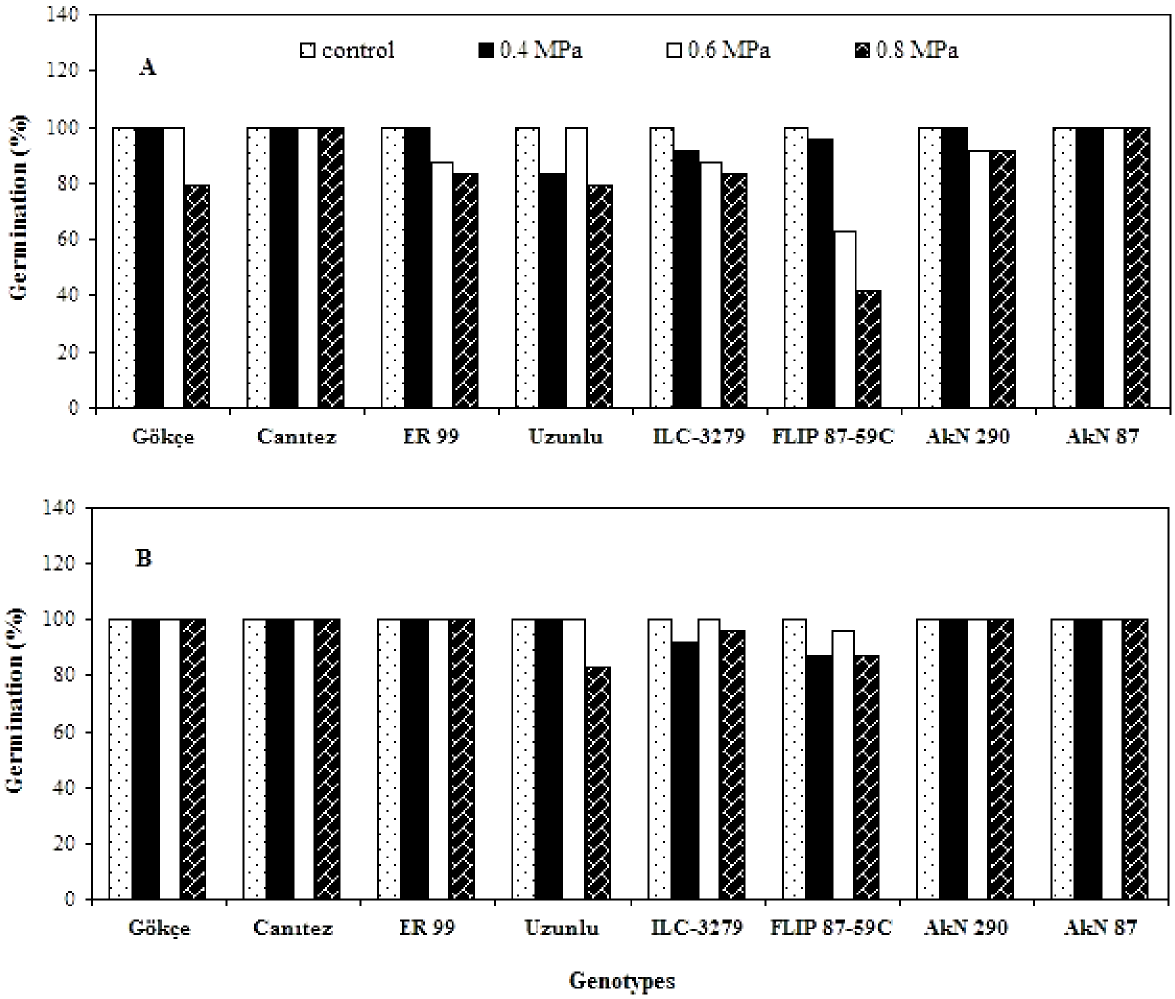

Figure 2. Percentage of germination of chickpea seeds exposed to different osmotic potentials of PEG (A) and NaCl (B) at the second sampling date.

3.2. Elongation of Roots and Epicotyls

The length of roots of all genotypes were found to be less than the controls at -0.4 MPa potential of both PEG and NaCl, except ILC-3279 and FLIP 87-59C for PEG treatment, and Uzunlu and FLIP 87-59C for NaCl treatment (Figures 3A and 3B). Also, root elongation of ER 99 showed the most significant decrease at -0.4 MPa osmotic potentials of both PEG and NaCl. Consequently, all treatments caused a decrease in root elongation in all genotypes according to their controls.

The length of epicotyls of all cultivars and lines decreased significantly with increasing PEG potentials (Figure 4A). There was no considerable epicotyl elongation in all genotypes at -0.8 MPa. The length of epicotyls of cultivars and lines except Gökçe, ER 99, ILC-3279 and AkN 290 was significantly increased by -0.4 MPa NaCl treatment compared to their controls (Figure 4B). But the epicotyl elongation of the line AkN 290 increased significantly at -0.6 MPa osmotic potential of NaCl. The lowest potential level of NaCl tested was found to be the most effective treatment for epicotyl elongation of all genotypes examined.

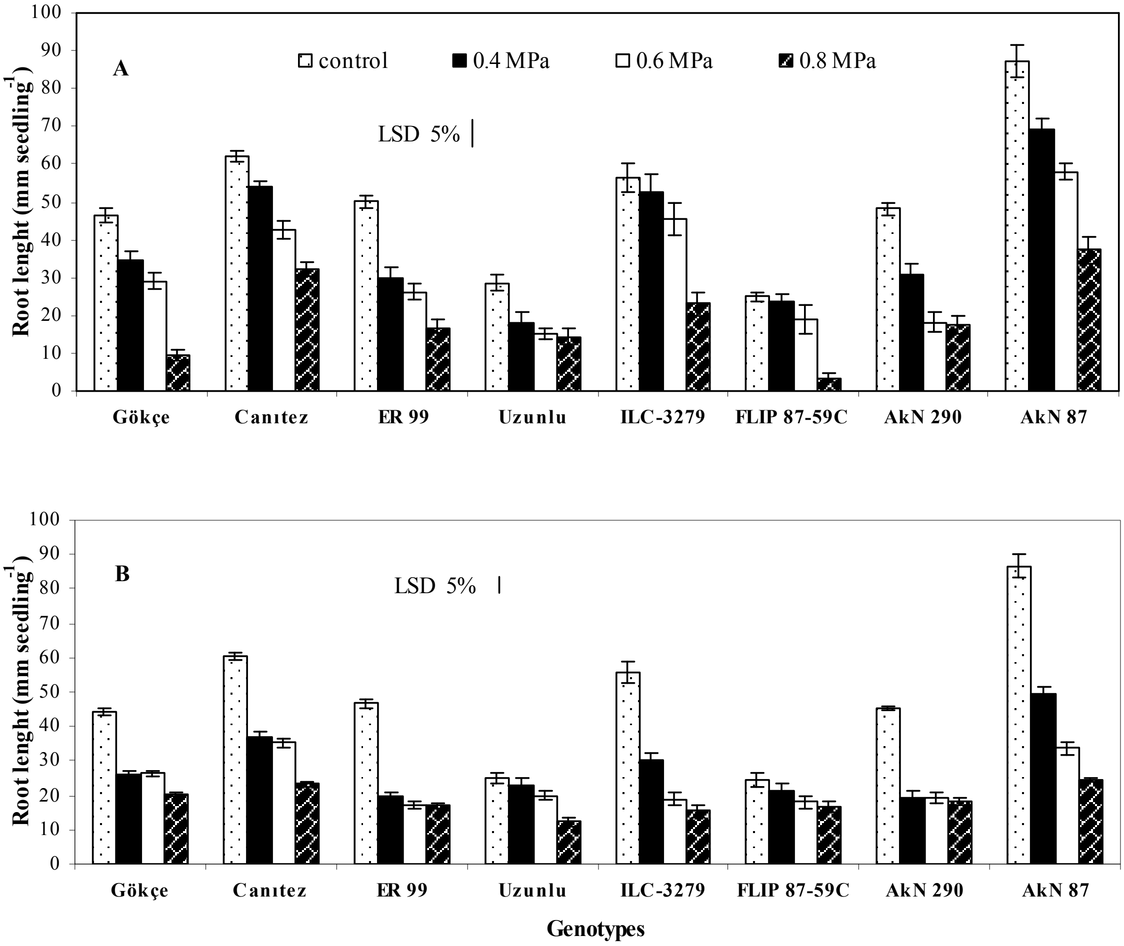

Figure 3. Length of root of chickpea seedlings exposed to different osmotic potential of PEG (A) and NaCl (B) at the second sampling date.

3.3. Total Tolerance Index Values of the Genotypes

Since there was no significant epicotyl elongation in different osmotic potentials of PEG treatments, reduction levels of root elongation of the genotypes were scored and evaluated, in order to determine the tolerance of all of the cultivars and lines to the treatments (Table 1). The tolerance index (%) was predetermined by taking the differences between the treated sample groups and their controls. The sample group showing the least reduction in root lenght was scored as 8, the one showing the highest reduction value was taken as 1 point in this index.

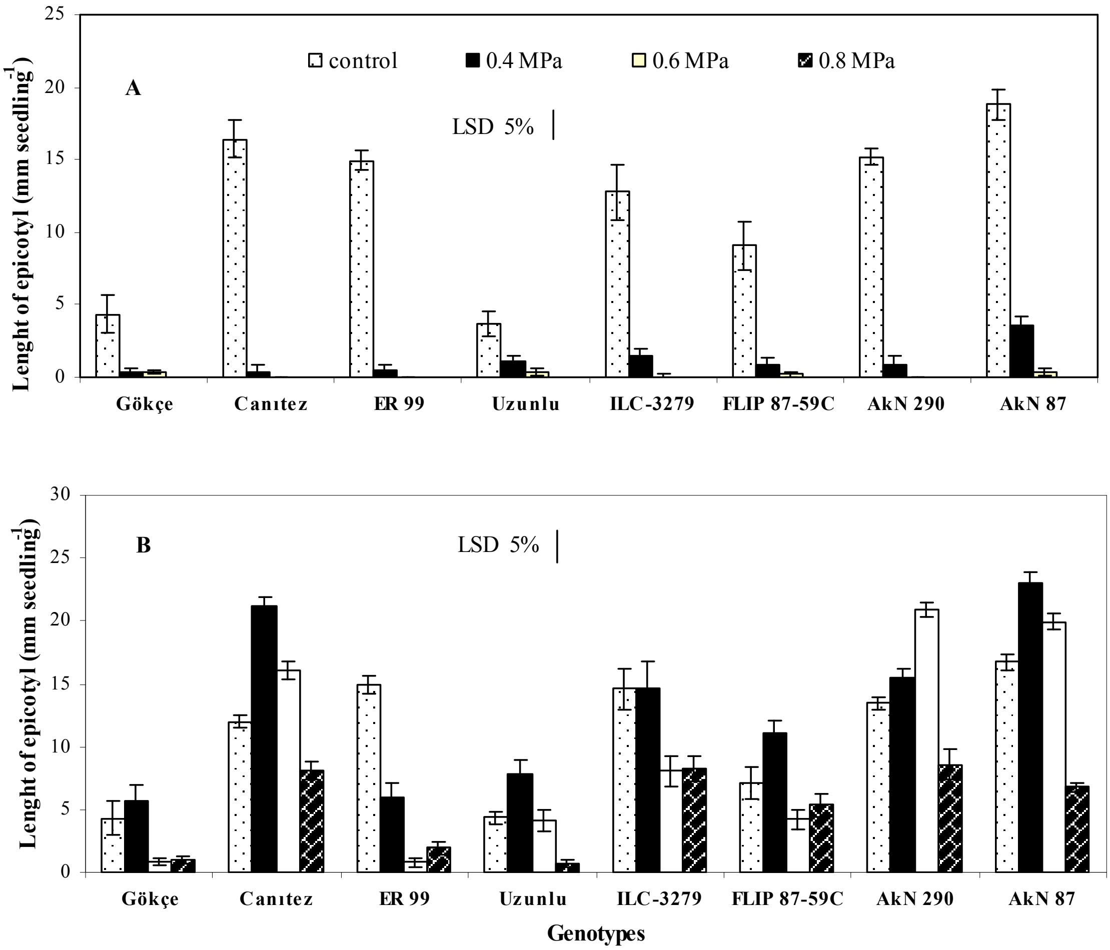

Figure 4. Length of epicotyl of chickpea seedlings exposed to different osmotic potentials of PEG (A) and NaCl (B) at the second sampling date.

The other genotypes are representing the intermediates in the index with their 2 to 7 score points depending on the damage they exposed. Then the genotypes were classified as tolerant, moderately tolerant and sensitive based on the sum of their scores. The same calculation procedure was applied for salt stress treatments to compare the effects of the drought treatments tested (Table 1).

To determine the drought intensities induced by different PEG potentials in early seedling stage, the genotypes were classified to 3 tolerance levels as tolerant (Canıtez and ILC-3279), moderately tolerant (AkN 87, FLIP 87-59C, Gökçe and Uzunlu) and sensitive (AkN 290 and ER 99). However, the classification of NaCl treated genotypes was found to be different from PEG treatment as tolerant (Uzunlu and FLİP 87-59C), moderately tolerant (Gökçe, Canıtez, AkN 290 and AkN87) and sensitive (ER 99 and ILC-3279).

Table 1. Total tolerance index (%) of chickpea cultivars and lines exposed to water deficit induced by PEG and NaCl.

Treatments	**Osmotic Potentials (MPa)**	**Genotypes**							
		Gökçe	**Canıtez**	**ER 99**	**Uzunlu**	**ILC-3279**	**FLIP-8759 C**	**AkN 290**	**AkN 87**
PEG	Control (0)	100 (8)*	100 (8)	100 (8)	100 (8)	100 (8)	100 (8)	100 (8)	100 (8)
	-0.4	74.62 (4)	87.13 (6)	60.06 (1)	62.79 (2)	93.50 (7)	94.54 (8)	63.59 (3)	79.34 (5)
	-0.6	62.50 (4)	68.55 (6)	52.42 (2)	53.29 (3)	80.39 (8)	75.76 (7)	37.44 (1)	66.62 (5)
	-0.8	20.08 (2)	51.53 (8)	32.79 (3)	50.53 (7)	40.78 (5)	13.80 (1)	36.41 (4)	42.76 (6)
Total score		18	28	14	20	28	24	16	24
NaCl	Control (0)	100 (8)	100 (8)	100 (8)	100 (8)	100 (8)	100 (8)	100 (8)	100 (8)
	-0.4	58.88 (5)	61.36 (6)	42.11 (1)	92.82 (8)	53.81 (3)	86.83 (7)	42.88 (2)	56.85 (4)
	-0.6	59.53 (6)	58.12 (5)	36.91 (2)	80.26 (8)	34.12 (1)	74.36 (7)	42.60 (4)	38.83 (3)
	-0.8	45.92 (6)	38.51 (4)	36.31 (3)	49.48 (7)	27.84 (1)	68.21 (8)	40.22 (5)	28.41 (2)
Total score		25	23	14	31	13	30	19	17

* total score of genotypes was given in the brackets

4. DISCUSSION

Water stress due to drought and salinity is probably the most significant abiotic factor limiting plant and also crop growth and development [26]. Salinity and drought stresses are physiologically related, because both induce osmotic stress and most of the metabolic responses of the affected plants are similar to some extent [13, 27]. Water deficit affects the germination of seed and the growth of seedlings negatively [20]. In in vitro conditions, different osmotic stress levels were applied by using PEG 6000 or NaCl. Kaya et al. [7], showed that PEG did not have a toxic effect in sunflower seeds. As the stress was removed, all seeds germinated. Therefore, PEG molecules affect the germination and growth by preventing the entry of water molecules into plant tissues. This type of stress is called physiological drought. In contrast, the ions Na+ and Cl- penetrate into plant cells and can be accumulated in the vacuole for the tolerant plants or in the cytoplasm for sensitive cultivars [28].

Seed germination is one of the most important stages in a plant life [17,29]. It is affected by not only genotypic characters (e.g. dormancy, integument thickness), but also environmental conditions [30]. Germination ratio of chickpea seeds was more affected from PEG potentials compared to NaCl potentials. In fact, the negative effect of NaCl is due to the delay of germination of the seeds. Similar results reported by Ghoulam and Fares [17]. For all species tested it was reported that there was a threshold potential under which germination does not occur [11]. However, in this study, neither PEG nor NaCl stress treatments have never reached the threshold level even at the -0.8 MPa for chickpea seeds.

The negative effects of drought and salinity on the growth of early seedling was much more than on the germination of chickpea seeds. Our results showed that, drought stress brought by PEG 6000 inhibited epicotyl elongation more than the root growth and decreased the shoot/root ratio. As generally accepted, the roots suffer first from exposure to environmental stresses, followed by their injury [15]. Thus, even slight root damage permits a large flux of ions and plant food reserve materials to the epicotyls. Under PEG or NaCl stress, the growth of epicotyls of drought tolerant cultivars (Canıtez for drought and Uzunlu for salt) less decreased with increasing concentrations compared with sensitive genotypes (ER 99). Drought and salt tolerance ability appears to be associated with decrease rate of water uptake during germination. This may be attributed to increased mobilization of reserve food materials from cotyledons to epicotyl. Similarly with this experiment, chickpea seedlings respond to PEG-induced drought stress by inhibiting epicotyl growth [31]. The decline in the seedling growth of various species as a result of drought was also reported by the other researchers [27,32,33]. Although drought and salinity stresses may promote similar morphological responses, this was not the case for the shoot/root ratio [34]. In general, all the plant organs are affected by salinity, though the extent of growth reduction of the different organs is not similar as very well known. In this study, at -0.4 MPa osmotic potential induced by NaCl, epicotyl/root ratio increased due to the higher epicotyl elongation and/or lower root elongation than the control plants and, roots of cultivars and lines were found to be more susceptible to increasing NaCl potentials compared to their epicotyls. Similar results have been reported by de Lacerda et al. [35] and Teixeira and Pereira [13] for negative effect of salinity on potato and sorghum seedling growth.

The assessment of the effect of these stresses on the germination and seedling development parameters in eight chickpea cultivars and lines allowed us to conclude that all of the considered parameters were affected by drought and salinity with varietal differences in their responses to PEG and NaCl which have similar osmotic potentials but different physiological mechanisms.

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