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SALINITY STRESS AS AN ABIOTIC FACTOR AT GERMINATION STAGE ON DRY BEAN (*Phaseolus vulgaris* L.) CULTIVARS

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Abstract
The study focused on germination parameters as the most critical stage of plant development in dry bean cultivars under different salt treatment. For two used cultivars (Akman-98, Karaman-2016) the data on germination percentage, mean germination time, seedling shoot length, seedling root length, seedling length and seedling vigor indexes were reached under the different salinity levels of irrigation water (0 mM, 25 mM, 50 mM, 100 mM, 150 mM, and 200 mM of NaCl) in Petri plates. The data indicates that these parameters could be used as screening the dry bean cultivars against salt stress levels. This study was carried out in a Completely Randomized Design (CRD) with a total of 12 treatments (6 salinity x 2 cultivars) for the germination and seedling data each comprising of three replicates. The results demonstrated that; i) From the overall observation of the germination characters, at 100 mM and over salt treatments showed decreasing in the germination percentage, the seedling length and the seedling vigor index in the mean of the cultivars, ii) Significantly decreasing in the seedling shoot length were observed in the mean of the cultivars at just after 25 mM and gradually at all salt concentrations, iii) Although the root length showed increasing in 25 and 50 mM treatments than the control plants, a sudden decrease occurred after 100 mM salt treatment in the mean of the cultivars and in each cultivars separately, iv) The mean germination time were sharply in increasing starting with 25 mM treatment in the mean of the cultivars, v) Except the seedling shoot length, in the mean of all the salt treatments between the cultivars did not show statistically differences in the calculated parameters, vi) Any correlation was found between the salt concentrations and the cultivars.

Keywords: abiotic stress, cultivar, dry bean, Phaseolus vulgaris, plant growth, salinity stress

1. INTRODUCTION

Salinity is one of the most important abiotic stress factors that negatively affect plant growth and development. Salt-effected soils are distributed in all countries in the world (Omuto et al., 2020). At the same time, pulses are grown in every continent and almost every country extensively, except Antarctica (FAO, 2022). This mean that has not only importance of appropriate environmental condition as climatic factor for yield of plant types, but also salt-tolerant genotypes must be chosen for successful and sustainable farming.

Numerous studies reported that there are high genetic variations in cultivated plants under any stress factors. The highly choosing stress-tolerant cultivars and understanding the stress-tolerance mechanism can be reached via study among cultivars.

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The salinity stress, among extensively grown field crops e.g. wheat genotypes (Ashraf and O'leary, 1996; Saqip et al., 2006), maize genotypes (Hoque et al., 2015; Luo et al., 2018) rice (Gerona et al., 2019) and potato genotypes (Harun-or-Rashid et al., 2020), were studied in scientific area in addition to salt stress effects on horticultural crops (Tahir et al., 2018; Abid et al., 2020).
Since they are widely grown in legumes such as lentil, peas, and chickpeas other than dry beans, some studies have been conducted on salt resistance. Some of these studies focused on the effects on germination, some of on seedling growth and some of them at field stages (Ouji et al., 2015; Yousef et al., 2020). In the first step there are studies worked on about the salt stress effect on germination, seedling and plant growth in addition to yield responses in *Phaseolus vulgaris* L. (Kaymakanova 2009; Asfaw, 2011; Cokkizgin, 2012; Kul et al., 2020). At the same time there are some studies about the salt effect on enzymatic and non-enzymatic antioxidant defence system in *Phaseolus vulgaris* L. (Taïbi et al., 2016), on physiological response (Gama et. al., 2007; Arteaga et al., 2020; Çirka et al., 2022), and on alleviation of salt stress (Stoeva and Kaymakanova, 2008; Desoky et al., 2019).
The objective of this study was to evaluate the salinity stress point of the highly similar two cultivars by germination and seedling growth in Petri. With results of the germination percentage, the mean germination time, the seedling shoot length, the seedling root length, the seedling length and the seedling vigor indexes, it can be revealed what the sensitivity point is.
The reason we used the two genotypes that are remarkably like each other was to reveal whether they show different reactions to salt stress even though they are highly similar.

2. MATERIALS AND METHODS

2.1. Plant Material

The seeds of two dry bean cultivars (Akman-98 and Karaman-2016), officially registered and produced in Transitional Zone Agricultural Research Institute, Eskişehir city, Türkiye, were used in this investigation (Figure 1a). The two assessed cultivars are commercially cultivated by dry bean growers. Both two cultivars are semi-climbing, breeding methods are selection, and their colors are white. Some of the morphological characters of the seed were given in Table 1. The seed harvest facilities of the cultivars were done about 4-5 months ago, before the study.

2.2. Experimental Design and Applications

The study was performed at laboratory condition of Plant Protection Department, Kocaeli University, Agricultural Faculty in December 2022. *Phaseolus vulgaris* seeds of the two cultivars were placed on one layer filter paper in glass petri plates 10 cm in diameter. Seed surface sterilization procedures were done with 2% NaOCl of technical quality for 10 minute (Figure 1b), washed three times with sterile distilled water and dried for 30 minutes before treatment. Salinity stress was applied by six different concentrations of NaCl (Molecular Weight:58,443 g/mol) solution in distilled water (0-, 25-, 50-, 100-, 150- and 200 mM). The cultures were placed in controlled plant growth chamber under 25°C and 16 h light/8 h dark cycle. A total of 10 seeds were laid down in each Petri dish (Figure 2a, b) and 10 ml of appropriate solution was added to each Petri plate. A bean seed was considered to have germinated when the radicle reached 1 cm in length (Figure 2c). The petri plates were protected with transparent nylon bag for protection the moisture. The study was lasted along 10 days and countings data was obtained daily (Figure 3). The evaluation on some measurements were done at the end of the 10th day (Figure 2a, b).

2.3. Analytical methods

The evaluated data were:

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Germination Percentage: calculated as; (number of germinated seeds / number of total seeds) x 100
Mean Germination Time: calculated as; ∑ (Ti x Ni) /∑Ni; Ni is the number of newly germinated seeds at time Ti
Seedling Shoot Length: calculated from shoot beginning point to the end
Seedling Root Length: calculated from tap root beginning point to the end
Seedling Length: calculated from end points of tap root and shoot
Seedling Vigor Index: calculated as; mean germination percentage x mean seedling length

2.4. Statistical analysis and data evaluation

This study was carried out in a Completely Randomized Design (CRD) with a total of 12 treatments (6 salinity x 2 cultivars) each comprising of three replicates. Each replicate consisted with 10 seeds that placed in each petri and totally 360 seeds were used and calculated. Statistical analysis was carried out using SPSS 16.0. A two-way ANOVA was done to determine differences among treatment groups for the parameters. The Duncan's Multiple Range Test was also done to determine if differences between individual traetments were significant (P≤0,05)

Table 1. The features of the sown seeds of Phaseolus vulgaris L. cultivars

Seed Characteristics*	Cultivars	
	Akman-98	Karaman-2016
Seed weight (g/1000 seeds)	334	403
Seed length (mm/seed)	12.08	12.15
Seed width at wide side (mm/seed)	7.63	7.71
Seed width at narrow side (mm/seed)	4.88	5.37

*The data obtained from measurement of 100 seeds a day before applications.

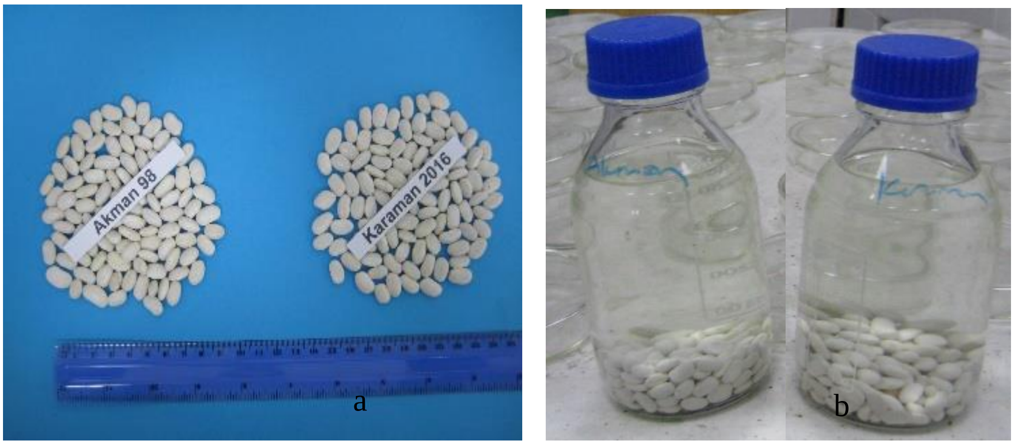

Figure 1. The study on salt treatment effects on Phaseolus vulgaris L. cultivars; (a) The samples of a hundred seeds of used cultivars each, (b) The sterilization step of the cultivars

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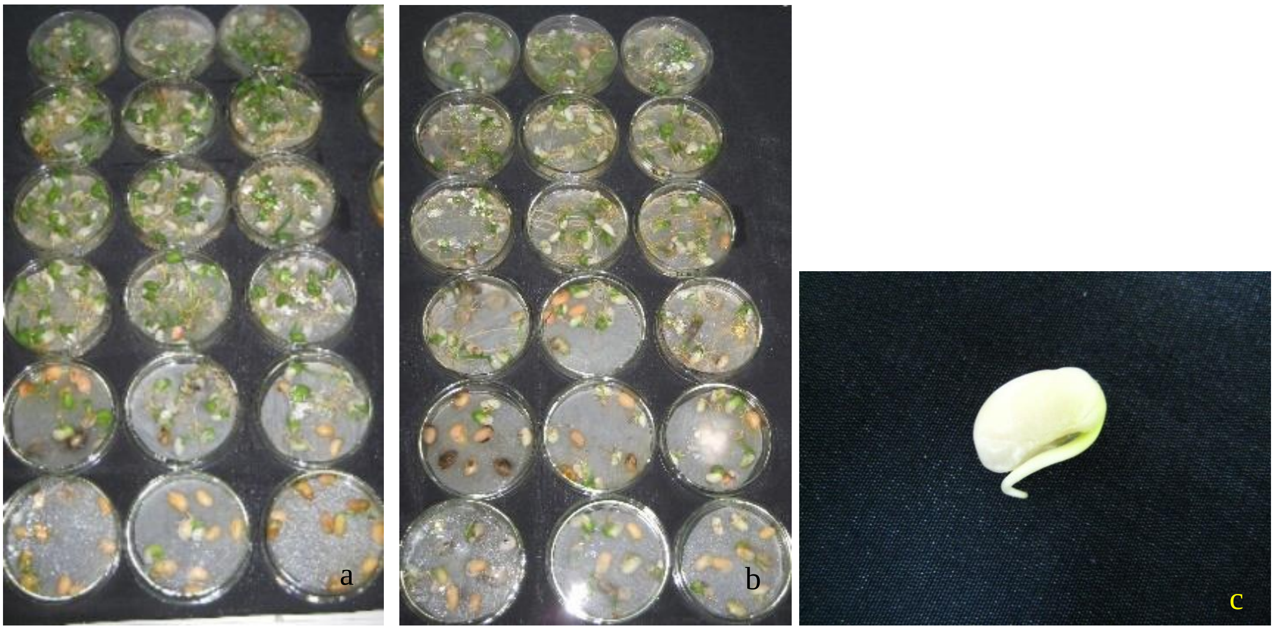

Figure 2. The study design of the two cultivars and six salt treatments with 3 replicates from 0 mM to 200 mM salt concentrations on -above to bottom- Phaseolus vulgaris L. cultivars; (a) Akman-98 at 10th day, (b) Karaman-2016 at 10th day, (c) The calculated germination sample that a tap root was at least 1 cm, and above

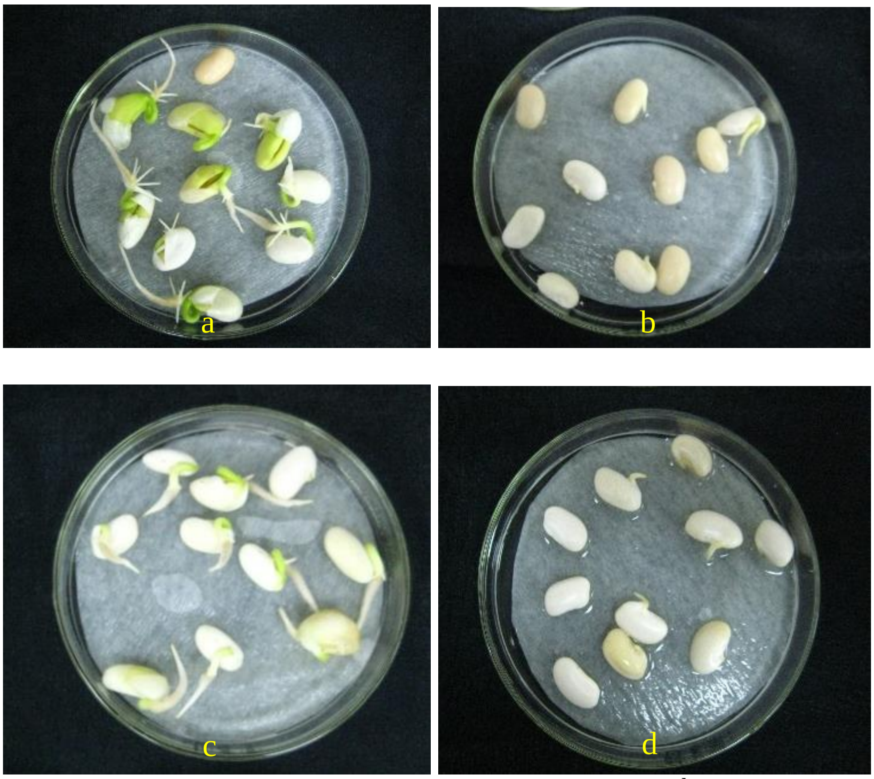

Figure 3. The salt effects on germination of Phaseolus vulgaris L. cultivars at 3rd day of treatment; (a) cv. Akman-98 in 0 mM, (b) cv. Akman-98 in 200 mM, (c) cv. Karaman-2016 in 0 mM, (d) cv. Karaman-2016 in 200 mM

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3. RESULTS AND DISCUSSIONS

The experiment was undertaken with an objective to study the effect of salinity stress on germination and seedling growth of the two dry bean cultivars. The seeds were treated with salt concentration on 15th December 2022 and full data were recorded along 10 days of germination and at the end of 10th day for seedling growth parameters. The main effects of the salinity were significant in all measured parameters. The salinity reduced the germination percentage, the seedling shoot and root length, the seedling length, and the seedling vigor index but increased the mean germination time in the two cultivars and at the different used salt concentrations. There were no significant differences between the cultivars and the salt concentrations interactions.

The data presented in Table 2 shows that the germination percentages in 0-, 25-, 50-, and 100 mM salt levels were not statistically different in cv. Akman-98 and in 0-, 25-, and 50 mM salt levels were not statistically different in cv. Karaman-2016. According to the mean of cultivars, 100 mM and higher concentrations have effectiveness on the parameter statistically (Figure 4a).

The data presented in Table 3 shows that the mean germination time highly affected by salt treatment. In the mean of the cultivars, even at 25 mM, salt treatments were in different statistical groups than control (Figure 4b).

The data on the shoot length was also highly affected by the salt treatment in the mean of cultivars just after 25 mM salt treatment (Table 4, Figure 5a). On the contrary to the shoot length, root length was more in 25- and 50 mM salt treatment than control (0 mM), but after 100 mM salt concentrations, main root started to decrease in both the two cultivars (Table 5, Figure 5b).

The seedling length affected by salinity at 100 mM and over treatments in both cultivars and mean of cultivars (Table 6, Figure 6a).

The seedling vigor index was higher in 50 mM salt treatment in cv. Akman-98 than control and the other salt application. The parameter was higher in 25 mM in cv. Karaman-2016 (Table 7, Figure 6b).

Various salts (NaCl, Na_2SO_4, $CaCl_2$, $CaCO_3$ and KCl) in water-agar solution with concentrations of 0, 100, 200 and 300 mM were applied to *P. vulgaris* var. *Djedida* at laboratory condition by Mansouri et al. (2019). After 10 days, the measured data indicated that length of tap root, number of secondary roots, seedling dry weight affected from salt concentration, salt type and their interactions. Final germination percentage was affected from salt concentration and salt type interaction. Inhibitory effects of NaCl, $CaCl_2$ and Na_2SO_4 salts on common bean were found very strong compared to those of $CaCO_3$ and KCl in the study.

Aghamir et al. (2016) applied various salts concentration as magnetized or non-magnetized saline water at concentration of 0.0, 25, 50, 75, 100 and 120 mM of NaCl to *Phaseolus vulgaris*. The study indicated that lenght, fresh and dry weight and tissue water content of root and shoot gradually were in decreasing with increasing salt concentration when ignoring remedy effect of magnetized saline water treatments.

Four different concentrations of salt (0.0, 50, 150, 250 mM of NaCl) to five genotypes of lentil (*Lens culinaris* L.) were applied by Ouji et al. (2015) in a petri study. According to the data percentage of germination, seedling shoot and root length, fresh shoot and root weight in the genotypes were significantly affected by salt concentrations, genotypes, and their interactions. They concluded that different responses after 150 mm NaCl concentration can be observed.

Kaymakanova (2009) applied two different salt types (NaCl, Na_2SO_4) to three bean (*Phaseolus vulgaris* L.) cultivars at a dose of 100 mM as petri study. The study indicated that salt tolerance is

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related with salt resources, and it can be different in cultivars. When only examined used NaCl, it is obviously seen that germination of only two cultivars affected by the concentration.
Al-Hurabi and Bafeel (2022) applied five different concentrations of salt (0.0, 50, 100, 150, 200 mM of NaCl) to pole bean (*P. vulgaris*). According to the data the germinated seed under control was 100%, whereas the germination percentage was decreased significantly by 97, 90, 87, 83 %, respectively at 50, 100, 150, and 200 mM NaCl.

Table 2. The effect of increasing NaCl on the final germination percentage (%) in Phaseolus vulgaris L. cultivars

Salt (NaCl) Concentration	Germination Percentage (%) in Cultivars		
	Akman-98	Karaman-2016	MEAN
0 mM	90.0 a*	96.7 a*	93.33±8.16 a**
25 mM	90.0 a	96.7 a	93.33±5.16 a
50 mM	100 a	90.0 a	95.00±5.47 a
100 mM	96.7 a	66.7 b	81.66±18.34 b
150 mM	73.3 b	53.3 b	63.33±16.33 c
200 mM	30.0 c	56.6 b	43.33±16.32 c
MEAN	80.0±25.67 a**	76.67±20.0 a	78.33±22.74

*Different lower cases in a column indicate significantly differences in the salt concentrations for the cultivar at least $P<0.05$ significance level, **Different lower cases in the last line and in the last column indicate significantly differences between cultivars for the mean of salt concentrations and significantly differences between salt treatments for the mean of cultivars respectively at least $P<0.05$ significance level.

Table 3. The effect of increasing NaCl on the mean germination time (day) in Phaseolus vulgaris L. cultivars

Salt (NaCl) Concentration	Mean Germination Time (day) in Cultivars		
	Akman-98	Karaman-2016	MEAN
0 mM	1.67 a*	1.86 a*	1.76±0.13 a**
25 mM	2.07 b	2.17 a	2.12±0.13 b
50 mM	2.40 c	2.71 b	2.55±0.20 c
100 mM	3.04 d	3.64 c	3.34±0.35 d
150 mM	3.82 e	4.25 d	4.04±0.40 e
200 mM	4.81 f	4.94 e	4.88±0.15 f
MEAN	2.97±1.11 a**	3.26±1.16 a	3.11±1.13

*Different lower cases in a column indicate significantly differences in the salt concentrations for the cultivar at least $P<0.05$ significance level, **Different lower cases in the last line and in the last column indicate significantly differences between cultivars for the mean of salt concentrations and significantly differences between salt treatments for the mean of cultivars respectively at least $P<0.05$ significance level.

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Table 4. The effect of increasing NaCl on the seedling shoot length (cm) in Phaseolus vulgaris L. cultivars

Salt (NaCl) Concentration	Seedling Shoot Length (cm) in Cultivars		
	Akman-98	Karaman-2016	MEAN
0 mM	7.38 a*	6.01 a*	6.69±1.63 a**
25 mM	6.33 a	4.06 b	5.20±1.57 b
50 mM	4.52 b	2.91 bc	3.71±0.10 c
100 mM	4.31 b	2.42 bc	3.37±1.23 c
150 mM	3.26 b	1.55 c	2.41±0.99 cd
200 mM	1.56 c	1.32 c	1.44±0.45 d
MEAN	4.56±2.06 a**	3.04±1.87 b	3.80±2.08

*Different lower cases in a column indicate significantly differences in the salt concentrations for the cultivar at least $P<0.05$ significance level, **Different lower cases in the last line and in the last column indicate significantly differences between cultivars for the mean of salt concentrations and significantly differences between salt treatments for the mean of cultivars respectively at least $P<0.05$ significance level.

Table 5. The effect of increasing NaCl on the seedling root length (cm) in Phaseolus vulgaris L. cultivars

Salt (NaCl) Concentration	Seedling Root Length (cm) in Cultivars		
	Akman-98	Karaman-2016	MEAN
0 mM	6.69 b*	8.24 bc*	7.47±1.90 b**
25 mM	8.87 ab	10.83 a	9.85±1.92 a
50 mM	11.05 a	9.65 ab	10.35±1.25 a
100 mM	6.55 b	6.31 c	6.43±0.87 b
150 mM	3.31 c	2.43 d	2.87±0.99 c
200 mM	1.22 c	1.92 d	1.57±0.70 c
MEAN	6.28±3.58 a**	6.56±3.62 a	6.42±3.55

*Different lower cases in a column indicate significantly differences in the salt concentrations for the cultivar at least $P<0.05$ significance level, **Different lower cases in the last line and in the last column indicate significantly differences between cultivars for the mean of salt concentrations and significantly differences between salt treatments for the mean of cultivars respectively at least $P<0.05$ significance level.

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Table 6. The effect of increasing NaCl on the seedling length (cm) in Phaseolus vulgaris L. cultivars

Salt (NaCl) Concentration	Seedling Length (cm) in Cultivars		
	Akman-98	Karaman-2016	MEAN[a]
0 mM	14.07 a*	14.24 a*	14.15 a**
25 mM	15.20 a	14.89 a	15.04 a
50 mM	15.57 a	12.55 a	14.06 a
100 mM	10.86 b	8.72 b	9.79 b
150 mM	6.57 c	3.99 c	5.28 c
200 mM	2.78 d	3.24 c	3.01 d
MEAN[b]	10.84±5.07 a**	9.61±5.01 a	10.22±5.01

*Different lower cases in a column indicate significantly differences in the salt concentrations for the cultivar at least *P<0.05* significance level, **Different lower cases in the last line and in the last column indicate significantly differences between cultivars for the mean of salt concentrations and significantly differences between salt treatments for the mean of cultivars respectively at least *P<0.05* significance level.

Table 7. The effect of increasing NaCl on the seedling vigor index in Phaseolus vulgaris L. cultivars

Salt (NaCl) Concentration	Seedling Vigor Index in Cultivars		
	Akman-98	Karaman-2016	MEAN
0 mM	1264 bc*	1366 ab*	1315 a**
25 mM	1368 ab	1433 a	1401 a
50 mM	1557 a	1129 b	1343 a
100 mM	1048 c	587 c	818 b
150 mM	482 d	211 d	347 c
200 mM	84 e	183 d	134 c
MEAN	967±549 a**	818±543 a	893±544

*Different lower cases in a column indicate significantly differences in the salt concentrations for the cultivar at least *P<0.05* significance level, **Different lower cases in the last line and in the last column indicate significantly differences between cultivars for the mean of salt concentrations and significantly differences between salt treatments for the mean of cultivars respectively at least *P<0.05* significance level.

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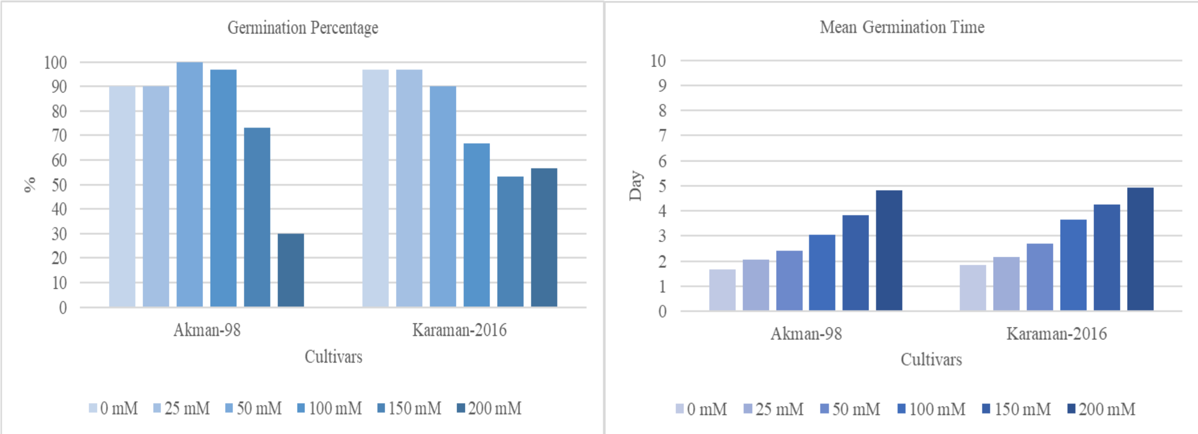
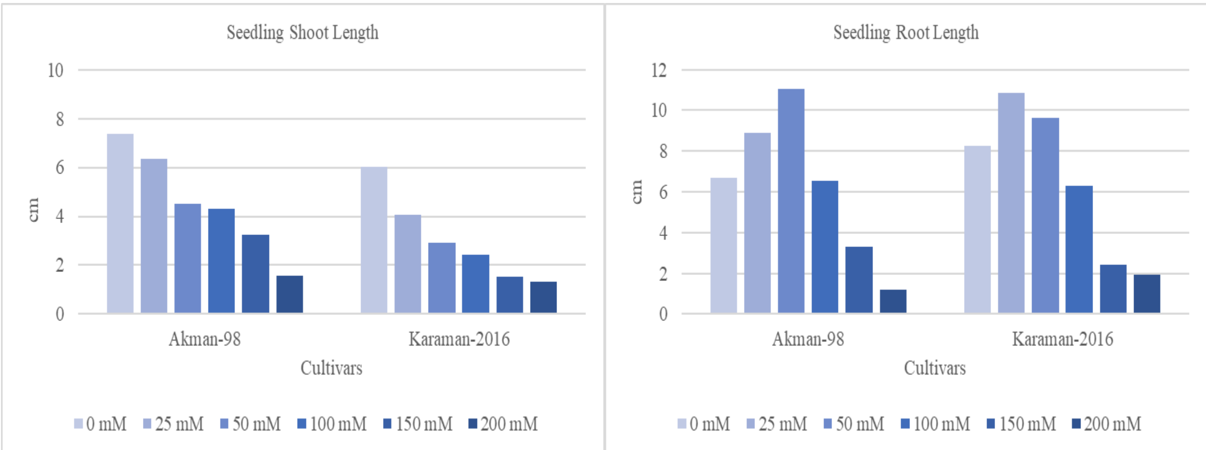


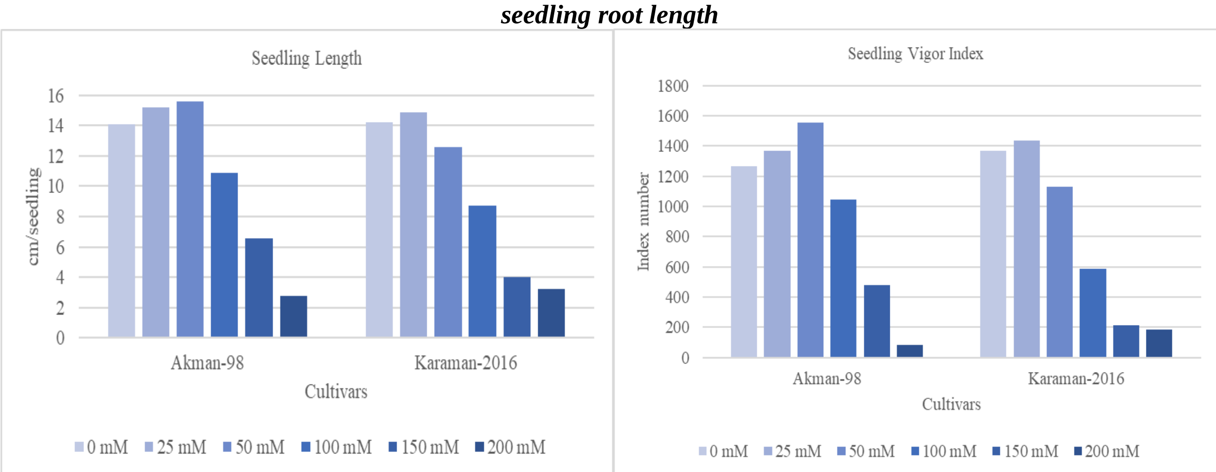
(a) (b)

Figure 4. The effect of increasing NaCl on Phaseolus vulgaris L. cultivars; a) on the final germination percentage, b) on the mean germination time



(a) (b)

Figure 5. The effect of increasing NaCl on Phaseolus vulgaris L. cultivars; a) on the seedling shoot length, b) on the seedling root length



(a) (b)

Figure 6. The effect of increasing NaCl on Phaseolus vulgaris L. cultivars; a) on the seedling length, b) on the seedling vigor index

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Asfaw (2011) applied 0, 2, 4, 8 and 16 dS/m salinity levels to fourteen *Phaseolus vulgaris* L. varieties in petri at laboratory condition. The data showed that variety x salinity level interaction was insignificant for Final Germination Percentage (FGP), Seedling Shoot Length (SSL), Seedling Root Length (SRL) and seedling Shoot-to-Root-Ratio (SRR) during germination. Seedling growth reflecting that all varieties responded similarly to salt stress with respect to the above parameters. In general, salt stress at 2 dS/m has enhanced growth with respect to FGP, SSL, SRL and SRR in some varieties. The study was also showed that Seedling Root Length (SRL) was more salt affected than Seedling Shoot Length (SSL) in the fourteen haricot bean varieties studied. It concluded that there are salt-sensitive and salt tolerant varieties.

The common bean (*Phaseolus vulgaris* L.) cultivars 'Carioca' and 'Mulatinho' were submitted to the germination under 10, 50, 100 and 200 mol.m^{-3} NaCl concentrations on germitest papers. Total germination of normal seedling changed between 0-91% in the first cultivar and 0-83% in the second one. The changing of fresh and dry weight of seedling changed in and affected by concentrations and cultivars (Dantas et al., 2007).

4. CONCLUSIONS

The main aim of this study was to recognize salt tolerance point of the similar dry bean cultivars. According to the results the study showed us, although the two dry bean cultivars remarkable similar each other, there were some differences at tolerance point in some of the parameters. This lead us, different genotypes or cultivars can be more tolerant or sensitive to the salt stress so studies should continue for obtain highly biologically diverse genotypes.

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