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Response of Oasian and exotic pepper (*Capsicum* spp.) cultivars from Tunisia to salt stress at germination and early seedling stages

Rabeb El Khaldi [1, 2*], Dhaouadi Latifa[1], Besser Houda[3]

1, *Regional Research Center in Oasis Agriculture of Degache, road of Tozeur Km1, 2260 Degache, Tunisia*
2, *UR13AGR09- Integrated Horticultural Production in the Tunisian Centre East, Regional Centre of Research on Horticulture and Organic Agriculture, University of Sousse, 4042, Chott –Mariem, Tunisia*
3, *High Institutes of Sciences and Techniques of Water, Gabes –Tunisia*

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***Corresponding author:**
Regional Research Center in Oasis Agriculture of Degache, road of Tozeur Km1, 2260 Degache, Tunisia
Email: rabebk@yahoo.com

ABSTRACT

Purpose: In Oasis areas, salinity becomes a real threat for sustainable agricultural production, and with the introduction of non-native varieties, salinity pressure is more expressed and has harmful outcomes to the local ecosystem and biodiversity. In this context the present study was conducted to compare salt stress behavior of local oasian and introduced cultivars. **Research method:** Seeds of two local oasian cultivars namely Djerid (Dj) and Nefta (Ne) and one exotic cultivar namely Cayenne (Ca) were treated with four salt concentrations (0; 2.5; 5 and 7g/l NaCl). Salt stress responses were evaluated using germination parameters and early seedling growth. **Findings:** Results revealed that the increase of salinity level had negatively effect on germination and early seedling for the three cultivars. However oasian cultivars were found to be the most tolerant to salinity. Ne cultivar showed the highest germination percentage and germination index and the lowest mean germination time at 7g/l NaCl concentration. Also, "Ne" performs better on seedling traits as indicated by the less effect of the high salinity on radicle and plumule length as well as plumule fresh weight, compared to the other cultivars. The introduced cultivar "Ca" was identified to be the most sensitive to salt stress. **Limitations:** No limitations were founded. **Originality/Value:** This study valorized the autochthone chili pepper cultivars by highlighting their salt stress tolerance. Thus, oasian genotypes could be very useful as genetic resources for the development of chili pepper cultivars with improved germination and seedling growth under salt-stress conditions.

INTRODUCTION

Salinity is considering as one of the most serious abiotic stresses causing significant damages in the crop growth, productivity and quality all over the world (Abdel Latef, 2010; Rozema & Flowers, 2008). In arid and semi-arid regions, the high evaporation level and the shortage of water with good quality, have led to an enlargement of saline land in agricultural areas and a reduction in yield of the most major crops (Bekmirzaev et al., 2011; Costa et al., 2011).
Salt stress affects all growth stages of the plant. Seed germination and seedling growth, critically important junctures in the plant life cycle, are known to be the most sensitive stages to salinity for the most plant species (Ashraf & Foolad, 2005; Cuartero et al., 2006; Sivritepe et al., 2003).

Some of the main effects of salinity on seeds are as delayed germination time, increasing of germination rate, and reduction of seedling growth (Kaveh et al., 2011; Thiam et al., 2013). High rates of salinity can cause a stronger physiological and biochemical alterations in germinating seeds and prevent, as consequence, seed germination through various factors, such as a reduction in water availability and mobilization of stored reserves (Ibrahim, 2016; Thiam et al., 2013; Wang et al., 2011).

Response of plants to abiotic stress, such as salinity, varies according to its species and /or varieties (Bertazzini et al., 2018). Na^+ and Cl^- ions accumulation in plant tissues is widely using to screening plants for salt tolerance (Sarabi et al., 2017). Germination and seedling growth under salinity stress are useful tools to select cultivars salt tolerance within a crop species (Ashraf et al., 1990).

In the oasis of Hamma-Tozeur (South West of Tunisia), local chili pepper (*Capsicum* spp.) is the most popular cultivated vegetable crop and it was considered as a territory oasis product with extremely economic importance. Due to water scarcity in this area, Continental Intercalaire (CI) and "Complex Terminal" (CT) aquifers, characterized by their highest salinity, were used in oasis cultures irrigation. As a consequence, salinity problems appeared and affected also glycophytes crops, such as chili pepper, from germination until plant growth and production.

Thus, the present study was undertaken to evaluate the germination and seedling growth responses of two local oasian and one exotic chili pepper cultivars to different levels of salinity.

MATERIALS AND METHODS

Plant material

Three chili pepper cultivars grown in the open-field conditions in the years of 2018 and 2019 were collected from the oasis of Hamma. Two local cultivars namely Djerid pepper (Dj); Nefta pepper (Ne) and one exotic introduction namely cayenne (Ca) were chosen for this study.

Germination assays

The germination study was conducted under laboratory conditions. Seeds were extracted separately by hand, air-dried and surface sterilized for 20 minutes in sodium hypochloride solution (5%), then they were rinsed 3 times with distilled water for 2 min.

After sterilization, 25 seeds of each cultivar were randomly distributed into Petri dish on Watman filter paper and then were wetted with 5 ml distilled water (control) or with saline water solution. Three different salt concentrations were prepared by dissolving NaCl in distillated water in concentrations equal to 2.5 g l^{-1}; 5 g l^{-1} and 7 g l^{-1}. The salt concentrations were chosen on the basis of the different water irrigation quality in the oasis. Petri dishes were sealed with Parafilm to avoid drying and contamination and then incubated in a completely randomized block design with three replications in a growth chamber at 25°C.

Germinated seeds were reviewed on daily basis. Germination was considered complete once the radicle protruded about 2 mm in length.

For the evaluation of salt tolerance during germination, several parameters were measured in this study:

Germination percentage (GP)

Calculated according to Ashraf and Foolad (2005) based on the following formula (1):

$$GP = \frac{\text{Total germinated seed} * 100}{\text{Total number of seed}} \quad (1)$$

Mean germination time (MGT)

Calculated according to the formula (2) developed by Ellis and Roberts (1981):

$$MGT = \Sigma\, Dn \,/\, \Sigma n. \quad (2)$$

Where (n) is the number of seeds germinated on day D, and D is the number of days counted from the beginning of germination.

Germination index (GI)

Germination Index (GI) was calculated as described by the Association of Official Seed Analysts (AOSA, 1983) as (1):

$$GI = \Sigma\, (Gt \,/\, Tt). \quad (3)$$

GI= [Number of germinated seeds in first count/ Day of first count] +…+ [Number of germinated seeds in final count/Day of final count).

Seedling growth

At the end of the germination test (10^{th} day), randomly selected five seedlings were taken from each Petri dish to measure radicles and plumules lengths (mm) and radicles and plumules fresh weights (mg).

Data analysis

Statistical analyzes are performed using Infostat Statistical Software (version 1.0). The main effects were salt treatment and cultivars as well as their interactions. Means were compared with LSD (Least significant difference, P≤0.05) values.

RESULTS

Germination study

From the results of the germination study, it was observed that the effect of salinity level was highly significant for all germination traits. No significant inter-cultivar differences were detected for germination percentage; however differences between cultivars were highly significant for MGT and GI (Table 1).

Germination percentage

The effect of different salt concentrations on germination ratio (%) was shown in Fig. 1. At the end of the trial, results revealed that the germination of the different cultivars was negatively affected by the increase of salt concentration.

Except for 5g NaCl l^{-1} treatment, no significant difference among local oasian cultivars was observed in terms of germination percentage.

At the lowest NaCl concentrations (0 g l^{-1} and 2.5 g l^{-1}), the average germination of Ca seeds resulted higher than that of both Dj and Ne seeds. However, at the highest concentrations the effect of increased salinity was not very marked for oasian cultivars, while Ca cultivar was shown to be the most sensitive to salinity stress. In fact, at 5 g l^{-1} and 7 g l^{-1} Dj and Ne showed intermediate germination levels (from 66.7% to 58.7 % respectively). Those values significantly decreased to 49.3 % and 33% for Ca seeds.

In terms of germination reduction, the Ne cultivar recorded the lowest reduction with values of about 24.5% at 5 g l^{-1} NaCl and 31.8% at 7 g l^{-1}. The strongest reduction of germination was observed at the highest salt concentration for Ca cultivar with the value of 65.8% (Table 2).

Table 1. Significance of variation (*p* values) according to two-way Anova testing the effect of salinity treatments, cultivars and their interactions (salinity × cultivars) for the different germination traits

Parameter	Salinity	Cultivars	Salinity × Cultivar
Germination %	0.0003	0.7473	0.3828
MGT	<0.0001	<0.0001	0.5876
GI	<0.0001	<0.0001	0.5412

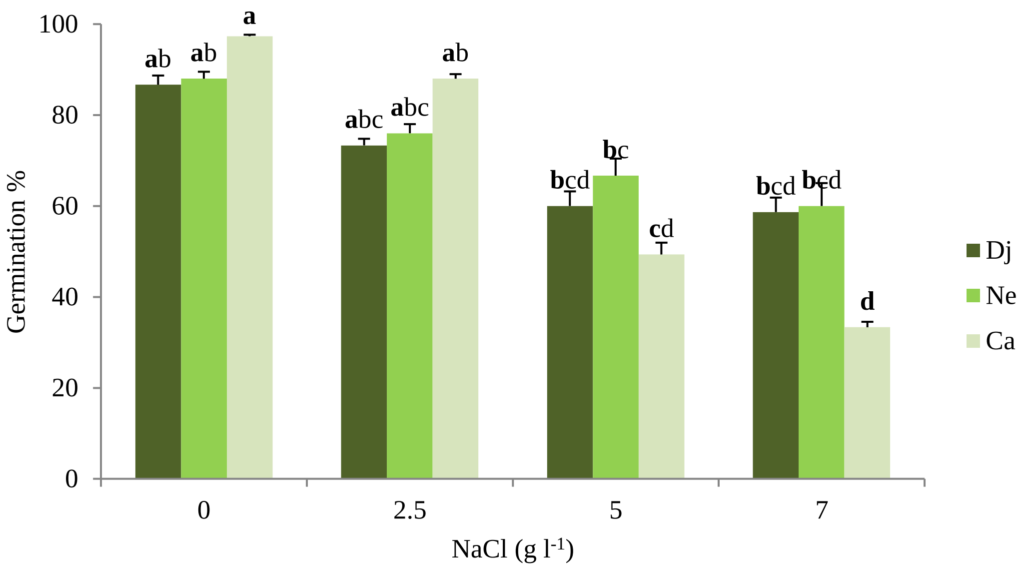


Fig. 1. Effect of salinity on germination percentage of the local oasian (DJ and Ne) and exotic (Ca) pepper cultivars.

Mean germination time

The effect of salinity for the mean germination time results, mentioned in Table 3, showed that increasing salinity delayed significantly the mean germination time for the 3 cultivars. The seeds that germinated under the lowest salinity concentrations exhibited reduced germinating time.

Mean germination time was found the highest for 7 g l^{-1} NaCl treated Ca seeds, followed by Dj.

The mean germination time of Ne seems to be the least affected by the increasing salinity compared to other cultivars.

Germination index (GI)

The germination index was significantly influenced by the different cultivars as well as by the different levels of NaCl concentrations. Ne cultivar was found to have the highest GI for all salt concentration treatments, as compared to other cultivars.

Although at 0; 2,5 and 5 g l^{-1} NaCl concentrations, Dj and Ca had a similar response in respect to GI values. At the highest salt concentration, Dj was shown to be more resistant (6.6) than Ca which showed the least value of GI (3.1) (Table 3).

Early seedling Growth

Analysis of variance (ANOVA) revealed that salinity and cultivars had a very high significant effect on all tested traits associated with early seedling growth (Table 4).

Table 2. Seed germination reduction (%) of the local oasian (Dj and Ne) and exotic (Ca) pepper cultivars at different NaCl concentration as compared to control

	NaCl (g l^{-1})		
Cultivar	2.5	5	7
Dj	15.4 ^{ab}	30.8 ^{abc}	32.3 ^{abc}
Ne	13.6 ^{ab}	24.2 ^{ab}	31.8 ^{abc}
Ca	9.6 ^{a}	49.3 ^{bc}	65.8 ^{c}

Means followed by the same letters are not statistically different at (P ≤0.05).

Table 3. Effect of salinity on mean germination time (MGT) and on germination index (GI) of the local oasian (Dj and Ne) and exotic (Ca) pepper cultivars

	NaCl (g l^{-1})							
Cultivar	0		2.5		5		7	
	MGT	GI	MGT	GI	MGT	GI	MGT	GI
Dj	6.7 ^{b}	18.8 ^{BCD}	6.9 ^{bcde}	14.3 ^{DEF}	7.3 ^{ef}	10.5 ^{EFG}	7.6 ^{f}	6.6 ^{GH}
Ne	6.1 ^{a}	27.4 ^{A}	6.1 ^{a}	22.3 ^{B}	6.8 ^{bc}	13.5 ^{EF}	6.8 ^{bcd}	11.2 ^{EFG}
Ca	6.9 ^{bcde}	19.4 ^{BC}	7.2 ^{cdef}	14.6 ^{CDE}	7.2 ^{def}	9.4 ^{FG}	7.7 ^{g}	3.1 ^{H}

Means followed by the same letters are not statistically different at (P ≤0.05).

Table 4. Significance of variation (*p* values) according to two-way Anova testing the effect of salinity treatments, cultivars and their interactions (salinity × cultivar) for the different early seedling growth traits

Parameter	Salinity	Cultivars	Salinity × Cultivar
Radicle length	<0.0001	<0.0001	0.2527
Plumule length	<0.0001	<0.0001	<0.0001
Radicle weight	<0.0001	<0.0001	0.1548
Plumule weight	<0.0001	0.0001	0.3852

Radicle and plumule length

Salinity treatments negatively affected radicle and plumule elongation of all cultivars with respect to controls ($p \leq 0.05$) (Fig. 2). Radicle and Plumule length reduction under salinity stress was showed in Table 5.

In the case of oasian cultivars, the radicle length varied for Dj, from about 3.62 cm at 0 g l^{-1} to 0.56 cm at 7 g l^{-1} NaCl (less than 84,53% of the length of the control) and for Ne between 5.82 cm at 0 g l^{-1} NaCl to 2.42 cm at 7 g l^{-1} NaCl (about 58,42% of the control length).

For Ca cultivar, the radicle length varied about 3.64 cm at 0 g l^{-1} to 0.7 cm at 7 g l^{-1} NaCl (80.77% of reduction of the length as compared to the control).

Radicle length was recorded significantly highest in Ne in all treatments compared to the two other cultivars

A similar trend in reduction of plumule length was also observed for the three cultivars. At the highest salt concentration, no significant difference between the plumule length of the oasian cultivars was detected ($p \leq 0.05$) (values varied from 0.12 cm to 0.4 cm). The Ca variety showed the lowest plumule length.

With respect to control, the strongest inhibitory effect was observed on both Dj and Ca cultivars (96.2%). For Ne, plumule length was reduced by 81.9%.

Table 5. Radicle and plumule length reduction (%) of the local oasian (Dj and Ne) and exotic (Ca) pepper cultivars at different NaCl concentration as compared to control

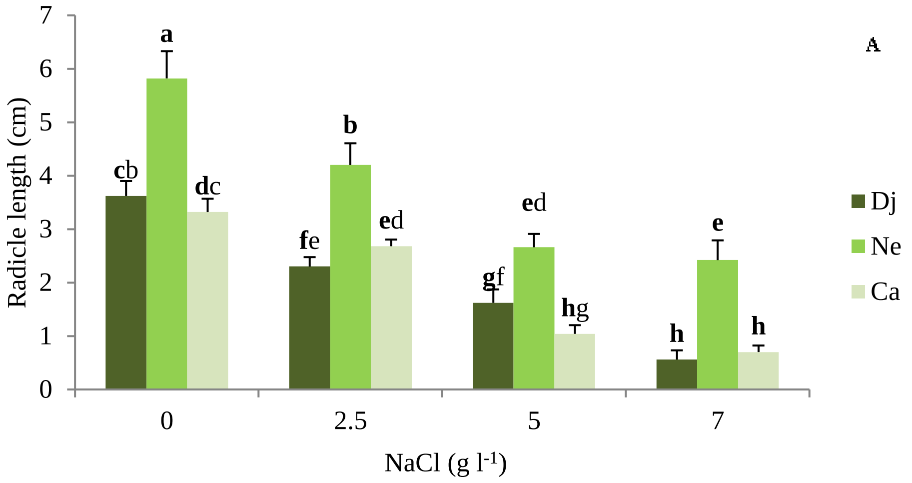
		NaCl (g l^{-1})		
	Cultivar	2.5	5	7
Radicle	Dj	36.5^{ab}	55.2^{bc}	84.5^{e}
	Ne	27.8^{a}	54.3^{bc}	58.4^{cd}
	Ca	26.4^{a}	71.4^{cde}	80.8^{de}
Plumule	Dj	50.6^{B}	90.5^{C}	96.2^{C}
	Ne	23.4^{A}	77.5^{C}	82.0^{C}
	Ca	35.0^{AB}	90.0^{C}	96.2^{C}

Means followed by the same letters are not statistically different at ($P \leq 0.05$).

Table 6. Radicle and plumule weight reduction (%) of the local oasian (Dj and Ne) and exotic (Ca) pepper cultivars at different NaCl concentration as compared to control

		NaCl (g l^{-1})		
	Cultivar	2.5	5	7
Radicle	Dj	33.3^{a}	41.7^{ab}	60^{cd}
	Ne	38^{a}	55.3^{bc}	62.7^{cd}
	Ca	56^{bc}	56.8^{bc}	60.8^{cd}
Plumule	Dj	22.1^{AB}	69.3^{CD}	75.5^{D}
	Ne	2.3^{A}	51.2^{BC}	62.3^{CD}
	Ca	41.5^{BC}	81.9^{D}	82.4^{D}

Means followed by the same letters are not statistically different at ($P \leq 0.05$).



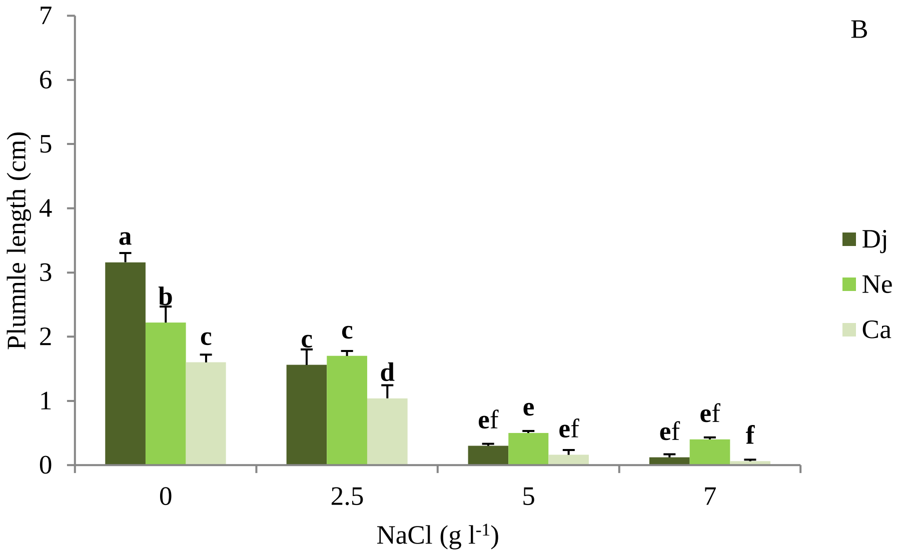


Fig. 2. Effect of salinity on radicle (A) and plumule (B) length of the local oasian (DJ and Ne) and exotic (Ca) pepper cultivars.

Radicle and plumule fresh weight

Data showed that the response of the different cultivars, in fresh weight of radicle and plumule, under the different salinity stress were significant ($P \leq 0.05$). Increasing in salt concentration reduced these traits (Fig. 3).

Under saline treatments, maximum fresh weight of radicle and plumule was obtained from Ne cultivar. The increase in salinity up to 7 g l^{-1} caused 62.7% and 62.3 % reduction of radicle and plumule weights compared with control, respectively (Table 6).

In the highest salinity level, there was no statistically difference among radicle weights of Dj and Ca cultivars. Values are between 9.6 and 9.8 mg respectively. However for plumule weight, Dj showed significant higher value than Ca.

The effect of the highest stress level in weight of plumule was shown for Ca with 75.5% of reduction (Table 6).

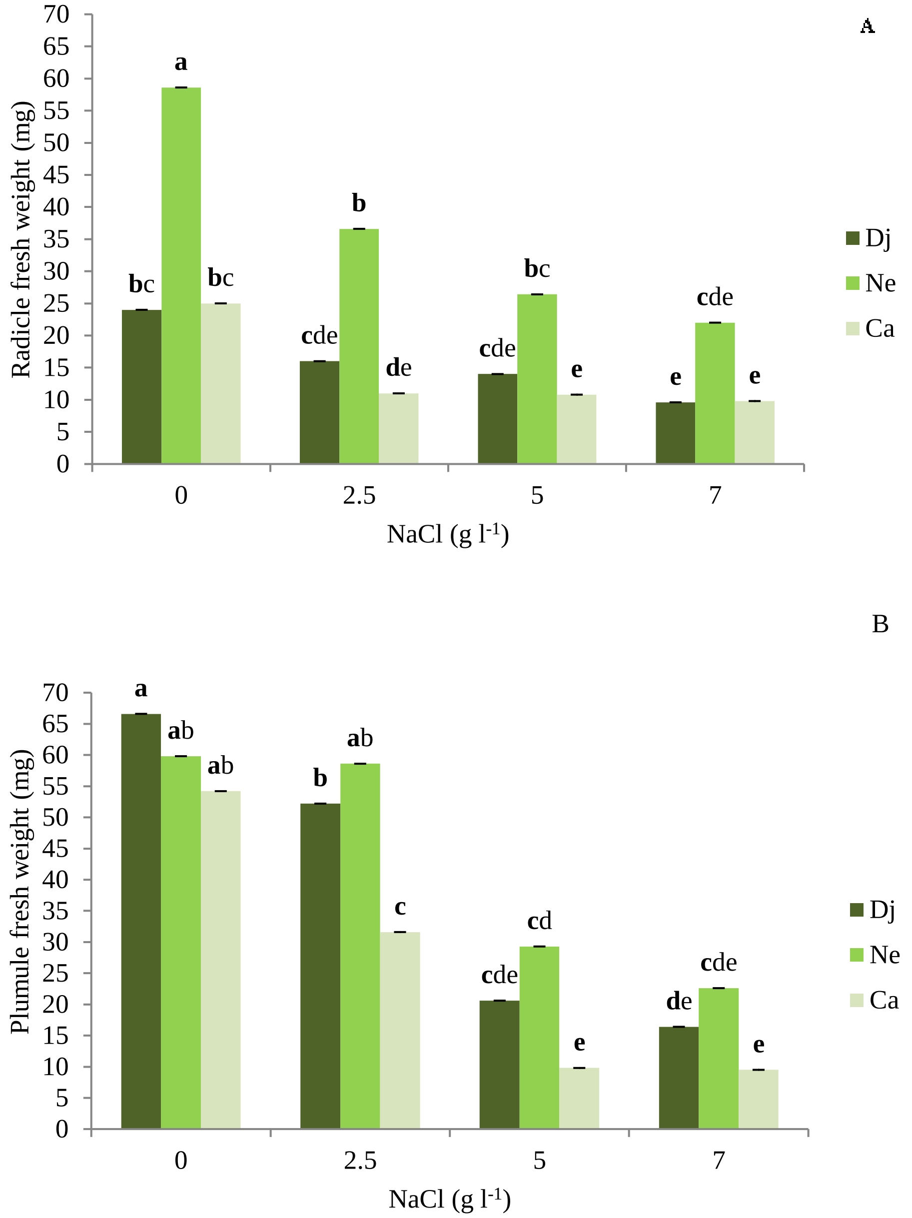


Fig. 3. Effect of salinity on radicle (A) and plumule (B) fresh weight of the local oasian (DJ and Ne) and exotic (Ca) pepper cultivars.

DISCUSSION

The main purpose of this study was to assess the effect of salinity on chili pepper cultivars through *in vitro* seed germination, and to investigate the genotypic variation in salt tolerance response during germination and early seedling growth. For this purpose, three cultivars named as “Dj “, “Ne” (local oasian cultivars) and “Ca” (exotic cultivar) were used with four different salt concentrations.

From the results of the germination study, it was observed that salt stress declined the germination percentage, the germination index and also delayed the emergence of seeds for the three chili pepper cultivars. However, a genotypic variation response of chili pepper seeds to salinity stress was observed. Under non saline conditions, “Ca” showed better seed germination performance as compared to oasian cultivars. Nevertheless, with the increase of salt concentration “Ca” exhibited the least stress tolerance capacity. “Ne” cultivar appeared to be the most salt resistant at germination stage.

Several studies have been conducted in genotype salt tolerance for different plant species such as: safflower (Karimi et al., 2011); sorghum (Almodares et al., 2007), wheat (Eskandari & Kazemi, 2011); tomato (Kazemi et al., 2014); pepper (Aloui et al., 2014; Kpinkoun et al., 2018; Yildirim & Guvenc, 2006).

Azhar and McNeilly (1988) and Krishnamurthy et al. (2007) attributed the difference in tolerance to salinity at the germination and seedling stage to the genetic diversities among genotypes and heredity differences between cultivars.

Yildirim and Guvenc (2006) reported that the variation between peppers cultivars on mean germinating time was due to common genes (or physiological mechanisms) that contribute to rapid germination under both stress and non-stress conditions. However according to Foolad (1996) and Foolad and Lin (1997), the genotypic variation in tomato germination response may be due to stress – specific genes which are inducible only under specific stress conditions.

The germination problems related to the loss of viability due to the salinity abiotic factor has been demonstrated in several studies. The reduction and/or inhibition of seed germination could be attributed to osmotic stress with slowing down of the water uptake (Welbaum et al., 1990) or to the accumulation of some specific toxic ions such as Na and Cl (Huang & Redmann, 1995). As our finding, many researches demonstrated that germination parameters significantly decreased as the level of medium salinity increased (Aloui et al., 20014; Kazemi et al., 2014).

Similar to the germination parameters, salinity affected early seedling growth of chili peppers genotypes. A clear reduction in radicle and plumule elongation and weight was observed. Among cultivars, “Ne” also showed better resistance in all NaCl treatments especially for radicle length. Our findings was supported by previous reports which indicated that radicle length is one of the most important characters in salt stress studies for assessing plant’s tolerance to salt stress (Kazemi et al., 2014).

Chakma et al. (2019) reported that under non salinity or low salinity conditions required cell enlargement was maintained due to suitable osmotic growth conditions, as consequence maximum growth was obtained as compared to the higher salinity levels.

CONCLUSION

Through the *in vitro* test carried out in the laboratory, the results of this study highlighted the negative effect of NaCl salt stress on chili pepper during germination and early seedling stages. A genetic variation within chili pepper cultivars in response to salt stress was shown. The exotic genotype showed poor performance under salt stress condition for all studies parameters. oasian “Ne” chili pepper was the more vigorous genotype and could be considered as useful genetic resources for the development of salt resistant cultivars.

Conflict of interest

The authors have no conflict of interest to report.

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