Journal of Stress Physiology & Biochemistry, Vol. 10 No. 1 2014, pp. 14-25 ISSN 1997-0838

ORIGINAL ARTICLE

Effect of Salt Stress (NaCl) on Germination and Early Seedling Parameters of Three Pepper Cultivars (*Capsicum annuum* L.)

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Received August 4, 2013

Salinity is one of the major environmental problem that lead to a deterioration of agricultural land and, as a result, to a reduction in crop productivity worldwide. This research tested the effect of different salinity levels on germination and early seedling growth of three pepper (*Capsicum annuum* L.) cultivars which were "Beldi", "Baklouti" and "Anaheim Chili". Experimental treatment included 7 concentrations of NaCl (0, 2, 4, 6, 8, 10 and 12 g/l). Results indicated that all investigate traits were affected by salt stress. Salt stress affected on germination parameters and radicle and plumule length. Fresh weight and dry weight of evaluated seedlings was also affected. "Anaheim Chili" cultivar was shown to be the most restraint cultivar to salt stress in comparison to "Beldi" and "Baklouti" cultivars.

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Key words: pepper cultivars , germination parameters, salt stress

Salt stress is certainly one of the most serious environmental factors limiting the productivity of crop plant (Ashraf, 1999). It is one of the major abiotic stresses, and approximately 800 million hectares of land are affected by high salt concentrations throughout the world (Munns, 2005). Both osmotic and ionic stresses can cause stunted growth and a reduced plant yield (Munns, 2002) and the irrigation with saline water is one of the principal factors that lead to salt accumulation and leads to a decrease in agriculture productivity. Germination of seeds is a complex process depending on genetic and environmental factors, such as temperature, light, and salinity (Barbour, 1988; Mahmoud, 1985). In fact, salinity is one of the most important factors limiting plant growth and delaying seed germination as well as final germination percentage (Rahman *et al*., 2000). Salinity can affect germination and seedling growth either by creating an osmotic pressure that prevents water uptake or by the toxic effects of sodium and chloride ions (Hopper et *al*., 1979).

Pepper (*Capsicum annuum L*.) is a glycophyte plant which can't survive under high salinity stress

or can survive with affected yields (Ibn Maaouia-Houimli *et al.,* 2008) . Certainly , salinity reduces performances especially in arid and semi-arid regions characterized by irregular and insufficient rainfall (Munns *et al.*, 2006). In Tunisia, pepper (*Capsicum annuum L*.) is an important crop considered as the fifth vegetable speculation after tomato, melon, watermelon, potato and onion. Also, Tunisia is the third largest producer of pepper after Nigeria and Egypt and the third largest exporter (in terms of tonnage) after Morocco and South Africa (Boughalleb and El Mahjoub, 2006). Many pepper cultivars are cultivated in Tunisia , in fields or under heated plastic tunnels, in costal and southern regions. The present study evaluates the effect of NaCl treatments on germination and early growth parameters in three pepper cultivars which are "Beldi", "Baklouti" and "Anaheim Chili" in order to understand the reaction of young seedling to salt stress and to select tolerant cultivar which can be used in future experiences.

MATERIALS AND METHODS

Seed material

This study was conducted under controlled environmental condition in the laboratory of the Department of Agronomy and crop science of the High Institute of Agriculture Chott Mariem , Sousse, Tunisia. Seeds of three peppers cultivars ("Beldi", "Baklouti" and "Anaheim Chili") were sterilized for 20 minutes in sodium hypochloride solution (5%), then they were rinsed 3 times with distilled water for 2 min. After sterilization under laminar flow, 20 seeds of each cultivar were transferred into sterile Petri dishes (100 x100 mm dimensions) between two layers of Watman filter paper and then watered with 10 ml of distilled water (for control) or 10 ml of a saline solution containing 2, 4, 6, 8, 10 and 12 g/l of NaCl and left to germinate at 25°C (Figure 1.A). The Petri dishes were arranged in a completely randomized block design with three replications. Germinated seeds were recorded daily during 14 days and the appearance of 2 mm or more of radicle was considered as germination.

Parameters measured

Parameters measured in this study were:

Germination percentage (GP) which was calculated based on the equation: GP = (Total germinated seed) / (Total number of seed) (Ashraf and Foolad, 2005).

Mean germination time (MGT) which was calculated according to the equation of Ellis and Roberts (1981) : MGT = Σ Dn / Σn. Where (n) is the number of seeds which were germinated on day D, and D is the number of days counted from the beginning of germination.

Germination Index (GI) which was calculated as described by the Association of Official Seed Analysts (AOSA, 1983) as: GI= ∑ (Gt / Tt). GI= [Number of germinated seeds in first count/ Day of first count] +…+ [Number of germinated seeds in final count/Day of final count).

The coefficient of velocity (CV) which was calculated using the following formula:

CV= 100× [ΣNi / ΣNi Ti] where Ni is the number of seeds germinated on i thdays, Ti is the number of days from sowing (AOSA,1983). Plumule and radicle length (cm) were measured with a graduated ruler. Then, radicle and plumule fresh weight (FW) and dry weight (DW) were determined with a precision balance "Mettler". In order to determine dry weight seedlings are separated and dried in an oven at 80°C for 48 hours.

Data analysis

Data were subjected to an analysis of variance, using SPSS 13.0 software and the difference

between means were compared by Duncan multiple range test at 5% level of probability. Differences were considered significant at the 5% level (means followed by different letters).

RESULTS

Total germination percentage

The results showed that different levels of salinity have significant effect on pepper seed germination. Many researchers have reported similar results (Garcia *et al.,* 1988 ; Ben Said, 2004). In all of cultivars, there was a decrease in germination percentage when the salt stress increase. While, in this experiment, cultivars had different response to the salinity (Figure 2). In control, seed germination percentage varied between 80% ("Baklouti" cultivar) and 90% ("Anaheim Chili" cultivar). At the highest salt concentration (12 g/l), the maximum germination percentage was observed in "Anaheim Chili" cultivar (55%) and the lowest one in "Baklouti" cultivar (5%). "Anaheim Chili" cultivar demonstrated better tolerance to salt stress than other cultivar for germination percentage.

Mean germination time

The results in Table 1 revealed that the increasing salinity delayed significantly the mean germination time (MGT) in all pepper cultivars. The highest MGT was observed at 12 g/l of NaCl in "Beldi" cultivar (9.25 days), and the lowest MGT was observed at 0 g/l of NaCl in " Anaheim Chili" cultivar (5.50 days). It has been reported that salinity delays germination (Mensah and Ihenyen, 2009; Murillo-Amador et *al*., 2002; Rahman *et al*., 2000). Similar result was also observed by Redondo-Gomez *et al*. (2008), where MGT of Gibraltar sea lavender (*Limonium emarginatum* L.) was higher in salt stress condition.

Germination index

According to Table 1, salt stress (NaCl) had significantly effect on germination index of all pepper cultivars "Beldi", "Baklouti" and "Anaheim Chili". The reduction gets stronger particularly at the highest level of salt stress (12g/l). Similar results were reported by Kaya *et al.* (2008) and Khan *et al.* (2009) working on hot pepper**.** Among the three pepper cultivar, the highest germination index values were observed in "Anaheim Chili" cultivar in all NaCl concentration**.**

Coefficient of Velocity

Considerable decrease was noted in Coefficient of Velocity (Table 1), depending on NaCl concentrations. In fact, coefficient of velocity varied from 14,58 ("Baldi" cultivar) to 18,22 ("Anaheim Chili" cultivar) in control. The addition of NaCl in water reduced this coefficient .However, this decrease was more pronounced for " Baldi " and "Baklouti" cultivars than " Anaheim Chili" cultivar. The minimum coefficient of velocity was found in the high salinity treatment (12 g/l) and registered in "Baldi" cultivar (10,83).

Radicle and plumule length

The effects of salinity stress on radicle and plumule length of three pepper cultivars have been showed in figure 3. Results showed a decrease in radicle and plumule length with an increase in salinity for the three pepper cultivars (Figure 1.B). Plumule length was more suppressed than radicle by salinity at all salt concentration levels. "Baldi" cultivar seems to be the most sensitive cultivar and "Anaheim Chili" the most tolerant one especially for the highest concentrations of 10 and 12 g/l of NaCl.

Production of fresh and dry matter

Increased salt stress level also reduced the production of fresh and dry matter in radicle and plumule for all cultivars, although the reductions

were the lowest in "Anaheim Chili" cultivar (Figure 4 and 5). The most reduction (length and weight) in radicle and plumule was observed when salinity reached 12 g/l of NaCl.

The highest dry weight of plumule and radicle was observed for "Anaheim Chili" cultivar. While "Baldi" cultivar showed highest reduction (22.75 %) of plumule dry weight (from 0 to 12 g/l) and had the highest reduction (50 %) of radicle dry weight.

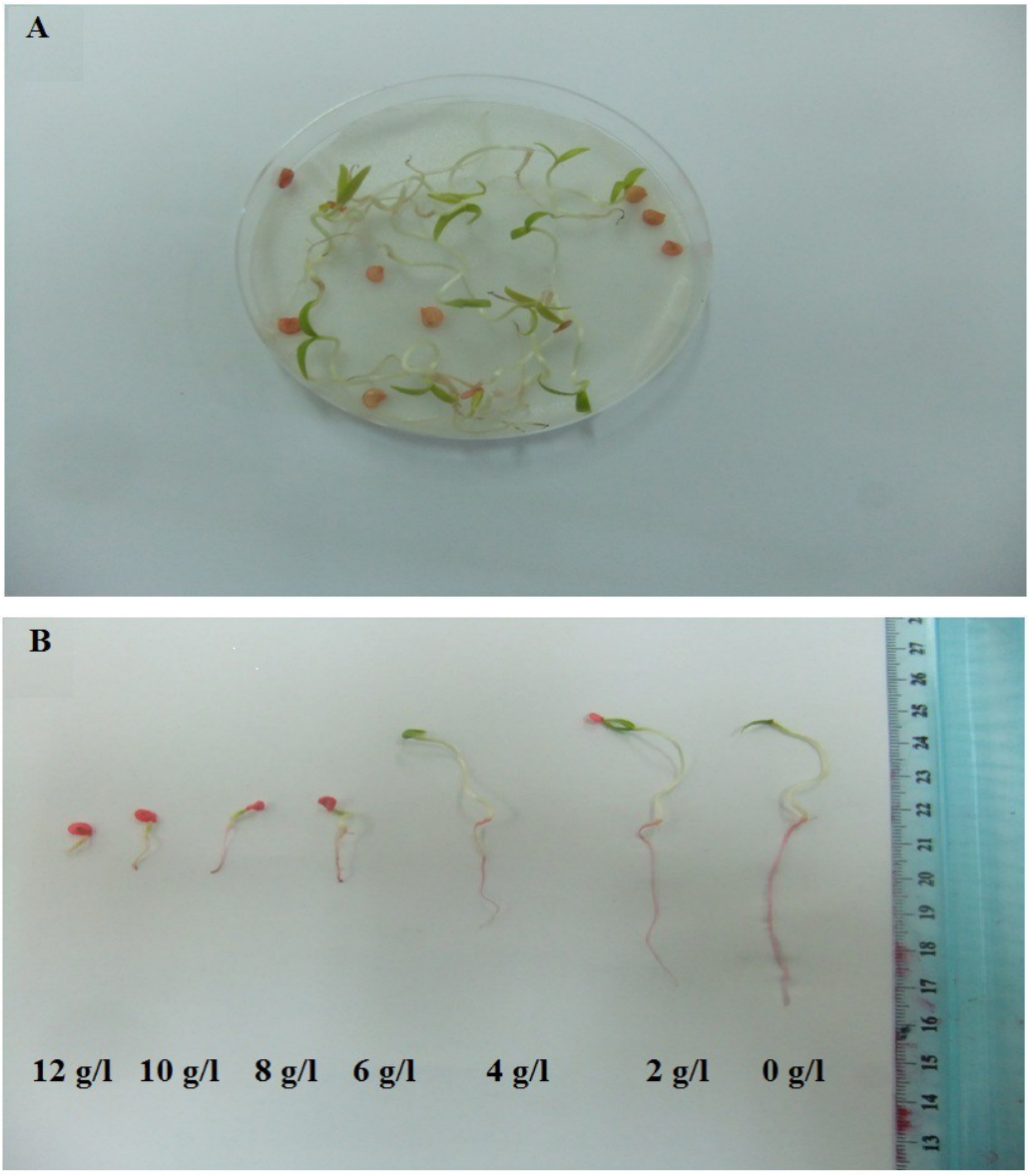

Figure 1. (A) Germination in Petri dish of pepper seeds. (B) Effect of salt stress on radicle and plumule length of Pepper seedlings ("Anaheim Chili" cultivar).

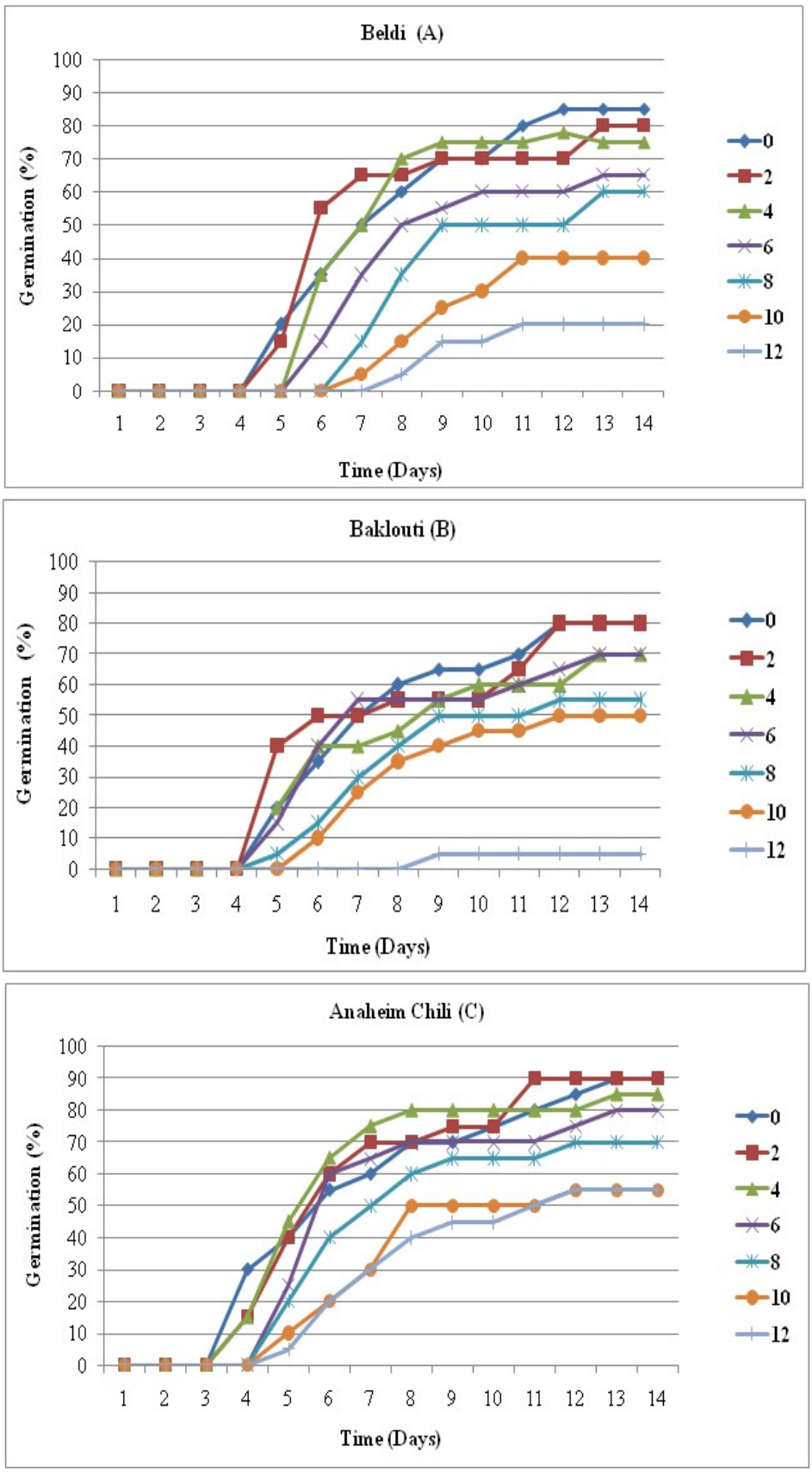

Figure 2. Effect of different salinity levels on Kinetics and total germination percentage of three pepper cultivars: "Beldi", "Baklouti" and "Anaheim Chili"

Table 1 : The effects of NaCl salinity on Mean Germination Time (MGT), Germination Index (GI) and Coefficient of Velocity (CV) of three paper cultivars : "Beldi" (BL) , "Baklouti" (BK) and "Anaheim Chili" (AC) .

		NaCl (g l^{-1})					
	Cultivar	**0**	**2**	**4**	**6**	**8**	**10**
MGT	**BL**	6.93 ^{e}	7 ^{de}	7.47 ^{cd}	7.86 ^{cd}	8.38 ^{bc}	8.62 ^{ab}
	BK	7.13 ^{e}	7.28 ^{de}	7.38 ^{cd}	7.57 ^{cd}	7.63 ^{bc}	8 ^{ab}
	AC	5.50 ^{e}	6.50 ^{de}	6.68 ^{cd}	6.71^{cd}	7.1^{bc}	7.26 ^{ab}
GI	**BL**	2.45 ^{ab}	2.47 ^{a}	2.19 ^{b}	1.32 ^{c}	1.16 ^{d}	0.89 ^{e}
	BK	2.85 ^{ab}	2.38 ^{b}	2.09 ^{b}	2.02 ^{c}	1.50 ^{d}	1.29 ^{e}
	AC	3.16 ^{ab}	3.07 ^{a}	3.1^{b}	2.58^{c}	2.05^{d}	1.59^{e}
CV	**BL**	14.58^{a}	14.28^{b}	13.41^{bc}	12.71^{cd}	11.96^{cd}	11.61^{de}
	BK	14.38^{a}	13.73^{b}	13.56^{bc}	13.22^{cd}	13.01^{cd}	12.4^{de}
	AC	18.22^{a}	15.58^{b}	15.08^{bc}	14.89^{cd}	14.48^{cd}	13.81^{de}

Means followed by the same letters are not significantly different according to Duncan test at 5% level.

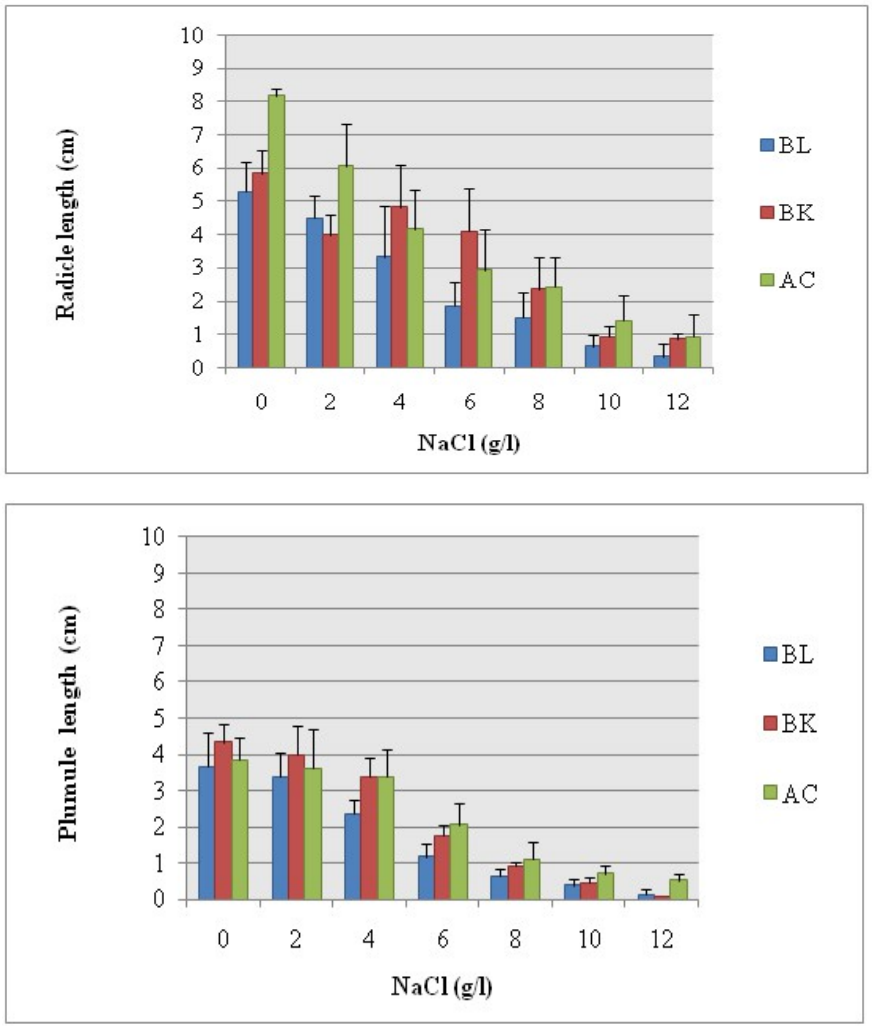

Figure 3. Effect of different level of salinity on radicle and plumule length of three pepper cultivars "Beldi" (BL), "Baklouti" (BK) and "Anaheim Chili" (AC).

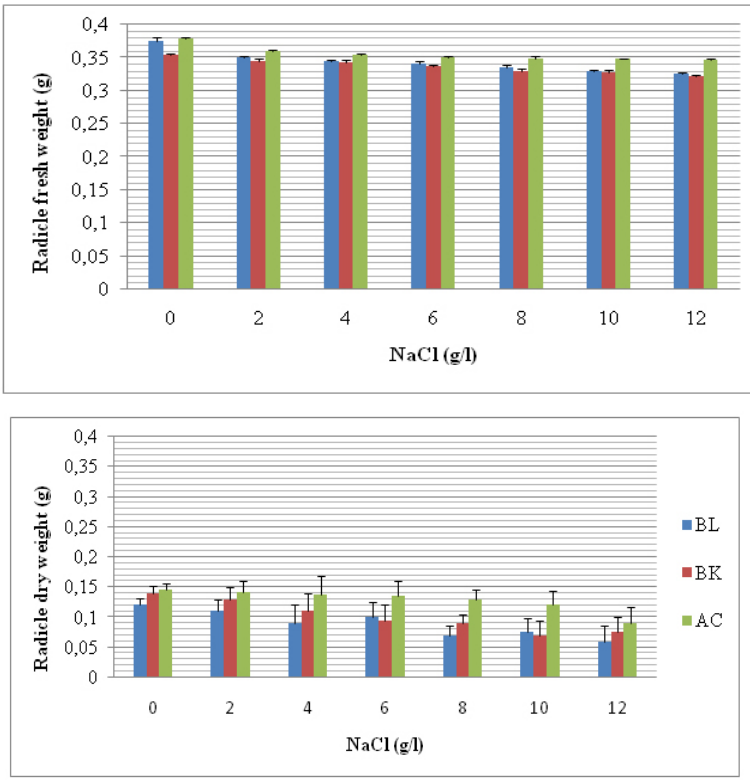

Figure 4. Effect of different level of salinity on radicle fresh and dry weight (g) of three pepper cultivars "Beldi" (BL), "Baklouti" (BK) and "Anaheim Chili" (AC).

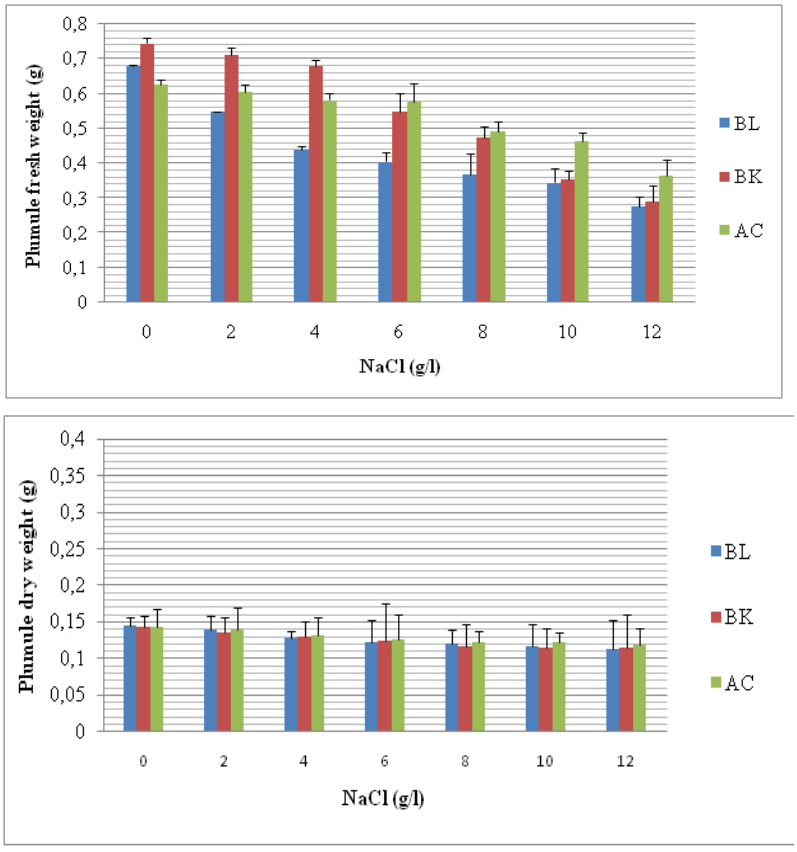

Figure 5. Effect of different level of salinity on plumule fresh and dry weight (g) of three pepper cultivars "Beldi" (BL), "Baklouti" (BK) and "Anaheim Chili" (AC).

DISCUSSION

According to Ayaz *et al.* (2000), the reduction in total seed germination under conditions of salt stress is due to some metabolic disorders. It seems that the decrease of total germination percentage is related to the reduction in water absorption into seeds (Hadas, 1977).

Gulzar and khan (2001) have reported that NaCl stress prevents water absorption by seeds and decreases significantly total germination percentage. Keshavarzi (2011) and Keshavarzi *et al.* (2011) have found the same result respectively on some savory and spinach cultivars. In fact salinity affects germination in two ways , first by decreasing the osmotic potential which retard or prevent the uptake of water and second by a toxic accumulation of ions which damages the embryo. These results have implications on the parameters of germination such as total germination percentage, germination index and the coefficient of velocity which are badly affected. In addition, germination is delayed and Mean Germination Time increased with salt stress. The same results were reported by Zapata *et al.* (2003) on lettuce (*Lactuca sativa* L.) and Chartzoulokis and Klapaki (2000) on pepper.

Plumule was more suppressed than radicle by salinity at all salt concentration levels. Similar results were found by Keiffer and Ungar (1997), Kaya *et al.* (2008) and Moud and Maghsoudo (2008), who showed that shoots of seedlings were more sensitive to salt stress than roots. This can be the result of a fast osmotic adjustment occurring

in roots. The gradual decrease in plumule length with increase in NaCl stress could be due to an inhibitory effect of NaCl salt in shoot growth. It seems that reduction in seedling fresh weight may be due to a decrease in water uptake by seedling under salinity. Similar results were reflected by Anantharaju and Muthiah (2007).

Also, Jabeen *et al.* (2003) reported decline in radicle growth under saline conditions. In addition, Khan *et al*. (1997) indicated that the plumule length significantly decreases with an increase in salt concentrations. These results demonstrated that the mechanism of tolerance to salinity is not yet fully developed in these young seedlings of pepper. and this characterize some sensitive plants in early seedling stages (Almodares *et al*., 2007). Salinity has both osmotic and specific ionic effects on seedlings growth (Dioniso-Sese and Tobita, 2000). Similarly, toxic ion accumulation (Na^+ and Cl^-) affect negatively the metabolism of plants (Grieve and Fujiyama, 1987). It has also been reported that salinity suppresses the uptake of essential nutrients like phosphorus (P) and potassium (K) (Nasim *et al*., 2008), which could affect adversely seedlings growth.

Similar results were reported by Munns (2002). Vicente *et al.* (2004) demonstrated that the reaction of plants to salt stress varied according to the stage of plant development. That 's why these pepper cultivar might be sensitive at early stage but it could be tolerant at later stage growth. However, based in these calculated parameters, the "Anaheim Chili" is the most tolerant cultivar and can be used in next selection programs.

CONCLUSION

In conclusion, this study demonstrated that seed germination of pepper varied according to the change in NaCl levels. The increase of salt stress decreased all studied parameters on germination and seedling growth and the increase of Mean Germination Time (MGT). We found that "Anaheim Chili" cultivar is the most resistant cultivar to salt

stress on germination stage. However, further studies are needed to assess the agronomic behavior of those cultivars during the later stages of the culture.

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