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ORIGINAL ARTICLE

Effect of sodium chloride on the germination of the seeds of a collection of carrot accessions (*Daucus carota* L.) cultivated in the region of Sidi Bouzid

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Seeds of ten accessions of carrot (*Daucus carota* L.) collected from seven areas in the region of Sidi Bouzid, Lessouda (three accessions: L_1, L_2 and L_3) Faid (one accession: F_4), El Ogla (one accession: E_5), Regueb (two accessions: R_7 and R_8), Lahweze (one accession: L_{10}) Garet Hdid (one accession: G_{11}) and Souk Jedid (one accession: S_{12}) were germinated at 20° C in the dark and in the presence of NaCl (0, 2, 4, 6, 8, 10, 12, 14 and 16 g/l) during 14 days. The results obtained showed that germination is possible until the highest concentration of salt (16 g/l) but germination and speed of germination decrease according to the concentration, so the accession L_3 was the most tolerant and accessions L_1, L_2 and S_{12} was the most sensitive.

Key words: Carrot, accessions, germination, NaCl, Sidi Bouzid

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Salinity affects 5% of cultivated land in the world (Munns *et al.*, 1999). In Tunisia, saline soils occupy an area of 1.5 million hectares, or about 25% of the total area of arable land in the country (Hachicha *et al.*, 1994). This salinity is caused by brackish water for irrigation, the aridity of the climate and the excessive intake of chemical fertilizers (Habib *et al.* 1994). Irrigation from dam waters presents a load in salts of 2 to 3 g/l, while those of wells contain 4 to 7 g/l (Hachicha and Braudeau, 1998). Indeed, the culture of carrot in the region of Sidi Bouzid, localized in the center of Tunisia and first production area of carrot, suffers especially at the stage of seed germination, the most sensitive phase to NaCl (Ben Ahmed, 1995, Misra and Dwivedi, 2004). In fact, salts reduce the faculty and/or energy of germination (Bayuelo-Jimenez *et al*., 2002) by increase in the osmotic pressure of the soil solution, which slows imbibitions and limit absorption of water necessary for the onset of the metabolic processes involved in germination.

Thus, the objective of this work is to study the effect of salt stress on germination of ten accessions of carrot grown in the region of Sidi Bouzid. And to subsequently identify which could be irrigated with brackish water unless his performance is significantly decreased.

MATERIALS AND METHODS

The plant material used consists of ten accessions of cultivated carrot, their seeds are collected in the region of Sidi Bouzid, from seven areas: Lessouda (3 accessions: L_1, L_2 and L_3) Faid (1 accession: F_4) El Ogla (1 accession: E_5) Regueb (2 accessions: R_7 and R_8), Lahweze (1 accession: L_{10}), Garet Hdid (1 accession: G_{11}) and Souk Jadid (1 accession: S_{12}). These seeds are disinfected in a solution of bleach to 50% for 20 minutes. They are then rinsed 4 times with distilled water and put to germinate in petri dishes of 90*14 mm in dimensions between two layers of blotter paper at the rate of 50 seeds/box. These boxes are, subsequently, placed in a germinator set at a temperature of 20 °C and total darkness for 14 days. Seeds of each accession are germinated in absence (NaCl =0 g/l, control concentration) or presence of NaCl (2, 4, 6, 8, 10, 12, 14 and 16 g / l), each salt concentration (including the control concentration) is represented by 150 seeds divided into three Petri dishes (50 seeds / petri dish). The control seeds are germinated in the presence of distilled water.

Germinated seeds are counted daily to determine the capacity and speed of germination. The length of the radicle is measured on the 14th days (end of the experiment) with a Vernier caliper. A seed is considered germinated when the radicle length exceeds one mm. The formula used to calculate the capacity of germination (CG) is as follows: CG (%) = TNGG / TNG X100, where TNGG: total number of germinated seeds; TNG: total number of tested seeds.

For each parameter measured, the averages of the various treatments and their ecartypes are determined. To release the effects of saline treatment, the results are submitted to analysis of variance using statistical software "SPSS 13.00". The comparison of the means was performed by the Student-Newman-Keuls test threshold probability of 5%.

RESULTS

Capacity of germination

Although it does not fully reproduce the behavior of plants in the field, capacity of germination in salt stress conditions, always gives a more likely or less precise behavior of varieties studied (Ben Naseur *et al*., 2001).

The observation results of the analysis of variance summarized in Table 1 shows that the main source of variation in the capacity of germination is the concentration of NaCl in the imbibing solution that represents 90% of the total variation of the test. It is followed by interaction accession x salinity and finally the accession. After fourteen days of germination, seeds germinated in the absence or presence of NaCl (fig. 1). In absence of salt, the rate of germinated seeds exceeds 70%, it varies from 77 (accessions F_4 and G_{11}) to 93% (accession S_{12}). But the seeds that did not germinate, may be dormant (embryonic or tegumental dormancy) or their health status is defective (Bosland and Votava, 2000; Ozçoban and Demir, 2002).

In the presence of salt (NaCl: 2 to 16 g/l), seeds germination is still possible even under the highest concentration (16 g/l), it is the case of accessions L3, F4, R8 and L10. In addition, the percentage of germinated seeds of ten accessions (L_1, L_2, L_3, F_4, E_5, R_7, R_8, L_{10}, G_{11} and S_{12}) decreases depending on the salt concentration (fig.2). Indeed, beyond the concentration 10 g/l NaCl, it dropped below 50% for the different accessions investigated. However, until the concentration of 8 g/l, the rate of seeds germination (80%), agronomically acceptable, concerned only the accession S_{12}. Which militates in favor for the exploitation of brackish water (salt: 4 to 5 g/l) used for crop irrigation, in this case culture of carrot (Mangal *et al*, 1989; Özturk. *et al.*, 2009 and Rode *et al.*, 2012).

Speed of germination

According to Figure 3, in absence of salt, the germination rate evolve according to a sigmoid curve which has three phases: a lag phase of two days, period of seed imbibition, increasing phase which run from 5 (accession S_{12}) to 10 days (accessions L_1 and L_2) characterizing the emergence of the radicle and a stationary phase, starting from the 6th (accession S_{12}), 8th (accessions F_4, E_5 and G_{11}), 9th (accessions R_7 and R_8), 10th (accessions L_3 and L_{10}) or 11th day (accessions L_1 and L_2) of setting in germination indicating germination of all non-dormant seeds.

In the presence of salt, the curve for the germination rate loses its sigmoid shape as well as the NaCl concentration increases, from 2 to 16 g/l. For example, with the highest concentration (16 g/l), this curve becomes linear for ten accessions studied. In addition, the first phase (lag phase) is extended with salt concentration, especially with the highest concentration (14 g/l), it is 4 (accessions L_3 and L_{10}) to 5 days (accessions L_1, R_7, R_8 and G_{11}) or even 6 days (accessions F_4 and E_5). Then salt prevents the imbibition of seeds and therefore delays the emergence of the radicle. Elongation of duration of seeds germination and the decrease in the speed of germination of all accessions, depending on the salt concentration, due to a delay in the establishment of the internal mechanisms of osmotic adjustment (Bliss *et al.*, 1986).

Length of radicle

Table 2 represents the analysis of variance of the length of the radicle for treatments ranging from 0 to 16 g/l NaCl shows that it is also the concentration of NaCl which is the main source of variation (72% of the total variation of the test); It is followed by interaction salinity x accession (14% of the total variation of the test) and finally by the accession. The results, presented in figure 4, show that the increase in NaCl concentration reduces the length of the radicle in all accessions. Indeed, in the absence of salt, the length of the radicle measured after 14 days ranged from 23 (accession F_4) to 94 mm (accession S_{12}). But, in the presence of salt in the germination medium (2 to 16 g/l), the length of the radicle decreases as the NaCl concentration (Allagui *et al*, 2005; Bhardwaj *et al*, 2010; Eskandari and Kazemi, 2011). For example, the concentration 16 g/l, where the measurement of the radicle is possible, caused a reduction of 98% (accessions L_3, R_8 and L_{10}) to 100% in the length (accessions L_2, G_{11} and S_{12}) compared with control plants.

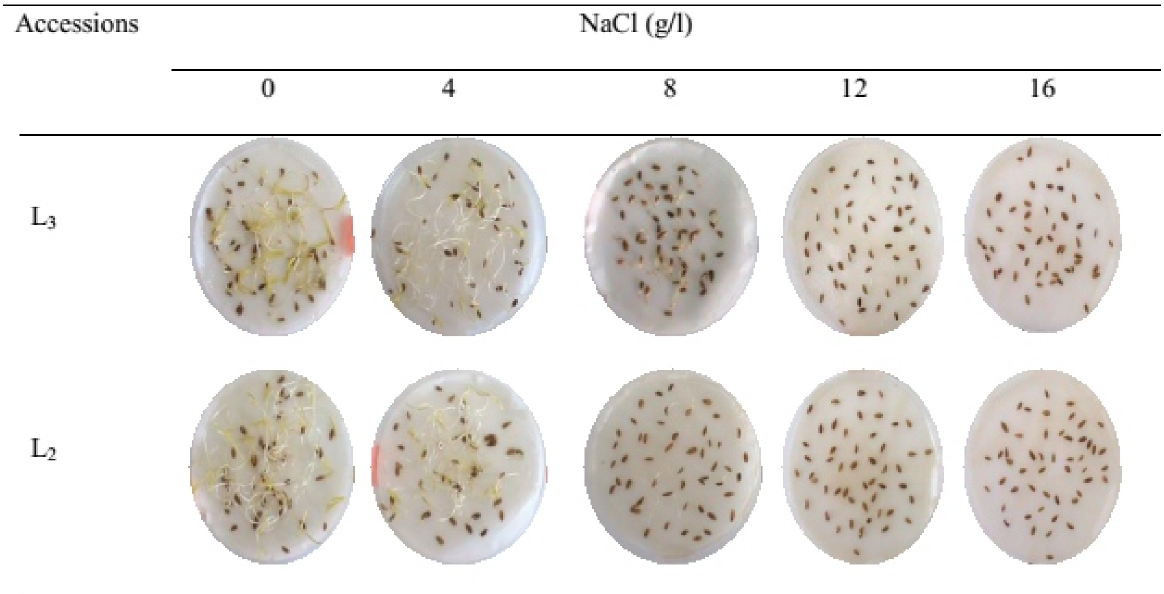

Figure 1. Seeds of two accessions of carrot grown in Sidi Bouzid, the most sensitive (L_2) and the more tolerant (L_3) germinated under different NaCl concentrations (0, 4, 8, 12 and 16 g/l) after 14 days of culture at 20 °C and total darkness.

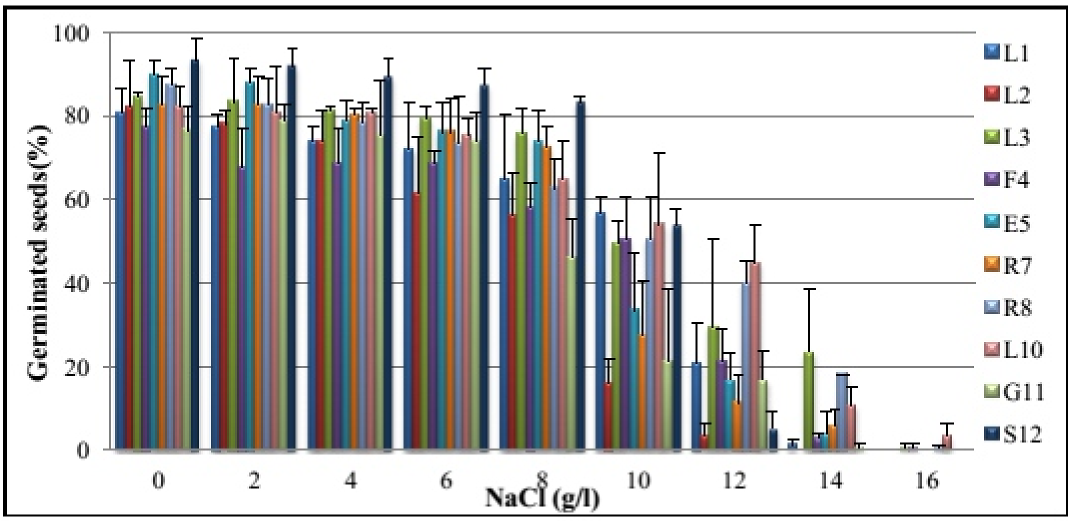

Figure 2. Effect of NaCl on the capacity of germination of ten accessions of carrot grown in Sidi Bouzid (L_1, L_2, L_3, F_4, E_5, R_7, R_8, L_{10}, G_{11} and S_{12}).

Table 1 Analysis of variance of the germination percentage parameter

Source of variation	Type II sum of squares	df	Mean square	F	Sig.
Total variance	299544,330	269	-	-	-
Treatment effect (1)	269145,496	8	33643,187	602,125	,000
Accession effect (2)	7184,922	9	789,325	14,288	,000
Interaction (1*2)	13156,578	72	182,730	3,270	,000
Error	10057,333	180	55,874	-	-

* The F-test is that of Fisher (at the risk of 5%).

Table 2 Analysis of variance of the length of radicle parameter

Source of variation	Sum of squares	df	Mean square	F	Sig.
Total variance	187494,776	269	-	-	-
Treatment effect (1)	134165,874	8	16770,734	225,311	,000
Accession effect (2)	14300,574	9	1588,953	21,347	,000
Interaction (1*2)	25630,244	72	355,976	4,782	,000
Error	13398,084	180	74,434	-	-

* The F-test is that of Fisher (at the risk of 5%).

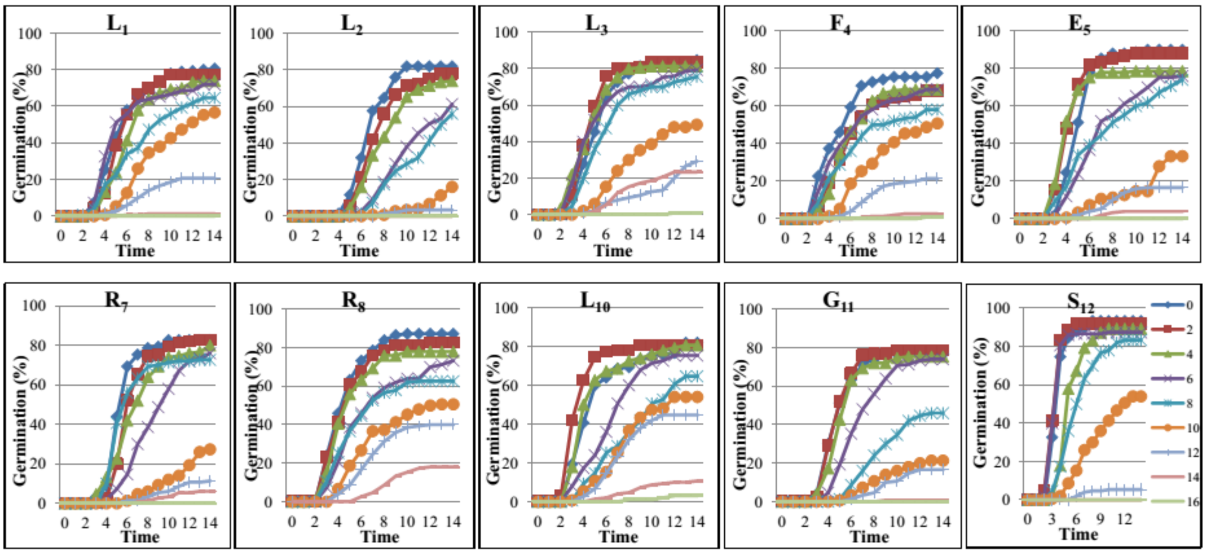

Figure 3. Effect of NaCl on the kinetics of germination ten accessions of carrot grown in Sidi Bouzid (L_1, L_2, L_3, F_4, E_5, R_7, R_8, L_{10}, G_{11} and S_{12}).

DISCUSSION

Germination is considered as a critical step in the cycle of development of the plant. Indeed, it affects the installation of seedling growth in the middle and probably his subsequent productivity (Tremblin and Binet, 1984). Our study shows that NaCl decrease the capacity of germination of ten accessions of carrot studied and slows their speed of germination. These effects are more marked when the concentration of salt is high. Agronomically, germination percentage should be higher than 50%, in this case the capacity of germination was significantly reduced from 10 g/l NaCl in accessions L_2, E_5, G_{11} and R_7 and 12 g/l NaCl in other accessions studied. This is in agreement with the results of Rode *et al.* (2012), which showed a reduction of 20 to 50% of the rate of germination with concentration 9 g/l NaCl. According to our results, the salt tolerance depends on accession, such note is reported in melon (Botia *et al.*, 1998), radish (Noreen and Ashraf, 2008), pepper (Ibn Maaouia *et al.*, 2011) and carrot (Rode *et al.*, 2012). Increasing of NaCl concentration in the imbibing solution resulted in a carrot seed elongation of germination period (two days for the control seeds) up to six days for the seeds of accessions F_4 and E_5 (NaCl: 14 g/l). This delay of germination is due to difficulties of water supply for the seeds (Bajji *et al.*, 2002) or excessive accumulation of ions Na^+ and Cl^- in the embryo, leading to alteration of metabolic processes of germination such as respiratory and mitotic activity and in extreme cases, death of the embryo by ion excess (Aghdhafna, 1990, Groom *et al.*, 1991.).

As for the length of the radicle, the results obtained show that the roots grow in the presence of salt, but for NaCl concentrations exceeding 4 g/l, all accessions respond negatively and in the same way. Thus, the minimum is reached at 16 g/l, there is still more visible in four accessions L_3, F_4, R_8 and L_{10}. This reduction in length would be due to a stop of the division and cell elongation at the level of the root (Fraser *et al.*, 1990).

Taking into account the results of the capacity and period of germination, it appears that at the germination stage accession L_3 is more tolerant to NaCl and accessions L_1 , L_2 and S_{12} the most sensitive. However, results of test of germination can not be extrapolated at later stages of the plant. Indeed, the work of Nerson and Paris (1984) and Botia *et al.* (1998) on melon, report opposite results between tolerance of studied varieties to germination and growth stage. Similarly, Maas and Poss (1989) and Maas and Grattan (1999) reported that most plants are more salt tolerant to germination then emergence and early stages of growth. Therefore, the study of germination under salt stress does not appear sufficient for detecting salt-tolerant genotypes. It is important to complete the work by stages of growth and fruiting.

CONCLUSION

The results reported in this study suggest that the carrot is a tolerant plant to the action of NaCl at the stage of germination. With salt concentrations which exceed 10 g/l, capacity and speed of germination are strongly affected. The depressive effects of salt are essentially of osmotic nature but at high concentrations (16 g/l NaCl) toxicity phenomena can be manifested. In fact, in the presence of 14 g/l of salt concentration, the accession L_3 has the best germination behavior. However, all three accessions L_1, L_2 and S_{12} prove to be the most sensitive.

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