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Effects of salt stress on the germination of two contrasting *Medicago sativa* varieties.

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Abstract

Selection strategies for increased salt tolerance in *Medicago sativa* must involve several growth stages, with the germination stage as a particularly important stage. At this stage, *M. sativa* is known to be more sensitive to salt than later growth stages. Cultivars differ significantly in their capacity to germinate under saline conditions. For this consideration and in order to analyze the response to salinity, initial screening at the germination stage for two varieties of *M. sativa* was undertaken. Seeds were germinated in Petri dishes under control treatment (0 mM NaCl) and four concentrations of NaCl (100, 150, 200 and 250 mM). Results from ANOVA showed that variation in root length (RL), fresh root weight (RFW) and shoot fresh weight (SFW) was explained by variety effect. The variation in hypocotyl length, (HL) SFW and RL was explained by the interaction of variety x treatment. Under salt stress, there was a major reduction (46, 66%) in final germination rate (GR) when seeds were geminated in 250 mM NaCl. The RL and HL were affected by salt stress and their respective values were reduced by more than 40% in 200 mM NaCl. The Californian variety was lesser affected by salt stress for RL and RFW while the local variety from El Hemma showed the lowest reduction for SFW. Further work is needed to asses these effects in later growth stages and in field conditions under salt stress.

1. INTRODUCTION

Salinity is a major abiotic stress that limits crop production (Ashraf, 2009). Hu and Schmidhalter (2005) reported that about 40000 ha of land becomes unavailable for agricultural production worldwide each year due to salinization, and approximately 50% of irrigated lands are currently affected by salinity (Elwan and El Shatoury, 2014). Salinity can affect seedling germination and growth by creating an osmotic pressure that prevents water uptake or by toxicity driven by sodium and chloride ion accumulation. Seed germination capacity is one of the most critical phases of plant establishment, and hence, is greatly influenced by salinity (Záborszky et al., 2002). Selection strategies for salt tolerant genotypes must involve several growth stages and great emphasis must be placed on the germination stage because it is a key factor in the lifecycle of a plant (Guan et al., 2009). According to Flowers and Flowers (2005), efforts to produce salt-tolerant crops have involved the use of artificial selection like in vitro selection and the development of transgenic plants, molecular biology techniques and conventional breeding approaches such as the domestication of halophytes and the manipulation of cultivated crop species through breeding programs (Ashraf, 2004). These approaches used the extent of genetic variation available within species. For some species, the genetic variation is a determinant factor that helps them to overcome environmental changes through the process of evolutionary adaptation (Mousavi-Derazmahalleh et al., 2018). *Medicago sativa* is the most cultivated perennial forage crop worldwide. It is an autotetraploid (2n = 4x = 32), a cross-fertilized species with higher genetic intra-population diversity compared to the inter-population diversity (Soltaniet al., 2012). It can be a candidate for the improvement of marginal degraded lands affected by salinity. In order to develop new *M. sativa* genotypes with high salt tolerance, the first important step is to lay out useful and substantial genetic variation in tolerance to salinity stress (Soltani et al., 2012). For this reason, the intention of this work is to analyze the responses of two contrasting varieties of *M. sativa* to salinity stress at the germination stage.

2. MATERIALS AND METHODS

Screening of two varieties of *M. sativa* was undertaken at the germination stage. These two varieties include the local Tunisian variety of El Hemma (selected by Prof Majid Mezni and Prof Hichem Ben Selem, and registered in 2009 in the National Catalog of Plant varieties JORT N°26 of 11/19/2011) and provided by Prof Aziza Zoghlami from the National Institute of Agronomic Research of Tunis (INRAT) and the Californian variety kindly provided by Mr. Mohamed Abdelhak Khorchani from the Tunisian Grain Company (COTUGRAIN). Ten seeds were germinated in each Petri dish with double-layer filter paper. The experimental treatments consisted of a range of five concentrations of NaCl (0, 100, 150, 200 and 250 mM). Three replicates per variety and per treatment were used. In each Petri dish, 5 mL of the appropriate concentration of NaCl were added every two days and placed in an incubator in constant darkness at 25°C. Germinated seeds were counted daily for 7 days. Seeds were considered to have germinated after radicle emergence. During the experiment, and when each Petri dish reached more than 70% of germination, seedling (with fully expanded cotyledonary leaves) were transferred to a phytotron (16h light/8h darkness, 80% humidity) until the end of the experiment, which lasted for 10 days. The measured physiological parameters were the final germination rate (%GR), root length (RL) (cm), hypocotyl length (HL) (cm), root fresh weight (RFW) (g) and shoot fresh weight (SFW) (g).

The two-ways variance analysis (variety and treatment) was performed using the GLM procedure. Estimated means of measured traits for the studied varieties was performed using MEANS procedure while their significance was determined using Duncan's test at the 5% level. Correlations between measured physiological parameters were calculated using the CORRESP procedure. All the above described analyses were performed using the SAS software (2002).

3. RESULTS AND DISCUSSION

3.1. Variation of parameters of germination under salt stress

The variation of measured parameters of germination for the studied varieties was explained by the effects of variety, treatment and the interaction of variety x treatment. The variation of root length (RL), seedling root fresh weight (RFW) and shoot fresh weight (EFW) was explained by variety effect (Table 1). Furthermore, the variability of hypocotyl length (HL), shoot weight (SFW) and RL was explained

Table 1. Effects of variety (var), treatment (trea) and the interaction of variety x treatment on the variation of measured parameters of germination for the two varieties of *Medicago sativa.*

Parameters	Means	MS (trea) df=4	MS (var) df=1	MS (var x trea) df=4	F (trea)	F (acc)	F (var x trea)
GR	88	3228.333	13.951	3.456	130.1***	0.54 ns	0.87 ns
HL	0.431	1.45817618	0.0000063	0.4775835	11.89***	0 ns	3.89*
RL	2.288	110.9892609	13.9508142	3.4561118	89.63***	11.27***	2.79**
SFW	0.011	0.00078521	0.00095417	0.00014964	19.23***	23.37***	3.66**
RFW	0.007	0.00081496	0.00012883	0.00004503	25.44***	4.02*	0.2324ns

MS: mean square, df: degree of freedom, ns: not significant (p >0.05), * : (p ≤0.05), ** : (p ≤0.01), *** : (p≤0.001).

by the interaction of variety x treatment (Table 1), suggesting that the studied varieties do not have the same behavior under the different treatments.

The variation of the final germination rate (GR) under salt stress is given in Fig. 1. The results obtained for the two varieties showed that maximum germination rate was observed under control treatment (0 mM) and under 100 mM, 150 mM and 200 mM NaCl. Similar results were reported by Soltani et al. (2012) in their screening of twenty *M. sativa* cultivars towards tolerance to salinity stress at the germination stage. They found that the maximum germination percentage was observed in control (0 mM) and 75 mM NaCl. While in 250 mM NaCl, the germination rate was severely affected since the capacity of seeds to germinate was reduced by more than 50%. Similar results were observed by Guan et al. (2009) who observed a decrease of 55% in germination for M. ruthenica under 200 mM NaCl. Furthermore, Soltani et al. (2012) found that the maximum reduction in germination rate for their studied cultivars was observed in 225 mM NaCl. According to El-Madidi et al. (2004), the inhibition of germination and emergence by salt stress is caused by an osmotic effect. This is in line with previous studies which suggest that the germination stage is the most sensitive stage of development to salt stress (El-Madidi et al. 2004).

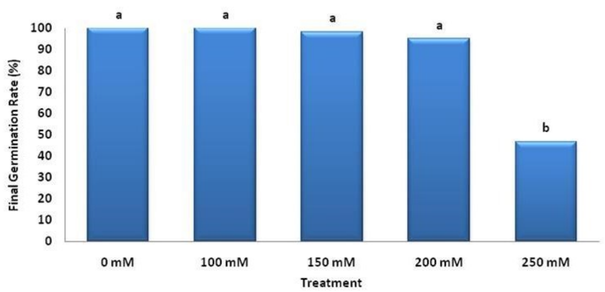

Fig. 1. Variation of the final germination rate (GR) of two *Medicago sativa* varieties under salt stress. Means with similar letter(s) under each treatment are not significantly different at 5% probability level according to Duncan's multiple range test.

The hypocotyl length (HL) (Fig. 2.) was affected by salt stress while the root length (RL) (Fig. 3.) was greatly reduced by increased salt concentration. A severe decrease for HL (approximately 81%) was observed in 250 mM NaCl. For RL, the major reduction (86.85%) was observed in 250 mM NaCl but the reduction (73.54%) was already more pronounced under 200 mM NaCl. Soltani et al. (2012) reported the importance of radicle length in providing an important clue to the response of plants to salinity. The same authors noted a reduction in presence of salt stress for radicle length, plumule length and seedling length for studied cultivars of *M. sativa*. The radicle length showed a reduction of 79% while the plumule length and seedling length decreased by 66% and 72%, respectively. These values of reduction of the analyzed parameters were obtained in 225 mM NaCl (Soltani et al. 2012), whereas highest levels of reduction were registered in 250 mM NaCl for our study. Bhardwaj et al. (2010) and Monirifar (2008) also confirmed that with increasing salinity concentration, radicle, plumule and seedling length decrease.

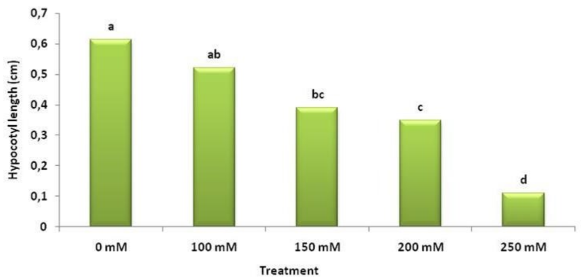

Fig. 2. Variation of hypocotyl length (HL) in two *Medicago sativa* varieties under salt stress. Means with similar or common letter(s) under each treatment are not significantly different at 5% probability level according to Duncan's multiple range test.

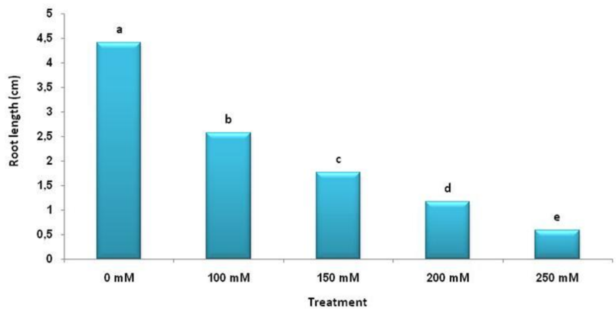

Fig. 3. Variation of root length (RL) in two *Medicago sativa* varieties under salt stress. Means with similar letter(s) under each treatment are not significantly different at 5% probability level according to Duncan's multiple range test.

Salt stress affected the shoot fresh weight (SFW) (Fig. 4.) while most reduction was observed under 250 mM NaCl. However, we

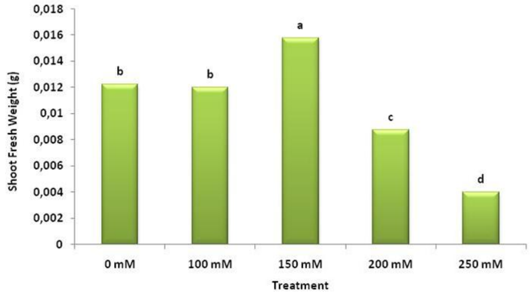

Fig. 4. Variation of the shoot fresh weight (SFW) in two *Medicago sativa* varieties under salt stress. Means with similar letter(s) in each treatment are not significantly different at 5% probability level according to Duncan's multiple range test.

observed an increase of shoot fresh weight in 150 mM NaCl compared to the control treatment. Such result was also reported by Parida and Das (2005) and Kurban et al. (1999) where the authors found that in Alhagi pseudoalhagi (a leguminous plant), total plant weight increased at low salinity (50 mM NaCl) but decreases under high salinity (100 and 200 mM NaCl). Salt stress also affected root fresh weight (Fig 5) while most reduction was observed under 250 mM NaCl. According to Shannon and Grieve (1999), salinity affects seedling morphology and plant development. The main effect is the reduction of growth rate, including reduction in length and mass of roots, which may become thinner or thicker. Salt stress also results in a considerable decrease in the fresh and dry weights of leaves, stems, and roots Parida and Das (2005).

Correlations between measured parameters of germination (Table 2), showed that the strongest and significant correlations were between root length (RL) and root fresh weight (RFW) (r= 0.555, p≤0.001) and between root length (RL) and hypocotyl length (HL) (r= 0.40, p≤0.001).

3.2. Analysis of the response of *Medicago sativa* varieties under salinity

The studied varieties showed similar values of final germination rate (GR) under salt stress (Fig 6). They exhibited high germination rates (95% and 96.7% for El Hemma and Californian, respectively) in control treatment and in low to moderate levels (from 50 mM to 200 mM) of salinity. The most pronounced decreases of GR under 250 mM NaCl were of 50% and 43.3% for El Hemma and Californian varieties, respectively. Several investigations of seed germination under salt stress have indicated that seeds of most

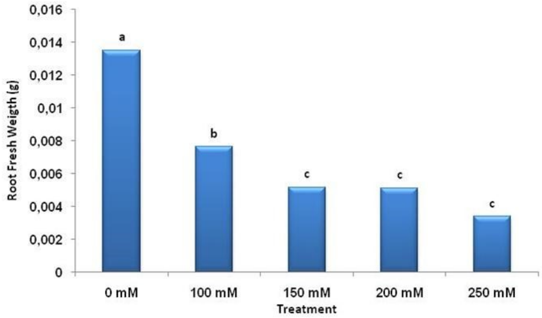

Fig. 5. Variation of the root fresh weight (RFW) of two *Medicago sativa* varieties under salt stress. Means with similar letter(s) in each treatment are not significantly different at 5% probability level according to Duncan's multiple range test.

Table 2. Correlations between measured parameters of germination for the two varieties of *Medicago sativa.*

	RL	HL	SFW	RFW
RL	1.00000			
HL	0.399***	1.00000		
SFW	0.084ns	0.283***	1.00000	
RFW	0.555***	0.220***	0.061ns	1.00000

ns: not significant (p >0.05), * : (p ≤0.05), ** : (p ≤0.01), *** : (p≤0.001)

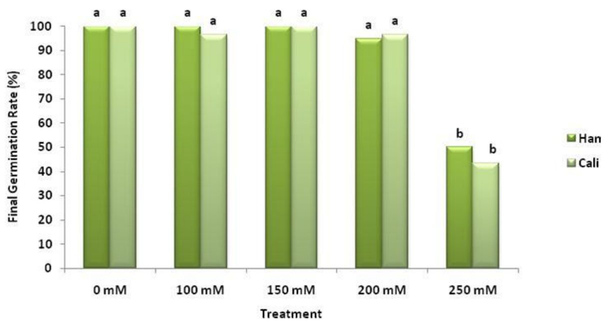

Fig. 6. Classification of *Medicago sativa* varieties based on the final germination rate (GR) under salt stress.

species achieve their maximum germination in distilled water (Torabi et al. 2011; Soltani et al. 2012) while Soltani et al. (2012) found that twenty alfalfa cultivars had a maximum of 68% germination rate under 225 mM NaCl.

The hypocotyl length (HL) was severely reduced for the two varieties in 250 mM NaCl (Fig 7). The El Hemma variety had the longest hypocotyls in the control condition while it is the most affected by salt stress. The Californian variety showed an increased hypocotyl length (by 29.5%) in 100 mM NaCl compared to El Hemma and it exhibited a moderate reduction of the hypocotyl length up to a concentration of 200 mM NaCl. Such difference in response to salt stress was also reported by Soltani et al. (2012), who observed a significant variation in salt tolerance among their studied cultivars of *M. sativa*.

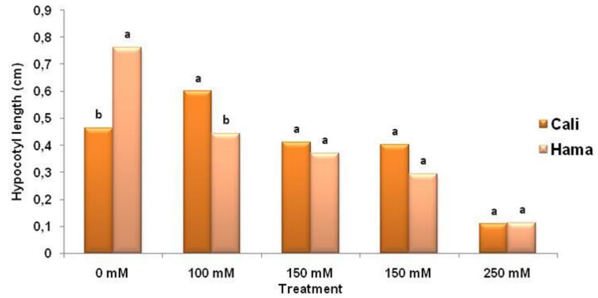

Fig. 7. Classification of *Medicago sativa* varieties for hypocotyl length (HL) under salt stress.

The two varieties of *M. sativa* showed similar root length (RL) under the control condition (Fig 8). The California variety was lesser affected for root length than El Hemma in 100 mM and 150 mM NaCl while both varieties exhibited similar behavior under excessive concentrations (200 mM and 250 mM) of NaCl. Similarly, Monirifar (2008) reported significant differences in responses among five ecotypes of *M. sativa* under different levels of NaCl.

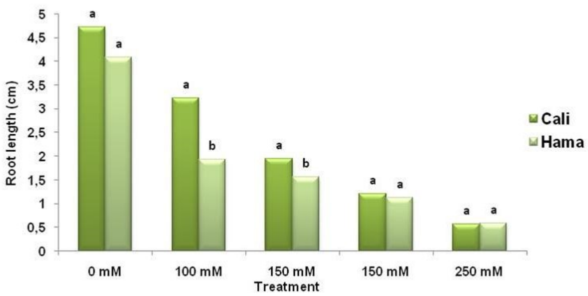

Fig. 8. Classification of *Medicago sativa* varieties for root length (RL) under salt stress. Means with similar letter(s) in each treatment are not significantly different at 5% probability level according to Duncan's multiple range test.

An increase of shoot fresh weight (SFW) of 35.93% and 63.35% were found for the Californian variety under 100 mM and 150 mM NaCl, respectively (Fig 9). Furthermore, only 13.04% of SFW augmentation was noted for the El Hemma variety under 150 mM NaCl. Under 200 mM NaCl, he SFW was slightly decreased (1.11%) for Californian variety while a high reduction (40.8%) was found for El Hemma. Moreover, the highest reduction values of SFW were found for both varieties under 250 mM NaCl. Similar finding was reported by Khan (2001), indicating that fresh and dry weights in Salicornia rubra increase with an increase in salt concentration. For this species, the optimal growth occurs at 200 mM NaCl and the growth declines with a further increase in salinity. Khan et al. (1999) showed that when Halopyrum mucronatum (a perennial grass) is treated with 0, 90, 180, and 360 mM NaCl, fresh and dry mass of roots and shoots peaks at 90 mM NaCl, and the maximum succulence is noted at 90 mM NaCl.

The Californian variety was least affected for root fresh weight (RFW) under 100 mM and 150 mM NaCl (Fig 10). Furthermore, the Californian and El Hemma varieties showed reduction values of 87.96% and 85.62%, respectively, under 250 mM NaCl. In general, osmotic stress caused by salinity is usually immediate and particularly detrimental to seed germination, emergence and seedling vigor (Masters et al. 2007). Moreover, genetic variation for salt tolerance was reported in *M. sativa* (Torabi et al. 2011; Soltani et al. 2012). It has also been reported that, under saline conditions, germination ability of seeds differs from crop to crop and even a significant variation is observed amongst different varieties of the same crop (Soltani et al. 2012).

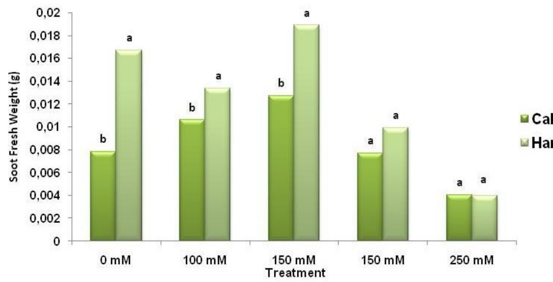

Fig. 9. Classification of *Medicago sativa* varieties for shoot fresh weight (SFW) under salt stress. Means with similar letter(s) in each treatment are not significantly different at 5% probability level according to Duncan's multiple range test.

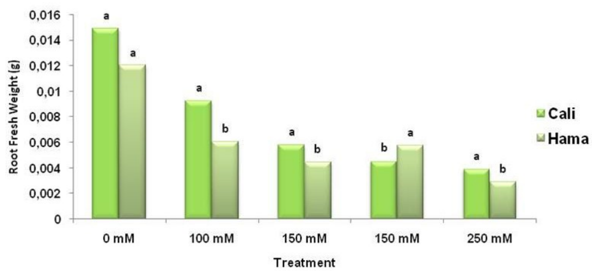

Fig. 10. Classification of *Medicago sativa* varieties for root fresh weight (RFW) under salt stress. Means with similar letter(s) in each treatment are not significantly different at 5% probability level according to Duncan's multiple range test.

4. CONLUSION

Overall, salt stress affected all measured variables for the two varieties, and the percentage of their reduction was more pronounced with augmented salinity level. A significant variation was also found in both studied varieties of *M. sativa* under salt stress for root length (RL), shoot fresh weight (SFW) and root fresh weight (RFW). The Californian variety was the least affected by salt stress for root length and weight (RL and RFW) whereas the local variety El Hemma was most tolerant for shoot fresh weight (SFW). The concentration of 250 mM NaCl caused a reduction of 50% for the germination rate of *M. sativa*. Further work is needed to asses these salinity effects in later growth stages and in field conditions under salinity constraint.

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