

SALINITY AND WATER DEFICIT EFFECTS ON SEED GERMINATION AND RECOVERY OF *LOTUS* POPULATIONS FROM NORTHERN TUNISIA

RIM HAJRI^{1,2*}, CHAYMA OUHIBI³, MOUNA MECHRI⁴, HOUDA KOURDA⁵ AND MONGI BEN YOUNES¹

¹ Pôl Régional de Recherche Développement Agricoles du Nord Ouest semi-aride, Tunisie.

² Institut Supérieur Agronomique de Chott-Mariem, Tunisie.

³ Unité de Physiologie et Biochimie de la Réponse des Plantes aux Contraintes Abiotiques, Faculté des Sciences de Tunis, Tunisie.

⁴ Institut Nationale des Grandes Cultures, Tunisie.

⁵ Banque Nationale des Gènes, Tunisie.

* Corresponding author's email: rimhajri@yahoo.fr

Abstract

Trials were set to assess salinity and water deficit effects on seed germination and recovery of populations of *Lotus creticus* L. and *Lotus ornithopodioides* L. species collected from different bioclimatic regions in northern Tunisia. Salinity treatments used were 0, 50, 100, 150, 200 and 250 mM of NaCl whereas water deficits of 0, -0.2, -0.6, -0.8 and 1.0 MPa were induced using polyethylene glycol 8000 (PEG-8000). Seeds of *L. creticus* L. collected from Tabarka region weighted 3.39 g/1000 seeds which were less than half of this weight for all other *Lotus* populations but having no effect on seeds germination capacity. *Lotus creticus* L. seeds collected from Seddine region showed 80% germination at 250 mM of NaCl compared to 55.7% for Tabarka populations. Seed germination of *L. ornithopodioides* L. was not affected by salinity regardless of population provenance indicating species tolerance to salinity. At -0.4 MPa water potential deficit, seed germination of *L. creticus* L. collected from Seddine region was reduced by 14% compared to 48.5% reduction for seeds from Tabarka provenance which would indicate its tolerance to water stress. *Lotus ornithopodioides* L. seed germination was reduced by 47% at -0.2 MPa and almost inhibited at -0.6 MPa water potential indicating species low tolerance to water deficit. Ungerminated seeds of *Lotus* species due to salinity and PEG-8000 treatments recovered their germination capacity when they were transferred to distilled water regardless of species and provenances indicating osmotic inhibition of seed germination for these two *Lotus* species.

Key words: Salt stress, Endophyte, Antioxidative enzymes, Osmotic adjustment.

Introduction

The *Lotus* genus includes both annual and perennial species, widely distributed throughout the world. It has been used as pasture and forage in the USA and in Europe. *Lotus* species are palatable, nutritious and non-bloating legumes for animals (Díaz *et al.*, 2005). Many species are also widely adapted to different environmental stresses (Escaray *et al.*, 2012). Several research reports indicated that *Lotus* species are tolerant to drought (Bañon *et al.*, 2004), acid soils (Campestre *et al.*, 2016), high salinity (Rejili *et al.*, 2007) and low soil fertility (Belesky, 1999). These important characteristics make *Lotus* species most widely used plants for ecological restoration of marginal soils. Therefore, *Lotus* species are regarded as promising forage legumes for arid and semi-arid areas. Although *Lotus* species use has been limited to high rainfall and waterlogging environments (Real *et al.*, 2008), genetic resources collected in the Mediterranean basin could provide useful information to identify germplasm adapted to dryland farming systems (Howieson & Loi, 1994). Previous preliminary projects reported promising *L. creticus* L. ecotypes as naturalized legume in arid land of Tunisia (Ferchichi, 1997). It was found to be adapted to diverse climatic and environmental constraints such as soil salinity (Paz *et al.*, 2012). *L. ornithopodioides* L. is an annual self reseeding pasture legume with a many interesting traits including deep root system, prolific seed production, tolerance to insects and low seed pod shattering compared to other species of the *Lotus* genus (Loi *et al.*, 2016). These features would make it an optimal *Lotus* ideotype (Howieson *et al.*, 2000). Natural presence landraces of *Lotus* found in

arid and semi arid regions is an indicator of their persistence in this environment where they have been going through drought and soil salinity stress that affect their growth and yield (Rejili *et al.*, 2009). Compared to other legume species, little information is available regarding *Lotus* seed germination under drought and salinity stress (Rejili *et al.*, 2009). Better understanding of *Lotus* species seed germination mechanism and physiology is important for selecting productive and adapted species useful for land reclamation and rehabilitation programs particularly for arid and semi-arid areas.

Given the important potential of these two *Lotus* species, their germination stage is determinantal? for successful seedling establishment. The objective of the present research was to investigate the response of two spontaneous populations of *L. creticus* L. and *L. ornithopodioides* L., frequently observed in northern Tunisia, to salinity and water deficit.

Material and Methods

Seed lot details: Fully ripened seeds of *L. creticus* L. and *L. ornithopodioides* L. populations were collected during June and July 2014 from different sites in northern Tunisia. In each site, an area of 0.5 ha was inventoried. Prospection and seed collections were conducted in joint collaboration with National Genes Bank in Tunisia. Weight of 1000 seed was determined for each species in each site through four replicates (International Seed Testing Association, 2003). Collection sites characteristics are presented in Table 1.

Table 1. Location and bioclimate of collected seeds of *Lotus* species

| Species | Location | Bioclimate | Average annual rainfall (mm) | Latitude | Longitude |
|-------------------------------|-----------------------|------------|------------------------------|--------------|--------------|
| <i>L. creticus</i> L. | Tabarka | Humid | 1000–1500 | 36°57'18",29 | 8°47'21",699 |
| | Parc national Seddine | Semi arid | 300–400 | 36°06'55",76 | 8°30'41",116 |
| <i>L. ornithopodioides</i> L. | Mnasriya | Humid | 1000–1500 | 36°33'43,840 | 8°42'04",044 |
| | Boulifa | Semi-arid | 300–400 | 36°09'49,86 | 8°72'61",150 |

Germinations tests: *Lotus* seeds were soaked in sulfuric acid (70%) for 30 min to break seed coat dormancy (Rejili *et al.*, 2009). Seeds were then surface sterilized with a 10 % sodium hypochlorite solution for 1 min and subsequently washed with distilled water to avoid fungal attacks. Germination trials were conducted in 9 cm sterile Petri dishes containing a double layer of filter paper. Six salinity treatments using solutions of 50, 100, 150, 200 and 250 mMNaCl were used to study species response to salinity stress. Another experiment was also conducted to investigate species response to drought stress using 0, -0.2, -0.4, -0.6, -0.8, and 1.0 MPa water potential induced by using polyethylene glycol 8000 (PEG-8000). Germination parameters were recorded on daily basis for each replicate of 50 seeds during 15 days under laboratory conditions. A seed was considered to have germinated when the emerging radicle elongated to 2 mm or more in length.

After 15 days non germinated seeds from all different salt and PEG solutions treatments were transferred to distilled water to study germination recovery. The germination of recovery was determined by counting the number of recovered seeds from total number of seeds using the following formula:

$$\text{Recovery (\%)} = (a - b) / c * 100$$

where, *a* is total number of seeds germinated after being transferred to distilled water, *b* is the number of seeds germinated in from osmotic treatments of solutes (salts and PEG) and *c* is total number of seeds.

Statistical analyses: Data were statistically analyzed using SPSS version 20.0 for Windows (2011). Analysis of variance (ANOVA) was carried out to test effects of main factors (Species, provenances and each treatment (salinity or water deficit stress) and their interaction on the rate and final percentage of germination. Duncan's test was used to estimate least significant range between means.

Results and Discussion

Seed weight and provenance effect on seed germination:

Investigated *Lotus* species 1000 seeds weight significantly varied depending on provenances. With an average seeds weight of 3.39 g, *L. creticus* L. collected from Tabaraka region was significantly higher ($p < 0.001$) compared 1.48 g for seeds collected from Seddine provenance. Similar results were also found for *L. ornithopodioides* L. seeds collected from Mnasrya region with highly significant

($p < 0.05$) seed weight compared to those collected from Boulifa location (Table 2).

Table 2. Average 1000 seeds weight of two *Lotus* species collected from several regions in northern Tunisia

| Species | Provenance | Mean 1000 seeds wei (g) |
|-------------------------------|------------|--------------------------|
| <i>L. creticus</i> L. | Tabarka | 3.39 ± 0.07 ^a |
| | Seddine | 0.91 ± 0.10 ^b |
| <i>L. ornithopodioides</i> L. | Mnasriya | 1.75 ± 0.04 ^a |
| | Boulifa | 1.28 ± 0.08 ^b |

Data are Means ± SD of 4 replications. Means followed by the same letter are not significantly different by Duncan's test ($p < 0.05$)

Analysis of variance test did not reveal significant differences of seed germination capacity between investigated *Lotus* species regardless of their provenances. Our results show that, despite significant variations in seed weight, no relationship can be deduced between these *Lotus* species seed weight and their rate of germination under optimal conditions (Fig. 1). Seed weight was negatively but not significantly associated with germination. Similar results have been reported for other species where relationships between seed weight and germination rate was negatively but not significantly associated with germination percentage (Liu *et al.*, 2007 and Atia *et al.*, 2010). However, several other authors reported significant relationship between provenances, germination rates and seed weight (Abbad *et al.*, 2004; Atia *et al.*, 2009 and Jacquemyn *et al.*, 2001).

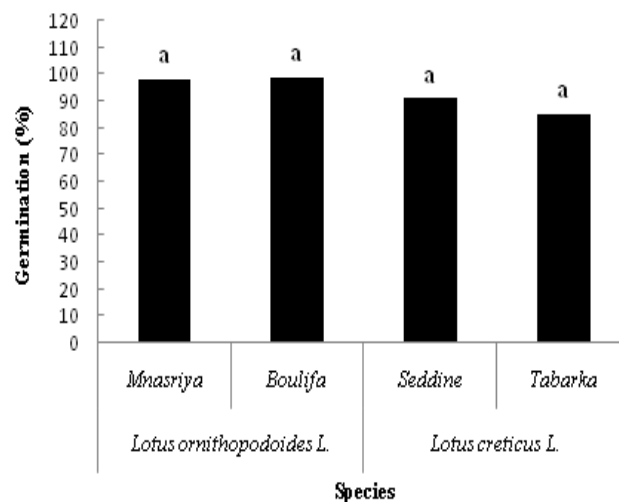


Fig. 1. Seed germination (%) of two *Lotus* species collected from different regions in Northern Tunisia. (Means with same letters are not significantly different ($p < 0.05$)).

Effect of salinity on germination: Provenance and salinity significantly ($p < 0.01$) affected *Lotus* species seed germination. At 50 mM, 100 mM and 150 mM, seed germination of *Lotus* populations collected from Tabarka and Seddine was similar to the check treatment with 0 mM NaCl. However, at 200 mM and 250 mM of NaCl *Lotus* seed germination was decreased for both provenances. At 200 mM and 250 mM, *L. creticus* seeds collected from Seddine region had 64.37% and 55.76% germination, respectively. Seeds collected from Tabarka region were more salt tolerant at these two salinity levels with germination rates percentages of 90.43% and 80%, respectively. Seed germination of *L. ornithopodioides* L. was not significantly affected by salinity levels regardless of populations provenances (Fig. 2).

The present study showed that high levels of NaCl decreased seed germination of two populations of *L. creticus* L. These results were similar to those reported by Rejili *et al.*, (2009) who indicated that at 300 mM of NaCl, germination of *L. creticus* L. seeds collected from Oued Dkouk region was reduced compared to inhibited

germination of seeds collected from Msarref region. Valiente *et al.*, (2007) also indicated that *L. creticus* L. seed germination remained at 80% level under 200 mM NaCl. Present germination trials also indicated that *L. ornithopodioides* L. was tolerant up to 250 mM of NaCl. These results are similar to those reported by Cristaudo *et al.*, (2008) who found that *L. ornithopodioides* L. species is able to germinate under up to 220 mM of NaCl treatment.

Bioclimatic origin such as Mnasrya which is a humid region compared to the semi-arid region of Boulifa had no effect on seed germination of the two *L. ornithopodioides* L. when they were exposed to salinity treatments. Similar results of no provenance effect on seed germination under saline treatments were also found for *L. creticus* L., collected from Tabarka (humid) and Seddine semi arid regions. These results are also similar to findings reported by Rejili *et al.*, (2009) and Atia *et al.*, (2010). Therefore, bioclimatic regions of Mnasrya, Seddine, Tabarka and Boulifa would have no effect on seed germination of these *Lotus* species.

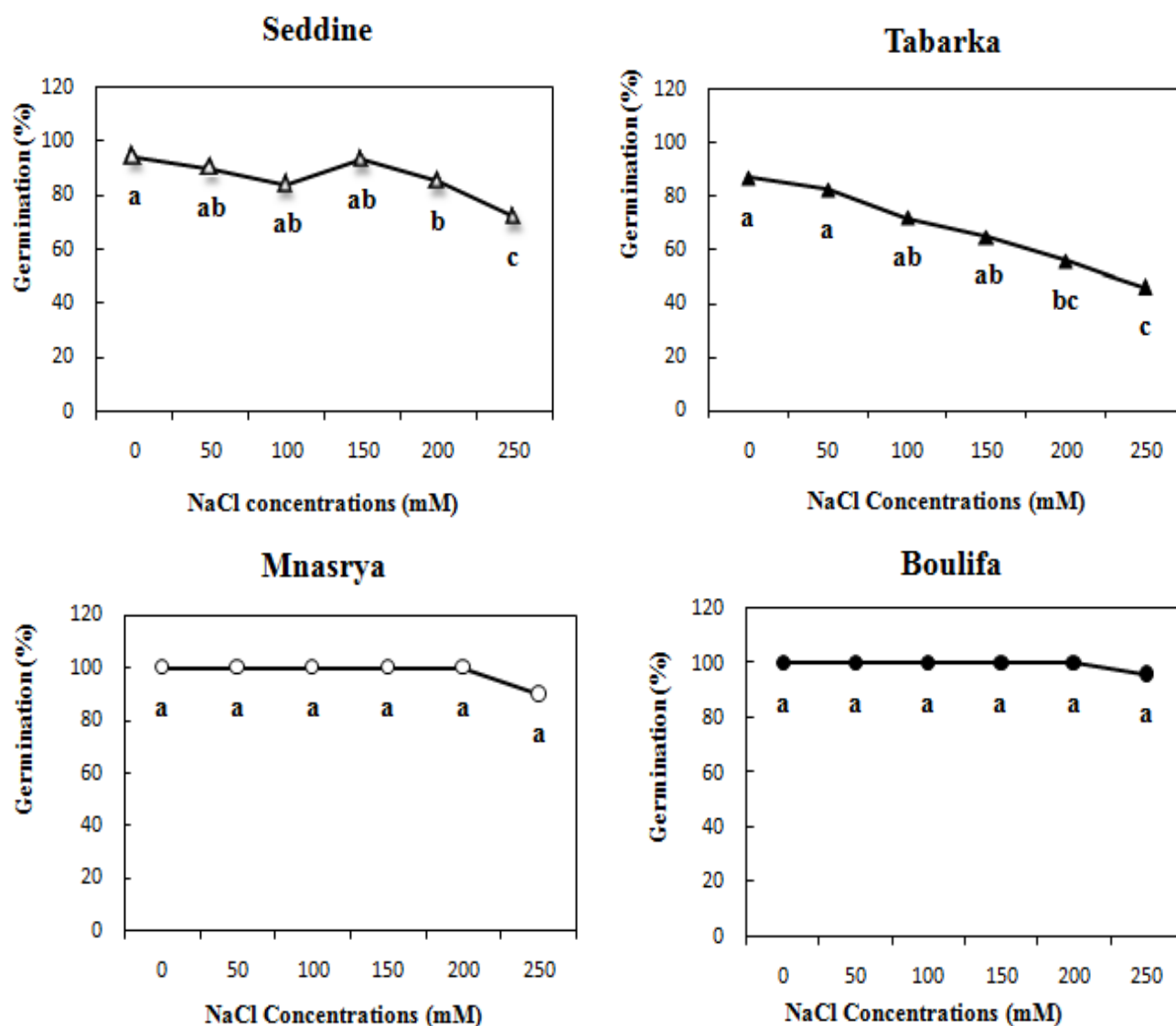


Fig. 2. Seed germination (%) of *L. creticus* L. and *L. ornithopodioides* L. seeds after 14 days of NaCl treatments (Means with same letters are not significantly different $p < 0.05$).

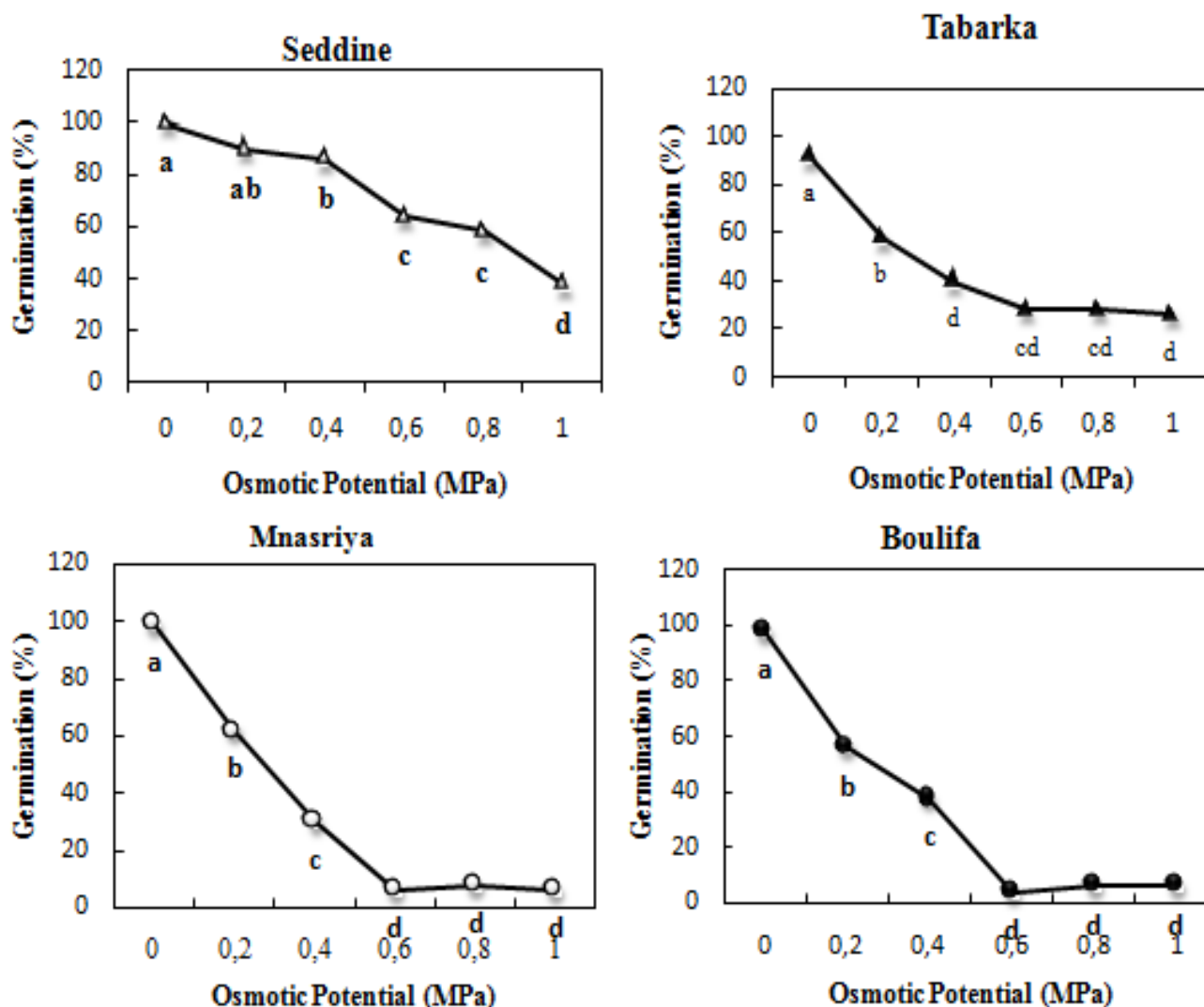


Fig. 3. Seed germination (%) of *L. creticus* L. and *L. ornithopodioides* L. seeds after 14 days of PEG-8000 treatments (Means with same letters are not significantly different $p < 0.05$).

Water deficit effect on seed germination: Water deficit trials conducted indicated significant effect ($p < 0.001$) on seed germination of *L. creticus* L. populations collected from Tabarka were compared to the same species seeds collected from Seddine region. Compared to the check treatment, a water potential stress of -0.2 MPa and -0.4 MPa reduced seed germination of Tabarka population by 29% and 48.5%, respectively, compared to 14% reduction for seeds collected from Seddine location. Pronounced effect for Tabarka genetic material was recorded at -0.6 MPa compared to -1.0 MPa for Seddine species (Fig. 3). These results indicated better tolerance of water deficit of *L. creticus* L. populations, collected from Seddine region. Induced water deficit indicated significant effect ($p < 0.01$) on seed germination of *L. ornithopodioides* L. populations regardless of their collection region of Boulifa and Mnasriya. A water potential stress of -0.2 MPa reduced germination for both the seeds collected from Boulifa and Mnasriya by more than 47% compared to the control treatment. These *L. ornithopodioides* L. populations seed germination was almost inhibited under -0.6 MPa osmotic potential treatments (Fig. 3). The presents results related to *L. creticus* L. collected from Tabarka region and *L. ornithopodioides* L. collected from Boulifa and Mnasriya

provenance were consistent with Talbi *et al.*, (2009) who reported that seed germination of *L. creticus* L. was significantly reduced by 87% at -0.7 MPa and inhibited at -1.0 MPa of water potential. *Lotus creticus* L. species collected from Seddine region showed interesting response of their tolerance to water deficit.

Effects of iso-osmotic agents on germination recovery: Present results indicate that NaCl salt has a depressive effect on the germination of *L. creticus* L. seeds, collected from Tabarka and Seddine regions. This inhibition can be osmotic and/or toxic and irreversible. High levels of salt reduce osmotic potential of the medium and prevent water absorption needed for seed germination (Hajlaoui *et al.*, 2016). High concentration of sodium chloride leads to Na^+ and Cl^- ions accumulation in the embryo which compromises metabolic processes of seed germination and may be lethal (Bajji *et al.*, 2002). Reversibility of NaCl effect can explain the origin of salinity depressive effect on seed germination. If it is due to osmotic origin, we expect germination recovery and sprouting of seeds once the osmotic constraint is removed. But if it is due to ionic toxicity, germination recovery will not occur.

Table 3 shows germination rates of *L. creticus* L. seeds from Tabaraka and Seddine regions when transferred into distilled water medium after salinity treatments. Although no significant effect was noticed, germination recovery occurred. These results show that the transfer of seeds in distilled water is followed by a recovery of germination. This recovery was not significant ($p < 0.05$) for both salt and provenance factors. At 100 mM of NaCl, Seddine and Tabaraka *L. creticus* L. populations had highest recovery rates with an average of 91.7% and 100%, respectively. These findings indicated that inhibited seed germination of these *Lotus* species was mainly due to osmotic effect rather than ions toxicity. Compared to the check treatment, germination recovery after salinity treatments was similar for both *L. creticus* L. regardless of their provenances. Hajlaoui *et al.*, (2007) reported that germination recovery occurred after salinity treatment using high doses of Na Cl (68 mM and 102 mM) and transfer of chickpea seeds in distilled water although it was lower compared to seeds directly set in 0 mM of NaCl. Nichols *et al.*, (2009) found that after exposure to a salinity level of 600

mM of NaCl, seed germination was irreversible for *Medicago polymorpha* L. and *Trifolium subterraneum* L.

Inhibited Seed germination of both *Lotus* species was higher under PEG-8000 treatment compared to salt stress. Our result agreed with the results reported for *Medicago sativa* L. (Dianati *et al.*, 2009) and *Glycine max* L. (Khajeh-Hosseini *et al.*, 2003). Seed germination inhibition under PEG-8000 treatment is mainly due to osmotic effects as compared to ionic effects under salt treatments (Dodd & Donovan, 1999).

Table 4 showed that when *L. creticus* L. and *L. ornithopodioides* L. seeds were transferred to distilled water after 14 days of PEG-8000 treatments, average seed germination recovery was increased for both species indicating that PEG-8000 had no toxic effects on seed germination. Merah (2001) and Khajeh-Hosseini *et al.*, (2003) indicated that PEG molecules did enter the seeds and found that there was no PEG toxicity during seed germination.

Table 3. Effects of salinity (50,100, 150,200 and 250 mM) on germination recovery of *L. creticus* L. seeds collected from Seddine and Tabaraka regions.

| Provenances | NaCl (mM) | | | | |
|-------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| | 50 | 100 | 150 | 200 | 250 |
| Seddine | 79,49 ^a | 91,67 ^a | 90,00 ^a | 81,67 ^a | 84,66 ^a |
| Tabarka | 65,00 ^a | 89,00 ^a | 75,00 ^a | 73,75 ^a | 66,67 ^a |

Data are Means \pm SD of 4 replications. Means followed by the same letter are not significantly different by Duncan's test ($p < 0.05$)

Table 4. Effects of PEG-8000 treatments (0.2, 0.4, 0.6, 0.8 and 1 MPa) on germination recovery of two *Lotus* species seeds collected from different regions in northern Tunisia.

| Species | Provenances | PEG-8000 (MPa) | | | | |
|-------------------------------|-------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 |
| <i>L. creticus</i> L. | Seddine | 41,66 ^b | 70,83 ^a | 83,75 ^a | 70,00 ^{ab} | 71,45 ^{ab} |
| | Tabarka | 85,00 ^a | 90,17 ^a | 85,60 ^a | 83,22 ^a | 80,84 ^a |
| <i>L. ornithopodioides</i> L. | Boulifa | 100,00 ^a | 93,66 ^{ab} | 85,41 ^{ab} | 87,47 ^b | 89,65 ^b |
| | Mnassriya | 86,36 ^a | 74,50 ^a | 78,89 ^a | 78,26 ^a | 76,53 ^a |

Data are Means \pm SD of 4 replications. Means followed by the same letter are not significantly different by Duncan's test ($p < 0.05$)

Conclusion

The present investigation results indicated that *L. ornithopodioides* L. and *L. creticus* L. collected from Seddine region were tolerant to salinity and water stress. Seed germination recovery after salinity treatments indicated that NaCl had an osmotic inhibition of these two *Lotus* species. Further field trials are needed for potential use of these species for rehabilitation of saline soils and arid environments.

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