

Diversity of Salt Tolerance During Germination in *Medicago ciliaris* (L.) and *Medicago polymorpha* (L.)

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Abstract

Variability of salt tolerance in eight wild populations of two annual *Medicago* species (*Medicago ciliaris* and *Medicago polymorpha*) was evaluated at germination stage using three treatments of salinity: 50, 100 and 150 mM of NaCl. Results showed that germination is clearly affected by high salt stress; their germination rate doesn't exceed 7% at 150 mM. The greatest variability in tolerance was observed at moderate salt stress (50 mM of NaCl) and the decrease in germination seems to be more accentuated in *Medicago polymorpha* than *Medicago ciliaris*. Whereas, the Tunisian population of *Medicago ciliaris* was the best tolerant in all ecotypes studied in this work. This population prospected on soils affected by salinity, exhibits a particular adaptability to salt environment, at least at this stage in the life cycle. This intra-specific variation in salt tolerance may be used to select genotypes particularly suitable for cultivation on lands relatively affected by salinity. On the other hand, when ungerminated seeds from NaCl treatments were transferred to distilled water, they recovered largely their germination at all the populations studied after only 2 days. This, indicate that the germination inhibition was related to osmotic stress rather than ion toxicity. In addition, seed germination in all populations tended to be extremely rapid than that observed in distilled water which indicate that this pretreatment raises dormancy.

Key words: Salt tolerance, Intra-specific Variation, Germination recovery, Annual *Medicago*, osmotic stress.

Introduction

In arid and semi-arid regions, soil salinity, often associated with the water constraint, constitutes one of the main environmental problems for development and crop productivity of plants. It causes reduction of cultivable area and combined with other factors, presents a serious threat to food stability in these areas (Kinet et al., 1998; Ashraf and Foolad, 2007). In the North Africa and the Middle East, 15 million hectares of arable lands are affected by salinity and this area is in continuous progression (Le Houérou, 1986; Lahmar and Ruellan, 2007). These vast regions are not exploited, except when they occasionally constitute a poor pasture land or irrigated domain with low yield. In Morocco, they correspond especially to arid and semi-arid regions where 80% of available water for irrigation contains salinity higher or equal to 5 g L⁻¹ (Choukrallah, 1991). In these marginal regions, plant biodiversity is reduced considerably and soil is dramatically degraded. Thus, a great number of plant species are threatened with disappearance and emergency measures must be taken to preserve the M. In front of this problem, the selection of salt tolerant species and varieties remains the best economic approach for exploitation and rehabilitation of salt-affected regions (Shannon, 1985; Alonso et al., 1999; Ghoulam et al., 2001). The effectiveness of such approach depends on the availability of genetic variation in relation with salt tolerance and its exploitation by screening and selection of the powerful plants under saline stress (Al-Khatib et al., 1992; Ali et al., 2007; Hussain et al., 2010). For many crop species, intraspecific variability for salt tolerance have been identified among cultivars and wild species (Al-Khatib et al., 1992; Chaudhary et

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al., 1996; Foolad, 1999; Mezni et al., 2002; Malcolm et al., 2003; Sibole et al., 2003; Raccuia et al., 2004; Hamrouni et al., 2008; Correia et al., 2010). These rustic resources are well adapted and constitute a potential reservoir useful to provide interesting materials in order to diversify and increase the productivity, particularly in pasture land affected by salinity. The majority of plant species of agronomic interest are arranged in glycophytes group, they present a large variation in salt tolerance behavior. The most sensitive species undergo growth reduction, often considerable at low salt concentrations. On the other hand, the tolerant species react to the harmful effect of salt by accumulation of toxic ions and a better mobilization of active organic compounds like glycine-betain and proline which enable to avoid withering and to limit growth reduction of plants (Khedr et al., 2003; Vinocur and Altman, 2005; Ashraf and Foolad, 2007). In this work, we focus on two annual species of *Medicago* chosen for their forage quality and their capacity to improve nitrogenation of salted land: *Medicago ciliaris* (L.) and *Medicago polymorpha* (L.). They grow naturally in a semi-arid to arid superior bioclimatic stages in Morocco. These species, classed as glycophytes, may be observed on relatively saline soils in association with halophytes (Abdelly et al., 2006; Merabet et al., 2006; Barrett-Lennard and Setter, 2010). In these areas, the halophytic species do not represent a good pastoral resource, at least for livestock because they are loaded with salt, which accounts for 15 to 30% of dry matter. On the other hand, medicos contain ten times fewer of NaCl (Abdelly et al., 1995). Annual species of *Medicago* are very appreciated and supported by the halophytes, which contribute periodically to the maintenance of low saline soil. However, their productivity can be reduced by 40% in salt concentration to 12 g L⁻¹ (Levigneron et al., 1995). The use of these species for rehabilitation and exploitation of lands affected by salinity can contribute to install, between the halophytes, an interesting potential of grassland for exploiting this ecosystem like grazing area. Successful establishment of plants often depends on germination success, especially those that grow in salt affected areas. Seeds of various halophytic or glycophytic species, show an important variability with their ability to germinate in various salt concentrations (Ben Naceur et al., 2001; Mensah et al., 2006; Rogers et al., 2008). Their germination decreases with the increasing salinity and in natural environment, they generally germinate in the period which follows strong precipitations, thus allowing a reduction of soil salinity level (Al-Khateeb, 2006; Gorai and Neffati, 2007). Usually, germination capacity under high salinity conditions is not always correlated with the ability of plant to grow under salt stress because both are controlled by different mechanisms (Malcolm et al., 2003; Yamaguchi and Blumwald, 2005). Although it's possible to find genotypes with similar tolerance at germination and during vegetative growth (Flowers, 2004; Dai et al., 2009). On top of that, in arid and semi-arid areas, high temperatures are added to salt effect and affect dramatically seed germination capacity. It decreases considerably when temperatures remain lower than 18°C and when they exceed 25°C (Itamar and Ray, 1988; El-Keblawy and Al-Shamsi, 2008). The aim of the present study was to assess salt tolerance variability at germination stage of *Medicago ciliaris* and *Medicago polymorpha*, until now

not estimated, in order to explore opportunities for selection and breeding salt tolerant genotypes. The study will also assess to determine if salt stress is induced by osmotic constraints or toxic effect of NaCl.

Materials and Methods

Plant Material

Seven wild populations prospected in the south-west of Morocco and one Tunisian population were studied: four populations of *Medicago ciliaris* and four populations of *Medicago polymorpha*. For each species, pods representative of each population were collected randomly on diverse regions reputed to be more or less affected by salinity. The four ecotypes of *Medicago polymorpha* are originating from the areas of Marrakech (Pmark), Taroudant (Ptar), Chtouka Ait Baha (Pchka) and Massa (Pmasa). Those of *Medicago ciliaris* are collected near Marrakech (Cmark), Taza (Ctaz), Tétouan (Ctéo) and Tunisia (Ctuni). This last population was collected near sabkha of Bouficha city and chooses in this work like control population for salt tolerance estimation.

Germination

Seeds from different pods (for limiting parental effect) were manually scarified and sterilized with 0.5% Sodium Hypochlorite solution (NaOCl) for 10 min, then rinsed with sterile distilled water several times, and briefly blotted on filter paper. Three replicates of 30 seeds from each accession were placed in plastic Petri dishes (90 mm diameter) on filter paper wetted with distilled water (control) and three salinity concentrations (50, 100 and 150 mM NaCl). Petri dishes were randomized in a precision incubator and maintained in the dark at 20±0.5°C. Seeds were considered to have germinated when their radicle reached at least 3 mm long. Germination response was recorded daily for 10 days. To test germination recovery performance after exposure of seeds to NaCl solutions, ungerminated seeds were transferred to distilled water and incubated for 3 days. Germination was estimated using both the Final germination percentage (FGP) and germination rate index (GRI):

$FGP = (N_t / N) \times 100$ where N_t is the total number of germinated seeds and N is the number of tested seeds.

$GRI = (G_1 / 1) + (G_2 / 2) + \dots + (G_x / x)$ where G is the germination percentage at each day after sowing, and 1, 2, ..., x represents the corresponding day of germination. The value of GRI was higher when seeds germinated earlier. This parameter described by Esehie (1994) and Weng and Hsu (2006) is a measure of seedling vigor and should involve not only germination but emergence characteristics.

Statistical Analysis

All values expressed as a percentage were arcsin square root transformed before performing statistical analysis to nor-

malize the data and to homogenize the variance (Turkington et al., 2002). These measures were submitted to a two ways analysis of variance (ANOVA) with populations and salinity treatments as factors followed by a Student–Newman–Keuls post hoc test. A difference was considered to be statistically significant when $P < 0.05$. All statistical analysis were performed with Statistica software Version 6.1 for Windows (Statsoft, 2001).

Results

Effect of Salinity on Seed Germination

For the tow species, ANOVA test showed significant differences for both population and NaCl concentration regarding seed germination ($P < 0.01$) (Table 1 and 2). The salinity x population interaction, which indicate a differential effect of salinity between populations, was highly significant at *M. ciliaris*, but no significant in *M. polymorpha* ecotypes ($F = 1.446$, $p < 0.210$) for final germination percentage (Table 1). However, for rate index this interaction is highly significant for the two species (Table 2).

Mean comparison at different salinity levels indicated that increase of salinity causes a decrease in seed germination percentage, which was higher in distilled water than in any NaCl concentration. In all populations, salt concentrations delayed the beginning of germination (Fig. 1) and reduced the final germination

percentages (Fig. 2).

The greatest variability in tolerance was observed at 50 mM of NaCl. At this saline stress, the decrease in germination seems to be less accentuated in *M. ciliaris* than *M. polymorpha* populations. However, in this last group, the decreasing was significantly different among populations: Pchka, which overlap with *Medicago ciliaris* genotypes, showed the highest final germination percentage (FGP = 50%) and (GRI = 8.58%). Whereas, the less tolerant populations, especially Ptar showed FGP and GRI values respectively 20% and 3.22%.

In *M. ciliaris*, which represent the most tolerant genotypes, significant differences were also recorded: Ctuni population originated from saline soil (Tunisia) showed very high final germination percentage (FGP = 100%) and revealed an earlier seed germination (GRI = 48.67%). The Cmark population can be regarded as moderately tolerant to salt stress (FGP and GRI closed, respectively, to 76.67% and 35.90%). The remaining populations Ctétó and Ctaz were the most sensitive genotypes to salinity and form a homogeneous group with the most tolerant genotype of *M. polymorpha* (Pchka).

At 100 mM, seed germination was more affected by salinity. However, the decreasing was also significantly different among populations. *M. ciliaris* seems to be more tolerant than *M. polymorpha* populations that react in the same way to this concentration (FGP never fell above 10%). Whereas, Ctuni was the best

Table 1. Variance analysis for final germination percentage of *Medicago ciliaris* and *Medicago polymorpha* populations.

Source of variation	<i>Medicago ciliaris</i>				<i>Medicago polymorpha</i>			
	d. f.	MC	F	p	d. f.	MC	F	p
NaCl	3	4.420	406.799	0.000	3	5.395	546.404	0.000
Population	3	0.182	16.775	0.000	3	0.037	3.775	0.019
NaCl x Population	9	0.088	8.120	0.000	9	0.014	1.446	0.210
Error	32	0.010			32	0.009		

df: Degree of freedom, MC: Mean square, F: ratio of variances, p: p-value.

Table 2. Variance analysis for germination rate index of *Medicago ciliaris* and *Medicago polymorpha* populations.

Source of variation	<i>Medicago ciliaris</i>				<i>Medicago polymorpha</i>			
	d f	MC	F	p	d f	MC	F	p
NaCl	3	1.188	739.030	0.000	3	0.873	382.456	0.000
POP	3	0.065	40.749	0.000	3	0.0348	15.279	0.000
NaCl x POP	9	0.020	12.797	0.000	9	0.0064	2.832	0.014
Error	32	0.001			32	0.0022		

df: Degree of freedom, MC: Mean square, F: ratio of variances, p: p-value.

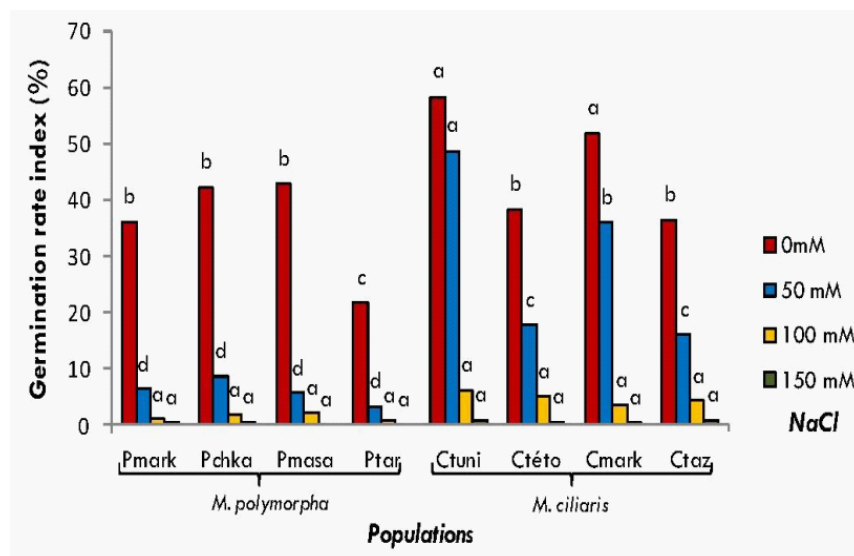


Figure 1. Germination rate index of *Medicago ciliaris* and *Medicago polymorpha* in various NaCl concentrations. At each concentration of NaCl, means of populations having the same letter are not significantly different ($p < 0.05$) (Newman-Keuls test).

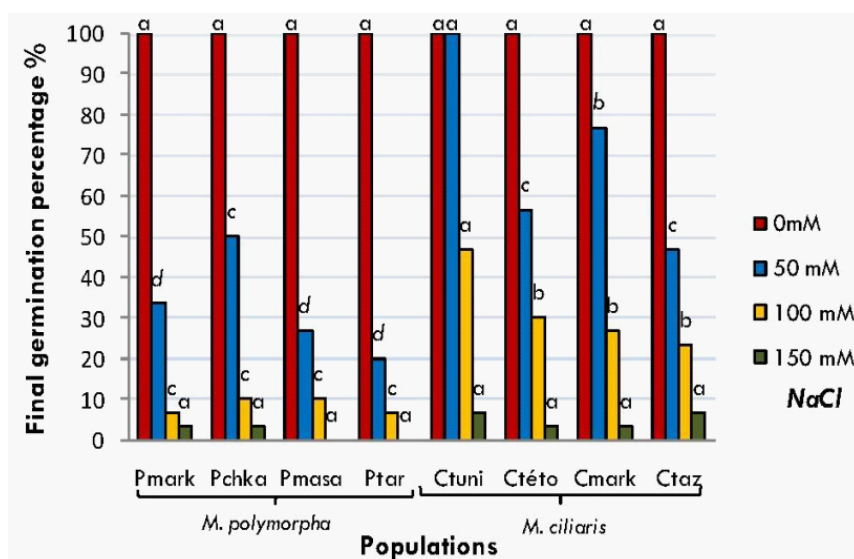


Figure 2. Final germination percentage of *Medicago ciliaris* and *Medicago polymorpha* in various NaCl concentrations. At each concentration of NaCl, means of populations having the same letter are not significantly different ($p < 0.05$) (Newman-Keuls test).

tolerant genotypes in all ecotypes studied in this work with FGP equal to 47%. This ecotype exhibits a particular adaptability to salt environment, at least at this stage in the life cycle.

High salt levels (150 mM) sharply reduced seed germination. At this NaCl concentration, all populations of the two species seem to be affected in the same manner, their final germination percentage does not exceed 6.67% and time to germinate gradually lengthened (FGI $< 0.85\%$).

This intra-specific variation in salinity tolerance in the two species, also reported in (Chérifi et al., 1993; Ibrar and Hussein,

2003; Rabhi et al., 2007; Nichols et al., 2009), may be used to select genotypes particularly suitable for cultivation on lands relatively affected by salinity; all the more since *M. ciliaris* and *M. polymorpha* are predominantly autogamous (Chérifi et al., 1993; Mizukami et al., 2006).

Recovery of Germination

When ungerminated seeds from NaCl treatments were transferred to distilled water, they recovered largely their germi-

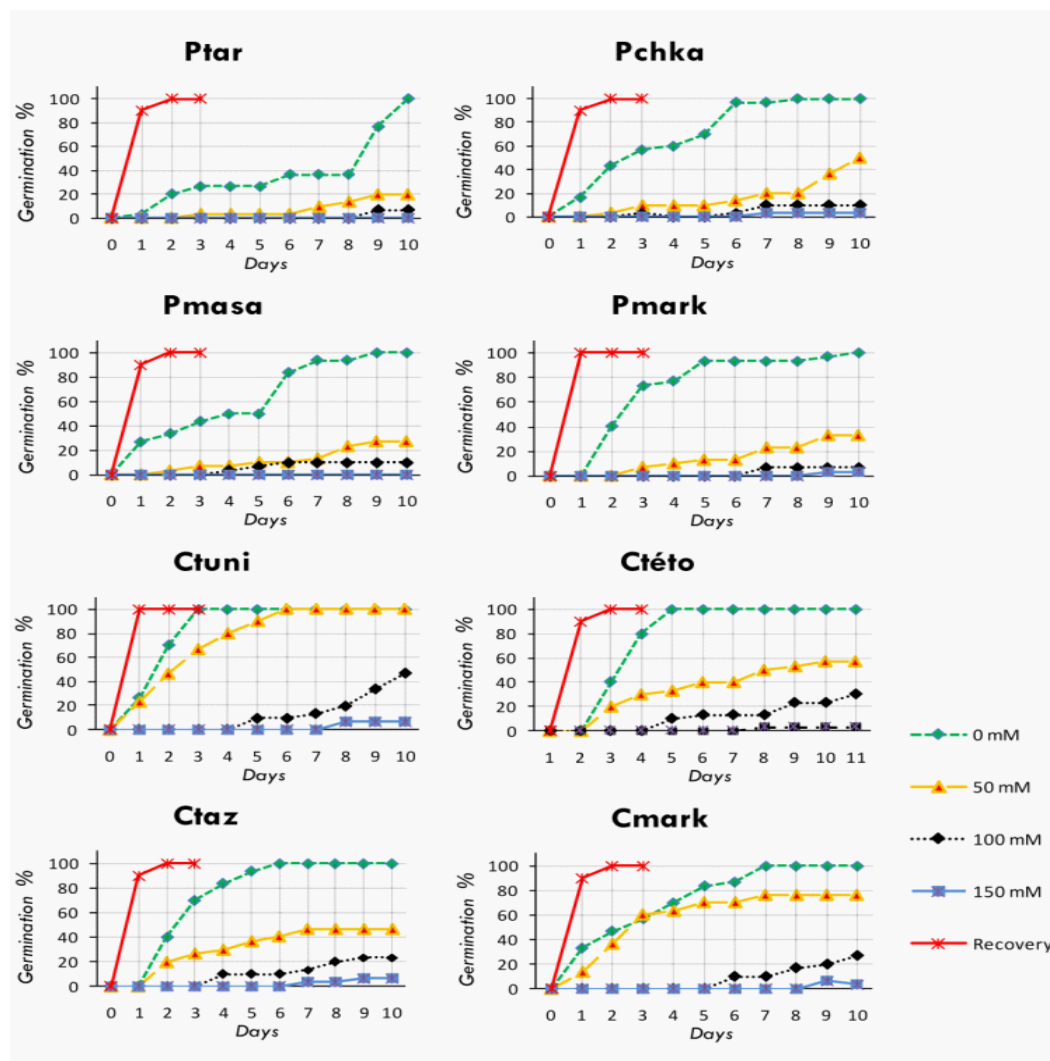


Figure 3. Cumulative germination percentages of different populations of *Medicago ciliaris* and *Medicago polymorpha* showing response both to varied salinity and to germination recovery.

nation at all the populations studied after only 2 days (Fig. 3). These observations indicated that NaCl caused a greatest osmotic effect on seed germination among the three chloride salt concentrations used in this research. In addition, seed germination in all populations tended to be extremely rapid than that observed in distilled water. This proved to be interesting because it improves the percentage and time of germination compared to the normal conditions. It seems that this pretreatment raises dormancy, which may be necessary in the case of populations with difficult germination like Ptara, Pchka, Pmasa and Cmark populations.

Recovery germination response has been demonstrated in several species including *Salsola affinis* (Wei et al., 2008), *Medicago ruthenica* (Guan et al., 2009), *Spartina alterniflora* (Li et al., 2010) and *Diplotaxis harra* (Tlig et al., 2008).

Our results, demonstrate that the recovery of germination is not a criterion of salt tolerance which distinguishes halophytes from glycophytes as reported previously (Khan and Ungar, 1984). It was probably due to a reversible osmotic effect that

induced dormancy. According to El-Keblawy and Al-Shamsi (2008) a high proportion of seeds remained practical and maintain the ability to germinate when salinity stress was removed. The reduction in germination is therefore attributed to the increased osmotic pressure environment preventing the seed imbibitions (Katembe et al., 1998) and mobilization of reserves for embryo's growth (Prisco and Filho, 1981). Thus, dormancy decrease the risk of seedling mortality when moisture is limited and salinity is augmented (El-Keblawy and Al-Rawai, 2005). High recovery germination speed revealed in the tow species, indicates seeds relative ability to avoid deterioration caused by prolonged exposure to unfavorable biotic factors (Grabe, 1976).

This situation constitutes an ecophysiological adaptive strategy to take advantage of favorable conditions, available for a short time, during the germination stage (Khan and Qaiser, 2006). It also, secure the long-term existence of seed bank helping the species in spreading germination over years (El-Keblawy and Al-Rawai, 2005), especially since the annual species

of *Medicago* have the ability to self-regenerate by a large number of seeds production having a staggered germination (Small and Jomphe, 1988; Cocks, 1995). Sowing in the late autumn and early winter would be recommended, to increase the probability of successful plant establishment in rehabilitation program of degraded lands, when salinity and temperature stresses are reduced after the first rains of the season (Guma et al., 2010).

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