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Effect of Salinity Stress on Seedling Development of Different Ecotypes of the Model Legume *Medicago truncatula*

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ABSTRACT

For the analysis of seedling growth under salinity stress, it will be useful to study root growth in comparison to shoot elongation under salt stress. In this study, we assessed the variability of eleven ecotypes of *M. truncatula* under salt stress (137 mM) of NaCl compared to the control. Several studies are focalised in root growth and development because it is the most sensitive part of the plant and controls rapid transmission information to other plant parts. In order to assess the level of root sensitivity, we measured the shoot length to root length ratio. The Results showed that Tru 131 ecotype, with a high ratio (Root more vigorous than shoot) is more tolerant to salinity stress than the sensitive ecotypes that had a low ratio. We consider that the most tolerant Algerian ecotype Tru 11 (Tru 131) can be cultivated in saline and semi-arid areas in Algeria and Mediterranean regions in order to improve the legumes productivity and ameliorate the nitrogen fixation.

Keys words: Salt stress, *Medicago truncatula*, model legume, root shoot ratio, seedling growth

INTRODUCTION

Salinity is an important abiotic stress which significantly affects seedling growth and seed quality. Legumes are very important plants both ecologically and agriculturally because they are able to interact symbiotically with rhizobia for biological nitrogen fixation (Spaink, 2000; Perret *et al*., 2000), which avoids the use of chemical fertilizers, affecting the rhizosphere and polluting the atmosphere. Among the *Medicago* annual species, *M. truncatula* is widely used as a model legume plant for understanding tolerance to abiotic stress (Young and Udvardi, 2009). This kind of legume is of great interest for sustainable agriculture and ecology. Seedling growth of many crop plants is the most sensitive to environmental stresses (Penmesta and Cook, 1997). Root and shoot length provide important indications of a plant's response to salt stress (Jamil and Rha, 2004). In certain plants, the root may be longer than the shoot and consequently this ratio may be lower compared to other plants (Snapp and Shennan, 1992). The aim of this study was to assess the response of seedling growth and especially root development to salt stress between eleven ecotypes of the plant model *Medicago truncatula.* The determination of the most tolerant ecotype is a great interest for sustainable agriculture and ecology in order to improve the productivity of this legume very important economically and environmental for nitrogen fixation and soil natural fertilization.

MATERIALS AND METHODS

Plant material, growth conditions and salt stress treatment: One hundred seeds of *M. truncatula* ecotypes (10 seeds per ecotype) provided by different institutes (Table 1), were used in two levels of salinity treatment (distilled water as control 0 and 137 mM) of NaCl solution (Amouri and Fyad-Lameche, 2012). Seeds were manually scarified and sterilized for 10 min in sodium hypo-chloride (6%) and then rinsed 3 times with distilled water for 2 min. Ten seeds were sown in Petri dishes and germinated on filter papers imbibed in distilled water or in sodium chloride solutions. The seeds of *M. truncatula ecotypes* were germinated in Petri-dishes (50 mm) on two layers of filter paper in an incubator maintained in the dark at 25±2°C. The filter papers were changed after 48 h in order to avoid salt accumulation (Rahman *et al*., 1996). During ten days of germination, root length, shoot length and seedling length were measured and the ratio (root length/shoot length) was calculated. In this study, we considered tolerant ecotypes that have a high ratio, i.e., their roots are more resistant than their shoot.

Data analysis: All statistical tests were carried out using the Statistical Analysis System Statistica 6.1 version. Statistical analysis was performed using two-way ANOVA (for $p<0.01$ and $p<0.05$). Based on the ANOVA results, a Duncan's multiple range tests for means comparison was performed, for a 95% confutation level, to test for significant differences among treatments (Duncan, 1955). Investigated traits of the ratio was subjected respectively to hierarchical cluster analysis using the procedure of Single-linkage clustering. The clustering of different ecotypes gives information on their similarity and dissimilarity in responses to salt stress, which facilitate the choice of the tolerant ecotypes to be involved in future breeding programs.

RESULTS AND DISCUSSION

Influence of salt stress on different parameters of seedling growth and development in *M. truncatula* ecotypes: Seedling development is critical stage for the establishment of plant populations under saline conditions (Bosque-Perez *et al*., 1998). The research presented here indicated differences in the root and shoot lengths of the eleven ecotypes studied. The data of two-way ANOVA based on Table 2, showed a significant difference between ecotypes in interaction with salinity treatment just for the root growth parameter studied, this data indicate the influence of salt stress at root level. Figure 1-3 showed that all parameters growth (root, shoot and seedling length) decrease at the concentration of 137 mM of NaCl compared to the control (Table 3). Under salt stress, the ratios vary between (0-1.79) (Table 4). High salinity may inhibit root and shoot elongation by slowing down the water uptake by the plant (Werner and Finkelstein,

Table 1: Information of different accession studied of *Medicago truncatula*

No. of accession	Code	Origin	Longitude	Latitude	Altitude
IG 53097	Tru 1	DZA (blida)	E02 41	N36 24	200
IG 53105	Tru 2	DZA (batna)	E06 28	N35 19	1,050
IG 53115	Tru 3	DZA (annaba)	E07 43	N36 55	730
IG 53945	Tru 4	DZA (bouarirej)	E04 58	N36 07	1,260
IG55917	Tru 5	SYR (hama)	E37 0200	N350100	500
IG 53965	Tru 6	LBN (zahe)	E3601	N3352	1,000
IG 53175	Tru 7	MOR (centre)	W07 28	N33 40	5
IG 53939	Tru 8	TUN (bajah)	E09 23	N36 30	400
IG 53326	Tru 9	JOR (al balqa)	E3546	N3159	550
Jemalong	Tru 10	(reference)	-	-	-
M. truncatula 131	Tru 11	DZA (IDG Belabess)	-	-	-

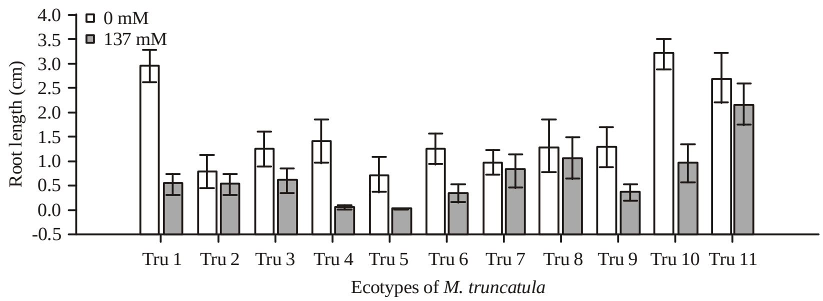

Fig. 1: Individual mean values of root length of *Medicago truncatula* ecotypes under control and salt stress treatment (Means±S.E)

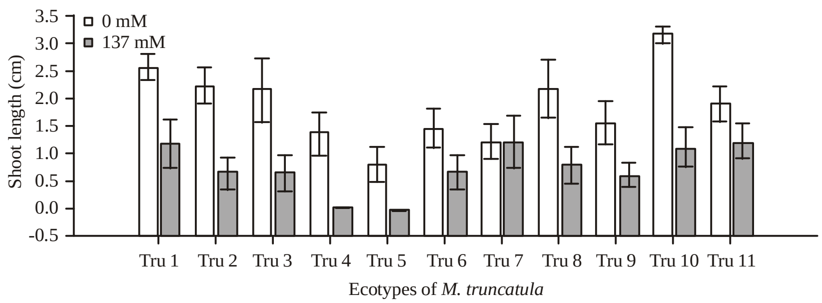

Fig. 2: Individual mean values of shoot length of different *Medicago truncatula* ecotypes under control and salt stress treatment (Means±S.E)

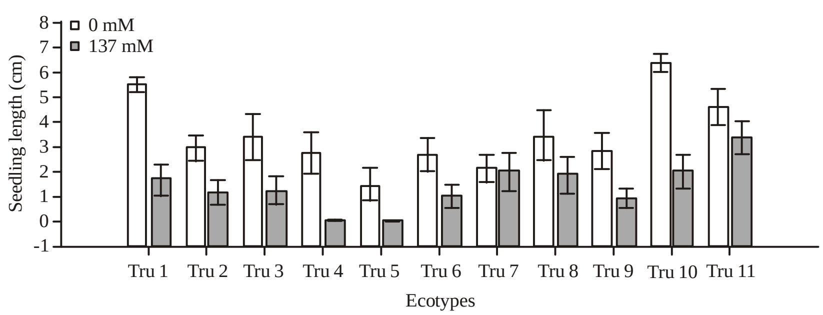

Fig. 3: Individual mean values of seedling length of *Medicago truncatula* ecotypes under control and salt stress treatment (Means±S.E)

Table 2: Two-way ANOVA of the effect of salt stress on different parameters of seedling development in *Medicago truncatula* ecotypes

	Root length			Shoot length		Seedling length	
Source of variation	df	F	p	F	p	F	p
Ecotypes	10	6.68	0.000**	3.67	0.000**	5.18	0.000**
Treatments	1	38.78	0.000**	53.76	0.000**	53.25	0.000**
Interaction	10	2.27	0.015*	1.17	0.310	1.48	0.149

*' **Significance at p<0.05, p<0.001, respectively, ns: Non significant, F: Coefficient of snedecor-fisher

1995). The ability of plants to tolerate salt stress varies with the stage of development in their life cycle (Khan *et al*., 2002) and seedling emergence is critical for the establishment of plant

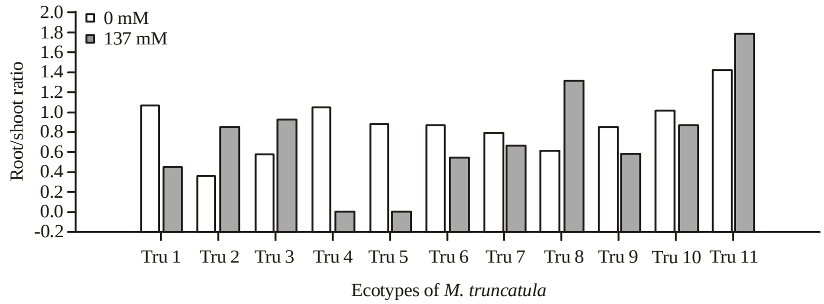

Fig. 4: Mean values of root to shoot ratio of *Medicago truncatula* ecotypes under control and salt stress treatment

Table 3: Classification of means simple effect of different salt stress levels on different parameters of seedling development in *Medicago truncatula* ecotypes

NaCl (mM)	Root length	Shoot length	Seedling length
0	1.596[a]	1.859[a]	3.455[a]
137	0.670[b]	0.722[b]	1.392[b]

Means with similar letters in each trait is not significantly different at 5% probability level according to Duncan's multiple range test

Table 4: Distances matrix between *Medicago truncatula* ecotypes under salt stress for the root to shoot ratio

Parameters	Tru 1	Tru 2	Tru 3	Tru 4	Tru 5	Tru 6	Tru 7	Tru 8	Tru 9	Tru 10	Tru 11
Tru 1	0.0	1.07	2.05	3.03	4.03	5.00	6.00	7.05	8.00	9.01	10.1
Tru 2		0.00	1.00	2.17	3.12	4.01	5.00	6.02	7.00	8.00	9.0
Tru 3			0.00	1.36	2.20	3.02	4.01	5.02	6.01	7.00	8.0
Tru 4				0.00	1.00	2.07	3.07	4.21	5.03	6.06	7.2
Tru 5					0.00	1.14	2.11	3.27	4.04	5.07	6.3
Tru 6						0.00	1.01	2.14	3.00	4.01	5.2
Tru 7							0.00	1.19	2.00	3.01	4.2
Tru 8								0.00	1.24	2.05	3.0
Tru 9									0.00	1.04	2.3
Tru 10										0.00	1.4
Tru 11											0.0

populations (Khan and Gulzar, 2003). The majority of the research indicates that most annual crops are tolerant at germination stage but sensitive during emergence and early vegetative development (Maas and Grattan, 1999). In this study, we used the parameter root to shoot ratio in order to have new and more information on reduction of root length compared to shoot length (Fig. 4). The roots are the most sensitive part of the plant and have the role as control centre with rapid transmission information to other plant parts (Blaha and Pazderu, 2013). So, it is very interesting to study root growth compared to shoot development under salt stress. The results showed a high ratio (1.79) in the ecotype Tru 131 (Tru 11) from Algeria, it's the most tolerant genotype (Fig. 4) with a highest root elongation (2.42 cm) (Fig. 1) and the best seedling development (Fig. 3). However, a low ratio was observed in the sensitive ecotypes like Tru 4 (DZA) and Tru 5 (SYR) (Fig. 4). Zahaf *et al*. (2012), analyzed the root transcriptome of two *M. truncatula* genotypes having contrasting responses to salt stress: TN1.11 (tolerant), sampled in a salty Tunisian soil and the reference Jemalong A17 genotype (sensitive). So, the tolerant genotypes showed increased root growth under salt stress as well as a differential accumulation of sodium ions, when compared to Jemalong A17. The same authors found that the root lengths of these two contrasting genotypes were significantly reduced between 90 and 150 mM NaCl treatments in comparison to control plants. In our studies, we observed a significant reduction of root length at 137 mM.

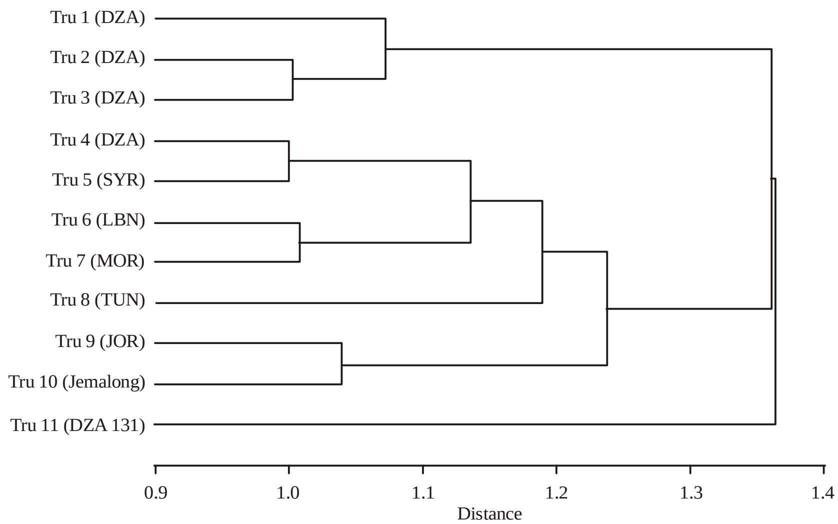

Fig. 5: Cluster analysis of *Medicago truncatula* ecotypes under salt stress treatment for the root to shoot ratio

Cluster analysis of different ecotypes of *M. truncatula* in relation to salt stress: The Results of cluster analysis (Single-linkage clustering) of different ecotypes of *M. truncatula* for the parameter root to shoot ratio under salinity treatment, arranged ecotypes into two groups (Fig. 5). The first cluster includes ten ecotypes (Tru 1-Tru 10) organized in two sub groups with low mean values for the ratio compared to the second group of clustering that contain just the tolerant ecotype Tru 11 (Tru 131) that have a higher mean values of ratio compared to the sensitive ecotypes. The highest distance was observed between the tolerant ecotype Tru 131 (Tru 11) and Tru 1 (DZA) with (d= 10.1) and the lowest distance was noted for example between Tru 4 (DZA) and Tru 5 (SYR) with (d = 1).

CONCLUSION

Analysis of the effect of salt stress on seedling development of eleven ecotypes of *M. truncatula*, showed a significant variation between different ecotypes at root level. A higher root shoot ratio was observed in the tolerant genotype (Tru 131) from Algeria compared to the others sensitive ecotypes like (Tru 2, Tru 3, Tru 4) from Algeria and Tru 5 (LBN), Tru 6 (MOR), Tru 9 (JOR) and Jemalong. This tolerance was explained by the resistance of root to salinity stress in the tolerant genotype (Tru 131). The biochemical and molecular investigation at root level for genes and protein expression provides a good comprehension of tolerance mechanism.

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