Journal of Stress Physiology & Biochemistry, Vol. 10 No. 4 2014, pp. 25-41 ISSN 1997-0838

ORIGINAL ARTICLE

Physiology and ion relations in response to salinity in *Trifolium isthmocarpum* Brot. and *Lotus ornithopodioides* L.

Kawtar Bennani, Najib Bendaou

Biology Department, Mohammed V University, Faculty of Sciences, B.P. 1014 RP, 10 000, Rabat, Morocco

*E-Mail: ***bennani.kawtar@gmail.com***

Received July 19, 2014

Salt tolerance in Moroccan ecotypes of *Trifolium isthmocarpum* Brot. and *Lotus ornithopodioides* L. were evaluated in greenhouse and compared with Australian cultivar : *Trifolium michelianum* Savi. Paradana. Plants were submitted to three irrigation treatments using concentrations 0 mM, 80 mM and 200 mM of NaCl, during 3 months. The effects of salinity on growth, dry matter production, shoot and root ion relations, leaf water relation and gas exchange were determined. Under stressed conditions, *T. isthmocarpum* was more salt tolerant ($p < 0.001$) with a greater survival rate and good dry matter production, than *L. ornithopodioides* and *T. michelianum*. To tolerate salinity, *T. isthmocarpum* and *T. michelianum* developed a Cl^- exclusion mechanism. However, the Cl^- exclusion was more important in *T. isthmocarpum* than *T. michelianum*. On the other hand, *T. isthmocarpum* accumulated more K^+ in shoots than other species, when exposed to moderate and high levels of salinity. At 200 mM of NaCl, the stress promoted a substantial degree of stomatal regulation; but, in spite of this, *L. ornithopodioides* showed signs of leaf tissue dehydration, decreases in relative water content and osmotic potential values.

Key words: Cl- exclusion, Lotus ornithopodioides, Plant physiology, Salt tolerance, Trifolium isthmocarpum

ORIGINAL ARTICLE

Physiology and ion relations in response to salinity in *Trifolium isthmocarpum* Brot. and *Lotus ornithopodioides* L.

Kawtar Bennani, Najib Bendaou

Biology Department, Mohammed V University, Faculty of Sciences, B.P. 1014 RP, 10 000, Rabat, Morocco

*E-Mail: bennani.kawtar@gmail.com

Received July 19, 2014

Salt tolerance in Moroccan ecotypes of *Trifolium isthmocarpum* Brot. and *Lotus ornithopodioides* L. were evaluated in greenhouse and compared with Australian cultivar : *Trifolium michelianum* Savi. Paradana. Plants were submitted to three irrigation treatments using concentrations 0 mM, 80 mM and 200 mM of NaCl, during 3 months. The effects of salinity on growth, dry matter production, shoot and root ion relations, leaf water relation and gas exchange were determined. Under stressed conditions, *T. isthmocarpum* was more salt tolerant ($p < 0.001$) with a greater survival rate and good dry matter production, than *L. ornithopodioides* and *T. michelianum*. To tolerate salinity, *T. isthmocarpum* and *T. michelianum* developed a Cl^- exclusion mechanism. However, the Cl^- exclusion was more important in *T. isthmocarpum* than *T. michelianum*. On the other hand, *T. isthmocarpum* accumulated more K^+ in shoots than other species, when exposed to moderate and high levels of salinity. At 200 mM of NaCl, the stress promoted a substantial degree of stomatal regulation; but, in spite of this, *L. ornithopodioides* showed signs of leaf tissue dehydration, decreases in relative water content and osmotic potential values.

Key words: Cl- exclusion, Lotus ornithopodioides, Plant physiology, Salt tolerance, Trifolium isthmocarpum

Salinity stress is a major abiotic stresses that limit forage legume growth, especially in arid and semi-arid regions (Ashraf and Harris, 2004). An understanding of the range of salinity, that various legumes can

tolerate, is central to their use in active programs for revegetation of saline lands (Mapfumo *et al*., 2008). Salt stress imposes two constraints: a hyperosmotic effect due to lower soil water potential and a hyperionic effect due to direct toxicity of ions over metabolism and nutrition of plants (Staudinger *et al*., 2012). Osmotic adjustment maintains the positive turgor required for stomata opening and cell enlargement (Torrecillas *et al*., 2003). Increased salinity has an inverse relationship with net photosynthesis rate and dry matter production (Lopez *et al*., 2002). Important mechanisms that allow plants to cope with salt stress are Na^+ exclusion and/or Na^+ compartmentalisation (Blumwald *et al*., 2000). In some species, response to salinity differs among ecotypes, and selection of salt tolerant ecotypes is an efficient means to cope with salinity (Gobilik *et al*., 2013). Furthermore, salt tolerance can depend on stress duration, phenological stage and annual or perennial plant (Melchiorre *et al*., 2009). In fact, perennial forage species have the advantage of an extended growing period. But, they must tolerate soil salinity concentrations, which peak in summer and autumn in a Mediterranean environment and which are avoided by annuals (Nichols *et al*., 2008). Thus, the present study was focused on salt tolerance of annual legumes. *Trifolium* and *Lotus* are two of the most important genera of the Fabaceae Family (Bulińska-Radomska, 2000; Arambarri, 2000). *Lotus* genus contains many species with salt tolerance potential (Schachtman and Kelman 1991). However, *Trifolium* species are generally recognised as being salt-sensitive (Mandal, 2014). Nichols *et al*. (2008) suggest that species do vary in their response, and that further research may be beneficial in identifying species that are suited to saline conditions. For example, *Trifolium isthmocarpum* Brot. present the superior tolerance to salinity compared to *T. subterraneum* L. and *T. purpureum* Loisel (Rogers and West, 1993). But no studies have detailed the mechanisms and physiological response to salinity of this species. *T. isthmocarpum* is found in a broad range of environment in Morocco and is known to tolerate coastal areas and clay soils.

This study aims to evaluate the response of Moroccan ecotypes of *T. isthmocarpum* and *L. ornithopodioides* compared to *Trifolium michelianum* to saline stress, for possible use in revegetation of saline areas. Also, to identify the tolerance mechanisms and physiological changes developed in these plants including observations on the effects of NaCl on osmotic adjustment, gas exchange, inorganic and organic contents and morphology.

MATERIALS AND METHODS

Plant materials and growth conditions

On the basis of their good survival and productivity, four Moroccan ecotypes of *T. isthmocarpum* (T1, T2) and *L. ornithopodioides* (L1, L2), were chosen from an evaluation trial conducted at the INRA's Guich experimental station, Rabat, Morocco (Mediterranean climate, latitude 34°03′ N, longitude 06°46′ W, elevation 10.5 m) (Bennani *et al*., 2010). One Australian check cultivar; *T. michelianum* cv. Paradana (Tm) was selected. It represented a well documented legume species grown successfully over a range of environments in

southern Australia (Mediterranean climate), and widely reported to have some salinity tolerance (Nichols *et al*., 2008).

Germinated seeds were planted individually in pots containing a substrate of black peat, sand and a clay–loam soil (1:1:1). Plants of the experimental species were sown in each pot, in a split-plot design using one control and two saline treatments and three replications. The experiment was conducted from October 2010 to February 2011 in a greenhouse. The environmental conditions during the experiment were 26 ±5°C (day) and 14 ±2°C (night), and the relative humidity ranged between 60% and 70%. The average maximum photosynthetically active radiation (PAR) was 820 µmol m^{-2} s^{-1}. Seven weeks after sowing treatments were imposed using irrigation water containing 0, 80 (moderate salinity), and 200 mM of NaCl (severe salinity) for 3 months. The NaCl treatments were imposed in increments of 40 mM/day until full treatments had been reached. During the experimental period the average electrical conductivities of the irrigation solutions were 1.05, 6.85 and 11.9 dS m^{-1}, respectively.

Measurements of growth

Two plants per replication were harvested at the beginning of the experiment (t0), and after 4 (t1), 8 (t2) and 12 (t3) weeks of salt treatments. Plants were gently washed with deionized water to remove soil from roots, and the plants were divided into shoots (stems and leaves) and roots. These were oven dried at 60 °C until they reached a constant mass to measure the respective dry weights. At the end of experimental period height and leaf area were measured (using a LI-2000 area meter; LICOR Biosciences, Lincoln, NE for leaf area). To compare the effects of the treatments on plant growth, the relative growth rate (RGR) was calculated according to Beadle (1993), as follows:

$$RGR = \frac{Ln(DW2/DW1)}{t2 - t1}$$, where:

DW1= dry weight of plant tissue (g) at harvest 1

DW2= dry weight of plant tissue (g) at harvest 2

ΔT= Difference in time between two harvests

Determination of inorganic and organic contents

Na^+, K^+ and Cl^- were analysed at the end of the experimental period in the shoots and roots of plants. The concentration of Cl^- was measured following titrametric method (Begum *et al*., 1992). The concentrations of K^+ and Na^+ were determined using a flame photometer (Jenway Ltd, model PFP7; Essex, UK). Values were calibrated using a reference plant tissue sample with known concentrations Na^+, K^+ and Cl^-. The K^+/Na^+ selectivity in the plant is expressed as $K^+ / (K^+ + Na^+)$ (Glenn *et al*. 1994).

Water relations

Surviving immature leaves for each replication and a sub-sample of three treatments were separated and weighed fresh and then dried (48 h at 70°C) to determine relative water content (RWC) (Inoue *et al*., 1993). The others were immediately frozen in liquid nitrogen, thawed, and osmotic potential (Ψ_{os}) was measured using a psychrometer (L-51, Wescor, Inc., Logan UT) and a HR-33T dew point microvoltmeter (Wescor, Inc.). Stomatal conductance (g_s) and the net photosynthetic rate (P_n) were determined on the same day and in the same plants as leaf osmotic potential,

using a gas exchange system (LI-6400, LICOR Inc., Lincoln, NE, USA). Measurements were made at midday on attached leaves.

Statistical analysis

The data were analyzed using the Statistical Analyses System (SAS, Inc., Cary, NC) software.

Significant differences between treatment means were determined using LSD test at the 0.05 probability level. ANOVA was used to identify overall significant differences and interactions between ecotypes, time and treatments.

RESULTS

Plant growth

Table 1 shows plant heights, leaf area, shoot and root dry weight in the control (0 mM of NaCl) and salt treatments. Under non-saline conditions, the ecotypes present a variation in dry weight production. T2 produce similar shoot dry weight to Tm (check cultivar) and more than other ecotypes. Under salt treatments, and especially 200 mM treatment, a significant decrease was observed in shoot dry weight production by 38 %, 44 %, 50 % and 58 % respectively for T1, Tm, L1 and L2 relative to control treatment. The treatment 80 mM of NaCl, had no significant effect (p> 0.05) on root dry weight between ecotypes. Whereas in 200 mM treatment, root dry weight was reduced by 22 %, 33 %, 53 %, 55 % and 56 % respectively for L1, L2, T2, T1, and Tm, relative to control treatment. Both salt treatments induced a significant decrease in leaf area. T2 was characterised by high height than other ecotypes in all treatments. Overall, L1 was most affected by salt treatment with height reduction by 43 % in 80 mM and 51 % in 200 mM. A significant difference in RGR was observed between plants and among salt treatments (p <0.001). T2 does not decrease RGR after 4 weeks (t1) in 80 mM of NaCl, and also appeared to resume growth rate after 12 weeks (t3) (Figure 1A). In 200 mM, L2 present an increased growth at t1, suggesting a halophytic-like response (Figure 1E). RGR of L1 and T1 decreased significantly in both salt treatments (Figure 1B and 1D).

Mineral content

Under salt stress, the sodium (Na^+) and chloride (Cl^-) concentrations of all ecotypes and check cultivar were increased by increasing salinity levels, compared to the control treatment (Table 2). The Na^+ and Cl^- contents were higher in shoots than roots. There was no significant difference in 80 mM between T2, L2 and Tm, and no significant difference between L1 and T1. In 200 mM treatment, L1 and L2 had more than twice the concentrations of Na^+ in their shoots compared to T2. However, T2 is the only that maintained relatively low concentrations of Na^+ and Cl^-. Unlike Na^+ and Cl^-, potassium (K^+) concentration was decreased by increasing salinity levels compared with the control treatments. The (K^+/ Na^+) selectivity (Table 2) showed that ecotypes with the highest concentrations of Na^+ in their shoots had significantly, lower ratios than those that excluded Na^+ from shoot tissue.

Water relations

Salinity induced a similar decrease (p <0.001) in the RWC in 80 mM and 200 mM treatments at the end of the experimental period. Differences were significant (p <0.001) between ecotypes under 0 mM

and 80 mM of NaCl, while under 200 mM, no differences were detected (p >0.05). The average of RWC for control plants was 70 % at t1. Whereas, in 80 mM, RWC decreased approximately by 17 % and 38 % relative to control treatment. T2 maintained the highest RWC in the experimental period. L1 showed the lowest RWC at the end of the experimental period (Figures 2A, 2B and 2C). Stressed plants, present a low leaf osmotic potential than unstressed plants (Figures 3A, 3B and 3C). Differences were significant at the beginning, middle, and end of the experimental period among treatments and between ecotypes (p <0.001). After 4 weeks (t1), osmotic potential was -0.83 MPa on average for control treatments, and respectively -1.79 MPa and -2.01 on average for 80 mM and 200 mM. At the end of experimental period (t3), osmotic potential declined in all treatments reaching on average -1.04 in control, and -2.68 in salt treatments. The stomatal conductance (g_s) and the photosynthetic rate (P_n) values decreased in both salt treatments and significant differences between treatments were evident (p <0.001) (Figures 4 and 5).

Table 1. Shoot and root dry weight (g plant^{-1}), height (cm) and leaf area (cm^2) measures at the end of the experimental period in 0 mM, 80 mM and 200 mM of NaCl for studied ecotypes. L1, L2 (*L. ornithopodioides*); T1, T2 (*T. isthmocarpum*); Tm (*T. michelianum*)

Treatment	Ecotypes	Shoot DW	Root DW	Height	Leaf area
0 mM	L1	18.65 ± 0.99 c	2.08 ± 0.50 d	22.50 ± 0.83 c	2.63 ± 0.72 c
	L2	29.11 ± 0.14 ab	4.30 ± 0.21 a	20.33 ± 0.33 c	3.07 ± 0.03 c
	T1	23.04 ± 8.68 b	2.83 ± 0.09 c	30.07 ± 0.70 b	7.00 ± 0.28 b
	T2	31.20 ± 7.23 a	2.90 ± 0.29 c	41.07 ± 3.03 a	8.89 ± 0.78 a
	Tm	33.77 ± 8.97 a	3.47 ± 0.37 b	32.67 ± 0.33 b	7.06 ± 0.09 b
80 mM	L1	11.08 ± 0.24 d	2.00 ± 0.17 a	12.67 ± 1.09 b	1.08 ± 0.28 c
	L2	20.54 ± 1.97 b	2.41 ± 0.32 a	15.35 ± 0.06 b	1.72 ± 0.72 c
	T1	15.01 ± 1.92 c	2.17 ± 0.16 a	18.33 ± 1.01 b	4.82 ± 0.03 b
	T2	28.79 ± 0.79 a	2.00 ± 0.25 a	33.50 ± 0.35 a	5.15 ± 0.78 a
	Tm	24.10 ± 0.10 b	2.44 ± 0.18 a	29.14 ± 1.35 c	5.17 ± 0.06 a
200 mM	L1	9.20 ± 0.03 d	1.62 ± 0.07 b	10.91 ± 0.01 c	1.06 ± 0.05 b
	L2	12.09 ± 0.36 d	2.87 ± 0.03 a	10.59 ± 0.01 c	1.03 ± 0.01 b
	T1	14.19 ± 0.31 c	1.22 ± 0.03 c	14.57 ± 0.01 b	3.39 ± 0.07 a
	T2	25.01 ± 0.02 a	1.36 ± 0.03 c	23.74 ± 0.13 a	3.37 ± 0.01 a
	Tm	18.80 ± 0.02 b	1.53 ± 0.02 b	19.38 ± 0.02 b	2.91 ± 0.02 a

Values represent means of 2 plants in three replications ± standard deviation.

Means followed by the same letter in each column are not significantly different according to Duncan's Multiple Range Test at 5 % level

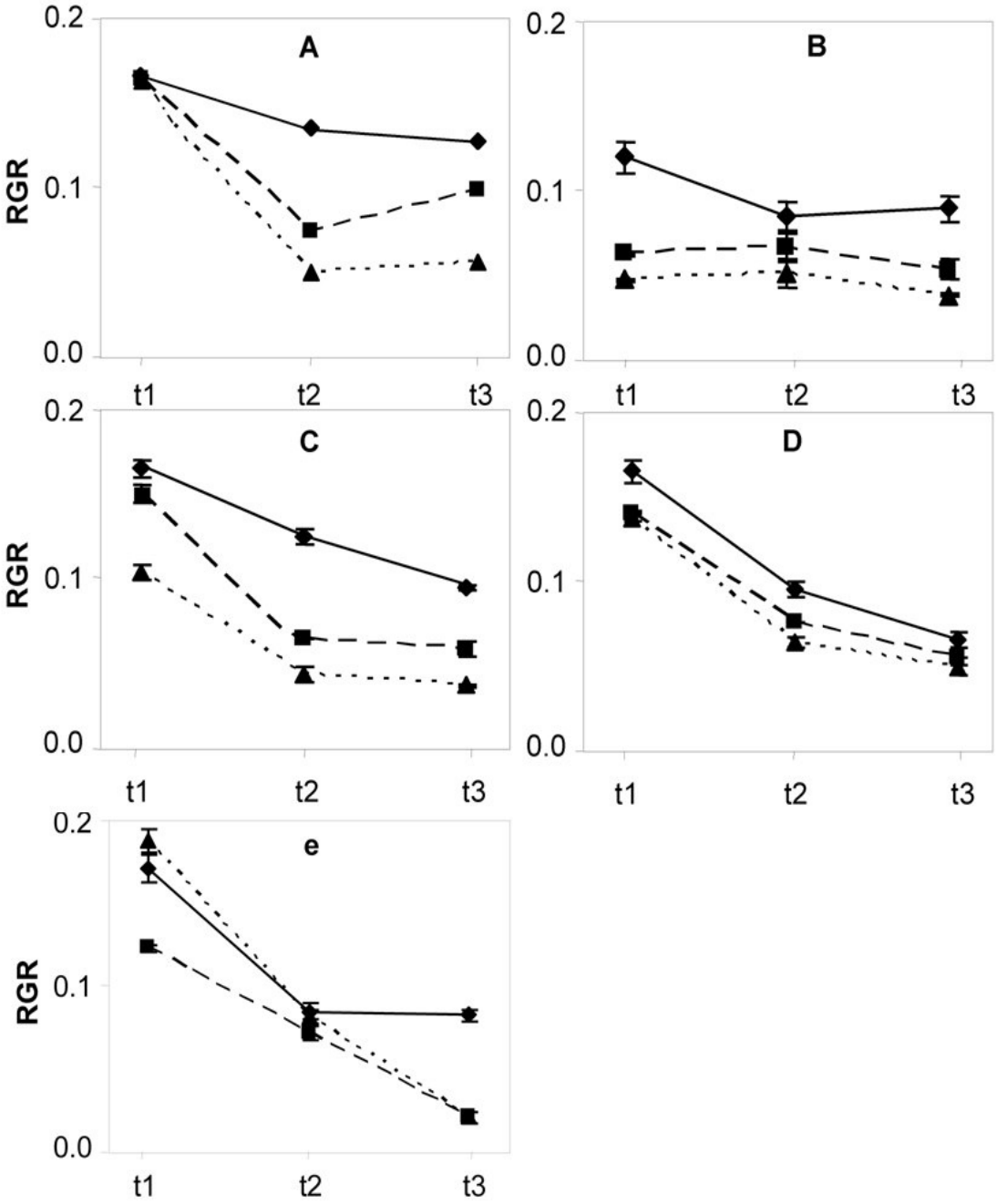

Figure 1. Relative growth rate (RGR) for ecotypes L1 (B), L2 (E), T1 (D), T2 (A) and Tm (C) in 0 mM (◆), 80 mM (■) and 200 mM of NaCl (▲), after t1 (4 weeks), t2 (8 weeks) and t3 (12 weeks). Bars represent the standard error of the means. Means are statistically different among treatments according to Duncan's Multiple Range Test at 5 % level.

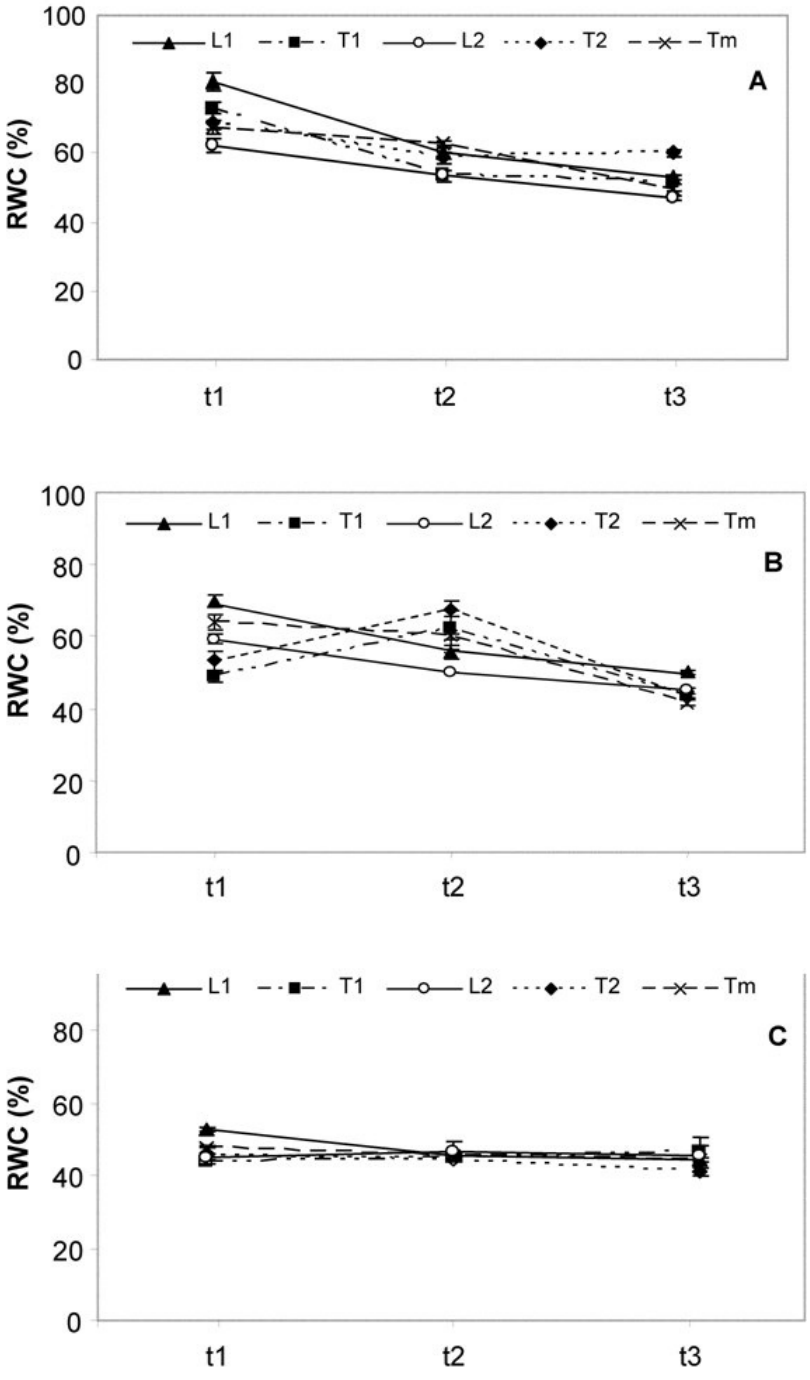

Figure 2. Leaf water content (RWC %) for ecotypes L1, L2, T1, T2 and Tm in 0 mM (A), 80 mM (B) and 200 mM of NaCl (C), at t1 (4 weeks), t2 (8 weeks) and t3 (12 weeks). Bars represent the standard error of the means. Each point is the mean of six measurements. Means are statistically different among treatments according to Duncan's Multiple Range Test at 5 % level.

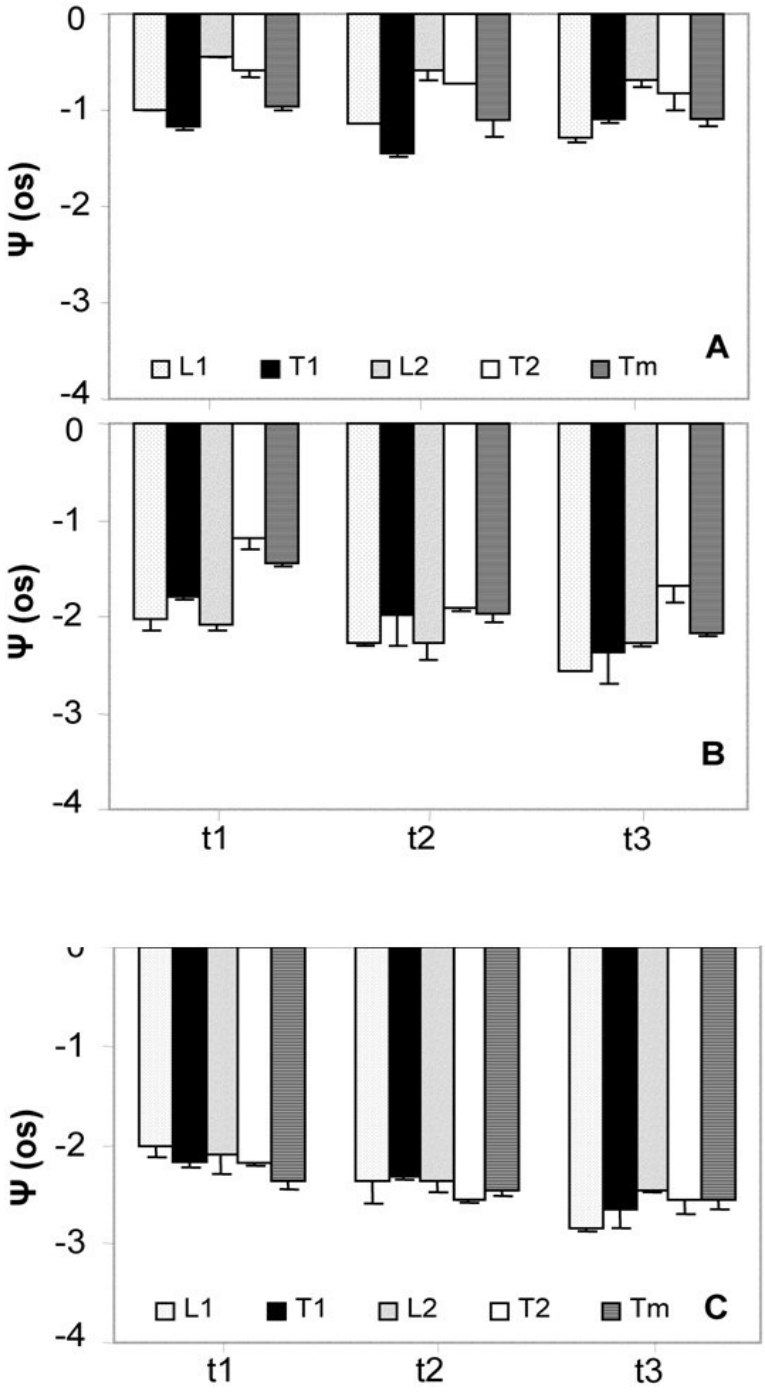

Figure 3. Leaf osmotic potential (Ψos) for ecotypes L1, L2, T1, T2 and Tm after application of different saline treatments: 0 mM (A), 80 mM (B) and 200 (C) mM of NaCl, at t1 (4 weeks), t2 (8 weeks) and t3 (12 weeks). Each point is the mean of six measurements. Bars represent the standard error of the means. Means are statistically different among treatments according to Duncan's Multiple Range Test at 5 % level.

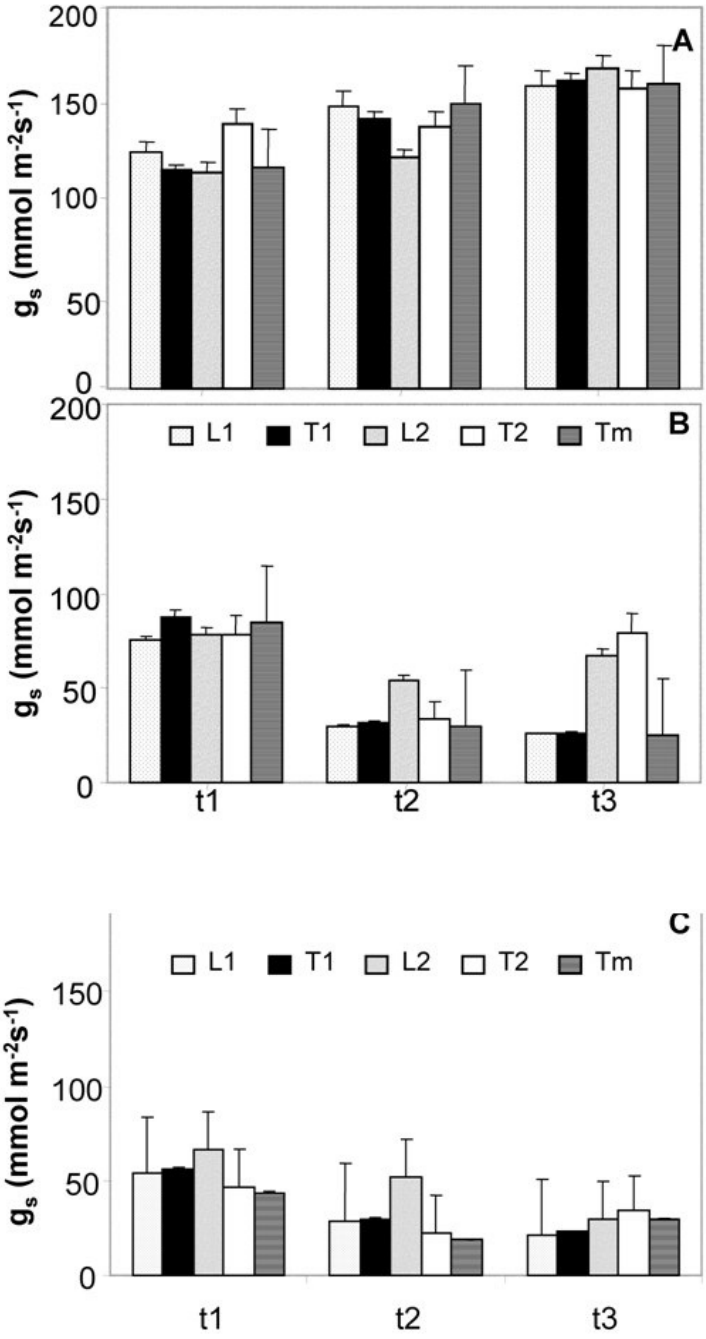

Figure 4. Stomatal conductance (gs) for ecotypes L1, L2, T1, T2 and Tm in 0 mM (A), 80 mM (B) and 200 (C) mM of NaCl at t1 (4 weeks), t2 (8 weeks) and t3 (12 weeks). Values are means of six measurements. Bars represent the standard error of the means. Each point is the mean of six measurements. Means are statistically different among treatments according to Duncan's Multiple Range Test at 5 % level.

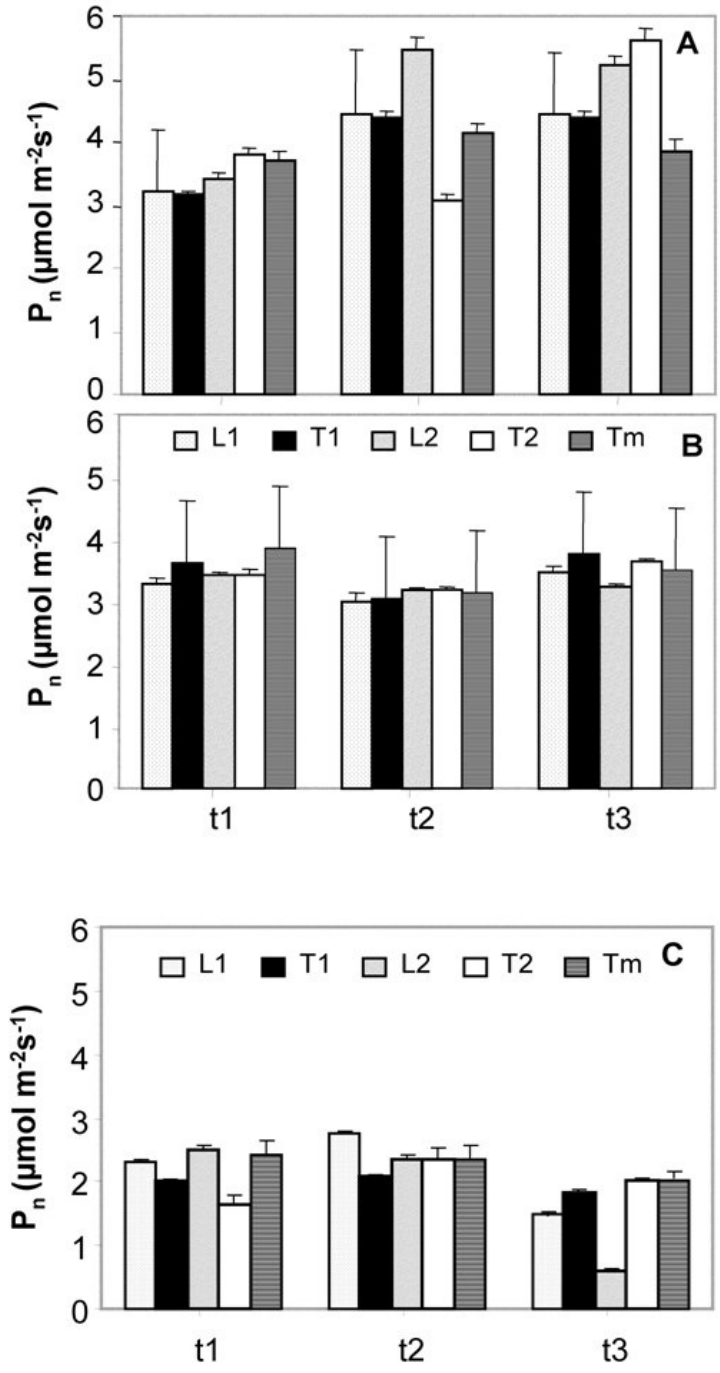

Figure 5. Net photosynthetic rate (Pn) for ecotypes L1, L2, T1, T2 and Tm in 0 mM (A), 80 mM (B) and 200 (C) mM of NaCl at t1 (4 weeks), t2 (8 weeks) and t3 (12 weeks). Values are means of six measurements. The vertical bars indicate standard errors. Means are statistically different among treatments according to Duncan's Multiple Range Test at 5 % level.

Table 2. Concentrations (mmol/g dry mass) of Na^+, K^+ and Cl^- and the K^+/ Na^+ selectivity ratio in the shoots of the ecotypes at the end of the experimental period in 0 mM, 80 mM and 200 mM of NaCl. L1, L2 (*L. ornithopodioides*); T1, T2 (*T. isthmocarpum*); Tm (*T. michelianum*)

Treatment	Ecotypes	Na^+	Cl^-	K^+	Na^+	Cl^-	K^+	Selectivity (K^+/ Na^+) in S2
		Shoots			Roots			
0 mM	L1	1.35 a	0.12 c	0.81 a	0.37 a	0.08 c	0.16 c	
	L2	1.03 b	0.16 b	0.98 a	0.06 b	0.12 ab	0.41 b	
	T1	1.46 a	0.20 a	1.06 a	0.08 b	0.10 b	0.89 b	
	T2	1.05 c	0.18 b	1.57 b	0.05 b	0.15 a	1.01 a	
	Tm	1.04 c	0.19 b	1.73 b	0.04 b	0.12 ab	1.08 a	
80 mM	L1	1.98 a	1.32 ab	0.79 c	0.83 a	0.27 d	0.10 d	
	L2	1.15 b	1.64 a	0.80 c	0.73 b	0.46 c	0.30 c	
	T1	1.82 a	1.05 b	0.98 c	0.86 a	0.39 c	0.55 c	
	T2	1.12 b	0.45 c	1.36 b	0.57 c	0.87 b	0.95 b	
	Tm	1.21 ab	0.57 b	1.41 a	0.52 c	1.14 a	1.01 a	
200 mM	L1	3.40 a	2.98 ab	0.76 c	2.58 a	1.64 d	0.09 c	15 c
	L2	3.35 a	3.26 a	0.68 c	2.42 a	2.80 b	0.25 b	15 c
	T1	2.32 b	2.14 bc	0.90 b	0.50 c	2.36 c	0.46 b	18 c
	T2	1.19 d	1.64 c	1.14 a	0.16 d	2.96 b	0.79 a	30 a
	Tm	1.94 c	2.54 b	0.91 b	1.34 b	3.08 a	0.80 a	22 b

Values represent means of 2 plants in three replications ± standard deviation.
Means followed by the same letter in each column are not significantly different according to Duncan's Multiple Range Test at 5 % level

DISCUSSION

This study showed a significant variability in salinity tolerance between *T. michelianum cv. Paradana* and Moroccan ecotypes of *T. isthmocarpum* and *L. ornithopodioides*. The variability occurred as growth responses and also traits associated with salt tolerance. *T. michelianum* cv. Paradana was chosen as check cultivar, because it reported to have some salinity tolerance (Nichols *et al*., 2008). *T. michelianum* is one of the current commercial forage legumes species used where salinity is expected (Rogers *et al*, 2009). However, our results indicate that is *T. isthmocarpum* that showed more productivity and good tolerance to salinity compared to *L. ornithopodioides* and Paradana. This highlights its potential for use in selection of productive cultivars for situations where salinity might be experienced. These differential effects of salinity on growth and metabolism may be due to osmotic inhibition of water availability, and disturbance of the uptake and translocation of nutritional ion (Niste *et al*., 2014). Overall, ecotype T2 was more salt tolerant than Tm (check cultivar) and other ecotypes. The morphological differences in plants due to exposure to saline conditions were expressed as a reduction in shoot and root dry weight, height and leaf area (Table

1). T2 was found to be tolerant to salinity up to 200 mM, as demonstrated by a less than 20 % of reduction in shoot yield relative to control treatment. Moreover, L2 was found to be tolerant of moderate level of salinity, although its production decreased significantly at 200 mM of NaCl. In fact, L2 exhibited high level of root dry weight compared to the check cultivar and other ecotypes. Grewal (2009) showed that one of the morphological mechanisms, associated with tolerance to salt stress is to develop a greater rooting depth and a greater total root dry weight. Leaf area of all treated plants decreased earlier than other morphological parameters under salinity effect. This behaviour confirms that, in general, the first symptom of salt stress in the plants is a restriction in leaf expansion (Parida and Das, 2005). The reduction in leaf area under saline stress can be considered as an avoidance mechanism, which minimise water losses when the stomata are closed, which happens to many species under osmotic stress (Chaves *et al*., 2008). Under saline conditions, it is known that the reduction in total leaf area can be explained by changes in cell wall properties, or a decreased photosynthesis rate (Rodríguez *et al*., 2005). In our study, it could be due to a decrease in the photosynthesis rate (Figure 5). Plant growth analysis showed also that the reduction in RGR might have been due to a direct effect of the stress on the stomatal closure and/or photosynthetic apparatus (Figure 4), indicating that photosynthesis could be the growth-limiting factor (Flexas *et al*., 2004). The salt stress induced stomatal regulation for all ecotypes, but in spite of this, the plants showed signs of leaf tissue dehydration, as was evidenced by their RWC values. However, in salt-treated plants the osmotic adjustments mean plants were able to maintain leaf cell turgor. Concentrations of chloride and sodium increased in the shoot and root, with increasing salinity in all species. But T2 was more capable of excluding both chloride and sodium from its shoots. The total concentrations of Cl^- and Na^+ present in the L2 were extremely high. An interesting finding in this study was the large difference in shoot Cl^- concentrations, between ecotypes, especially, between T2 and L2. T2 accumulated about half as much Cl^- in its shoots compared with L2 at NaCl concentrations from 80 to 200 mM (Table2). These differences in Cl^- were in contrast to Na^+ concentrations which only differed between plants in 200 mM of NaCL. This suggested that the low shoot concentration of Cl^- in ecotype T2 could be an important trait delivering salt tolerance. The Cl^- exclusion mechanism as a trait of salt tolerance was demonstrated in *T. michelianum* by Rogers and Noble (1991). The higher Na^+ and Cl^- levels were in shoots than in the roots of control and treated plants (Table 1). This indicated that in L2 an ion inclusion mechanism operated to salt tolerance. Preferential accumulation of either Na^+ and/or Cl^- has been reported to account for salt tolerance in plants and this capacity has been proposed as a trait of salt tolerance (Sangeeta *et al*., 1990). While Na^+ exclusion is an important determinant of salt tolerance, Cl^- 'exclusion' from shoots is also critical for plant survival in saline conditions (Hongtao *et al*., 2013). The capacity to include salts is considered a salt tolerance trait, when it is accompanied by the ability of plants to

compartmentalise NaCl in the vacuole, thus protecting salt-sensitive enzymes in the cytoplasm. Apparently, L2 treated with 200 mM of NaCl was unable to sequester ions efficiently intra or intercellularly, and the salts were accumulated and eventually become toxic leading to inhibition of growth. Furthermore, RGR showed high values at t1 in the 200 mM treatment, but with time this parameter decreased. The K^+/Na^+ selectivity ratio in the shoots can serve an indicator of crop species tolerance to salt stress (Chen *et al*., 2005). K^+/Na^+ ratio showed significant difference between salt treatments (p <0.001). It was very low in the control plants, increased substantially when plants were exposed to high level of NaCl. In the 200 mM treatment, T2 combined a favourable K^+/Na^+ selectivity ratio with greater relative salt tolerance, indicating its capability of maintaining a more favourable potassium concentration in its shoots under salinity stress. Under saline conditions, Na^+ competes with K^+ for uptake across the plasma membrane of plant cells. The competitive character of K^+ and Na^+ uptake was shown to be responsible of the differences in content of K^+ and Na^+ (Schachtman and Liu, 1999). High sodium concentrations are also detrimental to the uptake of potassium by the plant, and may result in reduced potassium levels (Hongtao *et al*., 2013). The seasonal trend in osmotic potential in stressed plants showed the capacity for osmotic adjustment of *Trifolium* plants. However, osmotic adjustment did not provide complete preservation of physiological processes despite turgor being maintained since there was a decrease in growth of plant in the 200 mM treatment. Osmotic adjustment was limited by capacity for salt accumulation in leaf tissue, and it was not enough to maintain the soil-plant osmotic gradient in plants treated with 200 mM of NaCl, where the stress induced dehydration.

CONCLUSION

The present study showed variability within Moroccan *T. isthmocarpum* and lotus ecotypes. The interesting finding that Moroccan ecotype T2 (*T. isthmocarpum*) was more tolerant than *T. michelianum cv. Paradana*. Overall, *T. isthmocarpum* species was more tolerant than *L. ornithopodioides* species in high level of salinity. Under NaCl treatment (200 mM), T2 accumulated half as much shoot Cl^- than L1 indicates better Cl^- 'exclusion' as an important trait for salt tolerance in this species. The response of *L. ornithopodioides* ecotypes to salinity shows an osmotic adjustment with ions inclusion when plants are treated with moderate levels of salinity (80 mM NaCl) which reduces stomatal conductance and consequently photosynthesis to maintaining the plant water balance. But when the salinity is long and severe (200 mM NaCl), the accumulation of ions in the shoot tissue seems to be more negative effect of the saline stress than a salt tolerance mechanism. These results open the way to test these ecotypes and evaluate their production under normal sward densities. The most productive ecotypes should be included in a breeding and seed production program to create Moroccan cultivars. While it is difficult to extrapolate results obtained under controlled conditions directly to the field where soil salinities may fluctuate widely throughout an irrigation cycle. Results from research

for *Melilotus* spp (Rogers *et al.* 2008) showing good correlation between glasshouse and field suggest that glasshouse studies are a useful first step in identifying salinity tolerant plant material.

ACKNOWLEDGMENT

We are grateful to National Institute of Agricultural Research of Rabat for Plant materials and for equipments, especially to Dr. Chawki Al Faiz (INRA). And to Dr. Mike Ewing for Centre for Legumes in Mediterranean Agriculture, University of Western Australia, for valuable comments on the manuscript and English editing.

REFERENCES

Arambarri AM. 2000. A cladistic analysis of the Old World species of *Lotus* L. (Fabaceae: Loteae). *Can. J. Bot.*, **78**, 351–360.

Ashraf M and Harris PJC. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.*; **166**, 3–16.

Beadle CL. 1993. Growth analysis. In: Hall DO, Scurelock JMO, Bolhàr-Nordenkampf HR, Leegood RC, Long SP, editors. Photosynthesis and Production in a Changing Environment: a Field and Laboratory Manual. Chapman & Hall, London, pp 36–46.

Begum F, Karmoker JL, Fattah QA and Moniruzzaman AFM. 1992. The effect of salinity on germination and its correlation with K^+, Na^+ and Cl^- accumulation in germinating seeds of *Triticum aestivum* cv. Akbar. *Plant Cell Phys*; **33**, 1009–1014.

Bennani K., Thami Alami I. and Al Faïz C. 2010. Évaluation morphologique et agronomique de soixante populations marocaines des genres *Trifolium* et *Lotus. Fourrages*, **204**, 239-245

Blumwald E, Aharon GS and Apse MP. 2000. Sodium transport in plant cells. *Biochem. Biophys. Acta*; **1465**, 140–151.

Bulińska–Radomska Z. 2000. Morphological relationships among 15 species of *Trifolium* occurring in Poland. *Genet. Res. Crop Evol.*, **47**, 267–272.

Chaves M. M., Flexas J. and Pinheiro C. 2008. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot., **103(4),** 551–560.

Chen Z, Newman I, Zhou M, Mendham N, Zhang G and Shabala S. 2005. Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. *Plant Cell Environ.*, **28**, 1230–1246.

Flexas J, Bota J, Loreto F, Cornic G and Sharkey TD. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biol.*, **6**, 269–279

Glenn EP, Olsen M, Moore D and Miyamoto S. 1994. How much sodium accumulation in necessary for salt tolerance in subspecies of the halophyte *Atriplex canescens*? *Plant Cell Environ.*, **17**, 711–719.

Gobilik J, Jerome V and Devina D. 2013. Preliminary selection of some ecotypes of *Cynodon dactylon* (L.) Pers. in Sabah, Malaysia, for turfgrass use. *J. Trop. Biol. Conserv.*, **10**, 51-66

Grewal HS. 2009. Response of wheat to subsoil

salinity and temporary water stress at different stages of the reproductive. *Plant soil*, **330**, 103–113.

Hongtao J., Pardob J. M., Batellic G, Van Oostend M. J., Bressane R. A. and Xia L. 2013. The Salt Overly Sensitive (SOS) Pathway: Established and Emerging Roles. *Molecular Plant*; 1-13.

Inoue Y, Morinaga S and Shibayama M. 1993. Non-destructive estimation of water status of intact crop leaves based on spectral reflectance measurements. *J. Crop Sci*., **62**, 462– 469.

Lopez, CML., Takahashi H and Yamazaki S. 2002. Plant-water relations of Kidney bean plants treated with NaCl and foliarly applied glycinebetaine. *J. Agron. Crop Sci*., **188**, 73–80.

Mandal H. K. 2014. Isolation of Salt Tolerant Strains of *Rhizobium Trifolii*. *Int. J. Agric. Food Sci. Technol*., **5**, 325-332

Mapfumo E, Behdani MA, Rengel Z and Barrett-Lennard EG. 2008. Growth and physiological responses of Balansa clover and burr medic to low levels of salinity. *Aust. J. Agr. Res*., **59**, 605–615.

Melchiorre M, Quero GE, Parola R, Racca R, Trippi VS and Lascano R. 2009. Physiological characterization of four model Lotus diploid genotypes: *L. japonicus* (MG20 and Gifu), *L. filicaulis*, and *L. burttii* under salt stress. *Plant Sci*., **177**, 618–628.

Nichols PGH, Craig AD, Rogers ME, Albertsen TO, Miller SM, McClements DR, Hughes SJ, D'Antuono MF and Dear BS. 2008. Production and persistence of annual pasture legumes at five saline sites in southern Australia. *Aust. J. Agr. Res*., **48**, 518–535.

Niste M, Vidican R, Rotar I, Stoian V, Pop R and Miclea R. 2014. Plant Nutrition Affected by Soil Salinity and Response of *Rhizobium* Regarding the Nutrients Accumulation. *ProEnviron*., **7**, 71 – 75

Parida AK and Das AB. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotox. Environ. S*., **60**, 324–349.

Rodríguez P, Torrecillas A, Morales MA, Ortuño MF and Sánchez-Blanco MJ. 2005. Effects of NaCl salinity and water stress on growth and leaf water relations of *Asteriscus maritimus* plants. *Environ. Exp. Bot*., **53**, 113–123.

Rogers ME and Noble CL. 1991. The Effect of NaCl on the Establishment and Growth of Balansa Clover (*Trifolium michelianum* Savi Var. balansae Boiss.) *Aust. J. Agr. Res*., **42**, 847–57.

Rogers ME and West DW. 1993. The effects of rootzone salinity and hypoxia on shoot and root growth in *Trifolium* species. *Ann. Bot*., **72**, 503–509.

Rogers ME *et al*. 2008. Diversity in the genus *Melilotus* for tolerance to salinity and waterlogging. *Plant Soil*, **304**, 89–101.

Rogers ME, *et al*. 2009. The influence of NaCl salinity and hypoxia on aspects of growth in *Trifolium* species. *Crop Pasture Sci*., **60**, 71–82.

Sangeeta Y.M., Irfan A. and Shamsul H. J. 2011. Causes of salinity and plant manifestations to salt

stress: A review. *J. Environ. Biol*., **32**, 667-685.

Schachtman DP and Kelman W. 1991. Potential of *Lotus* germplasm for the development of salt, aluminum and manganese tolerant pasture plants. *Aust. J. Agr. Res*., **42**, 139–140.

Schachtman DP and Liu W. 1999. Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants. *Plant Sci*., **4**, 281–287.

Staudinger C., Mehmeti V., Turetschek R., Lyon D., Egelhofer V. and Wienkoop S. 2012. Possible Role of Nutritional Priming for Early Salt and Drought Stress Responses in *Medicago truncatula*. *Frontiers Plant Sci*., **3**, 285. 1-13.

Torrecillas A, Rodríguez P and Sanchez-Blanco MJ. 2003. Comparison of growth, leaf water relations and gas exchange of *Cistus albidus* and *C. monspeliensis* plants irrigated with water of different NaCl salinity levels. *Horticult. Sci*., **97**, 353–368.