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**Differential Strategies of the Model Legumes *Lotus japonicus* and
Medicago truncatula in the Adaptation to
Salt Stress: Photosynthetic and Nutritional Responses***

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Abstract: In this research, *Lotus japonicus* and *Medicago truncatula* plants, forming determined and indeterminate nodules respectively, were subjected to NaCl stress to study changes in plant growth, nitrogen fixation, photosynthetic parameters and distribution and/or accumulation of organic and inorganic solutes. NaCl produced a significant inhibition of shoot growth in both legumes; meanwhile Nitrogen Fixation Rate (NFR) only was significantly inhibited in *L. japonicus*, indicating higher sensitivity of determined nodules to salinity. Optimum quantum yield of photosystem II (PSII), represented by the F_v/F_m ratio and pigments content, decreased in both legumes, mainly with 50 mM NaCl. The Na^+ percentage augmented in *M. truncatula* and *L. japonicus* shoot with 50 mM NaCl. NaCl also altered the K^+ concentration that decreased resulting in a decrease of K^+/Na^+ ratio in both legumes, more severe in *M. truncatula*. The results found in this study led us to conclude that the strategy against salt stress was different between these species, since amino acids and sugars accumulation can alleviate the effects of high Na^+ and low K^+/Na^+ in *M. truncatula* shoot, while in *L. japonicus*, the transport restriction of Na^+ to the shoot could be a strategy to avoid shoot Na^+ toxicity.

Key words: F_v/F_m , K^+/Na^+ , *Lotus japonicus*, *Medicago truncatula*, salt stress

INTRODUCTION

Legumes are of considerable importance for humanity and key for sustainable agriculture. Two model species, *Lotus japonicus* and *Medicago truncatula*, have been adopted internationally for legume genome sequencing and functional genomics research programs, because of the characteristics of these species (Stougaard, 2001). In spite of this, a few investigations about physiological and biochemical aspects of these plants have been done. The main biological differences between these species are: perennial growth and plant rejuvenation through side shoots, straight seed pods with easily accessible seeds and determinate nodulation in *L. japonicus* versus annual growth, solid spiral seeds pods and indeterminate nodulation in *M. truncatula*, thus the two models are complementary to some extent (Udvardy *et al.*, 2005).

Soil salinity is considered a significant factor affecting crop production and agricultural sustainability in arid and semi-arid region of the world, reducing the value and productivity of the affected land (Yamaguchi and Blundwald, 2005). The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution and specific Na^+ and Cl^- stress. High Na^+ content generally disrupts the nutrient balance, thereby causing specific ion toxicity despite disturbing osmotic regulation (Mengel and Kirkby, 2001; Ashraf and Harris, 2004). All of these cause adverse

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pleiotropic effects on plant growth and development and at physiological and biochemical levels (Munns, 2002). A better understanding of the mechanisms conferring salinity tolerance in plants is a key for developing selection and breeding strategies. However, despite of widespread research into the salinity tolerance of plants, mainly on water relationships, photosynthesis, and accumulation of various inorganic ions and organics metabolites, little is know on the metabolic sites and which salt stress damages plants and conversely, the adaptive mechanisms in plants to survive salinity stress (Munns, 1993; Munns *et al.*, 2002). High levels of Na⁺ inhibit Ca²⁺ and K⁺ absorption, which results in a Na⁺/K⁺ antagonism (Benlloch *et al.*, 1994). In brassicas, Ashraf and McNeilly (2004) suggested maintenance of high tissue K⁺/Na⁺ ratio as an important selection criterion for salt-tolerance. Plant species adapt to high salt concentrations in soils by lowering tissue osmotic potential accumulating inorganic solutes such as Na⁺ and K⁺ (Asch *et al.*, 2000), as well as organic solutes such as total soluble sugars, free amino acids and proline (Zhu, 2002).

It has been widely reported that photosynthetic capacity of chloroplast is depressed due to salt stress because of the reasons that salt stress leads to instability of the pigment protein complexes, destruction of chlorophylls and changes in the quantity and composition of carotenoids (Dubey, 1997). Besides, plants growing under saline conditions experience high light stress and photoinhibition is known to damage PSII (Ashraf and Harris, 2004). The measurements of chlorophyll fluorescence provide quantitative information about photosynthesis through non invasive means (Lichtenthaler, 1996). F_v/F_m ratio gives an estimate of the maximum quantum efficiency of PSII photochemistry (Baker and Rosenqvist, 2004) and has been widely used to detect stress induced perturbations in the photosynthetic apparatus (Sixto *et al.*, 2006; Weng and Lai, 2005).

The objective of the present study is to relate metabolic (organic and inorganic solutes) and photosynthetic alterations with the adaptation of two model legumes, (*Lotus japonicus* and *Medicago truncatula*) forming determined and indeterminate nodules respectively, to salinity.

MATERIALS AND METHODS

Plant Material and Experimental Treatments

Seeds of *L. japonicus* (cv. Gifu) and *M. truncatula* (var. Jemalong), were scarified by immersion in concentrated H₂SO₄ for 5 min washed with sterile water, surface sterilized by immersion in 5% NaClO plus Tween 20 for 20 min and germinated onto 0.8% water-agar plates at 28°C in the darkness. After 4 days, *L. japonicus* and *M. truncatula* seedlings were transferred to sterile vermiculite and watered with a nitrogen free nutrient solution supplied with NaCl (0, 25 and 50 mM). After 2 days, *L. japonicus* seedlings were inoculated with 1 mL of a stationary culture of *Mesorhizobium loti* R7A strain and *M. truncatula* seedlings with *Sinorhizobium meliloti* GR4 strain (c. 10⁹ cell mL⁻¹). Plants, in individual pots of about 300 mL, were grown in a controlled environmental chamber with a 16/8 h light-dark cycle, 23/18 °C day night temperature, relative humidity 55/65% and photosynthetic photon flux density (400-700 nm) of 450 μmol m⁻² sec⁻¹ supplied by combined fluorescent and incandescent lamps (Sylvania cool-white lifeline F96T12-CWVHO, Sylvania Ltd. Quebec, Canada). *M. truncatula* and *L. japonicus* plants were harvested 10 and 12 weeks after inoculation and nodules were frozen at -80°C for further analyses. Samples of leaves, stems and roots were dried at 70°C for 24 h and dry weight (DW) was estimated.

Nitrogen Fixation Assay

Nitrogen-fixation rate (NFR) was measured as the representative H₂ evolution in an open-flow system (Witty and Minchin, 1998) using an electrochemical H₂ sensor (Qubit System, Inc., Canada). H₂ production was continuously recorded on intact nodulated roots of plants. NFR was calculated from total nitrogenase activity (measured in Ar: O₂ atmosphere) and apparent nitrogenase activity (rate of H₂ production in air). Standards of high purity H₂ were used to calibrate the detector and values expressed on the basis of nodule dry weight (DW).

Chlorophyll Content and Fluorescence Parameters Analysis

To measure chlorophylls content, leaves were extracted with 95% ethanol in dark for 48 h until they were blanched. The concentrations of Chl a and Chl b were determined according to the procedure described by Wellburn (1994). Chlorophyll a fluorescence was measured using a portable pulse modulation fluorometer (OS5-FL, Opti-Sciences). Leaves were pre-darkened for 30 min, after dark adaptation, the fluorescence parameters F_0 , F_{max} and F_v/F_{max} were analyzed (Schreiber *et al.*, 1994). The minimum fluorescence (F_0) was obtained with modulated low intensity light ($<0.1 \mu\text{mol m}^{-2} \text{sec}^{-1}$) so as not to affect the variable fluorescence. The maximal fluorescence (F_{max}) was determined by a 0.8 sec long saturating light pulse ($180 \mu\text{mol m}^{-2} \text{sec}^{-1}$). The variable fluorescence (F_v) was determined by the difference between F_0 and F_{max} . F_v/F_{max} ratio, obtained from the F_v and F_{max} represents the activity of PSII and was used to assess the functional damage to the plants (Schreiber *et al.*, 1994). A single leaf per plant constituted each replicate.

Amino Acids, Proline and Total Soluble Sugars Determination

For amino acids, proline and soluble sugars determination, leaves (200 mg) were ground in ethanol (70% v/v) and centrifuged at 5000 g for 10 min. The supernatants were collected and stored at 4°C for free amino acids, proline and total soluble sugars determination. Proline content was measured according to the method described by Irigoyen *et al.* (1992) and free amino acids were determined using ninhydrin reagent (Yemm and Cocking, 1955). Standard curves, prepared with L-proline and L-asparagine, were used to estimate concentrations. The quantification of total soluble sugars was performed on the ethanolic extracts incubated with anthrone reagent at 100°C for 10 min and the absorbance measured at 625 nm according to Irigoyen *et al.* (1992).

Nutrients Determination

For the determination of sodium, potassium, nitrogen and calcium concentrations, dried samples of root and shoot were ground to pass a 20-mesh sieve and digested with a mixture of H_2SO_4 - H_2O_2 using microwave energy (Mingorance, 2002). Total nitrogen content was analyzed using the Kjeldahl method (Bouat and Crouzet, 1965). Sodium and potassium were directly measured by flame spectrophotometry using an EEL spectrometer according to CIETA (1969). Calcium was measured on acid-digested samples by atomic absorption spectrophotometry in a Perkin-Elmer Analyst 800 spectrophotometer equipped with a PE6017 lamp and measured at 422.7 nm.

Statistical Analyses

The experimental layout was a randomized complete block design. The growth values were means of five biological replicates per treatment. Four biological replicates were performed for the content of total soluble sugar, amino acids, proline, total pigments, nutrients and five replicates for fluorescence parameters. All results were subjected to analysis of variance with a least significant difference (LSD) test between means using a Statgraphics 5.0 (Statistical Graphics Corp., Rockville, MD, USA). The standard error (SE) and simple correlation coefficients were also calculated.

RESULTS

Growth and Nitrogen Fixation

The effect of two NaCl doses on growth of *M. truncatula* and *L. japonicus* during flowering stage (10 and 12 week after sowing) is showed in Table 1. Salinity stress reduced shoot dry weigh (SDW) of *M. truncatula* about 40 and 50% with 25 mM and 50 mM NaCl respectively and around 40% in *L. japonicus* with both salt doses. Regarding root-to-shoot ratio, it decreased proportionally in *M. truncatula* as salt doses increased (10 and 30% respectively), but in *L. japonicus* no significant

Table 1: Effect of NaCl treatments (mM) on shoot dry weight (g^{-1} plant), root shoot ratio, nitrogen fixation rate (NFR, $\mu\text{mol N g}^{-1}$ NDW h^{-1}) and shoot nitrogen concentration (N, %) in *M. truncatula* and *L. japonicus*

Parameters	<i>M. truncatula</i>			<i>L. japonicus</i>		
	0	25	50	0	25	50
Shoot dry weight	0.49 ^a	0.30 ^b	0.26 ^b	0.16 ^a	0.10 ^b	0.09 ^b
Root shoot ratio	0.32 ^a	0.28 ^a	0.23 ^b	0.33 ^a	0.34 ^a	0.33 ^a
NFR	67 ^a	65 ^a	58 ^a	146 ^a	124 ^a	72 ^b
N concentration	1.84 ^a	1.73 ^a	1.99 ^a	3.91 ^a	3.47 ^b	2.88 ^c

^{a-c}Means of each parameter within a row and legume followed by the same letter do not differ ($p \leq 0.05$) using the LSD test

Table 2: Effect of NaCl treatments (mM) on chlorophyll a and b contents (mg g^{-1} FW) minimum fluorescence (F_0), maximal fluorescence ($F_{m\max}$), variable fluorescence (F_v) and F_v/F_m ratio in *M. truncatula* and *L. japonicus*

Parameters	<i>M. truncatula</i>			<i>L. japonicus</i>		
	0	25	50	0	25	50
Chlorophyll a	0.38 ^a	0.33 ^a	0.17 ^b	0.74 ^a	0.63 ^b	0.50 ^c
Chlorophyll b	0.13 ^a	0.12 ^a	0.04 ^b	0.25 ^a	0.21 ^b	0.18 ^b
Chlorophyll a/b	2.95 ^a	2.82 ^a	4.14 ^b	2.77 ^a	3.22 ^a	2.71 ^a
F_0	168 ^a	177 ^a	170 ^a	117 ^a	123 ^a	112 ^a
F_m	968 ^a	948 ^a	843 ^b	670 ^a	678 ^a	575 ^b
F_v	800 ^a	771 ^a	673 ^b	554 ^a	555 ^a	462 ^b
F_v/F_m	0.83 ^a	0.81 ^b	0.80 ^c	0.83 ^a	0.82 ^{ab}	0.80 ^b

^{a-c}Means of each parameter within a row and legume followed by the same letter do not differ ($p \leq 0.05$) using the LSD test

differences were observed with salt treatments. In absence of saline conditions, *M. truncatula*'s SDW was about three fold higher than in *L. japonicus*'s SDW.

Concerning NFR showed a slight decline in *M. truncatula* without statistically significant differences under salt stress conditions; on the contrary, in *L. japonicus*, NFR showed 50% decline with the 50 mM salt dose. This parameter was more than double in *L. japonicus* in relation to in *M. truncatula* nodules in control conditions (Table 1).

The shoot nitrogen concentration remained stable in *M. truncatula* plants under saline stress; conversely, in *L. japonicus*, the N percentage decreased around 10 and 25% with 25 mM and 50 mM NaCl respectively. As in NFR, nitrogen content in *L. japonicus* was almost double than in *M. truncatula* (Table 1).

Chlorophyll Content and Fluorescence

NaCl treatments led to a decrease of chlorophyll content in both species, even more pronounced in *M. truncatula* plants where chlorophyll a declined 55% and chlorophyll b 70%, meanwhile in *L. japonicus* percentages decreased were 35 and 25% respectively. In control conditions *L. japonicum* showed double chlorophyll concentrations than *M. truncatula* (Table 2). So much chlorophyll a as chlorophyll b behaved similarly in *L. japonicus* showing chlorophyll a/b ratio no significant differences. Regarding fluorescence parameters (Table 2), F_0 remained stable in both legumes under NaCl treatments, but F_m declined with the higher salt dosage. Obviously, F_v had the same behavior that F_m because consist in the difference between F_m and F_0 which remained stable. Optimum quantum yield of PSII, represented by the F_v/F_m ratio, decreased in both legumes mainly with 50 mM NaCl dose. Nevertheless, *M. truncatula* also showed significant differences with 25 mM NaCl relative to non-salt treated plants.

Organic and Inorganic Solutes

Changes in the total soluble sugars, amino acids and proline content and other inorganic solutes in shoots of *M. truncatula* and *L. japonicus* treated with NaCl are shown in Table 3. Both legumes

Table 3: Effect of NaCl treatments (mM) on total soluble sugars, amino acids and proline contents (mg g⁻¹ FW) in shoot and potassium/sodium ratios (K⁺/Na⁺) in shoot and root of *M. truncatula* and *L. japonicus*

Parameters	<i>M. truncatula</i>			<i>L. japonicus</i>		
	0	25	50	0	25	50
Soluble sugars	8.69 ^c	13.08 ^b	15.86 ^a	9.18 ^b	11.40 ^a	10.86 ^b
Amino acids	1.44 ^c	1.74 ^b	1.99 ^a	2.49 ^a	2.13 ^b	2.46 ^a
Proline	0.18 ^c	1.70 ^b	3.90 ^a	1.03 ^a	0.40 ^c	0.86 ^b
K/Na ratio (shoot)	92.60 ^a	0.80 ^b	0.60 ^b	23.9 ^a	3.50 ^b	3.00 ^b
K/Na ratio (root)	64.20 ^a	1.20 ^b	1.30 ^b	6.30 ^a	2.40 ^b	1.70 ^b

^{a-c}Means of each parameter within a row and legume followed by the same letter do not differ ($p \leq 0.05$) using the LSD test

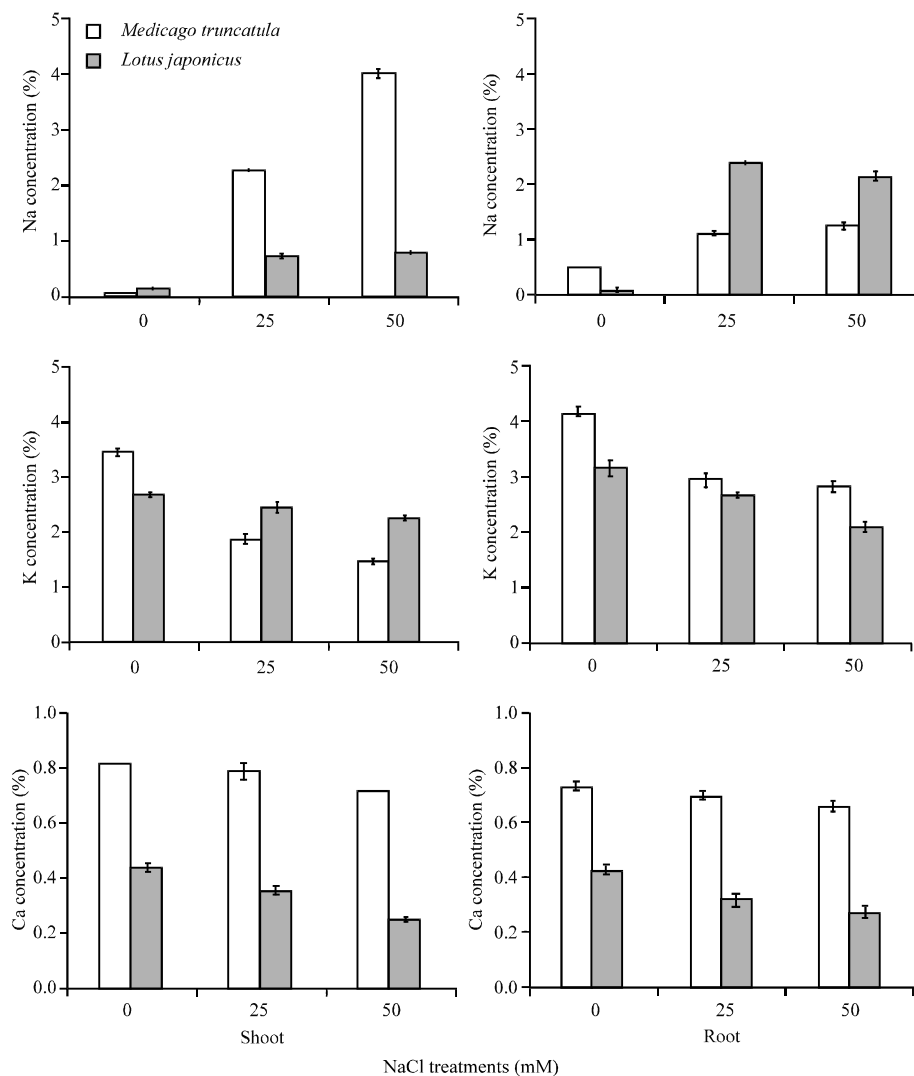


Fig. 1: Percentage of sodium, potassium and calcium in shoot and root of *M. truncatula* and *L. japonicus* treated with 0, 25 and 50 mM NaCl. Vertical bars represent \pm SE (n = 4) of four replicates

showed similar total soluble sugar contents in absence of salt treatments, although under salt stress conditions (50 mM NaCl), *M. truncatula* accumulated two fold higher total soluble sugars than control plants, meanwhile *L. japonicus* only accumulated approximately 20% more soluble sugars than non-salinized plants. On the other hand, saline conditions induced the accumulation of amino acids in shoot of *M. truncatula* plants (20 and 38%) and, more intensively of proline, which increased 9 and 22 fold with 25 and 50 mM NaCl, respectively. On the contrary, *L. japonicus* amino acids and proline content decreased with salt treatments, being lower values (about 15 and 60%) with 25 mM NaCl for amino acids and proline respectively.

NaCl treatments increased shoot and root sodium concentrations in both legumes as compared with non-salinized plants (Fig. 1), but increases were different in shoot and root depending on the specie. Na⁺ percentage augmented about 100 and 2 fold in shoot and root of *M. truncatula* respectively with 50 mM NaCl; however, in *L. japonicus* the Na⁺ increase was lower in shoot (7 fold) and higher in root (30 fold). To the contrary, potassium concentrations diminished with salinity in both legumes. Indeed, 50 mM NaCl resulted in a reduction of K⁺ by about 60% and 15% in shoot and root of *M. truncatula* respectively, and 32% in both organs of *L. japonicus*. The K⁺/Na⁺ ratio decreased in shoot and root of both legumes with the salt application (Table 3). In general, this parameter was higher in shoots than in roots, and under salinity the reduction was more severe in *M. truncatula*.

Concerning Ca²⁺ percentage, the tendency of this nutrient was to decrease with NaCl in both legumes so much in shoot as in root, being this effect more evident in *L. japonicus* (50%) than in *M. truncatula* (15%) with the higher salt dosage. In non salt-treated plants, Ca²⁺ concentration in shoot and root were two fold higher in *M. truncatula* than in *L. japonicus* (Fig. 1).

DISCUSSION

In this research, the effect of NaCl on model legumes *M. truncatula* and *L. japonicus* inoculated with *S. meliloti* and *M. loti*, respectively has been investigated. Salt stress diminished plant growth in both legumes, meanwhile nitrogen fixation only was inhibited by salinity in *L. japonicus* (Table 1), where the number and dry mass of nodules decreased with stress (data not shown), which has been reported in several legumes, such as *Phaseolus vulgaris* (Delgado *et al.*, 1994; Tejera *et al.*, 2006), *Pisum sativum* and *Vicia faba* (Codovilla *et al.*, 1999).

Plants growing under saline conditions accumulated more Na⁺, which generally cause specific ion toxicity by disrupting the nutrient balance and disturbing osmotic regulation (Ashraf and Harris, 2004). Present results (Fig. 1) showed a more significant increase of Na⁺ concentration in *M. truncatula* shoot and in *L. japonicus* root, which could be a strategy of *L. japonicus* to mitigate the shoot Na⁺ toxicity by regulating xylem solute composition and hence salt tolerance. Such regulation has been termed ion "exclusion" from the shoots (Munns, 2002) and has been detected in *Lotus tenuis* (Teakle *et al.*, 2007). Many physiological studies have demonstrated that Na⁺ toxicity is not only due to toxic effects of Na⁺ in the cytosol, but also because K⁺ homeostasis is disrupted possibly due to the ability of Na⁺ competing for K⁺ binding sites (Apse and Blumwald, 2002; Zhu, 2003). In this study, NaCl also altered the potassium (K⁺) concentration, which decreased in a lesser extend in *L. japonicus* shoot than in *M. truncatula* (Fig. 1), suggesting that selectivity for K⁺ over Na⁺ in *Lotus* is a contributory factor in the tolerance to the interactive effects of salinity. The reduction in K⁺ concentration with salt could inhibit growth by reducing the capacity for osmotic adjustment and turgor maintenance or by adversely affecting metabolic functions (Ashraf and McNeilly, 2004). The accumulation pattern of Ca²⁺ in *M. truncatula* and *L. japonicus* under salinity conditions showed a similar tendency in roots and shoots of *Vicia faba*, *Pisum sativum*, *Glycine max* and *Phaseolus vulgaris* (Codovilla *et al.*, 1995), which suggests the existence of a similar mechanism for Ca²⁺ accumulation. In addition, a reduction of Ca²⁺ concentration with salinity in *Arabidopsis thaliana* (Cramer and Jones, 1996) has been reported.

K^+/Na^+ ratio decreased with salt stress in both species, similarly to those reported in shoot of rice (Bohra and Dorffling, 1995) and chickpea (Tejera *et al.*, 2006). However, *L. japonicus*, capable of accumulating lower foliar Na^+ concentrations than *M. truncatula*, presented higher foliar K^+ concentration and higher K^+/Na^+ ratio under stress conditions. These characteristics have been demonstrated that enhance salt tolerance in tomato crops (Al-Karaki, 2000; Dasgan *et al.*, 2002). K^+/Na^+ ratio has been suggested as criteria of salt tolerance in brassicas (Ashraf and McNeilly, 2004). In addition, K^+/Na^+ ratio has been used as a nutritional indicator by a number of authors to select salt-tolerant plants, given the direct proportional relationship of this attribute with biomass production (Asch *et al.*, 2000; Sairam *et al.*, 2002).

M. truncatula accumulated more Na^+ in shoot (Fig. 1), thus, a higher damage in photosynthetic apparatus of this specie should be expected. Yellowing of the foliage is often one of the early symptoms of stress and it is associated with a concomitant decline in concentration of photosynthetic pigments (Webb and Fletcher, 1996). This was an obvious sign in our study correlated with the decrease in chlorophyll concentration and the Na^+ increase in shoots. The negative effect of salt on photosynthetic pigments concentration (Table 2) has been previously reported in Chickpea (Soussi *et al.*, 1998) and wheat (Sairam *et al.*, 2002). Salinity could affect chlorophyll concentration of leaves through inhibition of synthesis of chlorophyll or an acceleration of its degradation by chlorophyllase, which is more active under salt stress (Neera and Ranju, 2004). Impairment of the photosynthetic capacity is indicated by the measurements of chlorophyll fluorescence, one of the physiological parameters that have been shown to correlate with salinity tolerance (Monneveux *et al.*, 1990). The effect of different stressful environmental factors on PSII including salinity (Percival *et al.*, 2003) may be reflected by reduction in this ratio. The reduction of the quantum yield obtained in our experiment with 50 mM salt dose (Table 2) may result from a structural impact on PS II (El-Shintinawy, 2000), although Lu and Zhang (1998) found PS II to be highly resistant to salinity stress. It has been concluded that salinity affects reaction centers of PS II either directly (Masojidek and Hall, 1992) or via an accelerated senescence (Moradi and Ismail, 2007). Changes obtained in the F_v/F_m ratio in *L. japonicus* were followed by changes in the nitrogen content ($r = 0.99^{***}$) and plant biomass ($r = 0.81^{**}$). These results led us to suggest that salinity reduces the plant biomass production, likely due to an inhibition of nitrogen fixation and a reduction of plant photosynthesis.

Organic solutes, such as sugars and amino acids, have been proved to be helpful in osmoregulation in plant species, playing an important role in tolerance to salt stress (Bartels and Sunkar, 2005). Accumulations of total soluble sugars under salt stress was mainly detected in shoot of *M. truncatula* (Table 3), which is in accordance with results previously reported in chickpea leaves (Soussi *et al.*, 1998, 1999). In the same way, amino acids and proline accumulation was only observed in *M. truncatula* plants with NaCl supply. The accumulation of compatible solutes such as proline could be a consequence of damage produced by salt stress more than a protective strategy as occurs in *Lycopersicon esculentum* (Pérez-Alfocea *et al.*, 1996) and *Oryza sativa* L. (Lutts *et al.*, 1996). The role of proline in osmoregulation and salt tolerance generally has been questioned, and its concentration in many salt tolerant plants has been found to be higher than salt sensitive ones (Ashraf and Harris, 2004).

CONCLUSION

Finally, the results found in this study led us to conclude that the strategy against salt stress was different between these species, since amino acids and sugars accumulation can alleviate the effects of high Na^+ and low K^+/Na^+ in *M. truncatula* shoot, while in *L. japonicus*, the transport restriction of Na^+ to the shoot could be a strategy to avoid shoot Na^+ toxicity.

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REFERENCES

- Al-Karaki, G.N., 2000. Growth, water use efficiency and sodium and potassium acquisition by tomato cultivars grown under salt stress. *J. Plant. Nutr.*, 23: 1-8.
- Apse, M.P. and E. Blumwald, 2002. Engineering salt tolerance in plants. *Curr. Opin. Biotechnol.*, 13: 146-150.
- Asch, F., M. Dingkuhn, K. Dörfflin and K. Miezán, 2000. Leaf K/Na ratio predicts salinity-induced yield loss in irrigated rice. *Euphytica*, 113: 109-118.
- Ashraf, M. and P.J.C. Harris, 2004. Potentials indicators of salinity tolerance in plants. *Plant. Sci.*, 166: 3-16.
- Ashraf, M. and T. McNeilly, 2004. Salinity tolerance in *Brassica* oilseeds. *Crit. Rev. Plant. Sci.*, 23: 157-174.
- Baker, N.R. and E. Rosenqvist, 2004. Applications of chlorophyll fluorescence can improve crop productions strategies: An examination of future possibilities. *J. Exp. Bot.*, 55: 1607-1621.
- Bartels, D. and R. Sunkar, 2005. Drought and salt tolerance in plants. *Crit. Rev. Plant. Sci.*, 24: 23-58.
- Benlloch, M., M.A. Ojeda, J. Ramos and A. Rodríguez-Navarro, 1994. Salt sensitivity and low discrimination between potassium and sodium in bean plants. *Plant Soil.*, 166: 117-123.
- Bohra, J.S. and H. Dorffling, 1995. Salinity tolerance of rice (*Oryza sativa* L.) with reference to endogenous and exogenous abscisic acid. *J. Agron. Crop. Sci.*, 174: 79-86.
- Bouat, A. and C. Crouzet, 1965. Notes techniques sur un appareil semiautomatique de clorage de l'azote et de certains composés volatiles. *Ann. Agric.*, 16: 107-118.
- CIETA Comité Inter-Institutos para el estudio de técnicas analíticas de diagnóstico foliar, 1969. Métodos de referencia para la determinación de elementos minerales en vegetales. *Anal. Edaf. Agrobiol.*, 28: 409-431.
- Cordovilla, M.P., A. Ocaña, F. Ligeró and C. Lluch, 1995. Salinity effects of growth analysis and nutrient composition in four grain legumes-*Rhizobium* symbiosis. *J. Plant. Nutr.*, 18: 1595-1609.
- Cordovilla, M.P., S.I. Berrido, F. Ligeró and C. Lluch, 1999. *Rhizobium* strain effects on the growth and nitrogen assimilation in *Pisum sativum* and *Vicia faba* plant growth under salt stress. *J. Plant. Physiol.*, 154: 127-131.
- Cramer, G.R. and R.L. Jones, 1996. Osmotic stress and abscisic acid reduce cytosolic calcium activities in root of *Arabidopsis thaliana*. *Plant. Cell. Environ.*, 19: 1291-1298.
- Dasgan, H.Y., H. Aktas, K. Abak and I. Cakmak, 2002. Determination of screening techniques to salinity tolerance in tomatoes and investigation of genotype responses. *Plant. Sci.*, 163: 695-703.
- Delgado, M.J., F. Ligeró and C. Lluch, 1994. Effect of salt stress on growth and nitrogen fixation by pea, faba-bean, common bean and soybean plants. *Soil Biol. Biochem.*, 26: 371-376.
- Dubey, R.S., 1997. Photosynthesis in Plants Under Stressful Conditions. In: *Handbook of Photosynthesis*. Pessarakli, M. (Eds.). Marcel Dekker, New York, ISBN: 0824797086, pp: 859-875.
- El-Shintinawy, F., 2000. Photosynthesis in two wheat cultivars differing in salt susceptibility. *Photosynthetica*, 38: 615-620.
- Irigoyen, J.J., D.W. Emerich and M. Sánchez-Díaz, 1992. Water stress induced changes in concentrations of proline and total soluble sugar in nodulated alfalfa (*Medicago sativa*) plants. *Physiol. Plant*, 84: 55-60.

- Lichtenthaler, H.K., 1996. The Stress Concept in Plants: An Introduction. In: Stress of Life: From Molecules to Man. Csermely, P. (Ed.). Annals of the New York Academy of Sciences, New York, ISBN: 0801864313, pp: 156-189.
- Lu, C. and L. Zhang, 1998. Thermostability of photosystem II is increased in salt-stressed sorghum. *Aust. J. Plant Physiol.*, 25: 317-324.
- Lutts, S., J.M. Kinet and J. Bouharmont, 1996. Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. *J. Plant. Grow. Regul.*, 19: 207-218.
- Masojidek, J. and D.O. Hall, 1992. Salinity and drought stress are amplified by high irradiance in sorghum. *Photosynthetica*, 27: 159-171.
- Mengel, K. and E.A. Kirkby, 2001. Principles of Plant Nutrition. 5th Edn., Kluwer Academic Publishers, Dordrecht/Boston/London, ISBN: 1402000081 .
- Mingorance, M.D., 2002. Focused microwave-assisted digestion of vegetal materials for the determination of essential mineral nutrients. *Anal. Bioanal. Chem.*, 373: 153-158.
- Monneveux, P., M.E. Mekkaoui and X. Xu, 1990. Physiological Basis of Salt Tolerance in Wheat. Chlorophyll Fluorescence as a New Tool for Screening Tolerant Genotypes in Wheat Breeding. 1st Edn., Prospects and Future Approaches Varna, pp: 1-33.
- Moradi, F. and A.M. Ismail, 2007. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. *Ann. Bot.*, 99: 1161-1173.
- Munns, R., 1993. Physiological processes limiting plant growth in saline soil: Some dogmas and hypotheses. *Plant Cell Environ.*, 16: 15-24.
- Munns, R., 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.*, 25: 239-250.
- Munns, R., S. Husain, A.R. Rivelli, T. Condon and A.G. Lindsay *et al.*, 2002. Avenues for increasing salt tolerance of crops and the role of physiologically based selection traits. *Plant Soil.*, 247: 93-105.
- Neera, G. and S. Ranju, 2004. Growth, photosynthesis, nodule nitrogen and carbon fixation in the chickpea cultivars under salinity stress. *Braz. J. Plant. Physiol.*, 16: 50-74.
- Percival, G.C., G.A. Fraser and G. Oxenham, 2003. Foliar salt tolerance of Acer genotypes using chlorophyll fluorescence. *J. Arboricult.*, 29: 61-65.
- Pérez-Alfocea, F., M.E. Balibrea, A. Santa Cruz and M.T. Estan, 1996. Agronomical and physiological characterization of salinity tolerant in a commercial tomato hybrid. *Plant Soil*, 180: 251-257.
- Sairam, R.K., K.V. Rao and G.C. Srivastana, 2002. Differential response of wheats genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci.*, 163: 1037-1046.
- Schreiber, U., W. Bilger and C. Newbauer, 1994. Chlorophyll Fluorescence as a Noninvasive Indicator for Rapid Assessment of *in vivo* Photosynthesis. In: Ecophysiology of Photosynthesis. Schulze, E.D. and M.M. Caldwell (Eds.). Springer Verlag, Heidelberg, pp: 49-70.
- Sixto, H., I. Aranda and J.M. Grau, 2006. Assessment of salt tolerance in *Populus alba* clones using chlorophyll fluorescence. *Photosynthetica*, 44: 169-173.
- Soussi, M., A. Ocaña and C. Lluch, 1998. Effects of salt stress on growth, photosynthesis and nitrogen fixation in chick-pea (*Cicer arietinum* L.). *J. Exp. Bot.*, 49: 1329-1337.
- Soussi, M., C. Lluch and A. Ocaña, 1999. Comparative study of nitrogen fixation and carbon metabolism in two chickpea (*Cicer arietinum* L.) cultivars under salt stress. *J. Exp. Bot.*, 50: 1701-1708.
- Stougaard, J., 2001. Genetics and genomics of root symbiosis. *Curr. Opin. Plant. Biol.*, 4: 328-335.
- Teakle, N.L., T.J. Flowers, D. Real and T.D. Colmer, 2007. *Lotus tenuis* tolerates the interactive effects of salinity and waterlogging by excluding Na⁺ and Cl⁻ from the xylem. *J. Exp. Bot.*, 58: 2169-2180.

- Tejera, N.A., M. Soussi and C. Lluch, 2006. Physiological and nutritional indicators of tolerance to salinity in chickpea plants growing under symbiotic conditions. *Environ. Exp. Bot.*, 58: 17-24.
- Udvardy, M.K., S. Tabata, M. Parniske and J. Stougaard, 2005. *Lotus japonicus*: Legume research in the fast lane. *Trends Plant. Sci.*, 10: 222-228.
- Webb, J.A. and R.A. Fletcher, 1996. Paclobutrazol protects wheat seedlings from injury due to waterlogging. *Plant Growth Regul.*, 18: 201-206.
- Wellburn, A.R., 1994. The spectral determination of chlorophyll a and b, as well as total carotenoids, using various solvents with spectrophotometer of different resolution. *J. Plant Physiol.*, 144: 307-313.
- Weng, J.H. and M.F. Lai, 2005. Estimating heat tolerance among plant species by two chlorophyll fluorescence parameters. *Photosynthetica*, 43: 439-444.
- Witty, J.F. and F.R. Minchin, 1998. Methods for the continuous measurement of O₂ consumption and H₂ production by nodulated legume root systems. *J. Exp. Bot.*, 49: 1041-1047.
- Yamaguchi, T. and E. Blumwald, 2005. Developing salt-tolerant crop plants: Challenges and opportunities. *Trends Plant Sci.*, 12: 615-620.
- Yemm, E.W. and E.C. Cocking, 1955. The determination of amino acids with ninhydrin. *Analytic*, 80: 209-213.
- Zhu, J.K., 2002. Salt and drought stress signal transduction in plants. *Ann. J. Plant Biol.*, 14: 267-273.
- Zhu, J.K., 2003. Regulation of Ion homeostasis under salt stress. *Curr. Opin. Plant Biol.*, 6: 441-445.