African Journal of Biotechnology Vol. 10(65), pp. 14355-14365, 24 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1168
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Correlation between salt tolerance and genetic diversity between *Sulla carnosa* and *Sulla coronaria*

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Accepted 21 July, 2011

Sulla **constitutes an important genetic resource contributing to pastoral production, particularly in semi-arid regions. In Tunisia, seedlings of the southern species *Sulla carnosa* (Desf.) and the northern species *Sulla coronaria* (L.) were treated with NaCl (0, 100 and 200 mM) for 27 days. Salt treatments decreased leaf dry matter more in *S. carnosa* than in *S. coronaria*. *S. coronaria* accumulated less Na^+ and greater amounts of K^+ and showed greater K/Na selectivity, a trait which could be related to the maintenance of higher net K^+ uptake and transport in the presence of NaCl. Pigments were severely affected by salt stress in leaves of *S. carnosa* when compared with leaves of *S. coronaria*. In addition to these physiological characterisations, genetic diversity was measured between the two accessions using inter simple sequence repeat (ISSR) markers. Three ISSR primers generated a total of 63 DNA amplicons for *S. carnosa* and 64 DNA amplicons for *S. coronaria*, all of which were polymorphic between the two accessions. Correlations between the molecular and physiological data revealed statistically significant correlations between the salt response of these two *Sulla* accessions and two molecular markers B340 and B860, in roots and shoots, respectively. *S. coronaria* showed greater salt tolerance on the basis of growth and K/Na selectivity, making it a good candidate for inclusion in a future breeding programme.**

Key words: Salt constraint, *Sulla*, growth, ISSR markers.

INTRODUCTION

Salinity impairs plant growth through osmotic effects, specific ion toxicities and nutrient deficiencies (Wyn, 1981). Plant nutrition depends on the activity of membrane transporters that translocate minerals from the soil into the plant and mediate their intra and intercellular distribution (Epstein and Bloom, 2005; Marschner, 1995). High amounts of Na^+ in the growth medium perturb nutrient uptake in plants (Marschner, 1995; Grattan and Grieve, 1999; Ashraf, 2004). Selective ion uptake and differential ion compartmentalization are main features that explain disparities in tolerance to salinity between glycophytes and halophytes (Greenway and Munns, 1980). Na^+ compartmentalization is achieved by the action of Na^+/H^+ antiporters. The *Arabidopsis* AtNHX1 protein was the first Na^+/H^+ exchanger identified in plants (Gaxiola et al., 1999) and it mediates both Na^+ and K^+

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Abbreviations: AFLP, Amplified fragment length polymorphism; **ISSR,** inter simple sequence repeat; **PCR,** polymerase chain reaction; **RAPD,** random amplified polymorphic DNA; **RFLP,** restriction fragment length polymorphism; **SSR,** simple sequence repeat.

#These author contributed equally to this to this work.

coupled transport in vacuoles (Venema et al., 2002).

Salinity affects nutrient acquisition by interfering with K^+ uptake by ion carriers and ion channels (Maathuis and Amtmann, 1999). In *Arabidopsis thaliana*, passive uptake of K^+ into roots is mediated by K^+ selective channels located on the plasma membrane of root cells (Hirsch et al., 1998; Kim et al., 1998; Broadley et al., 2001; Gierth et al., 2005). Long distance transport of K^+ involves AKT2 channels, which secrete K^+ into the phloem in shoots (Lacombe et al., 2000) and SKOR, a shaker like outward channel which releases K^+ into the xylem of roots (Gaymard et al., 1998). The reduction in uptake of mineral nutrients (such as K^+) under saline conditions may occur due to competition with Na^+. However, maintaining a high cytosolic K^+/Na^+ ratio is a criteria of salt tolerance in species, such as barley and wheat, when they are confronted with a saline medium (Cuin et al., 2003; Dvorak et al., 1994; Gorham et al., 1990, 1997; Rubio et al., 1999). In *A. thaliana*, salt tolerance of ecotype *NOK2* also was related to a higher K^+/Na^+ selectivity (Kaddour et al., 2009).

Salinity modifies the level of secondary metabolites such as carotenoids (CAR) and chlorophylls (Agastian et al., 2000; Parida et al., 2002; Mahmoudi et al., 2010). Salt tolerance has been correlated to the maintenance of the level of these metabolites and the transcription of their genes (Mahmoudi et al., 2010). Indeed, steady levels of CAR are strongly correlated to salt tolerance of mungbean plants (Wahid et al., 2004) and sugarcane cultivars. In sugar cane, carotenoids may constitute a strategy for achieving salt tolerance along with limitations to the reduction of chlorophyll (Kanhaiya, 1996).

Sulla is a group of leguminous plants represented by six species of the *Sulla* and *Hedysarum* genii, which are spread across the mediterranean basin (Trifi-Farah et al., 2002; Choi and Ohashi, 2003). Accessions of *Sulla* are exploited for protection and enrichment of soil due to their N_2 fixation properties. Hence, *Sulla* constitutes an important genetic resource and contributes to pastoral production particularly in semi-arid regions because of its drought tolerance (Trifi-Farah and Marrakchi, 2002).

PCR-based molecular marker approaches are in demand when studying natural biodiversity because of their simplicity and requirement for only small quantities of sample DNA. Among PCR-based marker methods, AFLP (amplified fragment length polymorphism) (Karp et al., 1997), RAPD (random amplified polymorphic DNA) (Williams et al., 1990), SSR (simple sequence repeats or microsatellites) (Karlin and Burge, 1995) and ISSR (inter simple sequence repeats) (Zietkiewicz et al., 1994) are most commonly used. Genetic diversity between a number of *Sulla* accessions was revealed using AFLP, RFLP and rDNA ITS sequence variation (Trifi-Farah and Marrakchi, 2002; Marghali et al., 2005; Chennaoui et al., 2007). However, ISSRs have never been used to definite genetic variation between *Sulla* accessions. These PCR-based molecular markers involve amplification of DNA by a single 16 to 18 bp primer composed of a repeated non anchored sequence or a repeated sequence anchored at the 3' or 5' end by 2 to 4 arbitrary nucleotides (Zietkiewicz et al., 1994). ISSR markers are easy to handle, highly informative and repeatable.

Sulla coronaria (L.) is found growing wild in northern Tunisia, while *Sulla carnosa* (Desf.) (Choi and Ohashi, 2003) grows wild in southern Tunisia. The first objective of this study was to characterise growth, Na^+ and K^+ levels, nutritional parameters and transport and pigments in collections of these two *Sulla* species when treated with several NaCl concentrations (0, 100 and 200 mM). Our second objective was to describe genetic diversity between these two accessions using ISSR markers and to associate these molecular marker patterns with salinity-dependent and salinity-independent physiological data.

MATERIALS AND METHODS

Plant growth and salinity treatment

Seeds of *S. carnosa* and *S. coronaria* were collected from natural populations prospected throughout Tunisia. Seeds were scarified, then soaked in water for 24 h and put in pots containing a 1:2 (v:v) mixture of sand and peat. The pots were placed in a greenhouse and irrigated first with distilled water for 3 days, then for 47 days with a modified Long Ashton nutritive solution (Hewitt, 1966) diluted to 1/5th strength so that the final solution was composed of 1.5 mM $MgSO_4$, 1.6 mM K_2SO_4, 0.3 mM K_2HPO_4, 0.3 mM KH_2PO_4, 3.5 mM $Ca(NO_3)_2$, 2.0 mM NH_4NO_3, 0.04 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.05 µM H_3BO_3, 0.02 µM MoO_3 and 3 µM FeEDTA. Plants were harvested at day 50; thereafter, the remaining plants were irrigated for an additional 27 days with the same nutritive solution supplemented with NaCl (0, 100 or 200 mM) and then harvested again. For molecular analyses, seeds were scarified and then soaked in water for 24 h, transferred to Petri dishes and incubated in a greenhouse for 4 days at 25°C and a 12 h day length until root and cotyledon emergence.

Growth and elemental analyses

Fresh and dry matter of leaves and roots were determined. Roots were collected by submerging the whole root system three times in 120 ml distilled water to remove growth substrate from the roots. Fresh and dry matters were determined for leaves and roots. Dried roots and shoots of each plant were then separately digested in 0.1 N HNO_3. The concentrations of K^+ and Na^+ in the clear digests were measured by flame photometry (Jenway PFP7 butane air flame). The rates of ion net uptake (J_{upt}) and transport (J_{trp}) were calculated according to Pitman (1988) as:

$$J_{upt} = \frac{\Delta I_{tot}\, Ln\, (Wr_2/Wr_1)}{(t_2-t_1)\, \Delta_W}$$

Where, Δ stands for the difference between times t2 (day and t1 (days); Itot is the average amount of ion per plant (mmol) calculated

from ion concentrations (mmol g-1 DW) and plant dry weights (g) and Wr is the mean root dry weight (g).

$$J_{trp} = \frac{\Delta I_s \; Ln \; (Wr_2/Wr_1)}{(t_2-t_1)\; \Delta_W}$$

Where, Δ stands for the difference (in days) between time t_2 (day 77) and time t_1 (day 50); I_S is the average amount of measured ion per shoot (mmol) calculated from ion concentrations (mmol g^{-1} DW) and shoot dry weights (g) and Wr is the mean root dry weight (g).

Growth and ion data were presented as the means of eight plants for each treatment (one of three salinity conditions for each of two plant species). Significant differences between treatments were determined by multi-variate analysis of variance (ANOVA) and means were compared and separated with a LSD test (Statistica®, StatSoft France)) at $p \leq 0.05$.

DNA extraction and PCR amplification

DNA was isolated from frozen fresh seedlings according to the procedure described by Dellaporta et al. (1983) with minor modifications adapted to mini-extraction. PCR amplifications were conducted using a Crocodile III thermocycler (QBIOgène, France) and three ISSR primers (Table 1). Each amplification was performed in a total volume of 25 µl and contained: 30 ng of total cellular DNA, 200 mM of each dNTPs (DNA polymerization mix, Pharmacia, France), 1.5 mM $MgCl_2$, 60 pg of primers, 2.5 µl of 10x Taq DNA polymerase buffer and 1.5 U of Taq DNA polymerase (QBIOgène, France). Amplification conditions included an initial step of 5 min at 94°C, followed by 35 cycles (30 s at 94°C, 90 s at 45 to 60°C depending on the primer (Table 1) and 90 s at 72°C) and a final 5 min step at 72°C. ISSR PCR products were separated on 0.8% agarose gels and the DNA fragments were visualized by comparing ethidium bromide stained patterns and intensities with a DNA size marker of known concentration.

Each ISSR fragment (band) was scored as 1 when present and 0 when absent. Bands showing unambiguous polymorphism between salinity treatments and/or *S. carnosa* and *S. coronaria* were entered into a binary data matrix. Principal components analysis (PCA) was conducted on the ISSR patterns using XLSAS software (Statistical Analysis System, version 2009.3) (SAS, 2009). A Pearson correlation test and a Mantel test (Mantel, 1967) were used to test the statistical relationship between the presence or absence of specific ISSR amplicon patterns and the elements of the two matrices generated by the genetic, growth and physiological traits.

RESULTS

Effect of salt on growth and water content

The effect of NaCl (0, 100 and 200 mM) on the growth of the two *Sulla* species collected from different ecological locales is presented on Figure 1. After 27 days of treatment, 100 mM NaCl decreased plant dry matter equally in *S. carnosa* and *S. coronaria*. Differences were observed when plants were treated with 200 mM NaCl. Indeed, 200 mM NaCl decreased whole plant dry matter to < 20 and 40% of the untreated plant levels for *S. carnosa* and *S. coronaria*, respectively. Water content was much more variable in *S. carnosa* than in *S. coronaria* and masked any differences in shoot water content between the two *Sulla* accessions (Figure 2). Concerning the roots, a decrease in water content was observed only with 200 mM NaCl and was similar for both accessions (Figure 2).

Effect of salt on content, transport and selectivity of K^+ and Na^+

Since salinity can affect the rates of Na^+ and K^+ ion uptake and transport, the rates of ion uptake and transport were calculated for the two *Sulla* species by dividing the mean ion absorption per plant and per shoot over the course of the NaCl treatment by the mean root dry weight. Net K^+ uptake was identical for both accessions in the absence of NaCl treatment, although, K^+ transport was slightly higher for *S. carnosa* without salt (Figure 3). However, 100 mM NaCl decreased the net K^+ uptake and transport to a greater degree in *S. carnosa* when compared with *S. coronaria*. Both K^+ parameters showed even further decreases with exposure to 200 mM NaCl, but seemed to be totally inhibited by NaCl in *S. carnosa,* reaching negative values that suggested a loss of K^+ in this accession. K^+ accumulation was also significantly more reduced in both shoots and roots by NaCl treatments in *S. carnosa* as compared to *S. coronaria*, although, *S. coronaria* roots accumulated less of this ion overall in the absence of salt than *S. carnosa* (Figure 3).

Na^+ uptake was similar for *S. carnosa* and *S. coronaria* when treated with 200 mM NaCl, although, *S. carnosa* accumulated slightly higher amounts of Na^+ at 100 mM NaCl (Figure 4). Nonetheless, differences between the two accessions were observed for Na^+ accumulation, especially in the shoots. After 27 days of NaCl treatment, *S. carnosa* accumulated more Na^+ in shoots than *S. coronaria* (Figure 4). Indeed, *S. carnosa* accumulated 4.5 and 6.3 mmol g^{-1} DM with 100 and 200 mM NaCl, respectively whereas *S. coronaria* accumulated only 3.2 and 3.7 mmol g^{-1} DM, respectively when exposed to similar levels of salt. In roots, differences between the two accessions were less pronounced than those observed for shoots (Figure 4).

K^+ selectivity is estimated by the ratio between (K/K+Na) in the shoots or the roots and (K/K+Na) in the culture medium, and is a measure of the plant's ability to discriminate between these two ions, particularly when they are being absorbed by the roots. *S. coronaria* maintained a higher K^+ selectivity in the presence of 100 and 200 mM, NaCl when compared with *S. carnosa* for both roots and shoots (Figure 5).

Effect of NaCl treatment on chlorophyll and carotenoid content

Together with the impact of salt on growth, the photo-

Table 1. Percentage of polymorphic bands.

Sequence	Temperature (°C)	ISSR-PCR band			Size (pb)
		Total	Polymorphic		
			Number	Percentage	
$(AG)_{10}$ G	60	28	28	100	300-2090
$(AG)_{10}$ T	57	40	40	100	160-1950
$(TC)_{10}$ C	60	13	13	100	340-3900

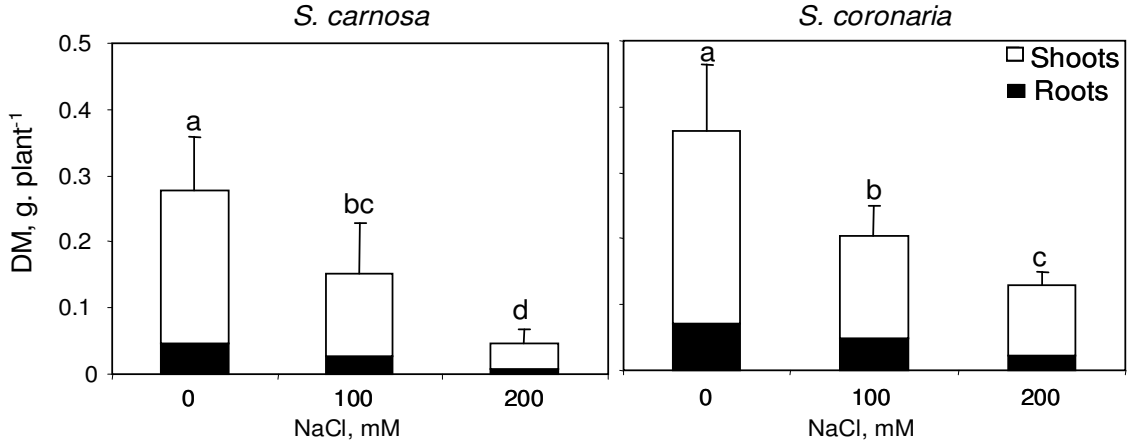

Figure 1. Effect of NaCl treatment on the growth of *S. carnosa* and *S. coronaria* shoots and roots. NaCl treatments (0, 100 and 200 mM) were applied to 50 day-old individual plants and lasted for 27 days. Data is expressed as means ± confidence intervals (n=8, $p \leq 0.05$). Statistically significant differences of the means are indicated by different letters for whole plant measurements (but not for individual shoot and root tissues).

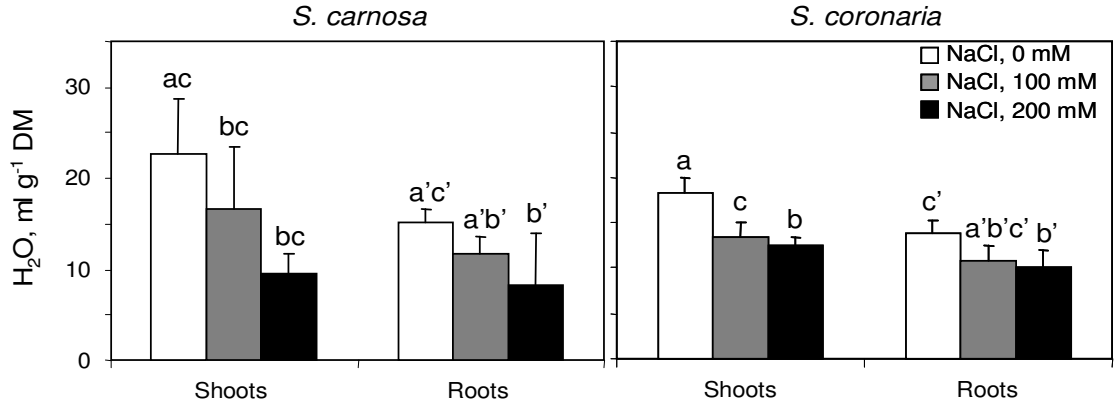

Figure 2. Effect of NaCl treatment on water content of *S. carnosa* and *S. coronaria* shoots and roots. NaCl treatments (0, 100 and 200 mM) were applied to a 50 day-old individual plants and lasted for 27 days. Data is expressed as means ± confidence intervals (n=8, $p \leq 0.05$). Statistically significant differences of the means are indicated by different letters (shoots) or prime letters (roots).

system of a plant is usually compromised under saline conditions unless sufficient anti-oxidants are present. *S. carnosa* and *S. coronaria* showed equivalent levels of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids (one type of anti-oxidant) in the absence of salt and when treated with 10 mM NaCl (Figure 6). *S. carnosa* leaves showed a decrease in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids when exposed to 200 mM NaCl, while these parameters were unaffected in *S. coronaria* leaves by the high salt concentration (Figure 6). The decrease in *S. carnosa* was estimated as 63% for chlorophyll a, 68% for chlorophyll

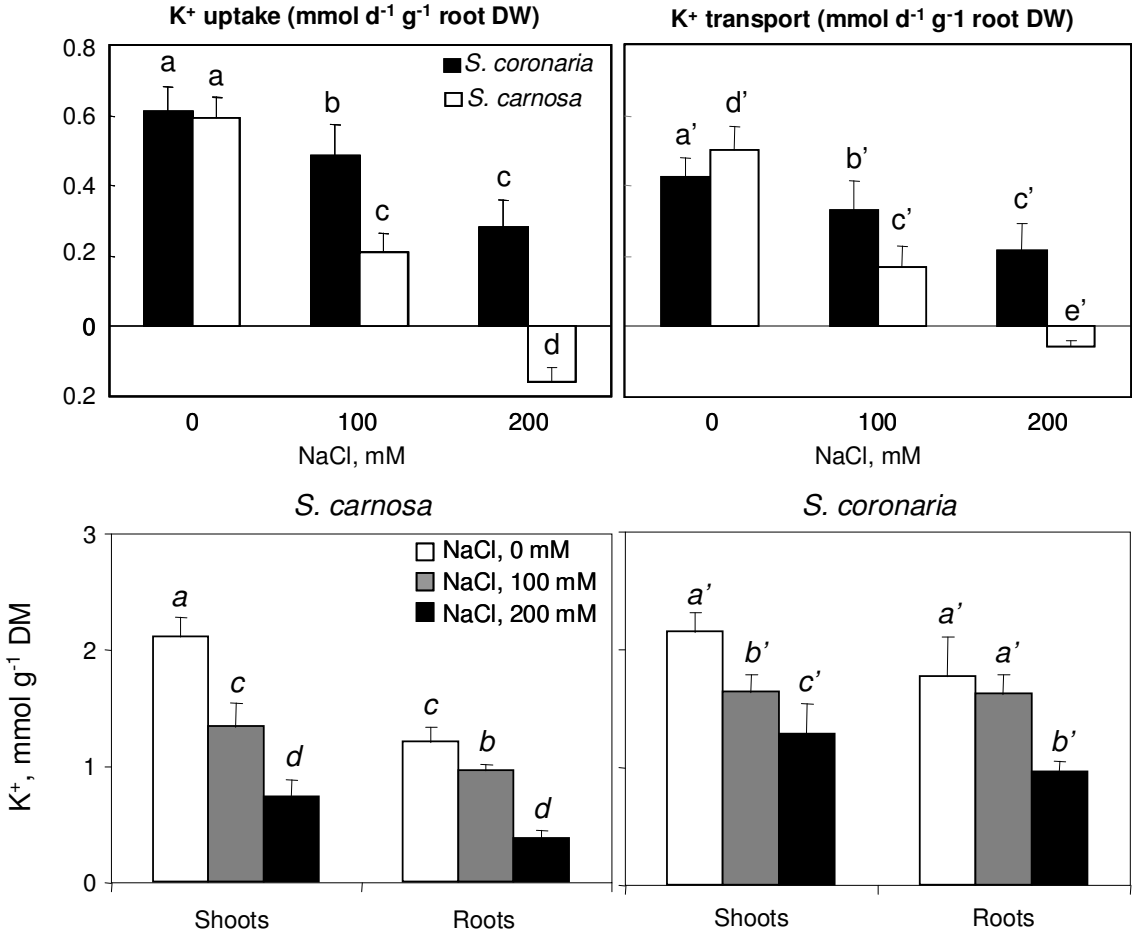

Figure 3. Effect of NaCl treatment on K^+ content, uptake and transport into roots and shoots of *S. carnosa* and *S. coronaria*. NaCl treatments (0, 100 and 200 mM) were applied to a 50 day-old individual plants and lasted for 27 days. Data is expressed as means ± confidence intervals (n=8, $p \leq 0.05$). Statistically significant differences of the means are indicated by different letters (K^+ uptake), prime (') letters (K^+ transport), italicized letters (K^+ content in shoots) or italicized prime letters (K^+ content in roots).

b, 64% for total chlorophyll and 46% for carotenoids when compared the non saline condition.

DNA profiling of *S. carnosa* and *S. coronaria*

Since physiological measurements indicated substantial differences in their responses to Salinity, DNA profiling was conducted on *S. carnosa* and *S. coronaria* to determine the degree to which these species were related. Three ISSR primers [$(AG)_{10}$ G, $(AG)_{10}$ T and $(TC)_{10}$ C], were used to analyse DNA polymorphism between *S. carnosa* and *S. coronaria.* Gel electrophoresis patterns of were used to analyse DNA polymorphism between *S. carnosa* and *S. coronaria.* Gel electrophoresis patterns of amplicons obtained using the three primers are illustrated in Figure 7 for 8 to 10 individual plants per *Sulla* species. For *S. carnosa,* a total of 64 ISSR loci were observed (only clear bands were scored), and all of them were polymorphic between the two accessions. For *S. coronaria*, 63 ISSR loci were scored, while for *S. carnosa*, all of these were polymorphic between the accessions (Figure 7).

Principal component analysis

Genetic variability between *S. carnosa* and *S. coronaria* was also studied by applying principal component analysis (PCA) applied to a data matrix developed for molecular marker data determined for the two accessions. Table 2 illustrates the percentage of variability obtained by the first three principal components, which in total explained 36.9% of the total variability (inertia) (1st

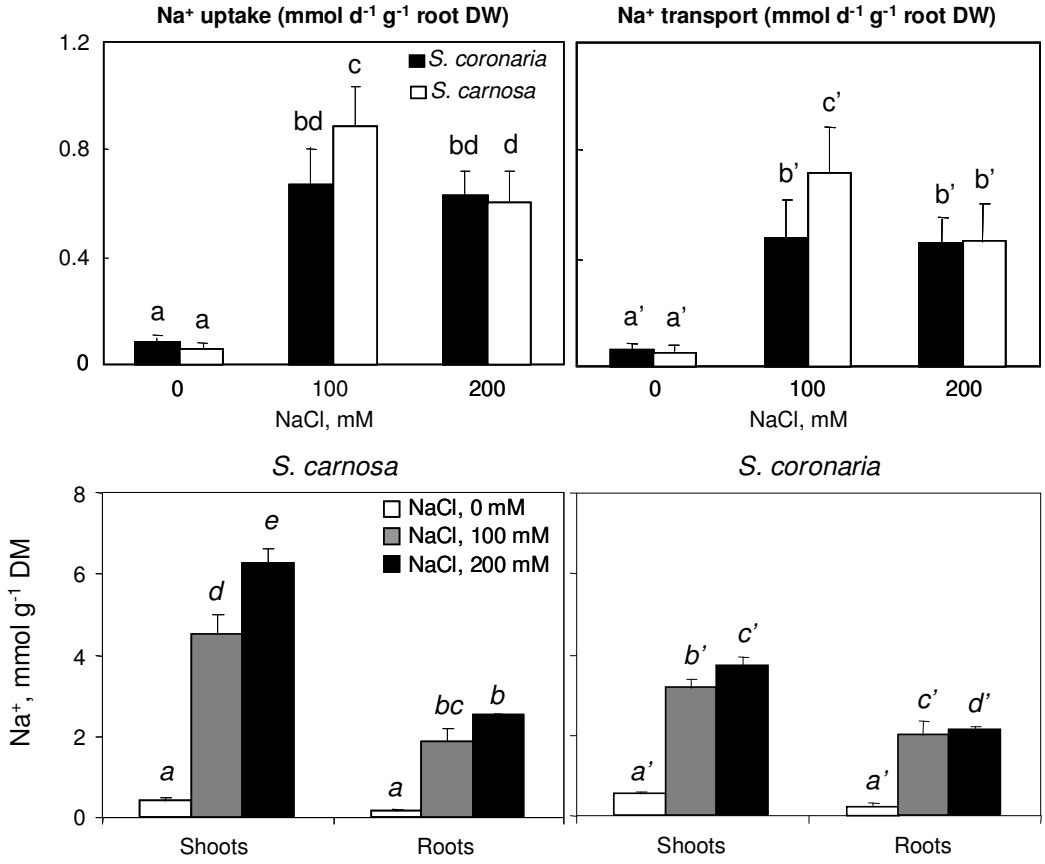

Figure 4. Effect of NaCl treatment on Na^+ content, uptake and transport into roots and shoots of *S. carnosa* and *S. coronaria*. NaCl treatments (0, 100 and 200 mM) were applied to a 50 day-old individual plants and lasted for 27 days. Data is expressed as means ± confidence intervals (n=8, $p \leq 0.05$). Statistically significant differences of the means are indicated by different letters (Na^+ uptake), prime (') letters (Na^+ transport), italicized letters (Na^+ content in shoots) or italicized prime letters (Na^+ content in roots).

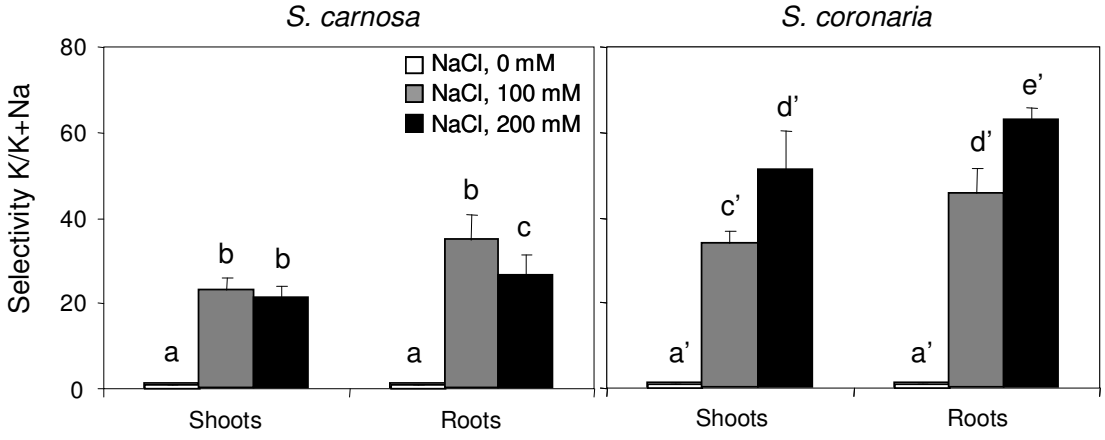

Figure 5. Effect of NaCl treatment on K^+/Na^+ selectivity of roots and shoots of *S. carnosa* and *S. coronaria*. K^+/Na^+ selectivity is estimated by the ratio between K/(K+Na) values in the specific organ and in the medium. NaCl treatments (0, 100 and 200 mM) were applied to a 50 day-old individual plants and lasted for 27 days. Data is expressed as means ± confidence intervals (n=8, $p \leq 0.05$). Statistically significant differences of the means are indicated by different letters (shoots) or prime letters (roots).

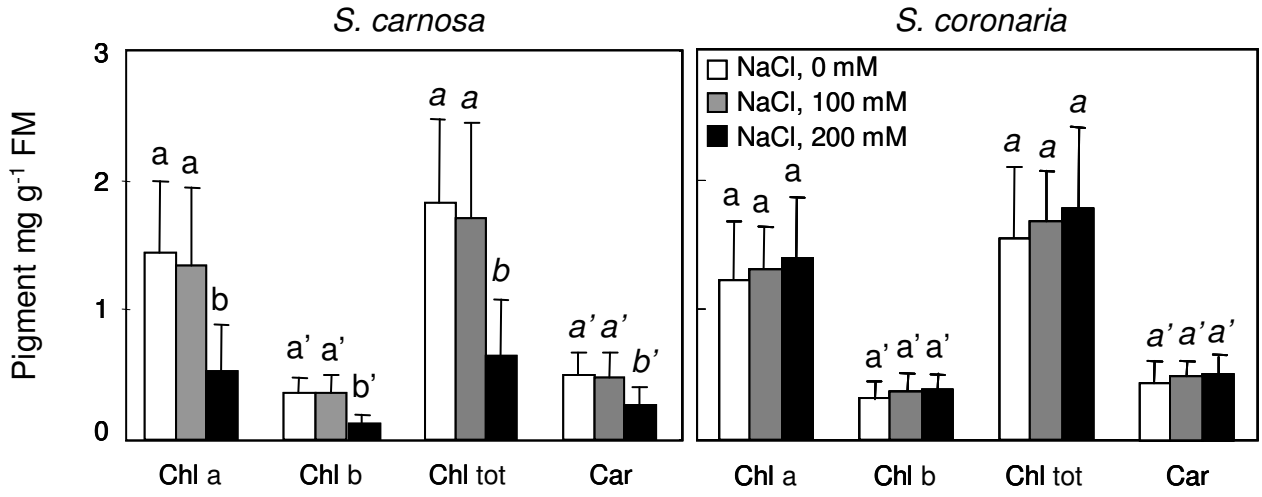

Figure 6. Effect of NaCl treatment on pigment content in the leaves of *S. carnosa* and *S. coronaria*. Chl a (chlorophyll a); Chl b (chlorophyll b); Chl tot (total Chlorophyll); Car (total carotenoids). NaCl treatments (0, 100 and 200 mM) were applied to a 50 day-old individual plants and lasted for 27 days. Data is expressed as means ± confidence intervals (n=8, $p \leq 0.05$). Statistically significant differences of the means are indicated by different letters (chl a), prime (') letters (chl b), italicized letters (total chl) or italicized prime letters (Car).

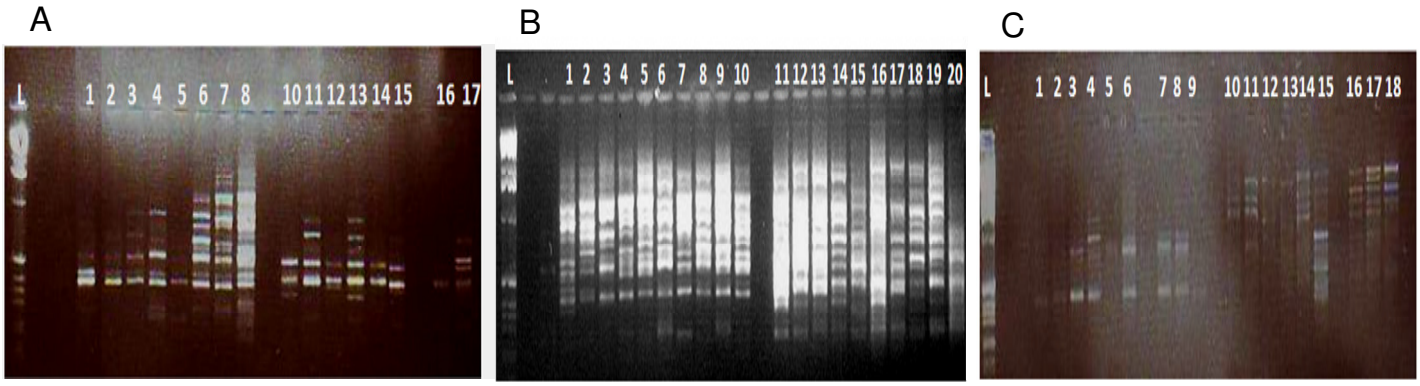

Figure 7. Representative PCR-based DNA amplification patterns using ISSR markers. (A): amplification with $(AG)_{10}G$ (lanes 1-8: *S.carnosa*; lanes 10-17: *S. coronaria*). (B): amplification with $(AG)_{10}T$ (lanes 1-10: *S. carnosa*; lanes 11-20: *S. coronaria*). (C): amplification with $(TC)_{10}C$ (lanes 1-9: *S. carnosa*; lanes 10-18: *S. coronaria*). DNA was run on 0.8% agarose gel and stained with ethidium bromide.

Table 2. Principal component analysis of *Sulla* ISSR amplicon bands.

Principal component	Absorbed inertia (%)	Cumulated inertia	Positive marker	Negative marker
1	B860	18.7	C2100 A1000 A1175	18.7 C2600 C3900
2	B310	28.3	B285 B500 B570	9.6 B420 B840
3	B200	34.9	A1090 B980 B1200	8.6 B1650 B1950

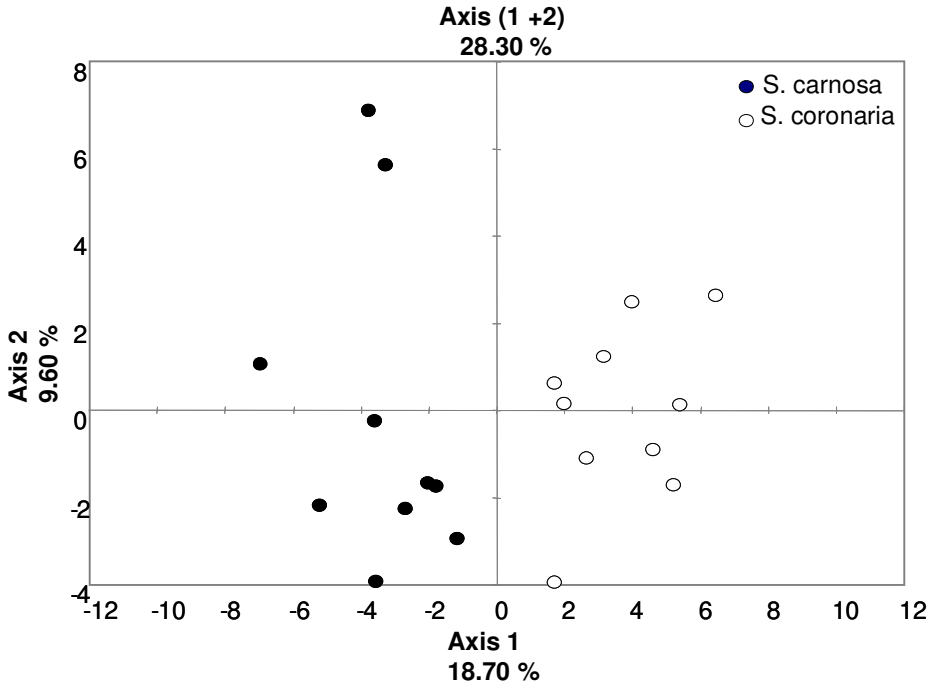

Figure 8. Principal component analysis plot illustrating the first two variability components (axes) for *Sulla carnosa* and *Sulla coronaria* ISSRs markers. *S. carnosa*: black symbols and *S. coronaria*: white symbols.

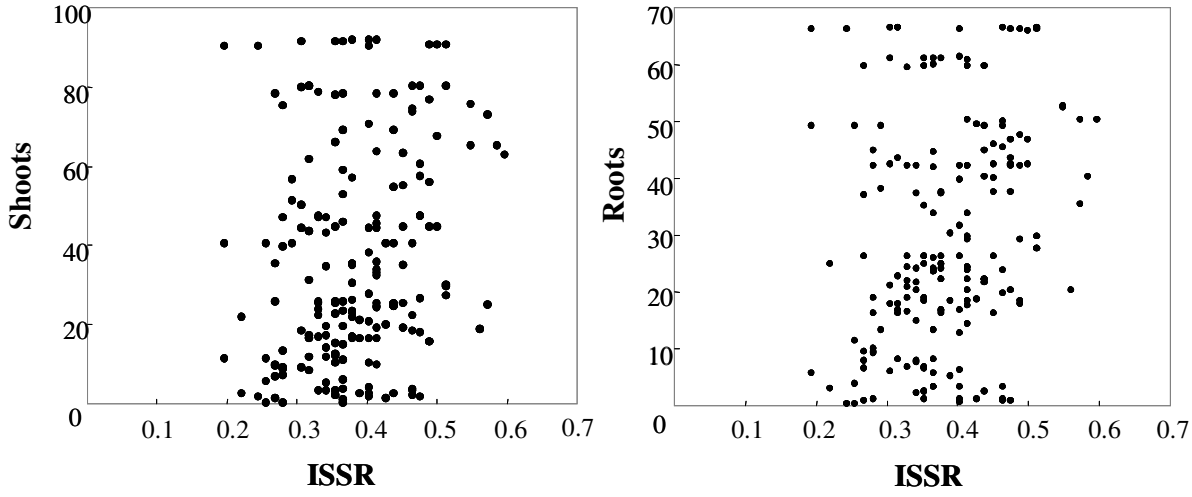

Figure 9. Correlation analysis between physiological data for shoots (left) and roots (right) and ISSRs markers according to a Mantel significance test. Statistically significant correlation between physiological and molecular data were obtained for shoots ($r= 0.213$, $0.003 < p < 0.05$) and roots ($r= 0.244$, $0.002 < p < 0.05$).

axis 18.7%, 2nd axis 9.6% and 3rd axis 8.65%. Graphic representation of dispersion generated by the first two axes (28.3% of total variability) revealed strong genetic diversity between *S. carnosa* and *S. coronaria* (Figure 8). In this representation, the presence of 9 specific amplicon bands (positive molecular markers) was attributed to *S. coronaria*, while the presence of negative molecular markers denoted *S. carnosa* (Table 2). In particular, positive markers C2100, C2600 and C3900 from axis 1 were attributed to *S. coronaria* and the negative markers

B860, A1000 and A1175 were attributed to *S. carnosa*.

Correlation analyses

Physiological and molecular data for both *Sulla* accessions were organized into a matrix and correlation coefficients determined using a Pearson correlation test. The correlation values between the different salt treatment (0, 100 and 200 mM), showed raised values varying between -0.918 and 1 in the roots and between –0.961 and 1 in the shoots (data not shown), therefore, molecular data showed significant correlation with physiological responses in both accessions. The obtained matrix showed that correlation coefficients increased with increasing salt concentration and varied for both shoots and roots, with positive values varying from 0.7 to 0.9 and negative values varying from -0.6 to 0.9.

Two molecular markers B340 and B860 were identified in roots and shoots after salt treatment using these correlations. The presence of these two markers were positively correlated to decreased Na^+ content and negatively correlated to increased DM, increased water content and increased K^+ content. The significance of these molecular markers to the overall physiological response of the two *Sulla* species was explored by a Mantel test. Statistical significance between physiological and molecular data were obtained for both shoots ($r= 0.213$, $0.003 < p < 0.05$) and roots ($r= 0.244$, $0.002 < p < 0.05$) (Figure 9), although, these overall correlations were relatively weak.

DISCUSSION

Salinity is one of the major stresses that severely limit crop production, especially in arid and semi-arid regions (Shannon, 1998). *Sulla* (*Sulla* and *Hedysarum* species) are leguminous plants with potential to be used as soil reclamation ground cover and livestock forage on arid lands. Their nitrogen fixing capacity reduces fertilizer inputs, and their moderate anthocyanin and proanthocyanidin content (Skadhauge et al., 1997) could protect against oxidative stress and impact positively on ruminant nutrition (Marles et al., 2003; Li et al., 2009). Two species of *Sulla* discovered growing in widely divergent ecological areas of Tunisia were investigated for their ability to withstand saline conditions The addition of NaCl 200 mM decreased plant growth more in *S. carnosa* than in *S. coronaria*. Reduction in plant growth can be related to excessive accumulation of Na^+ (Fernandez-Ballester et al., 1998). In shoots, *S. carnosa* accumulated more Na^+ than *S. coronaria*. When comparing NaCl sensitivity among barley genotypes, Flowers and Hajibagheri (2001) have found that the more sensitive variety of barley triumph accumulated more Na^+ in the shoot than the tolerant variety Gerbel.

To maintain cytosolic Na^+ homeostasis, excess ions must be removed from the cytosol either to the vacuole (intra-cellular compartmentalization), or be exported from the cell (Tyerman and Skerrett, 1999). In this study, Na^+ transport and uptake were compared between the two *Sulla* species and the results showed a higher Na^+ transport in S. c*arnosa* when compared with *S. coronaria*, at NaCl 100 and 200 mM. Na^+ transport was also compared between the relatively salt tolerant landrace line 149 and the salt sensitive cultivar Tamaroi. It was found that the major differences in Na^+ transport between the two varieties of durum wheat (*Triticum turgidum*) were; the rate of transfer from the root to the shoot (xylem loading), which was much lower in the salt tolerant genotype and the capacity of the leaf sheath to extract and sequester Na^+ as it entered the leaf (Davenport et al., 2005).

Salt concentrations of 100 and 200 mM decreased K^+ accumulation more in *S. Carnosa*. The decrease in K^+ accumulation could be related to a more inhibited K^+ transport in this accession. Potassium plays a number of important roles in plant growth and development. Molecular approaches have greatly advanced our understanding of K^+ transport in plants and a large number of genes encoding K^+ transport systems have been identified. In *A. thaliana* for example, at least 35 genes are thought to encode various K^+ channels or transporters (Qi and Spalding, 2004). The maintain of a high cytosolic K^+/Na^+ ratio is considered as a criteria of salt tolerance in many species (Cuin et al., 2003; Gorham et al.,1990; Rubio et al., 1999). Differences in Na^+ and K^+ accumulation between the two *Sulla* species, could explain the better K/Na ratio in *S. coronaria.* NaCl addition decreased plant salt tolerance, and is likely to be conferred by a large number of adaptive mechanisms. Among the most important of these is the ability of the plant to maintain a high K/Na ratio in the cytoplasm as well as to keep cytosolic Na^+ content below some crucial value (Greenway and Munns, 1980; Maathuis and Amtmann, 1999; Tyerman and Skerrett, 1999). In *A. thaliana*, salt tolerance of the *NOK2* ecotype was related to the maintaining of a higher K/Na ratio when compared with *Col* ecotype (Kaddour et al., 2009).

Salinity modifies the level of secondary metabolites, such as caraotenoids (carotenes and xanthophylls), having antioxidant properties and acting as accessory light harvesting pigments (de Pascale et al., 2001). Our results show a significant decrease in carotenoids and chlorophyll content in *S. carnosa*, while the level of these two metabolites remained unaffected by NaCl 100 and 200 mM in *S. coronaria*. The decrease in chlorophyll content is considered as an indicator of salt stress (Singh and Dubey, 1995). In mungbean, steady levels of CAR are strongly correlated to its salt tolerance (Wahid et al., 2004).

The ISSR markers measured diversity among accessions at DNA level, thus no interaction with environment

was expected (Terzopoulos and Bebeli, 2008). All the generated ISSR loci were polymorphic between the two accessions confirming that ISSR technique is very efficient to examine genetic diversity between these two accessions.

S. carnosa showed a large projection on the axis 1 and 2, in relation to its big diversity that could be explained by its large geographic distribution. Physiological characterization showed a sensitivity of *S. carnosa* to salt stress. Therefore, the important DNA polymorphism put in evidence in this accession seemed to be related more to its water stress resistance rather to its salt tolerance.

However, molecular markers found in *S. coronaria,* the less salt sensitive accession would be more in relation to its salt tolerance.

The implication of the molecular markers in the physiological response of *Sulla*, were explored by Mantel test. The results show that the statistically significant correlation between physiological and molecular data has revealed two molecular markers (B340 and B860) that are respectively identified in the roots and shoots after salt treatment. These two markers were positively correlated to salt response of *Sulla* accessions.

In conclusion, *Sulla coronaria* showed better salt tolerance on the basis of growth and K/Na selectivity that makes the local accession good candidates for a future breeding programme.

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