Tree Genetics & Genomes
DOI 10.1007/s11295-013-0693-x

ORIGINAL PAPER

Genetic and morphometric characterization of clones of *Prosopis alba*, Algarobia, selected for salt tolerance

Leandro Gabriel Roser · Laura Inés Ferreyra · Mauricio Ewens · Juan César Vilardi · Beatriz Ofelia Saidman

Received: 7 June 2013 / Revised: 8 October 2013 / Accepted: 20 December 2013

Abstract *Prosopis alba* is an important Argentinean species with a great potential for the production of timber and nontimber products. Many studies showed high salt tolerance of this species, which allows it to be used in afforestation and reforestation of saline soils. In this study, we applied the morphometric technique to characterize 21 salt-tolerant clones (ST). Twenty of these clones were studied by inter-simple sequence repeat (ISSR) and simple sequence repeat (SSR), and their molecular patterns were compared with those of 22 individuals selected for salt sensitivity (SS). Most morphological traits revealed highly significant differences among ST clones, and four out of 11 characters showed high heritability. ISSR analysis allowed detecting 89 loci, 91 % of them variable. ST versus SS groups differ significantly from each other by the frequencies of 22 of these loci, from which 12 were significant at the matrix level. Analysis of six SSR loci for the same groups indicated that all of them were polymorphic at the 1 % criterion. Allelic frequencies of SSR also showed highly significant differences between SS and ST groups. Analysis of coancestry between individuals within SS and ST groups and between groups indicated that the molecular differentiation between them cannot be explained solely on relationship grounds. Molecular groupings based on ISSR and SSR showed consistency to each other, as supported by the highly significant coinertia in the distribution of individuals in principal component analysis scatterplots. This work is the first contribution which tends to associate molecular patterns with life history traits and morphological differences in *Prosopis* clones.

Keywords *Prosopis alba* · Clones · ISSR · SSR · Morphometry · Multivariate analysis · Salt tolerance

Communicated by W. Ratnam

Electronic supplementary material The online version of this article (doi:10.1007/s11295-013-0693-x) contains supplementary material, which is available to authorized users.

L. G. Roser (✉) · L. I. Ferreyra · B. O. Saidman
Laboratorio de Genética de Especies Leñosas (GEEL), IEGEBA-CONICET, Departamento de Ecología, Genética y Evolución, FCEyN, Universidad de Buenos Aires, 1428, Buenos Aires, Argentina
e-mail: learoser@gmail.com

J. C. Vilardi
Laboratorio de Genética de Poblaciones Aplicada, IEGEBA-CONICET, Departamento de Ecología, Genética y Evolución, FCEyN, Universidad de Buenos Aires, 1428, Buenos Aires, Argentina

M. Ewens
Estación Experimental Fernández, Universidad Católica de Santiago del Estero, Santiago del Estero, Argentina

Introduction

The genus *Prosopis* (Leguminosae, Mimosoideae) includes about 45 species distributed in the arid and semiarid regions of America, Africa, and Asia. The Chaco eco-region in South America constitutes its main center of biodiversity (Burkart 1976), and many species in this area show high genetic and morphological variability (Palacios and Bravo 1981; Saidman et al. 2000; Ferreyra et al. 2004, 2007, 2010).

According to morphological criteria, mainly leaf, pod, and/or thorn shape and size, the genus has been divided into five sections, some of them subdivided into series (Burkart 1976). The section Algarobia includes the most important species from economic and ecological standpoints. The importance of the species of *Prosopis* is illustrated by the fact that in Argentina more than 100,000 tons of *Prosopis* logs are harvested annually for furniture, flooring, door, window, and shutter fabrication (Felker and Guevara 2003), and flour from the ground pods is used for human and livestock food uses

Published online: 10 January 2014

(Felker et al. 2003). Moreover, many species of the Algarobia section are also useful for the rehabilitation of saline soils in subtropical regions because of their high salt tolerance.

The negative impact of salinity on growth of plants in both irrigated and non-irrigated areas of the world's arid regions continues to be a major problem. Worldwide, more than 800 million hectares of land are estimated to be salt-affected (Rains 1991), and inevitably agricultural activities tend to aggravate this scenario (Flowers and Yeo 1995). For that reason, the need of increasing productivity in soils affected by salinity has encouraged the research on selection of genotypes with increased salt tolerance. Efforts to identify plants that are tolerant to salinity equal to seawater were pioneered by Epstein et al. (1979) for commercial crops such as wheat and barley (Velarde et al. 2003). More recently, Qadir et al. (2008) recognized some accessions of the genera *Acacia*, *Eucalyptus*, and *Melaleuca* as promissory for silvipastoral purposes in salty soils.

Argentina is considered the third country after Russia and Australia in terms of salinity-affected soils (Lavado 2008; Taleisnik et al. 2011). In this country, most work has been oriented to salt tolerance of forest native species, mainly those belonging to the genera *Prosopis* (Ahmad et al. 1994; Baker et al. 1995; Cony and Trione 1998; Velarde et al. 2003; Meloni et al. 2008a; Meloni and Martinez 2009), *Schinopsis* (Carnevale et al. 2004; Meloni et al. 2008b), *Vitis* (Cavagnaro et al. 2006), and forage shrubs such as *Atriplex* (Aiazzi et al. 2002; Taleisnik et al. 2011).

An initial work evaluating *Prosopis* for salt tolerance (Felker et al. 1981) found that a *Prosopis tamarugo* and a *Prosopis pallida* accession from Hawaii could grow at salinity equal to seawater. A later work (Rhodes and Felker 1988) that examined a broader germplasm base found that *Prosopis juliflora* from Senegal had the greatest combination of salt tolerance and rapid growth and that *Prosopis alba* would have sufficient biodiversity to select individual plants that could grow in salinity equal to seawater.

P. alba is one of the commercially most important species of the genus in Argentina. It has high genetic and morphological variability and adapts well to the desert areas of NW Argentina. Given the ever increasing harvest of *P. alba* for the Argentine furniture industry, efforts to establish *P. alba* plantations and the identification of salt-tolerant trees have been considered an important issue.

To capitalize on the genetic improvement on profitable traits such as higher salt tolerance, it is necessary to multiply asexually selected seedlings. In fact, as *Prosopis* is insect-pollinated and mostly outcrosser (Hunziker et al. 1986; Bessega et al. 2000, 2012; Felker et al. 2001), seeds from the same mother tree can be expected to have high probability of multiple male parents, resulting in high variability among the progeny. Thus, clonal propagation is an important tool for the genetic improvement of *P. alba*.

Previous works reported *P. juliflora* clones for erect form and fast growth in Haiti (Wojtusik et al. 1993), for biomass production and form in India (Harsh et al. 1996), and multipurpose clones of the *P. pallida*/*P. juliflora* complex in Peru (Alban et al. 2002). Some of the first *P. alba* progeny trials (Felker et al. 1983a, b) were conducted in southern California using half-sib families collected by Simpson and Solbrig in 1977. In 2001, Felker et al. obtained 12 individual clones of *P. alba* selected on the basis of pod production, pod flavor, and height growth from a progeny trial established in 1990 in Santiago del Estero Province, Argentina. These trees were originated from seeds collected from 57 individual trees (half-sib families) in eight northwestern Argentina provenances (Felker et al. 2001). Lately, Velarde et al. (2003) obtained 21 salt-tolerant clones of *P. alba* from nine provenances from a saline area of Santiago del Estero Province. Recently, Ewens et al. (2012) demonstrated highly significant differences among these clones after 5 years of growth in the field for biomass, diameter, and height.

Traditionally, the process of clone identification in many tree species has been based on morphological and phenological characteristics. In the particular case of species of the section Algarobia (mesquites and algarrobos) of the genus *Prosopis*, leaf, spine, and fruit morphology are the main criteria for species identification, and it is expected that they can be useful also for clone characterization.

In the last years, several molecular markers have also been successfully used in the genetic characterization of many tree species. Among many techniques, inter-simple sequence repeats (ISSRs) has shown to provide a powerful, rapid, simple, reproducible, and inexpensive means in the characterization of accessions and identification of cultivars and varieties (Senthil Kumar et al. 2009; Monte Corvo et al. 2011) and clones of forest tree species (De Pasquale et al. 2006; Chen et al. 2011).

Another technique for the analysis of genetic diversity is simple sequence repeats (SSRs) or microsatellites. The SSR markers are widely used because of its codominant nature, reproducibility, and high information content (UPOV 1995; Marcucci Poltri et al. 2003). Up to now, only six SSR loci are available to analyze *P. alba* variability (Mottura et al. 2005; Bessega et al. 2009).

Gailing et al. (2012) observed in the species of *Quercus* strong and significant correlations of leaf morphological differences with genetic distances analyzed by microsatellite markers. Their results showed leaf morphological differentiation between neighboring populations in contrasting environments, consistent with a pattern of isolation by adaptation. In a recent paper, Darquier et al. (2013) analyzed the genetic structure of *Prosopis flexuosa*, a close relative to *P. alba*, using both quantitative traits and molecular markers. They obtained evidence indicating that the variation in leaf size and shape is no neutral, and in most cases, these traits exhibit high geographical variation. This result suggests that leaf

morphological quantitative traits may be useful to characterize genotypes selected for adaptation to different environmental conditions, including salt tolerance.

In the present study, we analyzed the morphological variation for 11 leaf traits among the 21 salt-tolerant clones selected by Velarde et al. (2003) in order to determine if they are related with growing ability (productivity) under salty media.

We also compared the molecular patterns for ISSR and SSR between 20 salt-tolerant clones and 22 individuals selected for salt sensitivity. This work is the first contribution in *P. alba* in which molecular markers are analyzed to characterize genotypes selected on the basis of their ability to tolerate increased salinity conditions. The hypothesis was that molecular differences can be recognized and that this analysis will contribute to construct a starting point for future genomic analysis in this important natural resource in arid regions.

Materials and methods

Salt-tolerant individuals

The clones and individuals analyzed in this study were grown at the experimental facility of Estación Experimental Fernández (Universidad Católica de Santiago del Estero-Government of Province of Santiago del Estero, Argentina).

Twenty-one clones with increased salt tolerance (ST clones, Table 1) representing seven original families were vegetatively propagated by rooting cuttings, and they were planted in an experimental orchard with a randomized design with five blocks in January 2004 (for a thorough description of the methods of selection and propagation of clones, see Felker et al. 2008). The soil pH in the orchard ranged from 8.9 in block 1 to 10.2 in block 5 (Ewens et al. 2012). Each clone was represented by one individual (ramet) per block, in order to take into account block effects on the phenotypic differentiation among clones.

The 21 ST clones analyzed in this study were divided by Felker et al. (2008) into three groups according to their productivity (measured as differences in growing rate): high (H), medium (M), and low (L) (ESM-1, col 2). The proportion of each group within each family was recorded and compared among families by a Monte Carlo permutation test conducted with the package *stats* of the software R ver. 2.15.0 (R Development Core Team 2012).

Although all families derived from trees were identified taxonomically as *P. alba*, four of the ST clones (O3B4P7, O7B4P4, O7B4P6, O4B1P6) exhibit hybrid morphological characteristics, which suggest that the pollen donor might have been *Prosopis ruscifolia* or *Prosopis vinalillo* (considered a natural hybrid between those species).

Table 1 Individual codes and acronyms used in this work for the salt-tolerant and salt-sensitive trees. Salt-tolerant clones codes were taken from Felker et al. (2008)

Tolerant trees		Sensitive trees	
Tree	Acronym	Tree	Acronym
O2B4P1	ST1	M1B12P6	SS1
O7B3P11	ST2	M3B1P3	SS2
O7B4P4	ST3	M1B2P2	SS3
O6B1P12	ST4	M4B9P2	SS4
O6B1P11	ST5	M4B2P6	SS5
O7B4P11	ST6	M8B15P2	SS6
O4B1P6	ST7	M1B9P7	SS7
O9B4P14	ST8	M8B13P3	SS8
O3B4P8	ST9	M4B5P5	SS9
O6B4P4	ST10	M8B13P8	SS10
O3B1P13	ST11	M9B15P4	SS11
O3B4P7	ST12	M5B4P3	SS12
O8B3P15	ST13	M9B9P7	SS13
O3B4P12	ST14	M5B13P7	SS14
O7B4P6	ST15	M7B5P6	SS15
O6B1P13	ST16	M7B10P5	SS16
O7B3P3	ST17	M5B1P4	SS17
O7B3P10	ST18	M3B12P3	SS18
O8B3P14	ST19	M9B14P6	SS19
O7B3P8	ST20	M9B8P3	SS20
O7B4P8	ST21	M9B7P7	SS21
–	–	M7B3P5	SS22

Salt-sensitive individuals

Twenty-two individual seedlings which originated from seeds of Departamento de Robles, Santiago del Estero (Argentina) were selected in October 2007 for salt sensitivity (SS individuals, Table 1) and grown under greenhouse conditions.

For the selection of SS individuals, black plastic trays were divided into 128 cavities each of 30 cm^3 capacity, arranged in rows of 8 by 16. Each cavity was filled with a mixture of vermiculite/sand (1:1) and two 3-day-old seedlings were put on it. The trays were put inside a 163×114×15.5-cm cube. On a daily basis, the cube was inundated with a nutrient solution whose conductivity varied according to the experimental stage. The nutrient solution had the following concentrations (mg/l): N—190, P—35, K—210, Mg—45, B—0.2, Cu—0.1, Fe—1, Mn—0.5, Mo—0.005, Ca—150, Zn—0.15, S—70, B—0.5, and Mo—0.05. After a few minutes, the excess of the solution was drained to a 500-L tank from which the solution could be pumped to the refill the cube. After 4 days, only one seedling was kept in each cavity (in case both remained alive, one was eliminated). Air temperature was measured every day and the solution electric conductivity was recorded once a week.

The experiment was based on the ability of the seedlings to grow under different solution conductivities. Four height measurements (h_1 to h_4) were recorded at regular intervals as described in Fig. 1. Each seedling was measured from neck to apex with a ruler graduated at the nearest millimeter.

The ability to grow during the first phase was estimated as $a=h_2-h_1$, during the second phase as $b=h_3-h_2$, and during the third phase as $c=h_4-h_3$. For a salt-sensitive seedling, $b-a<0$ because growing stops after increasing medium conductivity. By contrast, the reduction in conductivity will determine that the seedling grows again during the third stage and $c-b$ should be positive. A difference $c-b<0$ indicates that the seedling is not able to recover under low conductivity conditions. In sum, if the ratio $(b-a)/(c-b)\leq 0$, the plant was considered as salt sensitive.

The selected SS plants were transferred into 5-L plastic pots until reaching enough size as to be used in DNA analyses without destroying the plant. Up to now, they are maintained under greenhouse conditions. Morphological traits in SS individuals were not recorded because they cannot be compared with ST clones, since they are on different stages of growth and are not planted in the field.

Morphometric methods

Morphometric data collection

All morphometric traits were recorded after drying the voucher material collected in the field. A total of 11 leaf morphology traits were measured and recorded for each individual plant: petiole length (PEL), number of pairs of leaflets per pinna (NLP), pinna length (PIL), number of pinnae (NPI), leaflet length (LEL), leaflet width (LEW), leaflet length/width (LEL/LEW), leaflet falcate (LEF), leaflet area (LEA), leaflet apex (LEX), and leaflet apex/total area (LEX/LEA).

Some traits are shown in Fig. 2. LEF and LEX were defined in Bessega et al. (2009). LEF is the ratio *l*/*f*, where *l* is the length of a straight segment from the base to the tip of the leaflet and *f* is the length from the same starting points but following the curve line that runs in the middle of the leaflet (Fig. 2b). LEX is the ratio *t*/*s*, where *t* is the area of the upper leaflet third and *s* is the area of a rectangle with the same dimensions (width and length) as the upper leaflet third (Fig. 2c).

PEL and PIL were measured with a ruler at the nearest millimeter. NPI and NLP were individually counted. LEL, LEL/LEW, LEF, LEX, and LEX/LEA were measured from scanned images with the software HOJA1.1 (available from the author upon request: A. Verga, INTA-IFFIVE, verga@gmail.com or arverga@yahoo.com.ar).

To account for among- and within-individual variation of quantitative traits and estimate heritability in salt-tolerant clones, we collected at random three leaves from each of two different strata (upper and lower) of the canopy. For LEL, LEL/LEW, LEF, LEX, and LEX/LEA measurements, 20 leaflets were randomly chosen from different leaves and areas of the canopy. For the other traits, one measurement was recorded per leaf. From each sampled tree (grown in the orchards), a voucher specimen was obtained and kept in Laboratorio de Genética de Especies Leñosas (GEEL), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

Statistical analysis of morphometric data

Morphological differences among clones were evaluated by individual ANOVA and multivariate analysis of variance (MANOVA) for salt-tolerant clones only. Wide-sense heritabilities (H^2) were estimated from variance components obtained by restricted maximum likelihood according to the following generalized linear model (GLM):

$$y_{ijk} = \mu + b_i + s_j + (b:s)_{ij} + I_k + \varepsilon_{ijk}$$

where y_{ijk} represents each individual observation, μ is the overall mean, b_i is the fixed block effect, s_j is the fixed stratum effect, $(b{:}s)_{ij}$ is the interaction between block and stratum

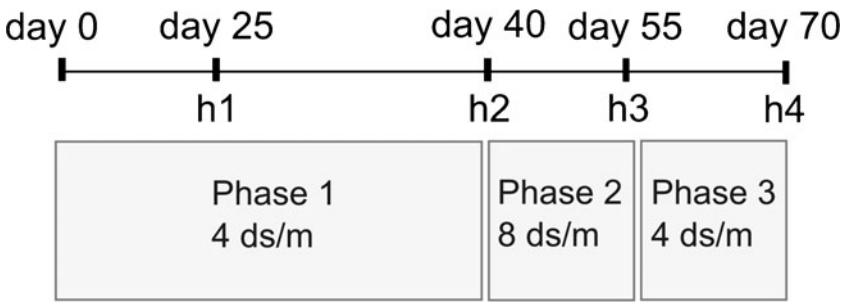

Fig. 1 Schematic representation of the hydroponic system to select SS individuals. From days 0 to 40 (phase 1), solution conductivity was low (4 dS/m); from days 41 to 55 (phase 2), the solution conductivity was 8 dS/m; and from days 56 to 70 (phase 3), the solution conductivity was reduced to 4 dS/m. Height measurements were taken at days 25 (h_1), 40 (h_2), 55 (h_3), and 70 (h_4). SS plants do not grow, grow slower, or undergo apex death during phase 2 and grow again during phase 3

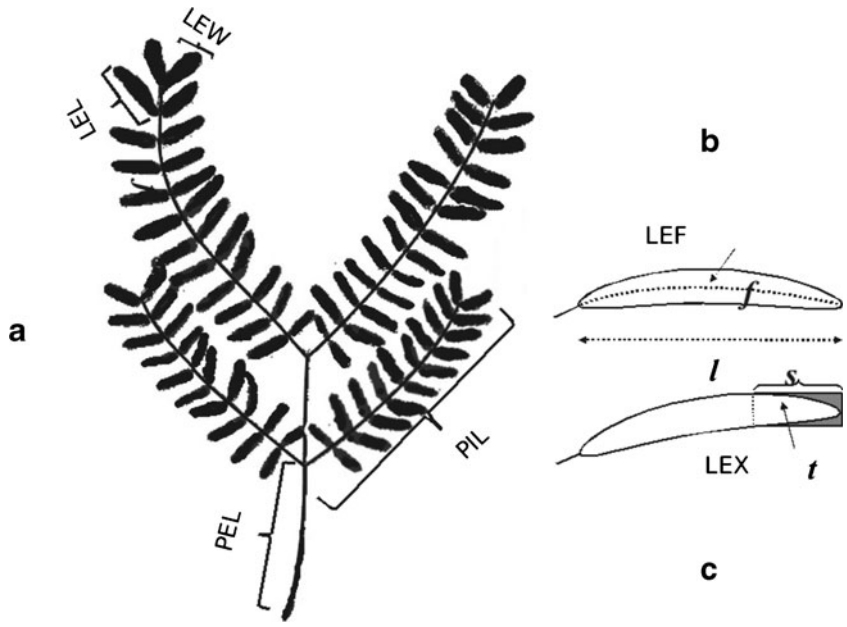

Fig. 2 Some leaf morphology traits measured and recorded for each individual plant. **a** Petiole length (PEL), pinna length (PIL), leaflet length (LEL), and leaflet width (LEW). **b**, **c** Description of measurements to estimate leaflet falcate (LEF) (**b**) and leaflet apex (LEX) (**c**). *l*=distance from the base to the tip of the leaflet; *f*= length from the base to the tip the leaflet following a curved line running along the middle of the leaflet; *t*=area of the upper leaflet third; *s*=area of a rectangle with the same dimensions as *t*

effects, I_k is the random clone effect, and ε_{ijk} is the random residual error effect.

Wide-sense heritability was estimated according to the expression:

$$\widehat{H}^2 = \frac{\widehat{\sigma}_b{}^2}{\widehat{\sigma}_b{}^2 + \widehat{\sigma}_w{}^2}$$

where $\widehat{\sigma}_b{}^2$ and $\widehat{\sigma}_b{}^2$ are, respectively, the estimated between and within (residual) clone variance components.

ANOVAs and MANOVA were conducted with the package *stats* of the program R. Variance component analysis was carried out by restricted maximum likelihood method (REML), which is a flexible method that can be applied in unbalanced designs, using the package *nlme* ver. 3.1-109 (Pinheiro et al. 2013) of R.

Molecular methods

ISSR analysis

Leaves were collected from each tree and were silica gel-preserved. DNA was extracted using DNeasy plant mini kit (Qiagen, Valencia, CA, USA), and samples were placed in a −20 °C freezer until analysis.

ISSRs were carried out following Zietkiewicz et al. (1994). Several primers were initially screened to identify well-amplified, polymorphic bands. The primers ISSR1 ($(AG)_8Y$), ISSR2 ($(AC)_8G$), and AE2 ($(CA)_8G$) had a higher level of bands per individual, showed high reproducibility among experiments, and were then selected and used in this work. PCR amplifications were performed in 50 μL of reaction mixtures containing the following reagents: 10–30 ng of template DNA, 0.8 μM of primer (Invitrogen), 0.3 U Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), buffer (Tris-HCl 10 mM, pH 8.3 and 50 mM KCl), 0.20 mM of each dNTP (Biodynamics), and 1.2 mM $MgCl_2$.

The PCR program used for amplification in a MyCycler Thermal Cycler System (Bio-Rad) was as follows: an initial heating at 94 °C for 90 s, 30 cycles of denaturing at 94 °C for 30 s, annealing at 48 °C for 45 s, extension at 72 °C for 90 s, and a final extension of 5 min at 72 °C. Re-amplification was performed routinely to ensure reproducibility of banding patterns. The usual cautions needed to prevent contamination of PCR experiments with previously amplified fragments were observed. Reliability of PCR products was tested by several controls that were routinely used, one without primer, the second one with no Taq DNA polymerase, and the third one with no genomic DNA. No amplification occurred in any of these negative controls. Re-amplification was performed routinely to ensure reproducibility of banding patterns. Each individual DNA sample was amplified three times and loaded in three different gels, and the same pattern was obtained. In each gel, we analyzed individuals from different morphologically predefined groups in order to avoid bias in the comparisons among groups attributable to experimental error.

ISSR PCR products were separated by electrophoresis at constant 60 W power for 3.5 h in a Model S2 apparatus (Gibco BRL Sequencing System, Life Technologies (Gaithersburg, MD, USA) through 4 % (*w*/*v*) polyacrylamide gel containing 5 M urea in 1× TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8). A 100-bp DNA ladder (Invitrogen) size marker was included twice in each electrophoresis run. Gels were stained with silver nitrate (Bassam et al. 1991).

SSR analysis

Six microsatellites, which had been developed for *Prosopis chilensis* exhibiting cross-species amplification (Mottura et al. 2005) and shown to be amplified successfully in *P. alba* (Bessega et al. 2009), were analyzed. We characterized the genotypes of the ST and SS individuals for all six microsatellites (Mo05, Mo07, Mo08, Mo09, Mo13, and Mo16).

The PCR amplifications were carried out in a 50-μL reaction volume containing 10–30 ng DNA, 0.6 μM each primer, 0.3 U Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), buffer (Tris-HCl 10 mM, pH 8.3 and 50 mM KCl), 0.2 mM dNTPs, and 1.5 mM $MgCl_2$. A PROGENE Techne thermal cycler (Techne Cambridge Ltd., Duxford Cambridge, UK) was used for amplifications, where the cycling profile was initial denaturation at 94 °C for 5 min followed by 30 cycles at 94 °C for 45 s denaturation, primer-specific annealing temperature (56–59 °C) for 45 s and at 72 °C for 45 s extension, and a final extension step at 72 °C for 10 min. Seven microliters of the PCR product was separated by electrophoresis in a Model S2 apparatus (Gibco BRL Sequencing System, Life Technologies (Gaithersburg, MD, USA)) through 6 % (*w*/*v*) polyacrylamide gel containing 5 M urea in 1× TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8). A 10-bp DNA ladder (Invitrogen) size marker was included twice in each electrophoresis run. Gels were stained with silver nitrate (Bassam et al. 1991).

Statistical analysis of molecular data

ISSR data analysis

Molecular patterns were obtained from 20 ST clones and the 22 SS individuals. ISSR patterns were coded as 1 or 0, respectively, for band presence or absence. ISSR band frequencies were compared between ST and SS clones by chi-squared test, estimating the probabilities by Monte Carlo simulations. Significance of *P* values was adjusted for multiple comparisons by the method of Benjamini and Hochberg (1995). This analysis was conducted with the package *stats* of the software R.

Multidimensional scaling (MDS) analysis

A matrix of pairwise Euclidean distances between individuals was obtained from the original dataset. From this matrix, a MDS analysis was conducted with the package *smacof* (De Leeuw and Mair 2009) of software R. The method implemented in this package is based on stress minimization. In order to examine the stability solution of the MDS, a jackknife on the configurations was performed. The result was plotted to show graphically if individuals tend to be grouped according to salt tolerance.

Between-group (BGA) analysis

The analysis of the association of ISSR patterns with the ability to tolerate high salinity conditions is complicated by the fact that there are more loci than samples (individuals analyzed). Most of the supervised classification methods are limited by the requirement of a greater number of cases (individuals or clones) than variables (genes). For example, to properly use conventional discriminant function analysis (DA), one must have more cases than variables, ideally by a factor of 10 or more. BGA (Dolédec and Chessel 1987) and DA are based on the same principle: find a linear combination of variables that defines a direction in the multidimensional space, along which maximizes the variance between groups (Truntzer et al. 2007). However, BGA allows to cope with the limitation related to the number of cases as it can be used when the number of variables exceeds the number of cases (Culhane et al. 2002). As an example, Ouahmane et al. (2006) applied successfully this approach to analyze the relationships between substrate-induced respiration (SIR) and five soil origins, where data matrix dimensions were 15 cases (soil samples)×33 variables (SIR responses). BGA was conducted with the package *ade4* (Chessel et al. 2004; Dray and Dufour 2007) of the software R.

Classification tree analysis

Classification tree analysis (Breiman et al. 1993) allows to identify structure in high-dimensional data settings. In order to compare ST and SS clones, we considered salt tolerance as the response variable (y) and ISSR patterns as the predictor variables (x_i). The method consists of a recursive partitioning to build a tree by first determining, among the set of all potential predictors, the variable x_i (locus) that is most predictive of the trait y (salt tolerance). Then the process is iterated until the complete tree is obtained (see Foulkes 2009, pp. 157–179). This analysis was conducted using the package *rpart* (Therneau and Atkinson 2011) of the software R.

SSR data analysis

For SSR, the dataset is a table of within-individual allelic frequencies of n rows and p columns, where n is the number of individuals, and p is the total number of alleles for all loci. Each cell represents the proportion (frequency) of alleles of each class in each individual genotype, and the possible values are 0, 0.5, and 1. As each individual bears two alleles, in a A_iA_i homozygote, the corresponding allele frequencies are 1 for A_i and 0 for any other allele. For a heterozygote (A_iA_j), allelic frequencies are $p_i=p_j=0.5$, and 0 for any other allele, and so on (see http://adegenet.r-forge.r-project.org/files/adegenet.pdf). Being frequencies, data sum to one for each locus. This matrix was built with the package *adegenet* (Jombart 2008) of the software R.

Multidimensional scaling and between-group analysis of SSR markers

The association of SSR patterns with salt tolerance was also analyzed by MDS and BGA applying the same approaches previously described in the ISSR analysis section.

Coancestry analysis

In order to determine whether possible associations between molecular patterns and salt tolerance may be attributed to the relationship among individuals within groups, coancestries within and among groups were estimated from SSR patterns applying the admixture *F*- model by Metropolis-Hasting (Karhunen and Ovaskainen 2012), with the package *RAFM* of the software R.

Consistency between ISSR and SSR datasets

The consistency between the molecular differentiation among groups obtained from ISSR and SSR datasets were evaluated by two methods. On the one side, the structures of Euclidean distance matrices obtained from ISSR and SSR datasets were compared by Mantel's (1967) test. On the other side, coinertia analysis (CIA) (Doledec and Chessel 1994; Culhane 2003; Dray et al. 2003) was applied to identify trends and study the common structure between the two datasets. This approach allows analyzing datasets that share one of the dimensions and which contain the same sample units by studying the global geometry of the clouds of points from both arrays, thereby reducing their dimensionality. The idea behind the CIA is to isolate axes in each space of new variables (defined for each array after the previous analysis). These new axes will be the ones to define the directions of maximum covariance between both datasets. The analyses were performed with the package *ade4* of the software R.

Results

Morphometric data analysis

Basic statistics of the morphological traits measured in the 21 ST clones is available in ESM-1. Average variation coefficient (cv) within each ST clone (ESM-1) takes the highest value for PEL (cv=0.440) and the lowest one for LEF (cv=0.028).

Individual ANOVAs to compare each trait among ST clones showed differences for all characters but PEL (Table 2). The MANOVA considering all traits demonstrates highly significant differences among clones (Wilks=4.50 10^{-5}, $P<2.20\ 10^{-16}$).

The analysis of components of variance and wide-sense heritability for each trait indicated that the characters with the highest heritability were LEL, LEW, LEA, and NLP (Table 2). In no case the confidence interval of H^2 include the zero, indicating significant heritability for all traits. The only exception might be PEL, where the H^2 value (0.014) was the lowest and the differences among clones were not statistically significant according to ANOVA.

Felker et al. (2008) classified the 21 original ST seedlings (from which the ST clones were obtained) in three productivity groups (H, M, and L). According to Table 2 in that paper, seedlings with different productivity were obtained within the same family. In fact, a homogeneity test comparing the distribution of H, M, and L classes among families yielded nonsignificant differences (chi-squared=11.360, P=0.600, based on 2,000 permutations). This result suggests that the variability in salt tolerance tends to occur within the original families.

Table 2 ANOVA results, variance components and wide-sense heritability for the morphometric traits analyzed in salt-tolerant clones of *Prosopis alba*

				Between clones			Within clones					
Character	*F*	*P*	*P* adj.	Est	Lower	Upper	Est	Lower	Upper	H^2	Lower	Upper
LEA	42.800	$2.20\ 10^{-16}$	$4.84\ 10^{-16}$	0.028	0.015	0.053	0.009	0.008	0.010	0.759	0.613	0.862
LEF	2.604	$1.55\ 10^{-03}$	$1.70\ 10^{-03}$	0.000	0.000	0.000	0.001	0.001	0.001	0.032	0.013	0.073
LEL	44.555	$2.20\ 10^{-16}$	$4.84\ 10^{-16}$	0.160	0.086	0.297	0.046	0.043	0.048	0.778	0.639	0.874
LEL/LEW	7.799	$2.26\ 10^{-11}$	$3.55\ 10^{-11}$	0.212	0.112	0.401	0.555	0.521	0.591	0.276	0.159	0.435
LEW	48.408	$2.20\ 10^{-16}$	$4.84\ 10^{-16}$	0.005	0.003	0.010	0.002	0.002	0.002	0.758	0.612	0.861
LEX	7.205	$1.28\ 10^{-10}$	$1.76\ 10^{-10}$	0.003	0.001	0.005	0.008	0.007	0.008	0.245	0.138	0.395
LEX/LEA	8.429	$3.88\ 10^{-12}$	$7.11\ 10^{-12}$	0.000	0.000	0.000	0.001	0.001	0.001	0.097	0.049	0.184
NLP	43.070	$2.20\ 10^{-16}$	$4.84\ 10^{-16}$	76.882	41.052	143.984	27.391	24.381	30.772	0.737	0.572	0.855
NPI	17.094	$2.20\ 10^{-16}$	$4.84\ 10^{-16}$	0.209	0.109	0.398	0.224	0.199	0.252	0.482	0.303	0.666
PEL	1.328	$1.89\ 10^{-01}$	$1.89\ 10^{-01}$	7.169	2.392	21.489	151.203	134.575	169.886	0.045	0.014	0.138
PIL	5.799	$1.10\ 10^{-08}$	$1.32\ 10^{-08}$	70.219	33.826	145.763	355.724	316.646	399.626	0.165	0.078	0.320

F ANOVA *F* value, *P P* value, *P adj. P* values adjusted for multiple tests by the Benjamini-Holchberg method, *Est* estimated variance, H^2 wide-sense heritability, *Lower* lower values for the 95 % confidence interval of the estimated value, *Upper* upper values for the 95 % confidence interval of the estimated value

The comparison of morphometric traits among groups of salt-tolerant clones with different productivity (Table 3) showed significant results for 6 out of 11 traits according to individual ANOVAs. The MANOVA showed highly significant differences among groups for the multivariate phenotype (Wilks=0.576, P=0.001).

ISSR data analysis

The analysis of ISSR markers allowed to detect 89 clearly identifiable and highly repeatable bands. About 91 % (81) proved to be polymorphic (see ESM-3). The comparison of band frequencies between ST and SS groups indicated that the differences were significant or highly significant for 22 loci at the individual level and 12 of them remained significant after correction for multiple comparisons (Table 4).

The comparison of ISSR band frequencies by chi-squared tests between ST clones and SS individuals revealed significant or highly significant differences at individual level for 22 loci (Table 4). When probabilities were adjusted for multiple comparisons, 12 loci showed significant differences between groups at the matrix level (Table 4).

Multidimensional scaling analysis

The MDS analysis for ISSR data (Fig. 3) showed that axis 1 tends to separate SS (on the right) from ST individuals (on the left). The individuals with hybrid characteristics are dispersed in the plot among the ST individuals and do not show a particular trend. The metric stress (based on 327 iterations) was also low (0.110), and the stability evaluated by jackknife was very high (between/total variance=0.990).

Between-group analysis

BGA based on ISSR data, considering three ST classes, high (H), medium (M), and low (L) productivity of salt-tolerant (ST) individuals, showed nonsignificant differences (Monte Carlo Test based on 9,999 permutations, P=0.334) among groups. When all three ST classes (H, M, and L) were pooled together and compared with SS individuals, the BGA (Fig. 4) produced a single principal axis. Highly significant differences between groups were shown (Monte Carlo test based on 9,999 permutations, P=1.00 10^{-04}). In this case, the loci with the highest contribution to the differentiation between salt-sensitive and salt-tolerant individuals were A2_17, A2_18, and A2_19.

Classification tree analysis

Class tree algorithm showed that the band that differentiated the groups with a highest degree of significance was A2_17, which was absent in all SS individuals and present in 70 % of

Table 3 Mean and standard deviation (in parentheses) of each trait for salt-tolerant clones of *Prosopis alba* with different productivity, together with results of individual ANOVAs and MANOVA comparing the phenotype among groups

Productivity	NPI	PEL	PIL	NLP	LEW	LEX	LEX/LEA	LEA	LEF	LEL/LEW	LEL	MANOVA
Low	1.870 (0.647)	26.040 (11.642)	92.888 (14.451) a	24.558 (12.016) b	0.270 (0.110) b	0.897 (0.052)	0.193 (0.013) a	0.358 (0.287) b	0.950 (0.012)	5.136 (0.592) a, b	1.404 (0.684) b	
Medium	2.144 (0.431)	24.516 (5.567)	92.615 (10.212) a	32.544 (7.590) a	0.201 (0.058) a	1.087 (0.703)	0.202 (0.009) b	0.180 (0.107) a	0.951 (0.009)	5.065 (0.516) a	0.999 (0.249) a	
High	2.244 (0.344)	24.996 (5.524)	101.907 (12.027) b	35.613 (3.843) a	0.186 (0.013) a	0.915 (0.023)	0.199 (0.005) a, b	0.160 (0.028) a	0.953 (0.006)	5.454 (0.579) b	1.009 (0.132) a	
P	0.069	0.646	0.017	0.000	0.001	0.292	0.001	0.000	0.747	0.064	0.001	0.001

Different indices indicate significant pairwise differences between means according to Tukey HSD tests

PEL petiole length, *NLP* number of pairs of leaflets per pinna, *PIL* pinna length, *NPI* number of pinnae, *LEL* leaflet length, *LEW* leaflet width, *LEL/LEW* leaflet length/width, *LEF* leaflet falcate, *LEA* leaflet area, *LEX* leaflet apex, *LEX/LEA* leaflet apex/total area

Table 4 Frequencies of the dominant phenotype of ISSR loci in salt-tolerant (ST) and salt-sensitive (SS) individuals of *Prosopis alba* and significance of the comparison between groups by chi-squared tests. Only loci that show significant differences between groups at individual level tests are listed

Locus	ST	SS	Chi-sq	*P*	*P* adj.
A2_17	0.700	0.000	23.100	$5.00\ 10^{-04}$	0.008
A2_18	0.650	0.000	20.710	$5.00\ 10^{-04}$	0.008
I1_11	1.000	0.409	17.116	$5.00\ 10^{-04}$	0.008
I2_2	0.350	0.955	17.230	$5.00\ 10^{-04}$	0.008
I2_27	0.350	1.000	20.710	$5.00\ 10^{-04}$	0.008
I1_10	0.300	0.818	11.486	$1.50\ 10^{-03}$	0.015
I2_1	0.550	1.000	12.600	$1.50\ 10^{-03}$	0.015
I2_31	0.400	0.864	9.808	$1.50\ 10^{-03}$	0.015
I1_17	0.550	0.955	9.451	$2.50\ 10^{-03}$	0.022
I2_24	0.850	0.409	8.636	$4.50\ 10^{-03}$	0.036
A2_19	0.350	0.000	9.240	$5.00\ 10^{-03}$	0.037
I2_3	0.600	0.955	7.821	$6.50\ 10^{-03}$	0.044
I2_23	0.900	0.500	7.843	$8.50\ 10^{-03}$	0.053
A2_c	0.400	0.818	7.769	$1.10\ 10^{-02}$	0.064
I2_5	0.950	0.636	6.121	$1.60\ 10^{-02}$	0.086
A2_11	0.950	0.636	6.121	$1.80\ 10^{-02}$	0.091
I1_6	0.750	1.000	6.243	$2.30\ 10^{-02}$	0.110
I1_19	0.800	0.455	5.301	$2.80\ 10^{-02}$	0.121
I2_16	0.400	0.773	6.041	$2.85\ 10^{-02}$	0.121
A2_2	0.800	0.455	5.301	$3.20\ 10^{-02}$	0.130
I1_7	0.700	0.955	4.887	$4.05\ 10^{-02}$	0.153
I2_15	0.550	0.864	5.050	$4.15\ 10^{-02}$	0.153

P chi-squared *P* value, *P adj.* chi-squared *P* value adjusted for multiple tests by the Benjamini-Holchberg method

ST clones. The three analyses (chi-squared tests, BGA, and class tree) were consistent in identifying A2_17 as the locus with a high contribution to the differentiation between SS individuals and ST clones.

SSR data analysis

The six analyzed SSR loci were polymorphic at the 1 % criterion, with four, four, seven, two, three, and four alleles for M008, M013, M007, M016, M009, and M005, respectively (see ESM-2).

Multidimensional scaling analysis

The MDS analysis from SSR data (Fig. 5) was consistent with the analysis based on SSR data. SS individuals tend to be on the left and ST individuals on the right of the plot, and no particular trend was observed regarding individuals with hybrid characteristics apart from being included within the ST group. The metric stress (based on 494 iterations) was low (0.070) and the stability evaluated by jackknife was high (between/total variance=0.870).

Between-group analysis

BGA based on SSR data, considering the tree classes (H, M, and L) of productivity of salt-tolerant (ST) individuals did not show significant differences (Monte Carlo test based on 9,999 permutations, P=0.422) among groups. When all three ST classes (H, M, and L) were pooled together and compared with SS individuals, the BGA (Fig. 6) showed highly significant differences between groups (Monte Carlo test based on 9,999 permutations, P=1.00 10^{-04}). The alleles with the highest contribution to the differentiation between salt-sensitive and salt-tolerant clones were MO09.1, M016.1, MO16.2, and MO09.2.

Coancestry analysis

The analysis of coancestry within and between groups considering salt-tolerant and salt-sensitive classes (Table 5) also

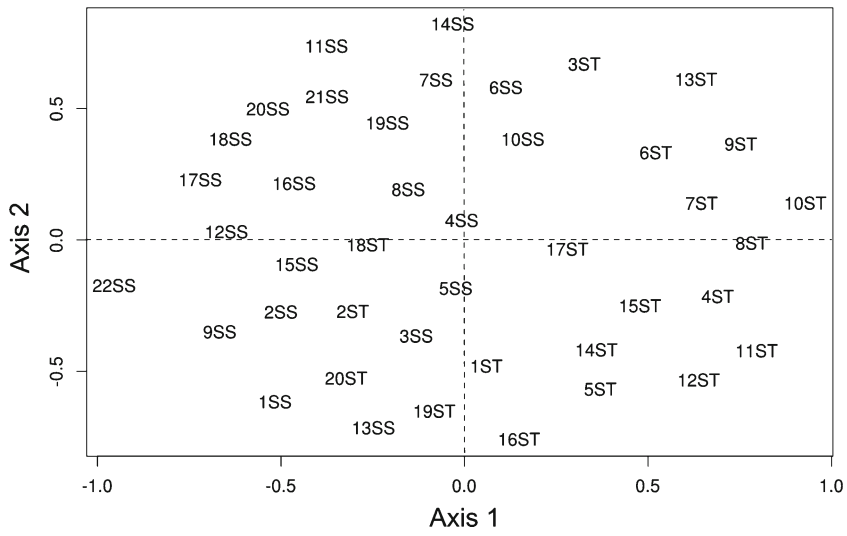

Fig. 3 Scatterplot of salt-tolerant (ST) and salt-sensitive (SS) individuals of *Prosopis alba* according to the two first axes of the multidimensional scaling (MDS) based on the Euclidean distance matrix from ISSR markers

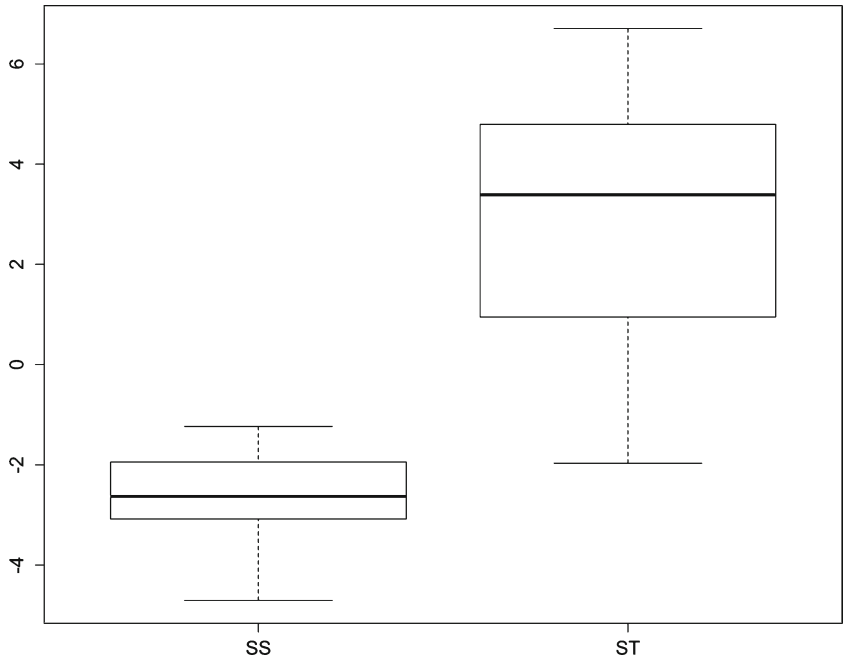

Fig. 4 Boxplot showing the distribution of salt-tolerant (ST) and salt-sensitive (SS) individuals of *Prosopis alba* individuals according to the first axis of the between-group analysis for the ISSR dataset. The *black horizontal line within each box* represents the median, and the *bottom and top limits of the box* indicate, respectively, the first and third quartile. The *vertical lines below and above the box* indicate the minimum and maximum values

suggested that variation in salt tolerance at a great scale occurs within families rather than among families. Although the highest estimate was observed within the salt-sensitive group, the coancestry within the salt-tolerant group is similar to that observed between groups. Moreover, confidence intervals of coancestry estimates widely overlap indicating that individuals within the tolerant group are not significantly more related to each other than to individuals from the other group.

Congruence between ISSR and SSR datasets

The structures of distance matrices between individuals obtained from ISSR and SSR are consistent according to the Mantel test (r=0.315, P=1.00 10^{-04}). Coinertia analysis performed between the principal component analyses (PCA) based on ISSR and SSR datasets (Fig. 7) also showed a significant result (RV=0.437, P=2.00 10^{-04} based on 9,999 permutations) indicating a common structure between the two datasets. The coinertia plot suggests that the most differentiated individuals in terms of salt tolerance and molecular patterns would be on the one side ST9 and ST12 and on the other side SS22.

Discussion

Afforestation and reforestation with salt-tolerant species, mainly with nitrogen fixing species, is one of the most attractive productive alternatives adopted in different areas of the

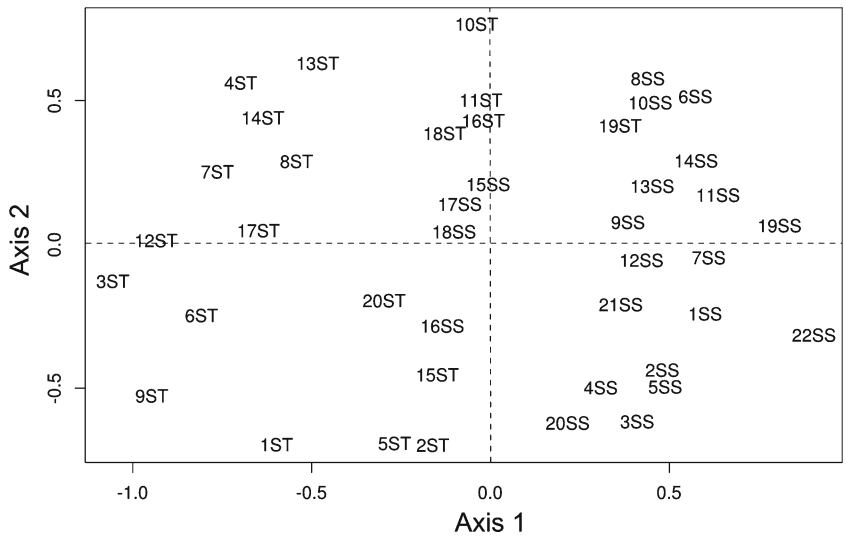

Fig. 5 Scatterplot of salt-tolerant (ST) and salt-sensitive (SS) individuals of *Prosopis alba* according to the two first axes of the multidimensional scaling (MDS) based on the Euclidean distance matrix from SSR markers

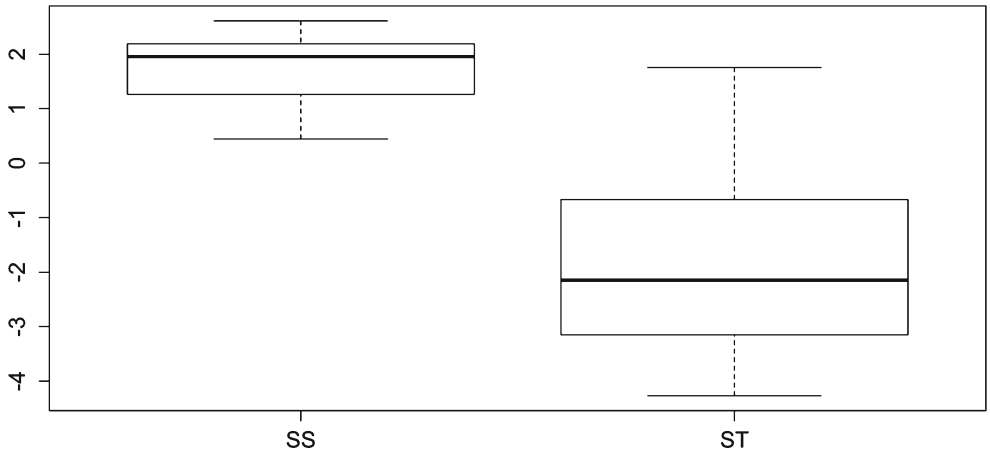

Fig. 6 Boxplot showing the distribution of salt-tolerant (ST) and salt-sensitive (SS) individuals of *Prosopis alba* individuals according to the first axis of the between-group analysis for the SSR dataset. The *black horizontal line within each box* represents the median, and the *bottom and top limits of the box* indicate, respectively, the first and third quartile. The *vertical lines below and above the box* indicate the minimum and maximum values

world for the restoration of degraded soils affected by salinity (Qadir et al. 2008). Nitrogen-fixing trees of the genus *Prosopis* are promising for these purposes in subtropical regions (Dutton 1989; Geesing et al. 2000) because of their high salt tolerance and the high economical value of the products that can be obtained from them (Felker et al. 2008).

The variety of adaptations to severe environmental conditions and the ecological and economic importance of shrubs and trees for rural dwellers confer on *Prosopis* species the potential to be used in the restoration of degraded areas (Cony 1995) leading these species to become the biological axis of new production systems (Roig 1993; Fagg and Stewart 1994).

Within this genus, the section Algarobia includes the most valuable multipurpose tree species, which can provide timber, firewood, charcoal, forage, human food (Pasiecznik 2001), as well as other services including gum production (Vilela and Ravetta 2005). Cony (1995) proposed that the use of multipurpose trees, such as *P. chilensis* and *P. flexuosa*, should be considered in afforestation programs in the Monte eco-region, whereas *P. alba* is one of the most promissory in the Chaco eco-region (Felker et al. 2001, 2008). Moreover, several studies on variability of quantitative traits estimated on experimental stands suggest that genetic variability (heritability) of different physiological and growth features in these species is high (Cony 1996; Cony and Trione 1998; Felker et al. 2001; Bessega et al. 2009, 2010), conferring them the potential to be included in genetic improvement programs.

Darquier et al. (2013) observed in *P. flexuosa* that the distribution of genetic variation for leaf morphometric traits does not agree with the expected under neutrality and that selection favored different optima in different sampling sites. Consequently, it is tempting to assume that adaptation to salty conditions may be reflected in leaf morphology.

Velarde et al. (2003) and Felker et al. (2008) selected and cloned *P. alba* individuals with the ability to grow under hydric stress and planted them in an experimental orchard in a randomized block design. In this paper, we analyzed these clones in order to assess the proportion of morphological variation explained by environmental and genetic causes.

Univariate analysis showed for most morphological traits highly significant differences among clones, which could not be explained by environmental causes. The only exception was petiole length (PEL) that exhibited high environmental variation as evidenced by its high coefficient of variation (cv) within clones.

The multivariate comparison considering all morphometric traits also demonstrated highly significant differences among clones. The analysis of components of variance and wide-sense heritability for each trait indicated that the characters with the highest heritability were LEL, LEW, LEA, and NLP. This result and the high morphological differentiation observed within the group of salt-tolerant clones indicate that these traits could be useful as morphological markers for clonal identification and to make phenotypic selection for quantitative traits of adaptive significance.

Significant differences among these clones were also detected by Ewens et al. (2012) for biomass, diameter, and height, with heritability estimates ranging from 0.45 to 0.59. Bessega et al. (2009) studied the heritability of several morphometric traits (including most leaf traits studied here) in an open pollinated trial established at San Carlos (Santiago del Estero), a place with similar environmental conditions and sited at about 38 km north from the clonal orchard studied

Table 5 Coancestry estimates between individuals belonging to the same (within) and different (between) salt tolerance groups of *Prosopis alba* individuals

	Median	Confidence interval	
		Lower	Upper
Within			
Tolerants	0.196	0.123	0.440
Sensitives	0.386	0.241	0.600
Between	0.145	0.084	0.257

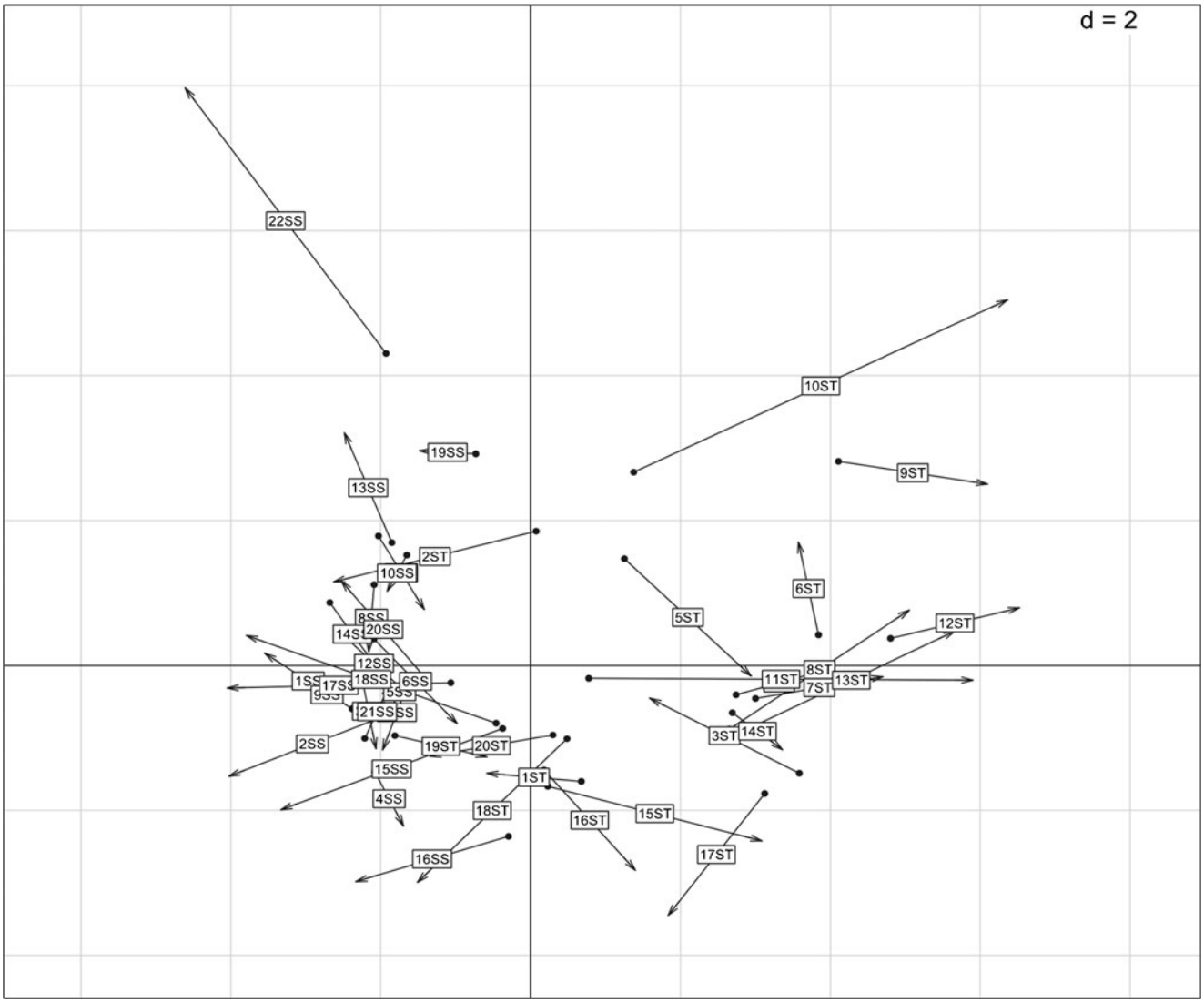

Fig. 7 Coinertia analysis of SSR and ISSR datasets of *Prosopis alba* clones. The *dots* represent projection of the individuals on the two first axes of the PCA based on SSR data, and the *arrowheads* represent the corresponding projection based on ISSR data. *Horizontal and vertical axes* correspond, respectively, to the first and second axes. *Each dot* and *arrowhead* is joined by a line, the length of which is proportional to the divergence between samples in the two datasets. *d* is the side length of a square in the grid

here. They obtained high heritability estimates for all leaf traits; however, some discrepancies can be observed with respect to our results. In particular, the heritabilities for LEX/LEA, PEL, and LEF were the lowest in our work, whereas they exhibit high values in the paper by Bessega et al. (2009). Such difference in genetic parameters between an open pollinated unselected trial and the group of selected clones suggests that salt tolerance is somehow related with leaf size and morphology.

The morphological variation analyzed here seems to be at least partially associated to productivity of ST clones, as significant differences were detected among H, M, and L classes within the ST group for six morphometric traits. This conclusion, however, should be taken with caution taking into account the low number of clones within the L and H groups. This group includes individuals from different families (Velarde et al. 2003; Felker et al. 2008), and the comparison of the proportions of H, M, and L classes within this group indicated a significant variability in salt tolerance within families. The presence of progenies with different levels of productivity within family arrays suggests that the physiological properties selected may be preserved by asexual propagation (rather than by family selection), without the risk of losing advantageous genetic combination as a consequence of segregation.

Estimation of genetic parameters of quantitative variation is difficult in forest species because of the complexity of the experimental design and its being time consuming. For this reason, in the last years, molecular markers are being increasingly used to assist classical morphological studies on

quantitative traits and to characterize clones and cultivars of domesticated species. Morphological markers have disadvantages that are limited in number and they do not often reflect genetic relationships because of interaction with the environment, epistasis, and the largely unknown genetic control of the traits. By contrast, DNA markers are found in abundance and are not influenced by the environment or developmental stage of a plant (Smith and Smith 1989), making them an ideal tool for genetic studies (Reddy et al. 2002; Kesari et al. 2010). This work is the first contribution which tends to associate molecular patterns with life history traits and morphological differences in *Prosopis* clones.

At the molecular level, highly significant differences in allele frequencies were observed between the different groups selected for salt tolerance. The salt-tolerant genotypes tend to cluster together in the MDS indicating that SSR and ISSR markers are efficient to differentiate between ST and SS genotypes based on their similarity matrix. This result is consistent with that of Zeng et al. (2004) in rice who observed significant differences in physiological traits related with salt tolerance among different clusters identified by microsatellite markers.

The between-group analysis comparing ST and SS clones showed that they can be clearly differentiated from each other. In particular, three ISSR loci (A2_17, A2_18, and A2_19) and four SSR alleles (MO09.1, M016.1, MO16.2, and MO09.2) accounted for most of the differentiation between these groups. Class tree analysis of the ISSR dataset was consistent with the BGA in identifying A2_17 as the locus with the highest contribution to the differences between groups.

The analysis of coancestry within and between SS and ST groups showed that the average relationships within the ST were similar to those recorded between individuals from different groups. This fact and the variation in salt tolerance within family arrays mentioned above indicate that the molecular differentiation between groups cannot be explained solely on historical or family grounds.

The coinertia analysis demonstrated high consistency between the results obtained from different molecular markers supporting the use of both ISSR and SSR as tools to help early selection of profitable physiological properties. The ability to select plant based on the genotype rather than the phenotype is extremely attractive to plant breeders because many associated problems with phenotypic selection will be avoided using DNA markers (Ibitoye and Akin-Idowu 2010). In particular, selection for superior *P. alba* genotypes is complicated by the need to select for multipurpose outputs that compete for photosynthate, i.e., fast trunk growth desirable for lumber production and high pod production (Felker et al. 2001).

As markers linked to traits can assist in selecting breeding progeny carrying desirable alleles (Ibitoye and Akin-Idowu 2010), the results here obtained are promising and encourage further molecular analysis. Up to now, the genomic information of *P. alba* is insufficient to conduct genome-wide association analysis as only a few genetic markers are available. The clones here analyzed are the only ones characterized for a particular physiological property. Important productivity traits such as wood properties in the species of *Prosopis* are being under study (Pometti et al. 2009, 2010), but there are no data at the population level to be related with molecular markers. In this paper, we present the first contribution of potentially useful molecular markers for the identification of genomic regions associated with different physiological properties in *P. alba*. The development of higher numbers of SSR and other markers is an initial step to genomic studies for clone identification, association tests, and genetic mapping. Also, sequencing the bands such as A2_17, A2_18, and A2_19 which showed apparent association with clone properties will contribute to identify possible genomic regions responsible for the profitable traits selected.

Acknowledgments This study was financed by Universidad de Buenos Aires (grant UBACYT 20020100100008 to B.O.S.), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (grant PIP No. 11220090100147 to J.C.V.), and ANPCYT (grant PICTO-OTNA 2011-0081). The salt-sensitive clones were obtained within the frame of Project "Ecología Molecular de Recursos Genéticos Forestales Nativos Proveedores de Servicios Ambientales y con Alto Potencial de Domesticación" (AEBIO2442). Instituto Recursos Biológicos (IRB)-INTA (Castelar), coordinated by Dr. Susana Torales. B.O.S. and J.C.V. are members of Carrera del Investigador Científico, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CIC-CONICET).

References

Ahmad R, Ismail S, Moinuddin M, Shaheen T (1994) Screening of mesquite (*Prosopis* sp) for biomass production at barren sandy areas using highly saline water for irrigation. Pak J Bot 26:265–282

Aiazzi M, Carpane P, Di Rienzo J, Arguello J (2002) Effects of salinity and temperature on the germination and early seedling growth of *Atriplex cordobensis* (Gandoger et Stuckert) (Chenopodiaceae). Seed Sci Technol 30:329–338

Alban L, Matorel M, Romero J, Grados N, Cruz G, Felker P (2002) Cloning of elite, multipurpose trees of the *Prosopis juliflora/pallida* complex in Piura, Peru. Agrofor Syst 54:173–182

Baker A, Sprent JI, Wilson J (1995) Effects of sodium chloride and mycorrhizal infection on the growth and nitrogen fixation of *Prosopis juliflora*. Symbiosis 19:39–51

Bassam BJ, Caetano Anollés G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 19: 80–83

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc B Met 57:289–300

Bessega C, Ferreyra LI, Julio N, Montoya S, Saidman BO, Vilardi JC (2000) Mating system parameters in species of genus *Prosopis* (Leguminosae). Hereditas 132:19–27

Bessega C, Saidman BO, Darquier MR, Ewens M, Leopoldo S, Rozenberg P, Vilardi JC (2009) Consistency between marker and

genealogy based heritability estimates in an experimental stand of *Prosopis alba* (Leguminosae). Am J Bot 96:458–465. doi:10.37327ajb.0800074

Bessega C, Saidman BO, Darquier MR, Ewens M, Felker P, Vilardi JC (2010) Accuracy of dominant markers for estimation of relatedness and heritability in an experimental stand of *Prosopis alba* (Leguminosae). Tree Genet Genomes 7(1):103–115. doi:10.1007/s11295-010-0318-6

Bessega C, Pometti CL, Ewens M, Saidman BO, Vilardi JC (2012) Strategies for conservation for disturbed *Prosopis alba* (Leguminosae, Mimosoidae) forests based on mating system and pollen dispersal parameters. Tree Genet Genomes 8(2):277–288. doi:10.1007/s11295-011-0439-6

Breiman L, Friedman J, Olshen R, Stone C (1993) Classification and regression trees. Chapman and Hall/CRC, Boca Raton

Burkart A (1976) A monograph of the genus *Prosopis* (Leguminosae subfam. Mimosoidae). J Arnold Arboretum 57:219–249

Carnevale NJ, Alzugaray C, Lopez D (2004) Effect of salinity on seedlings of two dominant tree species in the "Quebrachal" of *Schinopsis balansae* Engl. Rev Investig Fac Cien Agrarias 6:43–49

Cavagnaro JB, Ponce M, Guzmán J, Cirrincione M (2006) Argentinean cultivars of *Vitis vinifera* grow better than European ones when cultured in vitro under salinity. Biocell 30:1–7

Chen K, Ren P,Ying C, Jiang Q, Jia X (2011) Genetic relationships among *Jatropa curcas* L. clones from Panzhihua, China as revealed by RAPD and ISSR. Afr J Agric Res 6(11):2582-2585. Available online at http://www.academicjournals.org/AJAR

Chessel D, Dufour AB, Thioulouse J (2004) The ade4 package—I: One-table methods. R News 4:5–10

Cony MA (1995) Reforestación racional de zonas áridas y semiáridas con árboles de múltiples propósitos. Interciencia 20:249–253

Cony MA (1996) Genetic variability in *Prosopis flexuosa* DC, a native tree of the Monte phytogeographic province, Argentina. For Ecol Manag 87:41–49

Cony MA, Trione SO (1998) Inter and intraspecific variability in *Prosopis flexuosa* and *Prosopis chilensis*: seed germination under salt and moisture stress. J Arid Environ 40:307–317

Culhane AC, Perrière G, Considine EC, Cotter TG, Higgins DG (2002) Between-group analysis of microarray data. Bioinformatics 18(12):1600–8

Culhane AC, Perrière G, Higgins DG (2003) Cross-platform comparison and visualization of gene expression data using co-inertia analysis. BMC Bioinforma 15:1–15

Darquier MR, Bessega CF, Cony M, Vilardi JC, Saidman BO (2013) Evidence of heterogeneous selection on quantitative traits of *Prosopis flexuosa* (Leguminosae) from multivariate Q_{ST}-F_{ST} test. Tree Genet Genomes 9(1):307–320

De Leeuw J, Mair P (2009) Multidimensional scaling using majorization: the R package smacof. J Stat Softw 31(3):1–30

De Pasquale F, Siragusa M, Abbate L, Tusa N (2006) Characterization of five sour orange clones through molecular markers and leaf essential oils analysis. Sci Hortic-Amst 109:54–59

Dolédec S, Chessel D (1987) Rhythmes saisonniers et composantes stationelles en milieu aquatique I-Description d'un plan d'observations complet par projection de variables. Acta Oecol Oecol Gen 8:403–426

Dolédec S, Chessel D (1994) Co-inertia analysis: an alternative method for studying species-environment relationships. Freshw Biol 31:277–293

Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. J Stat Soft 22:1–20

Dray S, Chessel D, Thioulouse J (2003) Co-inertia analysis and the linking of ecological data tables. Ecology 84:078–3089

Dutton RW (1989) *Prosopis* sp: a justification for their future research and development. In: Dutton R (ed) A research and development strategy for *Prosopis*. Center for Overseas Research and Development, Durham, pp 33–40

Epstein E, Kingsbury RW, Norlyn JD, Rush DW (1979) Production of food crops and other biomass by seawater culture. In: Hollaender A (ed) The biosaline concept. Plenum, New York, p 39

Ewens M, Gezan S, Felker P (2012) Five year field evaluation of *Prosopis alba* clones on pH 9–10 soils in Argentina selected for growth in the greenhouse at seawater salinities (45 dS m^{-1}). Forests 3(1):95–113. doi:10.3390/f3010095

Fagg CW, Stewart JL (1994) The value of *Acacia* and *Prosopis* in arid and semi-arid environments. J Arid Environ 27:3–25

Felker P, Guevara JC (2003) Potential of commercial hardwood forestry plantations in arid lands and economic analyses of *Prosopis* lumber production in Argentina and the United States. For Ecol Manag 186(1–3):271–286

Felker P, Clark PR, Laag AE, Pratt PF (1981) Salinity tolerance of the tree legumes mesquite (*Prosopis glandulosa*, var torreyana, *P. velutina* and *P. articulata*) algarrobo (*P. chilensis*), kiawe (*P. pallida*) and tamarugo (*P. tamarugo*) grown in sand culture on nitrogen free media. Plant Soil 61:311–317

Felker P, Cannell GH, Osborn JF, Clark PR, Nash P (1983a) Effects of irrigation on biomass production of 32 *Prosopis* (mesquite) accessions. Exp Agr 19:187–198

Felker P, Cannell GH, Clark PR, Osborn JF, Nash P (1983b) Biomass production of *Prosopis* species (mesquite), Leucaena, and other leguminous trees grown under heat/drought stress. For Sci 29:592–606

Felker P, Lopez C, Soulier C, Ochoa J, Abdala R, Ewens M (2001) Genetic evaluation of *Prosopis alba* (algarrobo) in Argentina for cloning elite trees. Agrofor Syst 53(1):65–76. doi:10.1023/A:1012016319629

Felker P, Grados N, Cruz G, Prokopiuk D (2003) Economic assessment of production of flour from *Prosopis alba* and *P. pallida* pods for human food applications. J Arid Environ 53(4):517–528

Felker P, Ewens M, Velarde M, Medina D (2008) Initial evaluation of *Prosopis alba* Griseb clones selected for growth at seawater salinities. Arid Land Res Manag 22:334–345

Ferreyra LI, Bessega C, Vilardi JC, Saidman BO (2004) First report on RAPD patterns able to differentiate some Argentinean species of section Algarobia (*Prosopis*, Leguminosae). Genetica 121:33–42. doi:10.1023/B:GENE.0000019925.06080.c7

Ferreyra LI, Bessega C, Vilardi JC, Saidman BO (2007) Consistency of population genetics parameters estimated from isozyme and RAPDs dataset in species of genus *Prosopis*. Genetica 131:217–230. doi:10.1007/s10709-006-9132-3

Ferreyra LI, Vilardi JC, Tosto D, Julio N, Saidman BO (2010) Adaptive genetic diversity and population structure of the "algarrobo" [*Prosopis chilensis* (Molina) Stuntz] analyzed by RAPD and isozyme markers. Eur J For Res 129:1011–1025. doi:10.1007/s10342-010-0386-z

Flowers TJ, Yeo AR (1995) Breeding for salinity resistance in crop plants: where next? Aust. J. Plant Physiol 22:875–884

Foulkes AS (2009) Applied statistical genetics with R for population based association studies. Springer, New York

Gailing O, Lind J, Lilleskov E (2012) Leaf morphological and genetic differentiation between *Quercus rubra* L. and *Q. ellipsoidalis* E. J. Hill populations in contrasting environments. Pl Syst Evol 298:1533–1545. doi:10.1007/s00606-012-0656-y

Geesing D, Felker P, Bingham RL (2000) Influence of mesquite (*Prosopis glandulosa*) on soil nitrogen and carbon development: implications for global C sequestration. J Arid Environ 46:157–180

Harsh LN, Tewari JC, Sharma NK, Felker P (1996) Performance of *Prosopis* species in arid regions of India. In: Felker P, Moss J (eds) *Prosopis*: semi-arid fuelwood and forage tree: building consensus for the disenfranchised. Proceedings of an international symposium. Washington, DC. Center for Semi-Arid Forest Resources Publisher, TAMU Kingsville, Texas, pp 330

Hunziker JH, Saidman BO, Naranjo CA, Palacios RA, Poggio L, Burghardt AD (1986) Hybridization and genetic variation of Argentine species of *Prosopis*. For Ecol Manag 16:301–315

Ibitoye DO, Akin-Idowu PE (2010) Marker-assisted selection (MAS): a fast track to increase genetic gain in horticultural crop breeding. J Biotechnol 9(52):8889–8895

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405. doi:10.1093/bioinformatics/btn129

Karhunen M, Ovaskainen O (2012) Estimating population-level coancestry coefficients by an admixture F model. Genetics 192(2): 609–617. doi:10.1534/genetics.112.140871

Kesari V, Sathyanarayana VM, Parida A, Rangan L (2010) Molecular marker-based characterization in candidate plus trees of *Pongamia pinnata*, a potential biodiesel legume. AoB Plants 17:1–12. doi:10.1093/aobpla/plq017

Lavado R (2008) Visión sintética de la distribución y magnitud de los suelos afectados por salinidad en la Argentina. In: Taleisnik E, Grunberg K, Santa María G (eds) La salinización de suelos en la Argentina: su impacto en la producción agropecuaria. EDUCC (Editorial Universidad Católica de Córdoba), Córdoba, pp 11–16

Mantel NA (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

Marcucci Poltri SN, Zelener N, Rodriguez Traverso J, Gelid P, Hopp HE (2003) Selection of a seed orchard of *Eucalyptus dunnii* based on genetic diversity criteria calculated using molecular markers. Tree Physiol 23:625–632

Meloni DA, Matínez CA (2009) Glycinebetaine improves salt tolerance in vinal (*Prosopis ruscifolia* Griseb.) seedlings. Braz J Plant Physiol 21(3):233–241

Meloni DA, Gulotta MR, Martínez CA (2008a) *Prosopis ruscifolia* Griseb. (vinal) tolera concentraciones salinas equivalentes al agua de mar y excluye iones tóxicos de la parte aérea. Quebracho 16:32–40

Meloni DA, Gulotta MR, Martínez CA (2008b) Salinity tolerance in *Schinopsis* quebracho colorado: seed germination, growth, ion relations and metabolic responses. J Arid Environ 72:1785–1792

Monte-Corvo L, Goulão L, Oliveira C (2011) ISSR analysis of cultivars of pear and suitability of molecular markers for clone discrimination. J Am Soc Hort Sci 126:517–522

Mottuora MC, Finkeldey R, Verga AR, Gailing O (2005) Development and characterization of microsatellite markers for Prosopis chilensis and Prosopis flexuosa and cross-species amplification. Mol Ecol Notes 5:487–489

Ouahmane L, Hafidi M, Kisa M, Boumezouch A, Thioulouse J, Plenchette C, Duponnois R (2006) Some Mediterranean plant species (*Lavandula* spp. and *Thymus satureioides*) act as "plant nurses" for the early growth of *Cupressus atlantica*. Plant Ecol 185:123–134

Palacios RA, Bravo LD (1981) Hibridización natural en *Prosopis* (Leguminosae) en la región chaqueña Argentina. Evidencias morfológicas y cromatográficas. Darwiniana 23:3–35

Pasiecznik NM, Felker P, Harris PJC, Harsh LN, Cruz G, Tewari JC, Cadoret K, Maldonado LJ (2001) The *Prosopis juliflora-Prosopis pallida* complex: a monograph. HDRA, Coventry

Pinheiro J, Bates D, DebRoy S, Sarkar D and the R Development Core Team (2013) nlme: linear and nonlinear mixed effects models. R package version 3.1-109

Pometti C, Pizzo B, Brunetti M, Macchioni N, Ewens M, Saidman B (2009) Argentinean native wood species: physical and mechanical characterization of some *Prosopis* species and *Acacia aroma* (Leguminosae; Mimosoideae). Bioresour Technol 100(6):1999–2004

Pometti C, Palanti S, Pizzo B, Charpentier JP, Boizot N, Resio C, Saidman B (2010) Durability of five native Argentine wood species of the genera *Prosopis* and *Acacia* decayed by rot fungi and its relationship with extractive content. Biodegradation 21(5): 753–60

Qadir M, Tubeileh A, Akhtar J, Larbi A, Minhas PS, Khan MA (2008) Productivity enhancement of salt-affected environments through crop diversification. Land Degrad Dev 19:429–453

R Development Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing: Vienna. ISBN 3-900051-07-0. Available from: http://www.R-project.org

Rains DW (1991) Salinity and alkalinity as an issue in world agriculture. In: Choukr-Allah R (ed) Plant salinity research. New challenges. Institut Agronomique et veterinaire Hassan II, Agadir, pp 19–31

Reddy MP, Sarla N, Siddiq EA (2002) Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128:9–17

Rhodes D, Felker P (1988) Mass screening *Prosopis* (mesquite) seedlings for growth at seawater salinity. For Ecol Manag 24:169–176

Roig FA (1993) Aportes a la etnobotánica del género *Prosopis*. In: IADIZA (ed) Contribuciones Mendocinas a la Quinta Reunión de Regional para América Latina y el Caribe de la Red de Forestación del CIID. Conservación y Mejoramiento de Especies del Género *Prosopis*, Mendoza, Argentina, pp 99-119

Saidman BO, Bessega C, Ferreyra LI, Julio N, Vilardi JC (2000) The use of genetic markers to assess populations structure and relationships among species of the genus *Prosopis* (Leguminosae). Bol Soc Arg Bot 35:315–324

Senthil Kumar R, Parthiban KT, Govinda Rao M (2009) Molecular characterization of *Jatropha* genetic resources through inter-simple sequence repeat (ISSR) markers. Mol Biol Rep 6:1951–1956

Smith JSC, Smith OS (1989) The description and assessment of distances between inbred lines of maize. II. The utility of morphological, biochemical and genetic descriptors and a scheme for testing of distinctiveness between inbred lines. Maydica 34:151–161

Taleisnik E, Lopez Lauestein D (2011) Leñosas perennes para ambientes afectados por salinidad. Una sinopsis de la contribución argentina a este tema. Ecología Aust 21:3–14

Therneau TM, Atkinson BR, port by Brian Ripley. <atkinson@mayo.edu>(2011) rpart: recursive partitioning. R package version 3.1-50. http://CRAN.R-project.org/package=rpart

Truntzer C, Mercier C, Gautier C, Roy P (2007) Importance of data structure in comparing two dimension reduction methods for classification of microarray gene expression data. BMC Bioinforma 12: 1–12. doi:10.1186/1471-2105-8-90

UPOV (1995) Working group on biochemical and molecular techniques and DNA-profiling in particular. Third session. Identification methods based on molecular techniques. Wageningen, The Netherlands

Velarde M, Felker P, Degano C (2003) Evaluation of Argentine and Peruvian *Prosopis* germplasm for growth at seawater salinities. J Arid Environ 55:515–531

Vilela AE, Ravetta DA (2005) Gum exudation in South-American species of *Prosopis* L. (Mimosaceae). J Arid Environ 60:389–395

Wojtusik T, Felker P, Russell EJ, Benge MD (1993) Cloning of erect, thornless, non-browsed nitrogen fixing trees of Haiti's principal fuelwood species (*Prosopis juliflora*). Agrofor Syst 21:293–300

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeats (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176–1