Chapter 10

Genetic Breeding of *Prosopis* Species from the "Great American Chaco"

Diego López Lauenstein, Ingrid Teich, Edgardo Carloni, Mariana Melchiorre, Mónica Sagadin, Javier Frassoni, and M. Jacqueline Joseau

10.1 Use and Domestication of "Algarrobos": A Set of Multipurpose Tree Species

In the Argentine Chaco, "algarrobos" wood (mainly *Prosopis alba* and *P. nigra*, but also *P. chilensis*, *P. flexuosa*, *P. hassleri*, and other *Prosopis* of minor or local relevance), gives rise to a sawing industry that supports a significant number of jobs (Kees and Michela 2016). In this region there are 678 sawmills, in which approximately 4500 people work and consume 419,000 m^3 of native wood per year, and 40% of this volume comes from different species of *Prosopis*. Other important native species that supply raw material to these sawmills are *Schinopsis balansae*, *Schinopsis lorentzii*, and *Aspidosperma quebracho-blanco*. More than 85% of the establishments are micro-companies, and the rest are medium-sized companies (Min. Agro. 2015). The raw material for these sawmills comes exclusively from the native forest, which has led to a progressive environmental degradation, and also to a decrease in log quality. Moreover, the average yield of the sawmills does not exceed 35%, partly due to its low level of technification, but also due to the low quality and unevenness of logs from unmanaged forests.

The *algarrobos* have been intensively used in Argentina for more than 50 years for making high-quality solid wood furniture. Also, their wood has been traditionally converted in charcoal by means of craft methods with hemispherical brick kilns. According to official statistics, 1663 ton of charcoal were made with *Prosopis*

D. López Lauenstein (✉) · I. Teich · E. Carloni · M. Melchiorre · M. Sagadin
Instituto de Fisiología y Recursos Genéticos Vegetales, Unidad de Estudios Agropecuarios (IFRGV, UDEA) INTA-CONICET, Córdoba, Argentina
e-mail: lopez.diego@inta.gob.ar

J. Frassoni · M. J. Joseau
Facultad de Ciencias Agropecuarias – Universidad Nacional de Córdoba, Córdoba, Argentina

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wood in 2017 in Argentina (SAyDS 2019). However, the economic relevance of the *Prosopis* species from the Great American Chaco is not only related to its wood production. They are multipurpose trees, generally used for more than one product or service. They are suitable for silvopastoral systems, providing livestock with not only shade but also highly nutritious fruits for forage. In fact, their pods (regionally called "algarrobas") are also used for human consumption in many ways, from traditional food preparations (e.g., "patay") and beverages (e.g., "aloja" and "añapa") with economic relevance in the local markets, to food industry inputs traded regionally. A kind of flour is made by grinding the pods, which is used at home, in artisan bakeries and in cookie industries. A coffee substitute is also manufactured with ground pods. The official statistics have registered for 2017 the production in Argentina of 180 ton of algarrobas for human consumption, 67.63 ton of algarrobas for livestock forage, 30 ton of patay, 20 ton of algarroba flour and 2.5 ton of algarroba coffee (SAyDS 2019). Pods are collected from the natural forest, where each tree produces in average 60 to 120 kg per year, depending on the species, the site, and the year conditions (Galera 2000). Additionally, due to their tolerance to drought and salinity, and their ability to fix nitrogen in the soil, *Prosopis* species are regarded as a promising alternative for ecological restoration in degraded arid and semiarid environments.

The drastic reduction of *Prosopis* forests in recent decades has promoted interest in using the most productive species of the genus in afforestation, for a dual purpose of renewing the productive resource and recovering degraded ecosystems. Since the promotion of commercial plantations through the national subsidy granted by Law 25,080 for over 20 years, this interest has been translated into effective plantations (Fig. 10.1), that made *Prosopis alba* the second most planted native forest species in Argentina after *Araucaria angustifolia*. Currently, about 9000 ha are implanted with *P. alba* in Argentina, which are, in average, 12 years old with few having reached the rotation age. In most of this plantations silvicultural management was not done, and the origin of the genetic material (seeds) is unknown (Salto and Lupi 2019; Leandro Arce, personal communication).

These afforestation experiences have contributed to the domestication of the species by generating information on cultivation techniques and seedling production in the nursery. However, only in the last 10 years more specific silvicultural investigations begun in order to develop appropriate and modern methods for the cultivation of *P. alba*. Concerning seedling production on an industrial scale, the traditional system where seedling beds were at ground level, using polypots as "container" and substrates containing soil, is being replaced by modern technics with suspended seedling beds, using tray-pots and specific substrates appropriate for root development and optimal balance between root and aboveground biomass growth.

A mixture of composted pine bark, perlite, and vermiculite as substrate, in 125 cm^3 containers, resulted to be a good alternative for technical production in the nursery. Fertilization is necessary to produce seedlings under these conditions of substrate and containers. In this sense, a specific formulation has been developed for *P. alba*, which increases growth in diameter and height to reach the optimal size for transplant in 90 days (Salto et al. 2019a). This technological leap resulted in a

Fig. 10.1 Five-year-old afforestation of *P. alba* (Campo Durán provenance) located in Laguna Yema (Province of Formosa), with *Opuntia* sp. leaves drying between lines. (Photo: Diego López Lauenstein)

decrease of nursery time from 5 months with the traditional methods to 3 months with the modern technics, also reducing the volume of substrate, and the cost of both, production and movement of seedlings to the plantation. Information about edaphic characteristics for site quality determination for cultivating *P. alba* is also available. The best growth corresponds to soils with more than 50 cm of effective root exploration depth, good drainage, and light surface texture (loamy and/or silty sandy soils). On the contrary, in soils with somewhat poor drainage, shallow, heavy (clayey) soils, and that are in subnormal relief (depressed, waterlogged), the *algarrobos* are small in size (Kees et al. 2019). To initiate the plantation, site preparation depends on numerous factors, like previous use, type of soil, owner resources availability, and identification of possible soil limitations, among others. The usually used tools are a disc harrow, and if required, a subsoiler to improve the water infiltration. Planting should be done outside the frost period and coinciding with either the beginning or the end of the rainy season, which is concentrated in summer. The planting period can be longer, but always depending on water availability and favorable temperatures (MAGyP 2016).

In *P. alba*, the stem is formed by the succession of branching orders, of which three branches are generated, generally one aborts and one of the rest takes an orthotropic direction (upward) to continue with the trunk (Moglia and Giménez 2006). As a result of this branching model, young *algarrobo* plants without management show abundant branching, making it difficult to identify a main axis. Therefore, formative and lift pruning are essential to allow knot-free timber to form around the defect core. This management tends to be used for high-quality uses such as veneer, timber moldings, and furniture. On the other hand, planting density plays an essential role in branching degree, since denser plantations generate fewer branches and straighter stems. Zárate et al. (2019) tested different pruning treatments under a range of densities extending from 450 to 4500 plants/ha in a Nelder plot design, showing that growth up to 7 years in plantations at low densities (450, 560, and 750 pl/ha) have potential to achieve the highest bole volumes per plant and with few knots in the wood.

Another silvicultural treatment that should be used in any *Prosopis* plantation is thinning. The aim is to redistribute the growth of the stand in the best possible way, regulating the use of resources (water, light, and nutrients) in their growth space. The remaining individuals increase their growth in response to thinning, concentrating the stand growth on a smaller number of trees, but of higher quality, thus increasing the value of the produced wood. One of the limitations for decision-making is the opportune moment of thinning, which is carried out by evaluating the curves of current annual increment (CAI) and mean annual increment (MAI). For plantations in the Chaco region, the appropriate time for thinning ranges from 9 to 13 years. This variation is mainly due to the initial planting density (Gómez et al. 2019). Other factors that influence growth curves (CAI, MAI) are the seed provenance (genetics) and site quality (environment). Applying this silvicultural management, a rotation age of 25 years can be estimated, with diameters greater than 30 cm in the best quality sites (Kees et al. 2018).

10.2 Low-Intensity Breeding

Afforestation plans with *Prosopis* requires a large volume of seeds every year, and to supply this demand, fruits from the natural forest are collected. However, when collecting seeds, interspecific crosses that occur naturally within the genus must be avoided because can generate unevenness plantations, both in diameter and height growth, as well as great variation in morphological characters (e.g., multibranches or thorns). Like in all wild species, diversity is very large, which might constitute an advantage for breeding, but, at the same time, it forces to reduce variability in order to achieve discrete, more stable, and uniform genetic units. Therefore, the main objective of the low-intensity breeding of *Prosopis* is to assure the proper amount of seeds for seedling production, guaranteeing a high specific purity and uniformity of the seedlings. The genetic and ecophysiological knowledge available on *Prosopis chilensis*, *P. flexuosa*, and, more recently, *P. alba*, constitute the conceptual basis for advancing toward the implementation of seed stands for these purposes.

Given the alarming loss of forests that has occurred in Argentina during the last 35 years (estimated in 12 million ha in the Chaco region, e.g., Spensley et al. 2013, see Chap. 8), and the risk of losing biodiversity due to climate change, specific studies are required to identify and delimit seed production areas (SPA) for each species, and its subsequent transformation into seed stands. These actions aim to preserve existing variability and obtain base material with greater genetic uniformity and purity, avoiding hybridization between *Prosopis* species (Verga 2014 and see Chap. 9). At the same time, it seeks to respond to the suggestions of FAO (2014) to improve the sustainable use and management of forest genetic resources. Despite the intense degradation of *Prosopis* forests in Argentina, there are stands of good purity and silvicultural quality that, with a proper management, can be used in the short term as genetically stable seed stands. Accordingly, protocols were established together with the Argentine National Seed Institute (INASE; Res. 374/14) in order to systematize seed harvest for seedling production of the main *Prosopis* species. These protocols involve the morphological analysis of leaves of adult individuals, and the genetic analysis through biochemical markers (isoenzymes) of offspring (seeds) for the definition of purity thresholds (Verga 2014). So far 11 SPA (Fig. 10.2) of *Prosopis alba*, *P. flexuosa* and *P. chilensis* have been defined, which can be transformed into seed stands, some of which are already certified by INASE. This definition of SPA was a joint task of the Instituto Nacional de Tecnología Agropecuaria (INTA) and the National Germplasm Bank of *Prosopis* (BNGP) of the Facultad de Ciencias Agropecuarias de la Universidad Nacional de Córdoba (FCA-UNC). To identify these SPA, a joint analysis of information from databases of both institutions, bibliographic references, local informants, and satellite images was done. Then, field confirmation was performed accounting for several aspects: (1) specific

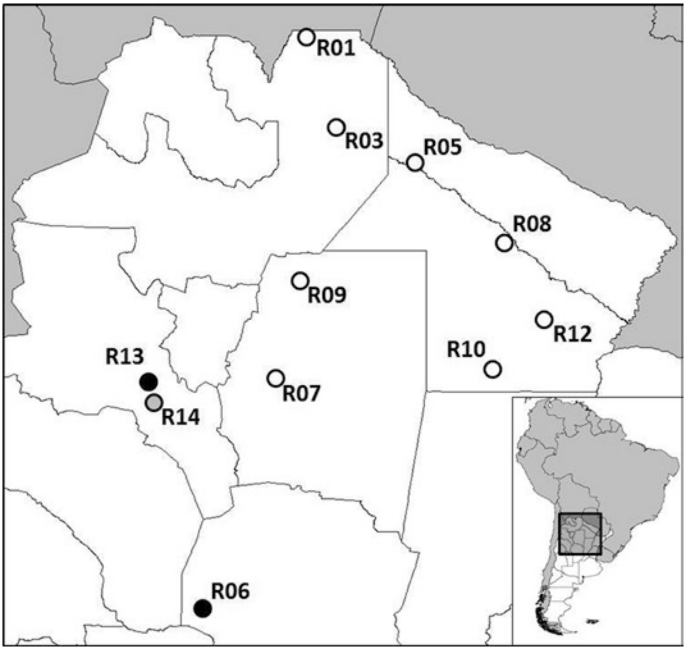

Fig. 10.2 Geographical distribution of *Prosopis* seed stands. White circles: *P. alba*; black circles: *P. flexuosa*; grey circle: *P. chilensis*. R01: Campo Durán, R03: La Unión, R05: Isla Cuba, R06: San Miguel, R07: Santiago del Estero, R08: Bermejito, R09: Chañar Bajada, R10: Villa Ángela. R12: Plaza, R13: Pipanaco, R14: Palampa

composition of the stand, i.e., predominance of the target species; (2) morphological characteristics and health status of the trees; (3) accessibility of the stand and (4) legal conditions and agreement of the landowner where the stand is located.

Considering the large interspecific hybridization rate of the genus and the need for the highest degree of purity for each seed source (i.e., regarding the target species), the first step to define a SPA is the accurate identification of the taxonomic status of the trees that compose the candidate stand. In this sense, there are two relatively simple and inexpensive methods to detect genetic variation of interspecific origin in the *Prosopis* complex: leaf morphology of adults and isoenzymatic analysis of seeds using the allozyme marker alcohol dehydrogenase (ADH). Due to the presence of species-specific alleles, the screening of the ADH isozyme in a sample of seeds allows to determine the proportion of interspecific crosses between the "white" algarrobos (*P. alba*, *P. hassleri*, *P. fiebrigii*, *P. chilensis*) and "black" algarrobos (*P. nigra, P. flexuosa*, *P. ruscifolia*) (Saidman 1986; Verga 1995). On the other hand, through morphological analysis of the leaves, it is possible to identify those algarrobos that are not the target species or that may presumably be of hybrid origin (Verga 2014). Both methods can be applied because algarrobos have a very strong correlation between their leaf morphological characteristics and their genetic basis, particularly that related to their specific origin (Saidman 1986; Verga 1995; Verga and Gregorius 2007; Ferreyra et al. 2013; Joseau et al. 2013).

The protocol establishes the sampling, at the selected stand, of all the *Prosopis* individuals with a diameter at the base greater than 5 cm. Each tree is identified, georeferenced and photographed to subsequently appreciate its morphological characteristics and estimate its allometric parameters. Leaves and fruits are harvested for morphological and genetic analysis (Figs. 10.3a and 10.3b), and for incorporation into the ACOR Herbarium (http://www.agro.unc.edu.ar/herbario) of the Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba (FCA-UNC). The first step is performing the foliar morphology analysis on five scanned

Fig. 10.3 Material used for the identification of trees in a natural stand for its transformation into a seed stand: (**a**) leaves, (**b**) fruits, and (**c**) a target tree of the seed stand of *P. alba* located in the Province of Salta. (Photos: Carmen Vega, Diego López Lauenstein)

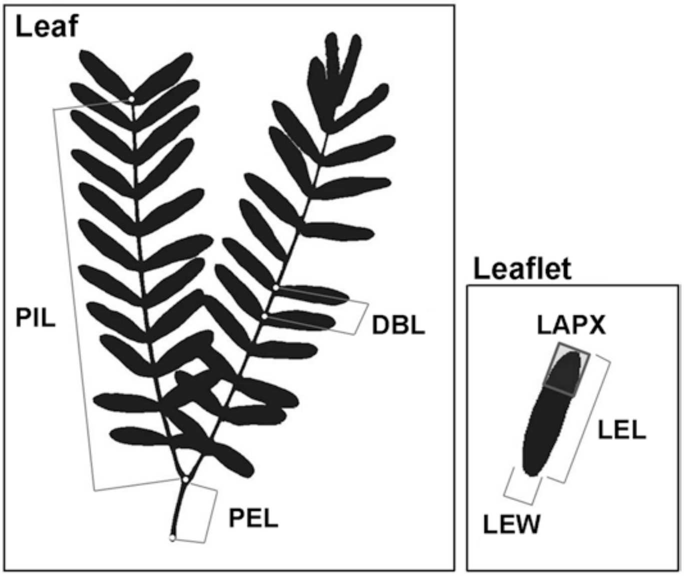

Fig. 10.4 Foliar morphological traits evaluated in *Prosopis alba* by means of the software Hoja 3.4. *PIL* pinna length, *PEL* petiole length, *DBL* distance between leaflets, *LAPX* leaflet apex, *LEL* leaflet length, *LEW* leaflet width

leaves for each tree (Fig. 10.3a) using the software Hoja 3.4 (Verga 2015). The most frequently used leaf traits are petiole length (PEL), number of pairs of pinnae (NPI), pinna length (PIL), number of pairs of leaflets per pinna (NLP), leaflet length (LEL), leaflet width (LEW), leaflet area (LEA), leaflet apex (LAPX), distance between leaflets (DBL), and the ratios LEL/LEW and LAPX/LEA (Fig. 10.4). Fruit morphometric characters usually include fruit length (FrL), fruit width (FrW), and fruit thickness (FrTh). *Prosopis* fruits are also characterized according to their shape (FSh), the fruit edge shape (FESh), and the color (FrC). To determine the taxonomical status a principal component analysis with the 13 leaf traits is carried out using each sampled tree as unit of analysis. Typical trees of each morphological group of *P. alba* (i.e., “chaqueño”, “santiagueño” and “salteño,” Verga et al. 2009) are included as reference. Once the species and the morphological group to which the stand belongs have been identified, a new principal component analysis is performed, and each tree is examined to detect possible outlier individuals. A threshold (3, −3) is set on the principal axes N° 1 and N° 2. For doubtful individuals (i.e., possible hybrids) a visual appreciation of the fruit is used.

Subsequently, the specific purity of the offspring (seeds) is evaluated using the ADH allozyme marker. A pool of seeds is harvested from at least 20 trees and not less than 10% of the individuals, and 100 seeds are analyzed by starch gel electrophoresis. The following two maximum allele frequency thresholds are set:

1. The SPA will not be suitable for certification as a seed stand if the heterozygous individuals (seeds) exceeds 10%.
2. The SPA will not be suitable for certification as a seed stand if the frequency of the allele corresponding to the “algarrobo negro” (*P. nigra*, *P. flexuosa*, *P. ruscifolia*) in the seed pool exceeds 2%.

The two combined analyses (allozyme and leaf/fruit traits) allow identifying, within each SPA, individuals to thin with the purpose of increasing the specific purity of the seeds from the seed stand.

Seed production in *Prosopis*, as in many forest tree species, is cyclic, so seeds are not available every year. For this reason, it is important to stock up on seeds during high production years to ensure their availability for seedling production. Due to their thick seed coat, the *Prosopis* seeds stored at 4 °C maintain a high germination power (80%) for at least 30 years (Verzino and Joseau 2005). The National Germplasm Bank of *Prosopis* (BNGP) currently stores 1650 accessions at −18 °C, corresponding to seeds from 1106 trees or stands. Other ex situ conservation collections are botanical gardens and field trials such as progeny and provenance trials (e.g., López Lauenstein et al. 2016; Capello 2019; Verzino et al. 2020).

To assess the adaptability of some of the already mentioned seed stands, three provenance trials were installed in 2011 in three sites in the semiarid region of Chaco: Laguna Yema (24° 16′ 23.53″ S; 61° 14′ 5.28″ W; 160 m asl), Leales (27° 11′ 12.54″ S; 65° 14′ 37.86″ W; 320 m asl), and Fernández (27° 56′ 16.06″ S; 63° 52′ 26.10″ W; 160 m asl) (Fig. 10.5). The three sites differed in the mean annual precipitation and the mean annual temperature (Laguna Yema, 703 mm and 22.7 °C; Leales, 965 mm and 19.9 °C; and Fernández, 575 mm and 20.8 °C, respectively). The genetic material evaluated in these field trials was seven provenances of *Prosopis alba* from different seed stands, two *P. alba* selections based on the evaluation of three progeny trials (povenances 6 and 7), and one *P. chilensis* selection from a natural forest of the Arid Chaco (López Lauenstein et al. 2019).

The performance of this small network of trials was evaluated with periodic measurements of survival, diameter at breast height (DBH), and total height. At the time of this publication, the latest records are Leales, 2018, 7 years old; Fernández, 2019, 8 years old; and Laguna Yema, 2016, 5 years old. For comparing between sites, the mean annual increment (MAI) was calculated, dividing the last record, both DBH and height, by the number of years of each plantation. The main results show significant differences between provenances in both DBH and height growth. In this sense, provenance N° 1 *Prosopis alba* “Campo Durán” had the highest MAI both in height and diameter (Fig. 10.6), standing out mainly in Leales (12.1 cm DBH and 6.24 m in height at 7 years old) and Laguna Yema (12.62 cm DBH and 6.04 m high at 5 years old). The selection of *P. alba* from the progeny trials and the selection of individuals from the natural stand of *P. chilensis* served as reference for the natural provenances of *P. alba*. Provenance N° 7 stands out and corresponds to a selection of 10 open-pollinated families carried out from the evaluation of more than 200 *P. alba* families from the entire Chaco region, in 3 progeny trials (López Lauenstein

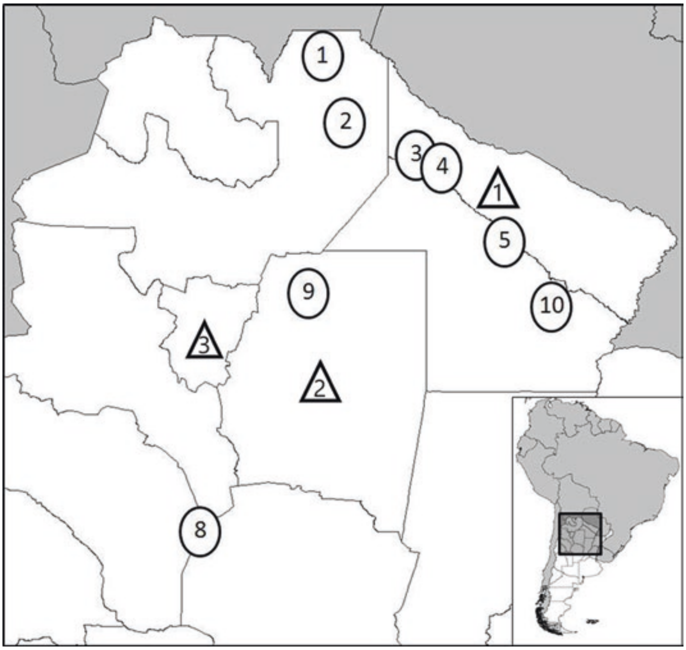

Fig. 10.5 Location of field trials (triangles) *1*, Laguna Yema; *2*, Fernández; *3*, Leales; and provenances (circles) *1*, Campo Durán; *2*, La Unión; *3*, Bolsa Palomo; *4*, Isla Cuba; *5*, Bermejito; *8, P. chilensis* selection; *9*, Chañar Bajada; and *10*, Plaza

et al. 2016). This evaluation was done 3 years after implanting the field trials and was based on growth and shape characters, highlighting the value of early selection and the high heritability of the chosen characters. It is worth noting that Campo Durán natural provenance, represented by open-pollinated seeds from unselected trees, performed better (or at least equivalently) than the progeny of the acute selection of 10 individuals out of 200 in a progeny trial. Likewise, provenance N° 8 (*P. chilensis* selection), as expected, showed the lowest growth in diameter and height in the three field trials. These results suggest a lack of adaptation of *P. chilensis* to the Semiarid Chaco region (it is a species of the Arid Chaco).

Although there is a clear (statistically significant) separation of treatments N° 1 and N° 8 (above and below, respectively) in terms of growth, the other provenances of *P. alba* did not show great differences between them in any of the three field trials. These provenances would have a high phenotypic plasticity to adapt to a wide variety of environmental conditions. In this sense, expanding the genetic base of recommended materials will increase the resilience of the plantations to climate change, e.g., different kinds of abiotic stress (saline, drought, thermal, etc.) and the occurrence of new pests or diseases.

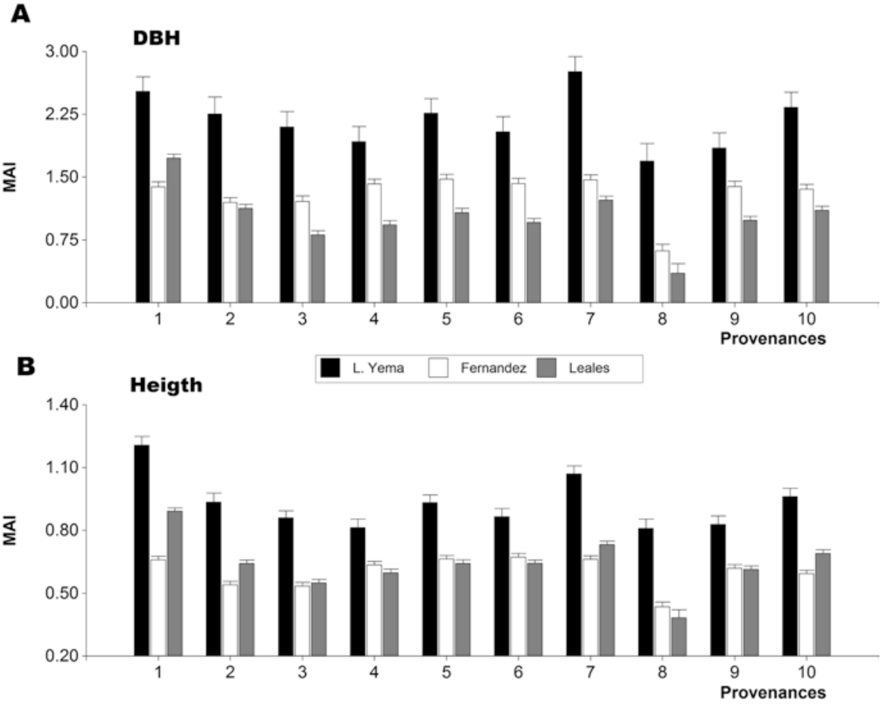

Fig. 10.6 Mean annual increment (MAI) in diameter at breast height (**a**) and height (**b**) for 10 *Prosopis* genetic materials (8 *P. alba* provenances, 2 *P. alba* selection, and 1 *P. chilensis* provenance; see Fig. 10.5) in three field trials: Fernández, Laguna Yema, and Leales

10.3 High-Intensity Breeding

The primary goal of *Prosopis* species breeding has traditionally been related to wood production. Growth traits (total height and diameter) and trunk shape and bole length have been the focus of improvement. However, as mentioned in the first section of this chapter, the objectives of different *Prosopis* breeding programs can be diverse. In addition to the wood volume yield, the objectives can be oriented to improve wood quality, fruits production and quality, resistance to pests and diseases, adaptability to marginal (e.g., dry) environments, or degraded (e.g., salinized) soils, among others. Improvement programs can contemplate one or more of the aforementioned goals, and in all cases, they must have the premise of maintaining a wide genetic diversity to guarantee that the genetic gains do not decrease, even in advanced generations.

Forest tree breeding has been successful at delivering genetically improved material for multiple traits based on recurrent cycles of selection, mating, and testing (Grattapaglia et al. 2018). Pedigree-based phenotypic selection, rather than genetic dissection approaches such as quantitative trait mapping and association genetics, is currently the main approach in *Prosopis* breeding programs of Argentina.

Initially, the genetic breeding strategy for *P. alba* was based on an adjustment of the "multiple populations" method proposed by Namkoong (1980), where genetic improvement and conservation of genetic resources are combined. This method consists of creating a base population conformed by the progeny of individuals from natural populations. Each base population is composed by subpopulations isolated from each other and located in different environments. These subpopulations are used as progeny trials and subsequently, after evaluation and selection, as seed orchards. In this way, each subpopulation is improved separately and, at the same time, passes through a process of differentiation from the others (due to the adaptation to the different environments). This allows for future recovering of genetic variability in stands arising by the mixture of seeds of the different subpopulations (Verga 2005).

Provenance and progeny trials of *Prosopis* in Argentina have been implemented since 1990 (Cony 1996; Felker et al. 2001; López Lauenstein et al. 2016), allowing the study of population performance in different environments and the estimation of genetic parameters related to traits such as height, diameter, stem shape, and growing rate. Progeny trials are a powerful tool in forest breeding programs (Zobel and Talbert 1984). Through the estimation of breeding values, family and/or individual rankings are made in order to carry out backward or forward selection. These progeny trials are then thinned according to the rankings (i.e., forward selection), to become seed orchards (Ruotsalainen and Lindgren 1998). Currently, *P. alba* breeding program in Argentina includes a network of three progeny trials established in 2008 (INTA net): Laguna Yema (24° 19′ 15.5″ S; 61° 17′ 31.7″ W; 161 m asl), Santiago del Estero (27° 56′ 45.1″ S; 64° 13′ 12.5″ W; 172 m asl), and Plaza (26° 56′ 3.6″ S; 59° 46′ 22.3″ W; 78 m asl). These trials include 217 open-pollinated families from ten different provenances, which cover a large part of the natural range of the species in the Argentine Chaco. This base population come from seeds collected during successive field campaigns between 2004 and 2007 (Verga et al. 2009) from phenotypically selected individuals (mother trees) in wild populations.

In this first phase of the program, the base population was constituted on the basis of the specific purity without considering differences by geographical origin. This decision was based on previous genetic studies in *P. chilensis* and *P. flexuosa* from the Arid Chaco region, where the main source of genetic variation was shown to come from hybridization processes (Verga 1995, 2005). In this species complex, there are no significant differences between populations that grow in dissimilar environments compared to the enormous variation within them as an effect of interspecific crosses and the presence of interspecific hybrids (Verga 1995). More recently, however, and based on leaf trait analysis and variation detected with molecular markers, three morphological groups within *P. alba* were determined, which could be considered subspecific taxonomic groups: *P. alba* "chaqueño," *P. alba* "santiagueño," and *P. alba* "salteño" (Verga et al. 2009, Verga 2014; Chap. 9). These groups show a separate geographic distribution and from their identification and evaluation through provenance trials, the breeding strategy was rethought. The new strategy considers dividing the current base populations into each subspecific taxonomic group and provenance. The objective is to advance in the installation of new progeny tests corresponding to each morphological group, developing each one as the base population of its own taxonomic group. In addition, and because there are traceability records of the seed used in the most recent plantations, plus trees are being evaluated and selected within the plantations to incorporate them into the base populations of each morphological group.

These two schemes are not excluding, and their simultaneous development can serve to face two challenges. On the one hand, with the original scheme (i.e., a mixture of provenances), a high genetic diversity is maintained improving adaptation to a large number of environments with high resilience to climate change. With the new scheme (i.e., separate morphological groups), on the other hand, the hope is to give a better response to the pursuit of specific objectives related to market demands and high growth rates. This new scheme began to be applied in particular to the outstanding population *Campo Durán*, which corresponds to the morphological group *P. alba* "salteño." Thus, in 2018, a progeny trial with 45 open-pollinated families from *Campo Durán* was installed in Sáenz Peña Experimental Station of INTA (26° 51′ 15.3″ S; 60° 25′ 16.8″ W). The experimental design includes the identification of the family and also of the seedlings from seeds of the same pod, since it is known that 64% of the seeds from the same fruit correspond to complete siblings (Bessega et al. 2012). This design allows to increase the accuracy in the estimation of breeding values from separately considering the treatments of complete siblings from those of half siblings.

Advanced forest genetic evaluation involves analyzing data from progeny tests using mixed linear models to estimate the best linear unbiased predictors (BLUPs) of tree breeding values (BVs). The high number of provenances and families in the INTA net not only allows these estimations but also contains ex situ and in vivo conservation material representative of the genetic variation of the species in the Argentine Chaco (Verga et al. 2009). In 2005, the first genetic markers for *Prosopis* species were published (Mottura et al. 2005); later, more molecular markers were developed (Suja et al. 2007; Bessega et al. 2013; Torales et al. 2013; Pomponio et al. 2015). These markers are mainly used for studies of genetic structure of the different *Prosopis* species and their interspecific hybrids (Chap. 9). Although the number of specific markers is still very limited, the challenge is to incorporate them into the breeding program through marker-assisted breeding and, at the same time, implement next-generation sequencing techniques for the development of large-scale markers (SNPs) and shorten the breeding cycle through genomic selection. With the current genomic resources, kinship matrices between individuals will be established, instead of using the theoretical values of the kinship relationships for the calculation of BVs, increasing the accuracy in their estimation (Marcucci Poltri and Gallo 2016; Grattapaglia et al. 2018).

As *P. alba* is a multipurpose resource (Galera 2000), its breeding process involves different strategies according to the product to be improved (wood quality, fruit production, etc.). One of the first evaluations of *P. alba* progeny trials considering its multiple uses included total biomass production, height, rate of pod production, and pod organoleptic characteristics (Felker et al. 2001). This study reported the evaluation of a 9-year-old progeny trial containing 57 open-pollinated families of 8 provenances from Northwestern Argentina established in 1990 in the Province of Santiago del Estero (27° 45′ S, 64° 15′ W, 200 m asl). The family-narrow-sense-heritability was 0.487 for height, 0.548 for aerial biomass production, and 0.244 for pod production. The mean values across families ranged from 10.7 kg/tree to 57.4 kg/tree for aerial biomass production, from 2.2 m to 3.6 m for height, and from 13 g/year to 874 g/year for pod production. Even though *P. alba* produces highly nutritious fruits for forage and human food with commercial value (Fagg and Stewart 1994), most studies have focused on traits related to wood production (Salto 2011; Cappa and Varona 2013; Carreras et al. 2016). Currently, ongoing studies seek to estimate breeding values at tree level for flowering and fruiting traits (flowering intensity, production of pods, nutritional quality of fruits) using the INTA net trials (Cisneros et al. in preparation).

The selection for wood yield requires improving traits like tree architecture, trunk shape, and bole length. After the progeny trials of INTA net were established in 2008, the families' early growth and shape were evaluated at 18 months of age. These studies reported significant interaction between provenance and environment in diameter (ranged from 1 to 8 cm) and total height (ranged from 1 to 3 m) and a negative correlation between tree form and the two growth traits (Salto 2011). This represents a constraint for the breeding program, as trees selected for fast growth would show low quality in terms of tree form. To overcome this limitation, Carreras et al. (2016) evaluated genetic parameters for several traits of economic importance in *P. alba*, including tree form (number of stems), height, diameter, and size increments using a multi-trait selection approach based on a selection index, with the goal of getting the maximum possible gain in all traits simultaneously (Bessega et al. 2015). They developed a breeding strategy for multiple trait selection taking into account the heritability of individual traits, the genetic correlation between them, and the increase of selected group kinship inherent to the selection process. This issue is important for breeding to preserve wide genetic diversity and prevent inbreeding depression in the following generations (Lindgren and Mullin 1997). They reported that although gain at individual trait level is reduced in comparison with the maximum potential, index selection allows significant gains for all three traits together and represents a suitable strategy to improve *P. alba* in order to establish clonal seed orchards. This study evaluated only one progeny trial of INTA net, and therefore no genotype x environment interaction was estimated. Currently, height, diameter, and tree form at different ages, measured in the three trials, are under analysis with individual tree mixed models (Borralho 1995) to predict breeding values and simultaneously estimate genetic and environmental effects (Cisneros et al. in preparation). Current efforts in *P. alba* breeding program aim at expanding the approaches to multiple uses (multipurpose selection) such as fruit production, forage, recovery of degraded areas, biomass production, and environmental services, among others.

10.4 Vegetative Propagation: A Useful Tool for Genetic Improvement

Agamic propagation constitutes a fundamental technique to be developed for the *P. alba* breeding genetic program, since it will allow the eventual installation of clonal seed orchards (López Lauenstein et al. 2016). In this way, ex situ conservation is also done by cloning representative samples of threatened natural populations. For these reasons, the INTA *P. alba* breeding program is developing asexual propagation methodologies through different in vitro tissue culture and grafting techniques. Also, different techniques of rejuvenation of vegetative material that allow the multiplication of *P. alba* through the use of mini-stakes are being tested.

The advantage of agamic propagation in forest genetic breeding is its ability to capture and transfer to the new individuals all the genetic potential of the mother plant, in a short period of time since it is not necessary to wait for the production of seeds to obtain their offspring. One of the vegetative propagation methods being tested is the in vitro culture of uninodal segments of 2-year-old seedlings from seeds of Campo Durán population (Fig. 10.7a, b). On the other hand, different types of grafts were tested from cuttings collected from the natural population and grafted on seedlings produced from seeds. The spike graft was the most appropriate in *P. alba*, in agreement with reports by other authors (Wojtusik and Felker 1993; Ewens and Felker 2003) (Fig. 10.7c–e). This technic allows us to propagate selected

Fig. 10.7 Vegetative propagation of *Prosopis alba*. (**a** and **b**), in vitro micropropagation from uninodal segments of seedlings obtained from seeds; (**c** and **d**), grafts of adult plants on juvenile seedlings; (**e** and **f**), mini-cuttings from Campo Durán population. (Photos: Edgardo Carloni)

individuals from the natural population and also adapt field materials to controlled conditions, looking to obtain uninodal segments to establish in vitro cultures.

Rooting of cuttings of rejuvenated material was also tested. Salto et al. (2012) selected 50 individuals of *P. alba* from the evaluation of a progeny trial installed in Laguna Yema. A multi-criteria selection index was calculated, considering the growth (diameter at the base and height) and the shape of the stem. The selection was made with the estimation of the individual improvement values at the third year of planting (Salto 2011). The 50 selected genotypes were coppiced in late winter (August) at a height of 20–25 cm, using a chainsaw. The stumps were sealed with a pruning bandage. The shoot harvesting campaigns took place at three moments in time (41, 61, and 110 days after coppicing). The length and diameter of shoots ranged from 15 to 70 cm and 2 to 6 mm, respectively. At the greenhouse, plant cuttings of 8–10 cm long were conditioned leaving two leaves reduced to a 50% in area. Then, the cutting bases were treated with indole-3-butyric acid at a 0.45 g/kg concentration as root inducer, using microbiological talc as vehicle. The rooting percentage of the plant cuttings brought from the field showed values ranging from 12.5% to 100% among genotypes (López Lauenstein et al. 2016).

Likewise, grafting adult plants on juvenile rootstocks provides rejuvenated material with the goal of establishing a propagation system for macro-cuttings. In *P. alba* and, as mentioned, in other species (Wendling et al. 2014), rejuvenated material is the most efficient way to achieve rooting of cuttings (de Souza and Felker 1986; Arce and Balboa 1991; Oberschelp and Marcó 2010). In recent years, de Souza et al. (2014) have implemented the mini-cuttings technique, which consists on the use of buds of mother plants under controlled conditions in order to generate a degree of rejuvenation and a lower lignification. These authors indicate that the mini-cutting have good rhizogenic capacity with rooting percentages ranged from 98% to 100%.

10.5 Beneficial Microorganisms

Soil microbiomes play important roles in terrestrial ecosystem regulation and functioning, impacting on productivity, diversity, and structure of plant communities. Different types of abiotic stress like drought, salinity, high temperatures, and low nutrient availability acting either alone or in combination have a strong influence on plant diversity, conditioning their survival. Usually plants coevolve with the biodiversity of soil microorganisms and mutual relationships between them exist. The use of rhizobia and mycorrhizal inoculants in nurseries is a strategy to improve the adaptation and survival of seedlings in transplanting to the field, both in wood production plantations and in restoration or ecosystem recovery plans. Therefore, isolation and characterization of specific strains of these microorganisms is highly valued in order to incorporate them into a technological package with the genetic breeding of plant germplasm.

10.5.1 *Rhizobacteria for* P. alba *Cultivation*

The symbiotic relationship between plants and rhizobacteria allows the incorporation of N_2 via biological fixation (NBF), inducing a set of systemic changes in the plant that contributes to efficient adaptive responses. Therefore, inoculation with rhizobacteria enhances plant survival during the development and establishment of forest plantations, especially in limiting environmental conditions (Zamioudis and Pieterse 2012).

In the Arid Chaco region of Argentina, the first described species with the ability to form root nodules in *P. alba* was *Mesorhizobium chacoense* (Velázquez et al. 2001). More recently, Chávez Díaz et al. (2013) reported that rhizobacteria belonging to *Mesorhizobium*, *Sinorhizobium* (Ensifer), and *Bradyrhizobium* genera, isolated from environments of five *P. alba* populations in the Chaco region, were also able to induce nitrogen-fixing nodules in this species. To obtain these isolates, soil was collected from *P. alba* seed stands in five localities in the Chaco: San Miguel-Córdoba (31° 45′ 59″ S; 65° 25′ 39″ W); Padre Lozano-Salta (23° 12′ 51″ S; 63° 50′ 39″ W), Isla Cuba-Formosa (24° 17′ 31″ S; 61° 51′ 10″ W), Bolsa Palomo-Formosa (24° 13′ 15″ S; 61° 57′ 42″ W), and Colonia Benítez-Chaco (27° 20′ S; 58° 55′ 60″ W). Seeds were grown under these substrates to capture the diversity of rhizobacteria, and after 40 days, fixing nodules were harvested form roots (Chávez Díaz et al. 2013) (Fig. 10.8a). To isolate symbiotic microorganisms, nodules were superficially disinfected and macerated under sterile conditions. The macerate liquid was streaked on LMA culture medium (Vincent 1970), and the plates were incubated in an oven for 3 to 5 days at 28 °C (Fig. 10.8b).

From the developed colonies, DNA extractions and amplification of randomly repeated fragments were performed by rep-PCR, using the primer BOX-A1 (Versalovic et al. 1994). A total of 100 isolates were analyzed, and amplification

Fig. 10.8 Rhizobacteria nodules in *Prosopis alba* roots (**a**) and developed colonies in Petri dish (**b**). (Photos: Mariana Melchiorre)

patterns were grouped according to a cluster analysis (UPGMA) by locality. Based on this, representative isolates from each site whose similarity was less than 60% were selected for subsequent analyses using the Dice coefficient (Di Rienzo et al. 2012). In total 33 isolates were selected: 14 from Bolsa Palomo, 11 from Isla Cuba, 5 from Padre Lozano, 1 from San Miguel, and 2 from Colonia Benítez.

Tolerance to water deficit was evaluated by cultivating the selected isolates in hyperosmotic solutions of polyethylene glycol (PEG) testing water potentials of −0.6 MPa and − 2 MPa. It was observed that isolates that tolerated greater water deficit, correlated with greater indole compounds (IAA) production. From this set of isolates, the 16S rRNA gene was analyzed. Alignment, sequence analysis and construction of a phylogenetic tree were performed using the 16S rRNA gene fragment (1163 bp) with the ClustalW alignment tool of the MEGA software and the UPGMA method (Tamura et al. 2011). The best results were obtained with two isolates from Bolsa Palomo (*Mesorhizobium* and *Sinorhizobium* (Ensifer)), one from Colonia Benítez (*Mesorhizobium*), and two from Padre Lozano (*Bradyrhizobium* and *Sinorhizobium* (Ensifer)) (Chávez Díaz et al. 2013); all these isolates were found to promote growth in *P. alba.*

In another study, Pozzi Tay (2016) evaluated drought tolerance of *P. alba* in symbiosis with rhizobacteria under greenhouse conditions. Using three isolates [N° 2 and N° 63 from Bolsa Palomo, (KC-759691, *Mesorhizobium* spp. and KC-759695, *Sinorhizobium* spp.) and N° 53 from Padre Lozano (KC-759699, *Bradyrhizobium* spp.)], a biofertilizer was elaborated. Seeds from two contrasting *P. alba* provenances with respect to precipitations were used: Santiago (27° 52′ 44″ S, 64° 9′ 16″ O, 579 mm average annual precipitation) and Campo Durán (22° 12′ 01″ S, 63° 40′ 33″ W, 1054 mm average annual precipitation). A factorial design with three factors of two levels each was used: provenance (Santiago and Campo Durán), biofertilization (with and without) and water stress (control and drought). Growth variables and physiological responses to stress were evaluated (Pozzi Tay 2016). Under drought stress, plants of Santiago with biofertilization conserved significantly more leaves (node with leaf/total node) than the uninoculated plants of both provenances ($p \geq 0.05$). Furthermore, the highest values of chlorophyll and proline content were recorded in biofertilized plants of Santiago. Biofertilized plants of both provenances have similar values in number and weight of nodules, but under drought conditions, these parameters were reduced, without distinction of provenances.

Summing up, the analysis of growth parameters and physiological variables allowed to discriminate the behavior of two *P. alba* provenances under drought conditions in symbiosis with specific rhizobacteria from the Chaco region. Both biofertilized provenances maintained higher levels of proline and chlorophylls under drought compared to those not inoculated. This result suggests that the establishment of symbiosis also gives adaptive advantages to water restriction. Future studies will seek to identify the selected rhizobacterial species by sequencing other housekeeping genes as well as to analyze a possible synergistic effect of the mix of microorganisms in the biofertilizer or the prevalence of any of them for the colonization of nodules and biological nitrogen fixation.

10.5.2 Mycorrhizae for **Prosopis alba**

Mycorrhizae are classified according to the characteristics of the infection and the mutual organisms that establish it. The most important group is the endomycorrhizae, which has been subdivided into several groups, the most significant being the arbuscular mycorrhizal fungi (AMF). About 96% of plants form this type of mycorrhiza and are the most abundant group of mycorrhizal fungi. The absorption of phosphate through AMF results in an increase in the absorption of inorganic phosphorus (which is an almost immobile element in the soil) and therefore in the growth of plants. Furthermore, the AMF-plant symbiosis increases the stability of soil aggregates in natural systems, acting as an adherent, agglutinating soil particles into more stable aggregates, therefore increasing water retention. In arid and semiarid ecosystems, plant establishment is increased when mycorrhizal plants are used, which have greater protection and tolerance to adverse soil and climate conditions (Begum et al. 2019).

In order to isolate, characterize, and obtain mixed native AMF inoculants that confer tolerance to abiotic stress (i.e., drought, salinity) in *P. alba* seedlings, a microbiological study was carried out (Sagadin et al. 2018). Soil was collected from two *P. alba* pure stands with contrasting edaphoclimatic characteristics: Colonia Benítez (CB) 27° 20′ 00″ S, 58° 55′ 60″ W (1300 mm of mean annual precipitation) and Padre Lozano (PL) 23° 12′ 51″ S, 63° 50′ 39″ W (650 mm) (Cabrera 1976). Soil AMF species were identified using *Medicago sativa*, *Sorghum bicolor*, and *P. alba* as trap plants. The predominant presence of the Glomeraceae family was recorded in the isolated inoculum of trap plants. Species such as *Funneliformis mosseae* and *Rhizophagus intraradices* have been identified, as well as *Claroideoglomus etunicatum* (Claroideoglomeraceae), frequently reported in association with the vegetation of arid and semiarid ecosystems.

To evaluate the performance of inocula in nursery, a comparative test was carried out with the application of fertilizer (Salto et al. 2019b). The inoculation was applied at the moment of sowing by inoculating 20 g of each inoculum (PL and CB) and a mixture of them (MIX). The fertilization treatments were fertilization (100%), diluted fertilization (25%), and without fertilization (NF) according to Salto et al. (2016). After 120 days of sowing, the following variables were measured: diameter at the base (DAB), total height (H), and number of leaves (NL). Percentage of mycorrhizal colonization was determined in a sample of five seedlings per treatment. On the other hand, 15 seedlings per treatment (120-day old) were transferred to the greenhouse and acclimatized during 10 days. Then, irrigation was suspended to approximately 10% of the soil water content. The recovery was evaluated in 145-day seedlings by watering them to their maximum capacity for 10 days and measuring resprouting capacity as number of plants with new green leaves.

The addition of 100% and 25% of fertilization solution to AMF inoculated treatments did not promote significant differences ($p = 0.4561$) in the percentage of mycorrhizal colonization, suggesting that the addition of fertilizer did not alter the capacity of colonization nor inhibited the formation of the different characteristic structures of AMF, such as arbuscules, vesicles, and hyphae. Furthermore, in unfertilized plants, AMF inocula promoted growth. The increased leaf production in inoculated plants suggests that mycorrhizae stimulate ontogeny and delay leaf senescence. In addition, increasing the fertilizer to 100% improved growth in the treatment with the PL inoculum, and on the contrary, the MIX and CB inocula had less effect than fertilization of 100% in most of the variables of increase. On the other hand, the resprouting capacity of *P. alba* after drought stress conditions varied with different levels of fertilization and with different types of inoculum. In this context, the uninoculated and unfertilized treatment exhibited the lowest resprouting rate of all the treatments. On the contrary, the effect of the treatment (AMF × fertilization) was significant ($p = 0.0198$) increasing the resprouting capacity. Subsequent comparisons indicate that the proportion of resprouted plants in PL_0% and PL_ 25% treatments were significantly higher (Fig. 10.9). These results demonstrated that the AMF inoculum isolated from the semiarid regions of the study area, such as PL, may have the potential to mitigate drought stress in *P. alba* seedlings compared to the inoculum isolated from wet areas (Salto et al. 2019b).

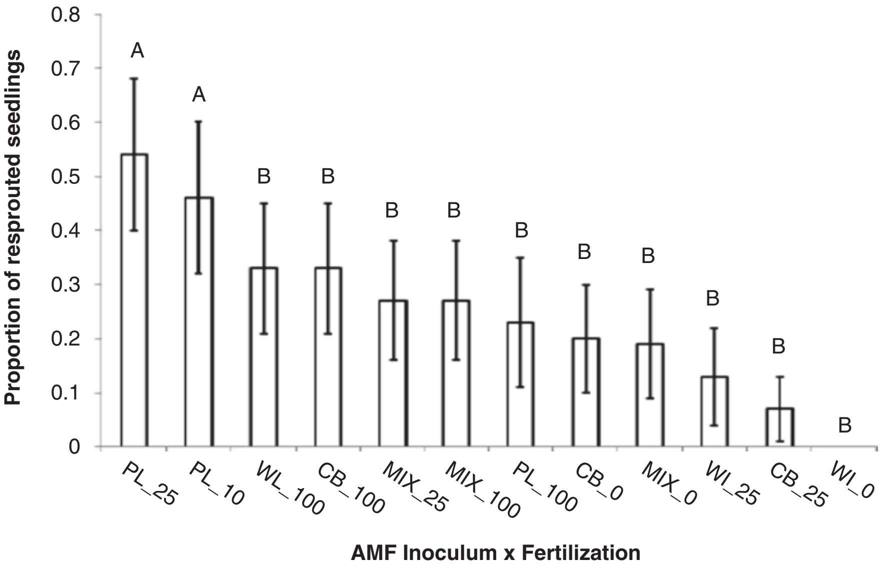

Fig. 10.9 Effect of different fertilization and AMF inoculation regimes on *P. alba* seedlings submitted to drought stress. Resprouting capacity is expressed as the proportion of resprouted plants after 15 days of drought stress. Mean values and their standard errors. Different letters indicate significant differences ($p \leq 0.05$) according to DGC’s test (Salto et al. 2019b). *PL* Padre Lozano inoculum, *CB* Colonia Benitez inoculum, *MIX* mixture of both inocula, *WI* without inoculum, 100 fertilization without dilution, 25 fertilization diluted to 25%, 0 without fertilization

References

Arce P, Balboa O (1991) Seasonality in rooting of *Prosopis chilensis* cuttings and in-vitro micropropagation. For Ecol Manag 40:163–173

Begum N, Qin C, Ahanger M, Raza S, Khan M, Ashraf M et al (2019) Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Front Plant Sci 10:1–15

Bessega C, Pometti C, 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 8:277–288

Bessega C, Pometti C, Miller J, Watts R, Saidman BO, Vilardi JC (2013) New microsatellite loci for *Prosopis alba* and *P. chilensis* (fabaceae). Appl Plant Sci 1:1–4

Bessega C, Pometti C, Ewens M, Saidman B, Vilardi J (2015) Evidences of local adaptation in quantitative traits in *Prosopis alba* (Leguminosae). Genetica 143:31–44

Borralho N (1995) The impact of individual tree mixed models (BLUP) in tree breeding strategies hardwood forestry. In: Proceedings of the CRC-IUFRO conference: Eucalypts plantations: Improving fiber yield and quality, 12–24 February, 1995, Hobart, pp 141–145

Cabrera AL (1976) Regiones Fitogeográficas Argentinas. Enciclopedia argentina de agricultura y jardinería, Tomo 2, fasc. 1. ACME, Buenos Aires

Capello R (2019) Banco de Germoplasma Forestal. Manejo de los Recursos Genéticos Forestales Nativos. Dirección de Recursos Naturales. Gobierno de la Provincia de Formosa

Cappa E, Varona L (2013) An assessor-specific Bayesian multi threshold mixed model for analyzing ordered categorical traits in tree breeding. Tree Genet Genomes 9:1423–1434

Carreras R, Bessega C, López C, Saidman B, Vilardi J (2016) Developing a breeding strategy for multiple trait selection in *Prosopis alba* Griseb., a native forest species of the Chaco Region in Argentina. Forestry 90:199–210

Chávez Díaz L, González P, Rubio E, Melchiorre M (2013) Diversity and stress tolerance in *rhizobia* from Parque Chaqueño region of Argentina nodulating *Prosopis alba*. Biol Fertil Soils 49:1153–1165

Cony M (1996) Genetic potential of *Prosopis* in Argentina for its use in other countries. Semiarid fuelwood and forage tree building consensus for the disenfranchised. Center for Semi-Arid Forest Resources, Texas, pp 6–3. 6–24

De Souza S, Felker P (1986) The influence of stock plant fertilization on tissue concentrations of N, P and carbohydrates and the rooting of *Prosopis alba* cuttings. For Ecol Manag 16:181–190

De Souza J, Bender A, Tivano J, Barroso D, Moroginski L, Vegetti A, Felker P (2014) Rooting of *Prosopis alba* mini-cuttings. New For 45:745–752

Di Rienzo J, Casanoves F, Balzarini M, Gonzalez L, Tablada M, Robledo C (2012) InfoStat versión 2012. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina

Ewens M, Felker P (2003) The potential of mini-grafting for large-scale production of *Prosopis alba* clones. J Arid Environ 55:379–387

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

FAO (2014) Plan de acción mundial para la conservación y la utilización sostenible y el desarrollo de los recursos genéticos forestales p comisión de recursos genéticos para la alimentación y la agricultura, 35 pp

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

Ferreyra L, Vilardi J, Verga A, López V, Saidman B (2013) Genetic and morphometric markers are able to differentiate three morphotypes belonging to section Algarobia of genus *Prosopis* (Leguminosae, Mimosoideae). Plant Syst Evol 299(6):1157–1173

Galera F (2000) Los algarrobos: Las especies del género *Prosopis* (algarrobos) de América Latina con especial énfasis en aquellas de interés económico. Graziani Gráfica, Córdoba, 269 pp

Gómez A, Rossi F, Bravo S (2019) Determinación del momento oportuno de raleo de algarrobo blanco en plantaciones: técnica alternativa para la medición de anillos de crecimiento. En: Avances en el conocimiento y tecnologías productivas de especies arbóreas nativas de Argentina / [Compiladoras]: Carla S. Salto; Ana María Lupi. Ediciones INTA, Buenos Aires, 78 pp

Grattapaglia D, Silva-Junior O, Resende R, Cappa E, Müller B, Tan B, El-Kassaby Y (2018) Quantitative genetics and genomics converge to accelerate forest tree breeding. Front Plant Sci 9:1693. https://doi.org/10.3389/fpls.2018.01693

Joseau J, Verga A, Días MP, Julio N (2013) Morphological diversity of populations of the genus Prosopis in the semiarid Chaco of Northern Cordoba and Southern Santiago del Estero. Am J Plant Sci 4:2092–2111

Kees S, Michela J (2016) Aspectos de la producción primaria y el mercadeo del algarrobo en Chaco, Argentina. Presidencia Roque Sáenz Peña, Argentina

Kees S, Ferrere P, Lupi A, Michela J, Skoko J (2018) Producción y crecimiento de las plantaciones de *Prosopis alba* Griseb. en la provincia Chaco. Revista de Investigaciones Agropecuarias 44:113–120

Kees S, López A, Zurita J, Brest E, Roldan M, Rojas J (2019) Identificación de las características edáficas determinantes de la calidad de sitio para el cultivo de *Prosopis alba*. En: Avances en el conocimiento y tecnologías productivas de especies arbóreas nativas de Argentina / [Compiladoras]: Carla S. Salto; Ana María Lupi. Ediciones INTA, Buenos Aires, 78 pp

Lindgren D, Mullin T (1997) Balancing gain and relatedness in selection. Silvae Genet 46:124–129

López Lauenstein D, Vega C, Luna C, Sagadin M, Melchiorre M, Pozzi E et al (2016) Subprograma *Prosopis*. In: Marcó MA, Llavallol CE (eds) Domesticación y mejoramiento de especies forestales. Unidad para el Cambio Rural. Ministerio de Agroindustrias. Presidencia de la Nación Argentina, Buenos Aires, pp 113–136

López Lauenstein D, Vega C, Verga A, Fornes L, Saravia P, Feyling M et al (2019) Evaluación de diez orígenes de Algarrobo para establecer sistemas silvopastoriles en el Chaco semiárido argentino. In: Proceedings of X Congreso Internacional sobre Sistemas Silvopastoriles. Asunción, 24–26 September, Editorial CIPAV, Cali

MAGyP (2016) Avances en la silvicultura del algarrobo blanco. https://www.agroindustria.gob.ar/sitio/areas/ss_desarrollo_foresto_industrial/biblioteca_forestal/publicaciones/_archivos/000000_Avances%20en%20la%20silvicultura%20del%20algarrobo%20blanco.pdf

Marcucci Poltri S, Gallo L (2016) Herramientas moleculares. In: Marcó MA, Llavallol CE (eds) Domesticación y mejoramiento de especies forestales. Unidad para el Cambio Rural. Ministerio de Agroindustrias. Presidencia de la Nación Argentina, Buenos Aires, p 201

Min. Agro (2015) Informe Nacional del Relevamiento Censal de Aserraderos. Ministerio de Agroindustria. República, Argentina. https://www.agroindustria.gob.ar/sitio/areas/ss_desarrollo_foresto_industrial/censos_inventario/

Moglia G, Giménez A (2006) Resultados preliminares de la arquitectura vegetal de *Prosopis alba* y *Prosopis nigra*. En: II Jornadas Forestales de Santiago del Estero: Forestación y Aprovechamiento Integral del Algarrobo. Santiago del Estero, 5 p

Mottura M, Finkeldey R, Verga A, 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

Namkoong G, Barnes R, Burley J (1980) Philosophy of breeding strategy for tropical forest trees, Tropical Forest Paper N°16. Commonwealth Forestry Institute, Oxford, 67 pp

Oberschelp G, Marcó M (2010) Efecto del ácido 3-indolbutírico sobre el enraizamiento adventicio y la altura de plantines clonales de *Prosopis alba* Grisebach. Quebracho Revista de Ciencias Forestales 18(1–2)

Pomponio MF, Acuña C, Pentreath V, López Lauenstein D, Marcucci Poltri S, Torales S (2015) Characterization of functional ssr markers in *Prosopis alba* and their transferability across *Prosopis* species. For Syst 24:2013–2016

Pozzi Tay E (2016) Simbiosis en *Prosopis alba* como estrategia para la mejora de su tolerancia a estrés hídrico. Graduate thesis, Fac. Cs. Exactas, Físicas y Naturales. Universidad Nacional de Córdoba

Ruotsalainen S, Lindgren D (1998) Predicting genetic gain of backward and forward selection in forest tree breeding. Silvae Genet 47:42–50

Sagadin M, Monteoliva M, Luna C, Cabello M (2018) Diversidad e infectividad de hongos micorrícicos arbusculares nativos provenientes de algarrobales del Parque Chaqueño argentino con caracteríticas edafoclimáticas contrastantes. Agriscientia 35:19–33

Saidman B (1986) Isoenzymatic studies of alcohol dehydrogenase and glutamate oxalacetate transaminase in four South American species of *Prosopis* and their natural hybrids. Silvae Genet 35:3–10

Salto C (2011) Variación genética en progenies de polinización abierta de Prosopis alba griseb. de la región chaqueña. Tesis de Maestría. Facultad de Ciencias Agrarias. Universidad Nacional de Rosario, Argentina, 75 pp

Salto C, Lupi A (2019) Avances en el conocimiento y tecnologías productivas de especies arbóreas nativas de Argentina / [Compiladoras]: Carla S. Salto; Ana María Lupi. Ediciones INTA, Buenos Aires, 78 pp

Salto C, Oberschelp J, Harrand L (2012) Recolección, acondicionamiento y transporte de material vegetal de Prosopis alba Griseb. para propagación vía estacas. En: Reunión Nacional del Algarrobo, 13–14 noviembre 2012, Córdoba, Argentina

Salto C, Harrand L, Oberschelp J, Ewens M (2016) Crecimiento de plantines de *Prosopis alba* en diferentes sustratos, contenedores y condiciones de vivero. Bosque (Valdivia) 37:527–537

Salto C, Harrand L, Oberschelp J, Ewens M (2019a) Efecto del tamaño de envase y calidad del sustrato utilizado sobre la calidad del plantín de *Prosopis alba*. En: Avances en el conocimiento y tecnologías productivas de especies arbóreas nativas de Argentina / [Compiladoras]: Carla S. Salto, Ana María Lupi. Ediciones INTA, Buenos Aires, 78 pp

Salto CS, Sagadin M, Luna C, Oberschelp G, Harrand L, Cabello M (2019b) Interactions between mineral fertilization and arbuscular mycorrhizal fungi improve nursery growth and drought tolerance of *Prosopis alba* seedlings. Agrofor Syst 94:103–111

SAyDS (2019) Anuario de Estadística Forestal de Especies Nativas 2017–2018. https://www.argentina.gob.ar/ambiente/bosques/estadistica-forestal

Spensley J, Sabelli A, Buenfil J (2013) Estudio de Vulnerabilidad e Impacto Del Cambio Climático En El Gran Chaco Americano. Ed. Programa de las Nacionales Unidas para el Medio Ambiente – PNUMA

Suja A, Venkataraman G, Parida A (2007) Identification of stress-induced genes from the drought-tolerant plant *Prosopis juliflora* (Swartz) D. C. Through analysis of expressed sequences tags. Genome 50:470–478

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Torales S, Rivarola M, Pomponio MF, Gonzalez S, Acuña C, Fernández P et al (2013) De novo assembly and characterization of leaf transcriptome for the development of functional molecular markers of the extremophile multipurpose tree species *Prosopis alba*. BMC Genomics 14:705

Velázquez E, Igual J, Willems A, Fernández MP, Muñoz E, Mateos PF et al (2001) Mesorhizobium chacoense sp., a novel species that nodulates *Prosopis alb*a in the Chaco Arido region (Argentina). Int J Syst Evol Microbiol 51:1011–1021

Verga A (1995) Genetic study of *Prosopis chilensis* y *Prosopis flexuosa* (Mimosaceae) in the dry Chaco of Argentina. Doctoral thesis, Abteilung für Forstgenetik und Forstpflanzensüchtung der Universität Göttingen, Alemania, 96 pp

Verga A (2005) Recursos Genéticos, Mejoramiento y Conservación de Especies del Género Prosopis. En: Mejores Árboles Para Más Forestadores: El Programa de Producción de Material de Propagación Mejorado y el Mejoramiento Genético en el Proyecto Forestal de Desarrollo Edición: Carlos A. Norberto. SAGPyA-BIRF

Verga A (2014) Rodales semilleros de *Prosopis* a partir del bosque nativo. Quebracho 19:125–138

Verga A (2015) "Hoja 3.6." Programa de distribución gratuita. Instituto de Fisiología y Recursos Genéticos Vegetales (IFRGV). Instituto Nacional de Tecnología Agropecuaria (INTA). Camino 60 Cuadras, km 5.5 X5020ICA, Córdoba, Argentina. E -mail: verga.anibal@inta.gob.ar

Verga A, Gregorius H (2007) Comparing morphological with genetic distances between populations: a new method and its application to the *Prosopis chilensis – P. flexuosa* Complex. Silvae Genet 56:45–51

Verga A, López Lauenstein D, López C, Navall M, Joseau J, Gómez C et al (2009) Caracterización morfológica de los algarrobos (*Prosopis* sp.) en las regiones fitogeográficas Chaqueña y Espinal norte de Argentina. Quebracho 17:31–40

Versalovic J, Schneider M, de Bruijn F, Lupski J (1994) Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods Mol Cell Biol 5:25–40

Verzino G, Joseau MJ (eds) (2005) El Banco Nacional de Germoplasma de *Prosopis*. Conservación de recursos forestales nativos en Argentina, 172 pp

Verzino G, Frassoni J, Joseau M, Clausen G, Navarro C (2020) Conservación ex situ, circa situ e in situ realizada por el Banco Nacional de Germoplasma de *Prosopis*, Córdoba, Argentina. Revista Nexo (NA-V7N1-9) (in press)

Vincent J (1970) A manual for the practical study of the root-nodule bacteria. Blackwell, Oxford

Wendling I, Trueman S, Xavier A (2014) Maturation and related aspects in clonal forestry. Part II: Reinvigoration, rejuvenation and juvenility maintenance. New For 45:473–486

Wojtusik T, Felker P (1993) Interspecific graft incompatibility in *Prosopis*. For Ecol Manag 59:329–340

Zamioudis C, Pieterse C (2012) Modulation of host immunity by beneficial microbes. Mol Plant Microbe Intract 25:139–150

Zárate M, Gyenge J, Gómez A (2019) ¿Cuál es el efecto de la poda y la densidad de plantación en el crecimiento de los algarrobos? In: Avances en el conocimiento y tecnologías productivas de especies arbóreas nativas de Argentina / [Compiladoras]: Carla S. Salto; Ana María Lupi. Ediciones INTA, Buenos Aires, 78 pp

Zobel B, Talbert J (1984) Applied forest tree improvement. Wiley, New York, 505 pp