Chapter 18
Contribution to the Domestication and Conservation of the Genetic Diversity of Two Native Multipurpose Species in the Yabotí Biosphere Reserve, Misiones, Argentina

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Abstract In this chapter we describe a strategy for domestication and conservation of the genetic variability of two native species of the Interior Atlantic Forest, or Selva Paranaense, *Peltophorum dubium* and *Enterolobium contortosiliquum*. Both species are leguminous trees, commonly used for forest restoration projects. In tropical and subtropical forests, tree species are vulnerable to habitat fragmentation and population reductions. The resulting negative genetic effects, such as loss of genetic variability and inbreeding depression, can affect the long-term survival of forest species, leading to their further vulnerability or extinction. While *in situ* strategies such as protected areas and biodiversity islands may be an option for biodiversity conservation, in practice there are many challenges to these strategies in humid

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subtropical ecosystems, where there is high tree species diversity and low abundance per species (0.1–1 trees/ha). Trees in remnants of forests, which can serve as biodiversity islands, must have high genetic diversity so that they can persist through time by adapting to disturbances. There is a great need to expand the genetic basis of collection of species' propagation material, so that the germplasm available for restoration programs includes the largest possible genetic diversity. To address this need, we established short- and long-term provenance and progeny trials and a vegetative reproduction methodology to produce propagation material to ensure the genetic diversity of these two species for domestication, including for restoration and enrichment. Our results lay the foundation for the conservation of genetic variability of *P. dubium* and *E. contortosiliquum* and contribute to the design of a possible biodiversity island strategy for these species.

Keywords Conservation strategy · Forest restoration · Genetic variability · Interior Atlantic forests · Native tree species · Selva paranaense · Vegetative propagation techniques

18.1 Introduction

The Yabotí Biosphere Reserve, with a total of 235,900 hectares, is located in the province of Misiones, Argentina and is part of the Atlantic Forest. The Atlantic Forest extends over the Atlantic coast of Brazil and is one of the most diverse ecosystems in the world, with about 15,000 species of vascular plants, of which at least 48% are endemic (Myers et al. 2000; Martini et al. 2007; Murray-Smith et al. 2009). The Atlantic Forest extends into the interior of Brazil and into the province of Misiones in the northeast of Argentina and eastern Paraguay, where it is known as Interior Atlantic Forest or *Selva Paranaense* (Paranaense forest) or *Selva Misionera.* The Interior Atlantic Forests, together with the northwest montane forest of the country known as the *Yungas* (mostly in the provinces of Salta, Jujuy and Tucumán), represent the most biodiverse regions of Argentina. The Atlantic Forest was included as one of the original biodiversity hotspots or critical zones in the widely cited publication of Ernst Mayr in 1988, along with 10 other regions of the globe, which have now been extended to 36 regions worldwide. Designation as a hotspot region should meet two strict criteria: a) it must contain at least 1500 endemic species of vascular plants (0.5% of the total vascular plants identified on Earth); and b) it must have experienced habitat loss of at least 70% of its original area. More than 50% of the world's plant species are endemic to the 36 biodiversity critical areas mentioned above and once covered 15.7% of the Earth's surface. Now, they are reduced to 2.3% of the Earth's surface and include many endemic species, which face a growing threat of extinction (Reed et al. 2011).

The growing population, the advance of the livestock agricultural frontier, and the extraction of timber trees have been the most frequent causes of the drastic decrease in surface area and consequent vulnerability of the 36 critical areas or hotspots. Given this situation, and as part of the commitments made on a global scale at the biodiversity convention in 1992 in Rio de Janeiro, the Global Strategy for Plant Conservation (GSPC 2010) agreement was signed in 2002 by more than 180 countries, with the aim of developing actionable policies to support plant conservation. GSPC Objective 8 requires that "at least 75% of threatened plant species are in *ex situ* collections and at least 20% available for recovery and restoration programs", as outlined by the United Nations Development Program (Reed et al. 2011).

The recognition of islands of biodiversity provides an important step for restoration and can contribute to reversing fragmentation and increasing biodiversity around the world (Montagnini et al. 2022). Several authors, such as Ratnam et al. (2014) and Thomas et al. (2014), have suggested strategies to increase resilience in forest restoration initiatives. Such measures include increasing tree population size and genetic diversity, maintaining forest cover in the landscape for genetic and geographical connectivity between tree populations, promoting genetic improvement of tree species, and enhancing the identification and protection of evolutionary shelters (Bhagwat et al. 2012; Pauls et al. 2013).

In tropical and subtropical forests, tree species are vulnerable to habitat fragmentation and population reductions. The resulting negative genetic effects, such as loss of genetic variability and inbreeding depression, affect the long-term survival of forest species, leading to their further vulnerability or extinction (Maina and Howe 2000). While *in situ* strategies such as protected areas and biodiversity islands may be an option for biodiversity conservation, in practice there are many challenges with these strategies in humid subtropical ecosystems, where there is high tree species diversity and low abundance (0.1–1 trees/ha of the same species).

In studies comparing fruits of the species *Enterolobium cyclocarpum* obtained from trees located in continuous forests and from isolated trees from pasture grazing areas, Rocha and Aguilar (2001) infer that habitat fragmentation alters the mechanisms through which plants regulate the quality of their offspring by increasing self-pollination rates in isolated trees and producing less fruit, which are then usually abortive. For this reason, isolated trees produce progeny that is less vigorous than the progeny of mother trees in continuous forests, which is another adverse effect of forest fragmentation. Therefore, intraspecific genetic variability is directly related to the resilience of the ecosystem and its ability to cope with abrupt changes in the climate and its habitat to ensure its long-term survival.

18.1.1 Importance of Genetic Diversity in Restoration and Conservation Programs

Genetic diversity is the basis of an organism's ability to adapt to changes in its environment through natural selection. Populations with little genetic variation are more vulnerable to the emergence of new pests or diseases, pollution, climate change, and habitat destruction due to human activities or other catastrophic events. The inability to adapt to changing conditions greatly increases the risk of extinction.

Reed and Frankham (2003) found that the correlation between genetic diversity at the population level and the adaptability of the population to changing environmental conditions was highly significant, concluding that the loss of heterozygosity[1] has a strong effect on the adaptability of the population. This is consistent with the provisions of the International Union of Conservation of Nature (IUCN) on the status of importance of the conservation of genetic diversity (McNeely et al. 1990). Genetic diversity is one of the three major components of biodiversity, but is still overlooked in most plans for conserving biodiversity. Therefore, the implementation of genetic criteria into the Red List assessment process will help to define more precisely the conservation status of the species (Bruford and Segelbacher 2018).

Meanwhile, there is also a commitment from the international community to restore hundreds of millions of hectares of degraded forest landscapes, following the Strategic Plan for Biodiversity 2011–2020, including the Aichi Biodiversity Targets (CBD 2018). However, the successes and failures of past restoration efforts remain in many cases undocumented and uncommunicated. Case studies show that failures may have been much more common than successes (Wuethrich 2007; Godefroid et al. 2011). The causes of restoration failures can be multiple. One reason, often ignored, is the inadequate consideration of the source and genetic quality of forest reproductive material (Godefroid et al. 2011; Le et al. 2012).

The impact of logging on the structure of forest species populations depends to a large extent on the degree of disruption and intensity of logging. The threat to genetic diversity posed by commercial logging correlates with the abundance of a species in each forest management unit. Natural regeneration, while allowing the transmission of genetic information to the next generation, does not guarantee adaptive and non-adaptive change of structures during the regeneration phase (Rajora and Pluhar 2003). When the number of seed trees left in the logged forest is low and dysgenic selection is heavily practiced according to the market demands, the tree phenotype left in the remaining population is skewed, leading to a gradual process of genetic erosion of the entire population. Without genetic diversity, evolution is impossible, and adaptation decreases, which can result in local extinctions. Processes such as natural selection, genetic drift,[2] and genetic flow[3] collectively affect the genetic diversity of populations and either promote or hinder local and wide-ranging adaptation.

Particularly in tropical and subtropical regions, the genetic diversity of tree species is a key component of the functioning of forest ecosystems (Ratnam et al. 2014). In tropical species, such as for example *Dipteryx odorata* (hermaphroditic and pollinated by insects), and *Bagassa guianensis* (dioecious and mainly wind-pollinated), studies of the impact of forest management have shown that selective

[1] The presence of different alleles at one or more loci are not the same (Acquaah 2012).

[2] The random fluctuations of gene frequencies in a population such that the genes amongst offspring are not a perfectly representative sampling of the parental genes (Schlegel 2010).

[3] Introduction of genetic material from one population of species to another, thereby, changing the composition of the gene pool of the receiving population (White et al. 2007).

logging induced an asynchrony in flowering, limited the flow of genes, and induced inbreeding, even in species that were cross-pollinated. In this manner, the regeneration of their offspring is affected with serious consequences, especially for tree species managed by selective logging. This is especially true in situations where the remaining forests have only few trees of reproductive age and lack pollinators, which contribute to the flow of genes (Ratnam et al. 2014).

18.1.2 Genetic Considerations for Restoration Programs for the Interior Atlantic Forest

The high intensity of some methods of logging, as mentioned above, can modify breeding patterns in residual trees and result in increasingly inbred seeds through self-fertilization or crossbreeding between closely related individuals (biparental inbreeding), compromising the role of a tree population as a source of seeds. The risk of inbreeding must be seriously considered in activities dealing with genetic resources, use of germplasm in practical forestry and tree improvement. In such cases, for restoration and/or enrichment programs, the use of germplasm obtained from similar ecological conditions, even if they are not from local sources, may be a better option than resorting to fragmented nearby forests or isolated trees (Navarro Pereyra 2002; Thomas et al. 2014).

Various general guidelines for seed collection that ensure a minimum of genetic diversity for restoration programs have been published, e.g., works by The Australian Network for Plant Conservation Inc. (Vallee et al. 2004), the University of California (Rogers and Montalvo 2004), the World Agroforestry Centre (ICRAF) (Kindt et al. 2006), and the Royal Botanic Gardens, Kew (2003), among others. It is essential that the collection of germplasm captures a representative sample of the genetic diversity of species to be used in restoration projects in a way that takes into consideration the natural distribution of the species as much as possible. A set of general guidelines for tree seed harvesting are intended to ensure a minimum level of genetic diversity. For example, as a general rule, a minimum of 30 randomly selected trees must be sampled for a completely cross-pollinated species (Rogers and Montalvo 2004).

In the province of Misiones, Argentina, the extraction of wood from forests and the harvesting and planting of yerba mate (*Ilex paraguariensis* A. St -Hil) began at the end of the nineteenth century. Forestry activities (mostly plantations of fast-growing exotic species such as *Pinus* spp) were consolidated by the end of the twentieth century (Ferrero 2005), along with plantations of tea, tobacco, and yerba mate monocultures, and more recently livestock production. In the area of study (Yabotí Biosphere Reserve) historically the selective cutting of timber species was done using low impact management practices, such as the Minimum Cutting Diameters (MCDs) that were established by provincial legislation.

The selective cutting method following the MCD requirement for forest harvesting is widely used in tropical and subtropical ecosystems. However, in many cases, this type of management is suited to industrial processing technologies and market demands, while not considering the reproductive biology of the individual trees, and the appropriate number of remaining seedlings, among other factors, to ensure the persistence in perpetuity of the harvested species. Therefore, a management program focused exclusively on the MCD does not result in ecologically sustainable forest management in the long term (Montagnini et al. 1998; Putz et al. 2000; Jennings et al. 2001; Sist et al. 2003).

Among the species that are vulnerable and/or threatened by excessive habitat fragmentation and extraction in the Interior Atlantic Forest, *Peltophorum dubium* (Spreng.) Taub and *Enterolobium contortosiliquum* (Vell.) Morong, are two important species that have been long used in restoration projects. These species are both hermaphrodites and pollinated by insects. Mori et al. (2013), who studied the reproductive system in natural populations of *P. dubium,* observed that the crossings were not random, and that the species was not self-incompatible, but presented a typical system of mixed mating, combining crosses with self-fertilization. Inbreeding was also detected in both the parental generation and in its offspring, although they observed evidence for natural selection between the juvenile and adult phase against inbred individuals.

There is a great need to expand the genetic basis of species propagation material, so that the germplasm available for restoration programs includes the largest possible genetic diversity. In this chapter, we propose a strategy for domestication and conservation of the genetic variability of two native species, *Peltophorum dubium* and *Entorolobium contortosiliquum*, to provide germplasm for enrichment and/or restoration projects of native forests. Our methods could also be used for other species, to produce propagation material with genetic diversity for domestication, enrichment, and/or restoration programs. This work in progress is expected to contribute to a strategy for establishing or enriching biodiversity islands.

18.2 Case Study: Provenance and Progeny Trials for the Domestication and Conservation of Genetic Diversity of *Peltophorum dubium* (Caña Fístola) and *Enterolobium contortosiliquum* (Timbó) in the Yabotí Biosphere Reserve, Misiones, Argentina

The species, provenance, and progeny trials aim to ascertain genetic variation at different levels: between species, between geographical regions, between stands and between individuals, and they are the first logical step of genetic improvement for any species (White et al. 2007). Therefore, as a case study, we proposed to assess and evaluate the genetic resources of two native species of the Interior Atlantic Forests, *P. dubium* and *E. contortosiliquum,* as a source of germplasm for enrichment and/or restoration projects of native forests. The species, *P. dubium* and *E. contortosiliquum,* both belonging to the legume family, are of great interest to the local forest industry due to the quality of their wood, which has led to high levels of extraction and in turn, genetic erosion. Currently, there is also an increasing interest in planting them in small- and medium-sized farms, in compliance with provincial regulations. These species can facilitate the production of honey, have good properties for restoration of degraded areas, can be used in agroforestry or silvicultural consociated systems, and have dendro-energy potential. Local towns have high demand for these species for use as ornamentals in landscaping projects in urban areas, since they are representative of the Misiones Forest (Eibl et al. 1998; Barth et al. 2008; Alexandre et al. 2009; Eibl et al. 2017). As seen, local communities consider both as multipurpose species.

Initial field trials to assess their behavior under different silvicultural conditions, have identified *P. dubium* as a heliophyte species, with rapid initial growth and good behavior in open monoculture plantations. In the west Chaco, field trials have shown that *P. dubium* can reach 45 cm in diameter at 20 years of age (Gómez and Cardozo 2003). In Misiones, plantation trials of *E. contortisiliquum* have shown that this species can reach 25 cm in diameter at 14 years of age (Montagnini et al. 2006), demonstrating fast growth even when genetic material with little or no domestication was used. Similar results have been obtained in forest enrichment experiments carried out under canopy of native forest San Pedro, Misiones (Eibl et al. 1993) and in San Ignacio in plantations under the canopy of *Pinus* spp. stands (Crechi et al. 2016).

The main goal of the present case study was to develop a working strategy (Fig. 18.1) for domestication and conservation of genetic variability of two native species of the Interior Atlantic Forests, *P. dubium* and *E. contortosiliquum,* which contemplated the following activities (short, medium and long term): 1) selection and marking of seed trees using the mother seed tree method (White et al. 2007) covering the ranges of both species in Argentina, and subsequent seed harvest from these mother trees; 2) study of morphometric characteristics of fruits and seeds of provenance and progeny of *P. dubium* and *E. contortosiliquum*; 3) establishment of provenance and progeny trials of *P. dubium* and *E. contortosiliquum* in the greenhouse (short-term) and in the field (long-term) to analyze the genetic diversity of their adaptive characteristics; and 4) development of mini-clonal gardens of provenance and progeny of *P. dubium* and *E. contortosiliquum* for the conservation and propagation of propagules with genetic diversity, for domestication, enrichment and/or restoration projects.

Below, we describe each of the activities[4] that were carried out.

[4]The studies developed in the activities described in this chapter were carried out using appropriate statistical designs and analyses, which will be described in future publications.

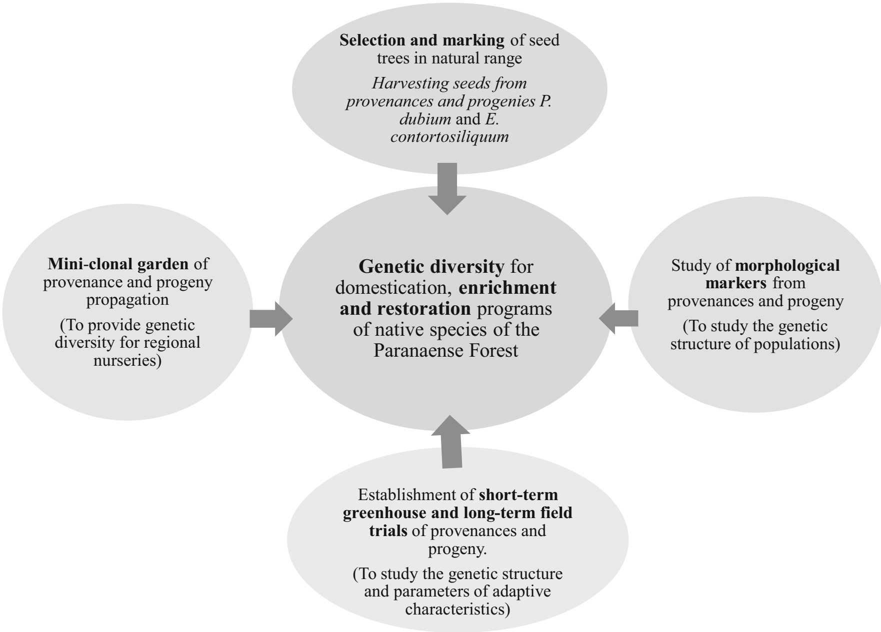

Fig. 18.1 Proposed strategy for the domestication and conservation of genetic diversity of *P. dubium* (Caña fístola) and *E. contortosiliquum* (Timbó) in the Yabotí Biosphere Reserve, Misiones, Argentina (Own source)

18.2.1 Selection and Marking of Seed Trees and Harvest of Seeds from Provenances and Progenies of P. dubium *and* E. contortosiliquum

A survey of seed trees, and seed harvests were carried out in much of the natural distribution range of these species in Argentina. Seeds were harvested from individuals in native populations of the northwestern and northeastern region of Argentina (latitudes 23° 28′ to 32° 08´ South, and longitude 54° 30′ to 65° 18´ West), over an area of 1,025,000 km^2. We were able to harvest seeds from 54 trees of *E. contortosiliquum* and 35 trees of *P. dubium* from the provinces of Misiones, Corrientes, Entre Rios, Formosa, Tucuman, Salta, and Jujuy.

The information survey in the field and subsequent processing of the data were carried out as follows: 1- Identification and contact with owners who possessed isolated individuals or remnant forest with the species under study. 2- Visit to the property to be able to identify, mark and take the following data from the seed trees: diameter at breast height (DBH) (cm), shaft height (meters), total height (meters), treetop diameter (meters), phenological state, sanitary state, environmental variables and other observations; photograph of the selected tree; information on roads to

Fig. 18.2 Seed and fruits of one progeny of *P. dubium.* (Photo: Y. López)

reach the site and location coordinate data with GPS. The information was processed using a Geographic Information System (GIS).

18.2.2 Study of Morphometry of Fruits and Seeds, from the Provenances and Progenies of P. dubium *and* E. contortosiliquum

A minimum of 2000 seeds per tree were harvested from each geo-referenced tree. The fruit and seeds morphometric variables of weight, width, length, number of seeds per fruit, number of seeds per kg of fruits, and number of fruits per kg were measured for *P. dubium,* from the material collected, as described in point 2.1.1 (Fig. 18.2).

Seeds of *P. dubium* and *E. contortosiliquum* (Fig. 18.3) were also used to study the germination power, seedling morphometry and survival in the nursery under controlled conditions of temperature and humidity, and they were also used for the field progeny trials. The collection sites, i.e. the provinces, are the *provenances* and the progeny from each tree registered in this study, are *progeny groups*. The morphometric characteristics of fruits and seeds, as well as germination and seedling growth, were evaluated and measured in the Vegetative Propagation Laboratory of the Faculty of Forestry Sciences (UNaM), in Eldorado (Misiones).

Fig. 18.3 Seed and fruits of one progeny of *E. contortosiliquum.* (Photo: Y. Lopez)

18.2.3 Establishment of Short-Term Greenhouse, Long-Term Under-Canopy Enrichment Field Trials, and Mini Clonal Gardens of P. dubium *and* E. contortosiliquum

18.2.3.1 Short-Term Greenhouse Trials

To perform germination power tests, the seeds of each provenance and progeny of each species were scarified with sandpaper (150 granulometry) and disinfected with sodium hypochlorite (NaClO) at 50% (v/v) for 15 minutes, with 4 subsequent rinses with distilled water. The seeds were germinated in 90 cm^3 HIKO™ trays, in winter with semi-controlled conditions of humidity (micro spray irrigation) and temperature (between 20 and 30 °C). The substrate used was composted pine bark, with application of Plantacote Plus slow release fertilizer™ (3 kg/m^3).

From each offspring, seed batches of between 25 to 40 seeds were taken, and germinations counts were made after 4, 6, 10, 24, 25 and 31 days. Within 10 days of planting the germination and emergency stabilized, therefore this was considered the period to assess the germination capacity. Seedling morphometry and survival were assessed at 60 and 120 days. Seedling height (cm), neck diameter (ND, mm), and number of internodes were measured.

18.2.3.2 Long-Term Under Canopy Enrichment Field Trial of *P. dubium* and *E. contortosiliquum*

For the establishment of the under-canopy long-term field trial of provenances and progenies of *P. dubium* and *E. contortosiliquum,* the site selected is in the Municipality of El Soberbio, Province of Misiones, in the Yabotí Biosphere Reserve (latitude 27º08’32′‘South and longitude 53º58’57′‘West), belonging to the Company El Moconá SA and managed by Puerto Laharrague SA.

The site has a subtropical climate that corresponds to a Cfa classification according to Köppen (1936), without a dry season, with average temperatures ranging from 12 to 31 °C and with more than 2000 mm of annual precipitation. Soils are deep, red Ultisols classified as Kandiudults. For the preparation of the site, the strips were opened mechanically and manually, ranging from 3 to 3.5 m wide and 90 to 190 m long, with 20 m of undisturbed area between strips.

Seedling establishment was done in a completely randomized block design with 20 replicate blocks. For each 3 m-wide enrichment blocks on the opened strips of forest, the distance between blocks was 20 m and the seedlings were set in rows with 3 m between seedlings (Fig. 18.4). The following characteristics were evaluated:

Fig. 18.4 Long term field trial establishment in enrichment blocks of *P. dubium.* (Photo: P. Thalmayr)

survival at the first, second and third year, diameter at breast height (DBH), total height, and trunk shape. The morpho-physiological variables (leaf size, stomatal density, photosynthesis, transpiration, phenology), will be evaluated at the fourth, seventh year and at half of the rotation age. The field experiments are being maintained up to 3 years post-establishment, with periodic control of weeds and ants, and replacement of failed seedlings.

18.2.3.3 Mini-Clonal Gardens of Provenances and Progenies of *P. dubium* and *E. contortosiliquum*

To ensure the long-term conservation and production of provenances and progenies of *P. dubium,* a mini-clonal garden was established, using seedlings produced for the short-term greenhouse experiment, as described above. The methodology described by Niella et al. (2013, 2014, 2016a, b) was used for the mini-stumps management and rooting of mini-cuttings (Figs. 18.5 and 18.6).

Fig. 18.5 Greenhouse mini-stumps garden (*P. dubium*). (Photo: F. Niella)

Fig. 18.6 Rooted mini-cutting (*P. dubium*). (Photo: F. Niella)

18.3 Results and Discussion

18.3.1 Morphometry of Fruits and Seeds from Provenances and Progenies of P. dubium *and* E. contortosiliquum

For *P. dubium,* the morphometric variables of weight, width, length, number of seeds per fruit, number of seeds per kg of fruits, and number of fruits per kg showed significant differences among provenances and progenies (Table 18.1, Figs. 18.2 and 18.3). These data agree with the results of a study of morphological variation of fruits and seeds of *Prunus nepaulensis* Steud. in Meghalaya, India (Shankar and Synrem 2012). The weight of the fruit showed the greatest variation (37.94%) among provenances. Positive correlations were found between fruit weight and length, and lower correlations among weight, fruit width, and number of seeds per fruit (data not shown).

Significant differences among provenances and progenies of *P. dubium* were observed for all seed morphometric variables (Table 18.2). Of these, seed length varied the most. Seed weight was positively correlated with germination percentage.

In this study, the association between the size of the *P. dubium* seeds, expressed in terms of length and width, with the size of the seedlings with 4 months of growth was verified. These data agree with other studies that demonstrate a correlation between seed size with seedling size, verifying that larger seeds produce more

Table 18.1 Means of seed morphometric variables for *P. dubium* provenance and progenies

Provenances	Progenies	REP	PF (g)	AF (mm)	LF (mm)	N° SEM	Sem kg^{-1}	Fruits kg^{-1}
MISIONES	CF31	100	0.23a	15.70b	71.72bc	1.45bcde	6.21 h	4.76i
MISIONES	CF23	100	0.20abcd	14.37 cd	80.51a	1.56bcd	7.40fgh	5.27ghi
MISIONES	CF32	100	0.20bcde	14.87de	68.84cde	1.42cdef	7.12fgh	5.65fghi
MISIONES	CF25	100	0.17efg	17.28a	66.53def	1.21efg	7.81efgh	6.40 cdfg
FORMOSA	CF22	100	0.22ab	13.09 fg	67.75cdef	1.70ab	7.84efgh	5.13hi
FORMOSA	CF21	100	0.19bcde	14.16 cd	65.11efg	1.60abcd	9.62 cd	6.18cdefg
FORMOSA	CF24	100	0.16fgh	12.72 g	64.18 fg	9.91bc	9.91bc	7.46ab
FORMOSA	CF20	100	0.15gh	12.84 g	64.62efg	11.39ab	11.39ab	7.08abc
FORMOSA	CF30	100	0.14 h	14.66c	70.58bcd	1,16 g	8.05defg	7.73a
JUJUY	CF34	100	0.21abc	15.88b	67.84cdef	1.67cbc	7.71efgh	5.27ghi
JUJUY	CF17	100	0.18cdef	15.55b	74.38b	1.55abcd	8.52cdef	5.85efgh
TUCUMÁN	CF28	100	0.16fgh	13.56ef	71.01bc	8.77cdef	8.76 cdef	6.72bcde
TUCUMÁN	CF33	100	0.17efg	14.08cde	70.38bcd	1.8a	11.68a	6.18cdefg
SALTA	CF27	100	0.18cdefg	15.88b	67.71cdef	1.17 fg	6.77gh	5.97defgh
CORRIENTES	CF29	100	0.17defg	13.15 fg	61.76 g	1.37 cdefg	9.24 cde	6.85abcd

Adapted from Tuzinkievicz (2019)
REP (replications). Means with a common letter are not significantly different ($p > 0.05$). Test: Tukey Alpha-0.05. PF (g) (fruit weight in grams), AF (fruit width in mm), LF (fruit length in mm), No. SEM (number of seeds per fruit), Sem kg^{-1} (seeds per kg) and fruits kg^{-1} (fruits per kg)

Table 18.2 Means of seed morphometric variables for *P. dubium* provenance and progenies

Provenance	Progenies	REP	PS 1000 (g)	Sem kg^{-1}	AS (mm)	LS (mm)
MISIONES	CF31	100	60.20a	16.79i	4.49bc	10.16a
MISIONES	CF23	100	51.40 cd	20.06fgh	3.98ef	9.48bc
MISIONES	CF32	100	55.71b	1824hi	4.06def	9.20 cd
MISIONES	CF25	100	54.21bc	19.11ghi	4.04def	8.77ef
FORMOSA	CF22	100	51.22bcd	19.42fghi	4.52ab	9.30bc
FORMOSA	CF21	100	50.46cde	20.49fgh	4.76a	8.37 fg
FORMOSA	CF24	100	49.93de	20.27fgh	4.24 cd	8.77ef
FORMOSA	CF20	100	33.14j	32.30a	3.56 h	8.50efg
FORMOSA	CF30	100	35.44ij	29.35b	3.85 fg	8.87de
JUJUY	CF34	100	46.58ef	21.89ef	4.02def	9.29bcd
JUJUY	CF17	100	40.99gh	24.76 cd	4.02def	8.13 g
TUCUMÁN	CF28	100	48.33def	21.68efg	3.88f	9.68b
TUCUMÁN	CF33	100	37.85hi	27.27bc	4.18de	9.61bc
SALTA	CF27	100	41.27gh	24.95 cd	4.15de	8.76ef
CORRIENTES	CF29	100	44.43 fg	23.16de	3.60gh	9.24 cd

Adapted from Tuzinkievicz (2019)
PS 1000 (g) (weight of 1000 seeds in grams). Sem kg^{-1} (seeds per kg). AS (mm) (seed width in mm). LS (mm) (seed length in mm). REP (number of replications). Means with a common letter are not significantly different ($p > 0.05$). Test: Tukey Alpha-0.05

vigorous seedlings, and with better survival. (Frazao et al. 1985; Haig and Westoby 1991; Pastorino and Gallo 2000; Ursulino Alves et al. 2005).

For the species *E. contortosiliquum*, the studies of fruit and seed morphometry are still in process.

18.3.2 *Short-Term Greenhouse, Long-Term Under Canopy Trials, and Mini Clonal Gardens of Provenances and Progenies of* P. dubium *and* E. contortosiliquum

18.3.2.1 Short-Term Greenhouse Trials

For *P. dubium* the results of germination capacity tests stabilized within 10 days of planting. Seeds from the Jujuy and Entre Ríos provinces had the highest germination rates. The greatest variation in germination was recorded for seeds from Misiones and the smallest for seeds from Jujuy. Germination power and survival rate presented high coefficients of variation both within and between progenies from different provenances, except progenies from Jujuy (Tuzinkievicz 2019). Seed weight was positively correlated with germination rate. The emergence speed in this study was an average of 6.67 AD (average days), compared to the results observed by Espínola Areco and Rodriguez Espínola (2010), where the *P. dubium* seeds registered an emergency speed of 6.85 AD.

At 60 to 120 days of growth, *P. dubium* seedlings showed significant differences among provenances and progenies for seedling height (HT cm) (P value: 0.001) and root collar diameter (RCD mm) (P-value: 0.001). Progenies CF10 and CF6 from the Corrientes provenance had the highest mean heights at 60 and 120 days of nursery growth. Progenies CF13 and CF15 from the Misiones provenance had the lowest mean heights at 60 and 120 days of nursery growth. The Misiones provenance had both the minimum and maximum mean RCD, with progenies CF26 having the maximum RCD at 60 days and CF31 at 120 days, while CF13 showed the minimum RDC at 60 and 120 days of growth. Overall, positive correlations were found between seedling height at 120 days and seed width and length, and between fruit width and seedling height of seedlings at 60 days, suggesting that the larger the fruits and seeds, the larger the seedlings.

While there were no significant differences in seedling survival among provenances, there were significant differences in survival among progenies (Table 18.3). Nevertheless, Misiones presented the highest number of progenies mortality. Survival was negatively correlated with seedling size at 120 days of growth, suggesting that as the plants grew, the survival in the nursery decreased if the seedling density in the tray was high. According to Villagra (2012), *P. dubium* seedlings are characterized by a high light requirement and fast growth. Therefore, since the tray was not thinned, competition could have been one of the problems. This also agrees with work on the evaluation of containers for the production of *Eucalyptus globulus* Labill, in which they concluded that in some cases, such as those obtained for *P. dubium*, the greenhouse low survival rate could be explained by the accelerated growth of the seedlings, which suppress the growth of other individual seedlings (Molina et al. 1992).

Significant differences were found among progeny for germination and seedling growth of *E. contortosiliquum*, with seedling survival per progeny ranging from 0 to 77% at 60 days of growth (Table 18.4). Preliminary results showed that on the third day after planting, more than 50% of progeny had at least one seedling germinated, and that the average germination rate was 23.3% after 120 days of growth (Buchweis 2019). The germination rate in general was low for the species (23%), which could be attributed to the fact that many of the seed trees from which the seeds were collected were isolated. This is in accordance with findings by Rocha and Aguilar (2001), who studied the reproductive biology of *E. cyclocarpum*, a species of the same genus, and concluded that trees that grow in continuous forests were almost six times more likely to produce more seeds than those from isolated trees. Moreover, the seeds from the continuous forests are more vigorous than those of trees that grow isolated, probably due to increasing self-pollination rates in isolated trees, thereby producing less fruit, which are usually abortive.

Significant differences among progenies were found, both for seedling height and RCD. The Misiones province had the progenies with the highest mean seedling heights and RCDs followed by progenies from the Corrientes and Tucumán provinces. The overall mean seedling height was 37.3 cm, mean RCD was 5.48 mm, and mean survival was 44.85% after 120 days of growth (Buchweis 2019).

Table 18.3 Mean greenhouse (%SUVGH) and field (%SUVFIELD) survival capacity at 12 months for *P. dubium* provenances and progenies

Provenances	Progenies	%SUVGH	%SUVFIELD
MISIONES	CF 12	30ab	80ab
MISIONES	CF13	0b	NA
MISIONES	CF14	0b	NA
MISIONES	CF15	0b	100a
MISIONES	CF16	0b	NA
MISIONES	CF 18	57.5ab	90ab
MISIONES	CF 19	7.5b	100a
MISIONES	CF 23	42.5ab	71ab
MISIONES	CF 25	60ab	75ab
MISIONES	CF 26	45.7ab	81ab
MISIONES	CF 31	65ab	86ab
MISIONES	CF 32	50ab	86ab
MISIONES	CF 35	40ab	75ab
CORRIENTES	CF 6	50ab	80ab
CORRIENTES	CF 7	63ab	85ab
CORRIENTES	CF8	0b	100a
CORRIENTES	CF 9	11.4b	82ab
CORRIENTES	CF 10	66.7ab	90ab
CORRIENTES	CF 11	5ab	100a
CORRIENTES	CF 29	30ab	80ab
ENTRE RIOS	CF 1	14.3b	92ab
ENTRE RIOS	CF 2	16b	50b
ENTRE RIOS	CF 3	65ab	85ab
ENTRE RIOS	CF 4	80a	63ab
ENTRE RIOS	CF 5	62.5ab	81ab
FORMOSA	CF 20	15b	69ab
FORMOSA	CF 21	14b	80ab
FORMOSA	CF 22	60ab	80ab
FORMOSA	CF24	0b	100a
FORMOSA	CF 30	5b	100a
JUJUY	CF 17	42.5ab	40b
JUJUY	CF 34	55ab	60ab
TUCUMÁN	CF 28	12.5b	65ab
TUCUMÁN	CF 33	62.5ab	45b
SALTA	CF 27	65ab	62ab

Means with a common letter are not significantly different ($p > 0.05$). Test: Tukey Alpha-0.05

18.3.2.2 Long-Term Under Canopy Enrichment Field Trials

One year after establishment of the field trial, the survival rates of *P. dubium* progenies differed significantly, with a mean of 79% (Table 18.3, Fig. 18.4). Progenies of *E. contortosiliquum* showed no significant differences in field survival

Table 18.4 Mean greenhouse (%SUVGH) and field (%SUVFIELD) survival capacity at 12 months for *E. contortisiliquum* provenances and progenies

Provenance	Progenies	%SUVGH	%SUVFIELD
Misiones	TB1	45	89ab
Misiones	TB2	22.5b	NA
Misiones	T28	75a	80ab
Misiones	T29	77.5a	70bc
Misiones	T30	55ab	70bc
Misiones	T31	10b	NA
Misiones	T32	15b	NA
Misiones	TB8	7.5b	NA
Corrientes	TB3	0	NA
Corrientes	TB4	0	NA
Corrientes	TB5	35ab	78ab
Corrientes	TB6	5	NA
Corrientes	TB7	0	NA
Tucumán	TB9	52.5ab	67bc
Tucumán	TB10	25ab	78ab
Tucumán	TB11	32.5ab	NA
Tucumán	TB12	17.5b	NA
Tucumán	TB13	0	NA
Tucumán	TB14	0	NA
Tucumán	T15	62.5ab	100ab
Tucumán	T16	22.5b	NA
Tucumán	T17	5b	NA
Tucumán	T18	5b	NA
Tucumán	T19	22,5b	NA
Jujuy	T21	5b	NA
Jujuy	T22	5b	NA
Jujuy	T23	5b	NA
Jujuy	T24	47.5ab	60bc
Jujuy	T25	12.5b	NA
Jujuy	T26	15b	NA
Salta	T20	45ab	70bc
Formosa	T27	15b	NA

Adapted from Buchweis (2019)
Means with a common letter are not significantly different ($p > 0.05$). Test: Tukey Alpha-0.05

capacity, with a 70% mean survival (Table 18.4, Fig. 18.7). According to Widiyatno et al. 2014, the variable of survival rate could be used to select the best family for breeding programs in the future because this variable showed the adaptation of the family toward the extreme condition in their early establishment.

Fig. 18.7 Seedling at the first-year evaluation field trial (*E. contortosiliquum*). (Photo: R. Buchweis)

18.3.2.3 Mini-Clonal Gardens of Provenances and Progenies of *P. dubium* and *E. contortosiliquum*

A mean of 927 mini-cuttings/m^2/year was produced for both species, based on three collections per year; with the mean survival and rooting capacity of mini-cuttings both above 85% after 120 days of growth in greenhouse conditions (Niella et al. 2014). This technique offers great opportunities for improving the quality of planting stock, through its capability to produce any quantities of plantation materials in homogenous quality at any time (Yasman and Natadiwirya 2001).

18.4 Conclusions

At this stage of the study, it can be concluded that:

1. In spite of the low density of individuals/ha, the number of seed trees selected for germplasm collection of the species *P. dubium* and *E. contortosiliquum* met the

minimum of 30 randomly selected trees to be sampled according to Rogers and Montalvo (2004).
2. Fruits and seeds of *P. dubium* showed differences among provenances and progenies, and positive correlations between morphometric variables of fruits and seeds and the height and survival of seedlings at 60 and 120 days.
3. Short-term greenhouse trials showed significant differences among provenances and progenies of *P. dubium* for height at 60 and 120 days, and positive correlations between fruit and seed size and height. Field survival, 12 months after establishment differ significantly among progenies of *P. dubium;* nevertheless, no difference was observed on *E. contortosiliquum*.
4. The strategies presented in this case study agree with the ex-situ conservation and population improvement methods proposed by Navarro Pereyra (2002), which include: vegetative propagation of mother trees; import of seeds from other populations outside the one to be improved; establishment of conservation gardens that include at least twenty non-related progenies, and development of low intensity breeding programs for the tropical species.
5. The presented results are preliminary and long-term studies are required for both species. However, this study lays the foundation for understanding genetic variability in *P. dubium* and *E. contortosiliquum* and provides methods for the propagation of germplasm to ensure genetic diversity of these species for domestication, enrichment, and/or restoration programs. It will also contribute to delineation of a proper design for a biodiversity island strategy for *P. dubium* and *E. contortosiliquum.*

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