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Cloning of elite, multipurpose trees of the *Prosopis juliflora/pallida* complex in Piura, Peru

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Abstract

The nitrogen fixing trees of the *Prosopis juliflora/P. pallida* complex are among the most adaptable and fastest growing trees in the truly tropical arid regions and have become naturalized in semi-arid tropics in Latin America, the Caribbean, Hawaii, Sahelian Africa, the Indian subcontinent and northern Australia. The trees have been regarded both as a serious weed and as a valuable resource for firewood and dune stabilization. Unfortunately the introductions into Sahelian Africa and the Indian subcontinent were from trees that had non-palatable pods. Despite their widespread occurrence, seed from a genetic improvement program is not available. Peruvian *Prosopis* has rapid growth, erect form and high survival of in Haiti, Cape Verde and India. An evaluation of form, diameter at breast height, pod production and pod palatability was conducted in a 10 year old plantation of 1,800 trees in Piura, Peru and seven were selected that had: more than 20 cm DBH; an erect form; 100% of branches with pods; and pods with a very sweet flavor. Scions and budwood were taken from these trees and successively grafted onto greenhouse grown stock plants to be used for clonal multiplication. For the first time we report successful chip budding grafting of *Prosopis*. This is the first report of Peruvian clones that have been selected for high production of highly palatable sweet pods.

Introduction

The nitrogen fixing trees of the genus *Prosopis* are an important component of many agroforestry systems worldwide. Perhaps none of the 44 species of *Prosopis* (Burkart, 1976) has been as blessed and as maligned as *Prosopis juliflora*. While *P. juliflora* has its origins in the true tropics of the Western Hemisphere (Caribbean, Mexico, Venezuela and Peru), it has been introduced to practically all the worlds' arid zones. In India, *P. juliflora* was declared the Royal Plant of the State of Jodhpur (Muthana and Arora, 1983) while in the Sudan and Australia it is officially classified as a noxious weed with prohibitions to propagate it. Due to the total lack of leaf drop with onset of winter temperatures and total lack of frost hardiness of *P. juliflora* and *P. pallida* (as compared to other species such as *P. alba*, *P. glandulosa*), Felker (1990) has proposed that these tropical interbreeding species be treated together as a complex.

While *Prosopis* pods were extensively consumed by indigenous peoples in the native ranges of *Prosopis* in Peru (Diaz, 1994), North America (Felger, 1977) and Argentina (D'Antoni and Solbrig, 1977), the *Prosopis* pods in both sub-Saharan Africa and India have bitter astringent tastes and are occasionally consumed by livestock but not humans (Felker, P., unpub obs., Felger, R. S. pers comm., 1996). We have found considerable variability in the sweetness of the pods in the native range, some pods being very sweet and highly palatable while others are astringent, bitter and acidic. Evidently the introductions into sub-Saharan Africa and India were from seed sources of the non-palatable type.

The selection of clones of multipurpose trees is more complicated than for trees that will only be used for single purposes such as lumber or pulp. For lumber trees, a single trunk with minimal branching is ideal. In contrast, a tree needs to have a branched canopy with many locations for pod production. In addition, there is competition for photosynthate for trunk and pod production so that trees with high pod production would be expected to have lower production of vegetative biomass in the form of timber.

The evaluation of selection parameters for multipurpose trees is also complicated. While tree diameter and height can easily be measured in the field on hundreds of trees, precise measurements of characteristics such as pod production and pod palatability is much more time consuming and/or complicated. Therefore we have chosen to measure the easily quantifiable characters e.g., height and diameter by precise traditional methods and to employ visual ranking (based on verifiable criteria) to assess pod production and pod palatability characters.

Materials and methods

The 9 ha plantation of 1,800 trees was established in January 1988 on the grounds of the Universidad de Piura, Peru (5°10′11″ S; 80°36′51″ W) with a spacing of 10 by 5 m (200 trees ha^{-1}). The soil texture was sand – 95.7%, silt – 2.6% and clay – 1.6%. The soil was alkaline (pH = 8.4), nonsaline (EC = 0.5 mmhos), and low in organic matter (0.12%), nitrogen (0.006%), K_2O (102 kg ha^{-1}) and P_2O_5 (13 mg L^{-1}). Due the low mean annual rainfall (66 mm excluding Niño years of 2000 mm yr^{-1}), the seedlings were drip irrigated for the first four years until the trees reached permanent groundwater at a depth of 10 m The seedlings were purchased from the forestry nursery of the Ministry of Agriculture in the city of Catacaos and were of unselected genetic stock collected from trees near the city of Piura. The mean annual temperature, rainfall and class A pan evaporation were 24 C, 66 mm yr^{-1} and 1825 mm yr^{-1} respectively.

Despite various taxonomic works on *Prosopis juliflora* and *P. pallida* (Burkart, 1976; Ferreyra, 1987), considerable confusion exists on the taxonomy of the trees in northern Peru. While specimens can be identified that fit the description of *P. pallida* or *P. julifora*, the presence of interspecific hybrids blurs these distinctions. Therefore we have chosen to identify these clones with a unique field location and a description of their economically useful characters.

In August 1998, 22 plots representative of the entire plantation were systematically selected for a detailed forest inventory. Each of these plots contained four rows of 10 trees per row (40 trees). The plots were separated by three rows of trees. A total of 880 trees of the 1,800 trees in the plantation were measured (49%). The following measurements were made; trunk height to first branch, diameter at breast height (DBH), total tree height and canopy diameter. In addition, the form was scored as: 1 = tree with multiple main stems below 1.2 m in height and branch angles greater than 30 C; 2 = intermediate; and 3 = only one stem to minimum of 1.2 m in height and branch angles 30° or less.

In January 1999, a second evaluation of the entire plantation of 1800 trees was conducted and 3.7% of the trees were found to have a DBH greater than 20 cm. These trees were further evaluated for pod production and pod quality. Fruit production was ranked as 0, 1, 2, 3, 4 with 0 being without fruit; 1 = 25% of branches with fruit; 2 = 50% of branches with fruit; 3 = 75% of branches with fruit; and 4 = 100% of branches with fruit. Following pod abscission, the ground surface under a tree with a rank of 4 was covered with about 1 cm of pods equivalent to about 35 kg $tree^{-1}$. The fruit flavor was ranked as 0 = very bitter; 1 = bitter; 2 = sweet; or 3 = very sweet. The field was divided into plots, columns and rows to aid in the measurement of tree evaluation.

In August 1999, scions and budwood were taken from the mature trees to graft or bud onto unselected genetic stock in the UDEP greenhouse. Double English grafts and chip budding were conducted according to the technique of Hartman et al. (1996). Scions about 4 mm in diameter containing three to four nodes, were used and then wrapped with clear plastic tape.

Results

The mean total height, height to the first branch, canopy diameter and diameter at breast height in 1998 of 5.8 m, 1.7 m and 11 cm respectively, were quite respectable for 10 years growth at this low rainfall (Table 1). More promising is the fact that the great variability in the population permitted the possibility for selection for individual trees with outstanding performance. For instance, approximately 5% of the population had a diameter at breast height twice the population mean.

The 3.7% of the 1800 trees that had diameter at breast height greater than 20 cm in 1999 was used as the basis for further selection for pod characteristics (Table 2). It is striking that slightly less than half of this population (44.8%) had no pods at all. Similarly, while we have observed that all these Peruvian pods have high sugar content, a very high percentage of these large trees (78%) had pods that had a bitter or very bitter taste. Therefore it is not surprising that previous introductions to Africa and India were from trees that had pods unpalatable to humans. With respect to tree form (with potential for fine lumber production), 79% of the trees did not have an erect growth form. An example of one of the seven cloned trees (Figure 1) illustrates the narrow branch angle and erect form of this *Prosopis*.

Out of the total of 67 trees with a diameter greater than 20 cm, only 15 trees had the highest pod production rank (100% of the branches with pods). Only 10 of these 15 trees had good form that would be suitable for lumber and of these, seven had pods that were sweet or very sweet. Thus only seven trees had; a diameter greater than

Table 1. Frequency distribution of diameter at breast height (DBH), height to first major fork, total height and canopy diameter for 880 of the 1,800 *Prosopis juliflora/pallida* trees in 1998, Piura, Peru.

	DBH diameter Class					
DBH (cm)	< 5.0	5–10	10–15	15–20	20–25	> 25
% of pop.	2.14	32.83	43.27	17.11	3.14	1.51
95% Confidence Int.	1.01	3.26	3.44	2.62	1.21	0.85
	Height to first fork Class					
Height first fork (m)	< 1.0	1.0–1.5	1.5–2.0	2.0–2.5	> 2.5	
% of pop.	8.44	17.13	33.75	19.40	21.28	
95% Confidence Int.	1.93	2.62	3.29	2.75	2.85	
	Total height class					
Total height (m)	< 2.0	2–4	4–6	6–8	8–10	> 10
% of pop.	0.60	8.16	28.33	44.90	15.37	2.64
95% Confidence Int.	0.52	1.86	3.06	3.38	2.45	1.09
	Canopy Diameter class					
Canopy diameter (m)	< 2.0	2–4	4–6	6–8	8–10	> 10
% of pop.	1.68	11.03	23.26	36.69	21.34	6.00
95% Confidence Int.	0.87	2.13	2.87	3.27	2.78	1.61

95% of the observations are obtained when the confidence interval is added to, or subtracted from, the mean.

Table 2. Frequency distribution of pod production, pod flavor and form for Prosopis *juliflora/pallida* trees with diameter at breast height > 20 cm (3.7% of the 1,800) in 1999, Piura, Peru.

	Percentage of trees with diameter > 20 cm diameter at breast height				
Percentage of branches with pods	0% with pods 44.8%	25% with pods 16.4%	50% with pods 8.9%	75% with pods 7.5%	100% with pods 22.4%
Pod flavor	Very bitter 17.9%	Bitter 59.7%	Sweet 7.5%	Very sweet 14.9%	
Form	Weeping 17.9%	Intermediate 61.2%	Erect 20.9%		

Figure 1. Ten year old cloned tree P6C6R3 at Universidad de Piura.

20 cm, an erect form, 100% of the branches occupied by pods and pods that were sweet or very sweet. These clones ranged from 137 to 170%, 47 to 100%, 116 to 166% and 190 to 327% times the the population mean for total height, height to first fork, canopy diameter and breast height, respectively.

All these clones had spines tat varied from about 5 to 10 mm in length, depending on the level of stress experienced by the plant (more stress, greater spine length) and whether or not the shoot originated from a recently cut surface (greater spine length).

At the end of August 1999, scions and budwood were taken from the elite trees for grafting and budding onto unselected *P. pallida* in the UDEP greenhouse. A total of 166 double English grafts (Figure 2 a,b) yielded 68% success while 80 budded plants (Figure 3 a,b) yielded 55% success. Intense red anthocyanin pigmentation in the immature tissue makes the scion and bud clearly distinguishable from the rootstock. As the only other work grafting (Wojtusik and Felker, 1993) failed to achieve success with budding, this is the first report of successful budding in Prosopis. At present about 40 stock plants of each clone are being grown under drip irrigation in the UDEP greenhouse where they produce about 1200 cuttings clone^{-1} per five week harvest cycle. Cuttings from these rejuvenated plants are being used to produce rooted cuttings (Klass et al., 1984), as this is technique is more efficient for mass propagation.

Discussion

While clones of *Populus*, *Eucalyptus* and *Salix* are widespread in standard forestry operations, we are not aware of clones being used in agroforestry applications. As long as clones serve traditional multipurpose agroforestry objectives-both economic and environmental, we see no reason why clones are not appropriate in agroforestry systems. Due to the multipurpose requirements of agroforestry tree species, it is more difficult to obtain all of the desirable characteristics- lumber quality, fruit production/quality, and site adaptability in one plant, than in species where only lumber is desired. Thus when plants are located that have all the desirable characteristics, cloning is useful to capture all these characteristics in one plant. As *Prosopis* is obligately outcrossed, the trees would be expected to have allelic variation similar to F1 hybrids. Thus seedlings from 'elite plus trees' would be equivalent to F2 progeny and would be highly variable due to independent segregating genes. Thus the only way to capture these elite trees is by cloning (in contrast self fertile *Leucaena* produces true to type seeds). As Felker et al. (2001) has measured narrow sense heritability estimates of 0.487 for height, 0.548 for biomass production and 0.244 for pod production in *Prosopis alba*, and as Oduol et al. (1986) has measured intraclass correlations (similar to heritability coefficients) of 0.6 for pod protein and 0.3 for pod sugar for *Prosopis* in plantations, there is good reason to believe the characteristics we have selected are under strong genetic control.

Table 3. Characteristics of the seven elite *Prosopis pallida* clones selected in Piura, Peru.

Plot, column, row	Pod production rank	Pod flavor rank	Form rank	Total height (m)	Height to first fork (m)	Canopy diameter (m)	Diameter at breast height (m)
P4C15R2	4	Sweet	3	10	1.1	10	0.36
P6C6R3	4	Sweet	3	10	1.3	8	0.32
P6C1R12	4	Sweet	3	10	1.7	8	0.21
P8C13R2	4	Sweet	3	10	1.6	9	0.27
P9C2R2	4	Very sweet	2	9	1.1	10	0.23
P5C3R8	4	Very sweet	2	10	1.2	8	0.23
P1C14R9	4	Very sweet	2	8	0.8	7	0.24

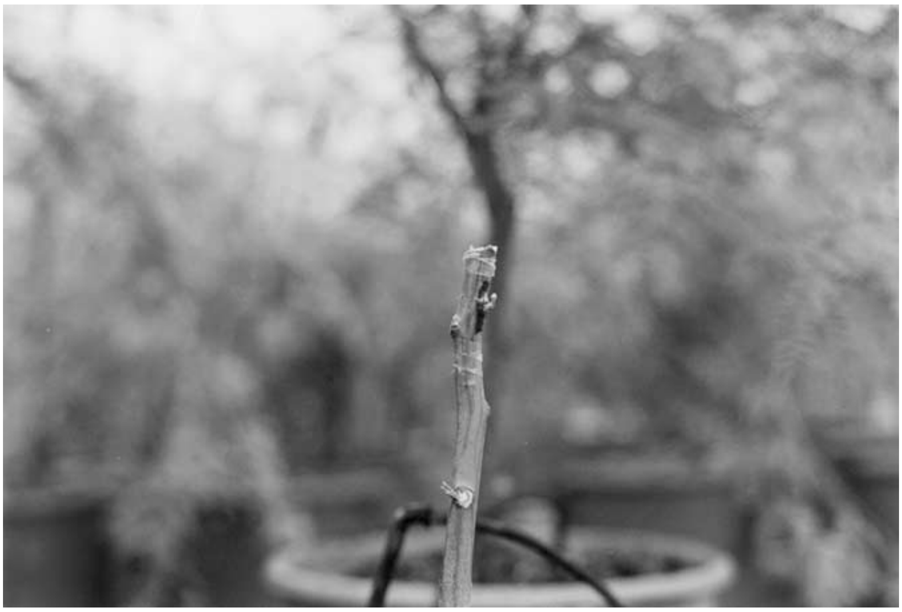
The pod production (0 = none and 4 = maximum), pod flavor (very bitter, bitter, sweet, very sweet) and form (1 = multiple branched, 2 = intermediate and 3 = single trunk to 1.2 m in height and branch angles < 30°) were ranked as described in the materials and methods.

(a)

(b)

Figure 2 a, b. Example of double English graft and grafted plant that yielded 68% success with *Prosopis pallida*.

(a)



(b)

Figure 3 a, b. Example of bud and a budded plant that yielded 55% success with *Prosopis pallida*.

In comparison to the Peruvian *Prosopis* clones made in Haitian (Wojtusik et al., 1993) and Indian field trials (Harsh et al., 1996), based on only a few families, these clones originated from seed from many families in this center of biodiversity and thus represent a much broader germplasm base. Moreover the Wojtusik et al. (1993) and Harsh et al. (1996) clones did not consider pod production or pod palatability characteristics. The mean diameter growth of 1.1 cm year^{-1} compares favorably with *Prosopis* diameter growth rates of 1.25 to 2.5 cm year^{-1} reported for *P. glandulosa* (Patch and Felker, 1997) and *P. alba* (Felker et al., 1989) in Texas.

In the past, clones of *Prosopis* have only been envisioned for use in seed orchards or in special high value applications around homes, due to the low percentage success in rooting cuttings and the low production rate (10 plants hr^{-1} with 6 mm scions) of grafted plants. Due to the much greater ease in propagation of *Prosopis* from seed than clonal propagation, the production of plants from seed is the only viable option for the many rudimentary tree nurseries in developing countries. Thus we felt the principle utilization of these clones would be in seed orchards. As *Prosopis* is self-incompatible (Hunziker et al., 1986), seed from a clonal seed orchard theoretically would consist of hybrids between the various clones. Since a mature tree can produce 40 kg of pods, from which it is possible to obtain 2.5 kg of cleaned seed at 25,000 seed kg^{-1}, a one-hectare seed orchard of 100 trees could produce more than 6 million seed per year.

However, recent successes in rapidly grafting 1.5 mm diameter, 30 day old seedlings (Ewens and Felker, 2002) and recent improvements in rooting of difficult clones with intensive management of the environment of the cuttings, may make clonal agroforestry plantations of commercial scale feasible. There is additional cause to believe commercial scale plantations from rooted cuttings may be possible with this species as it is the easiest of the Prosopis species to root (Leakey et al., 1990).

These clones could also be useful to convert weedy, non-productive stands of P. juliflora into highly productive stands by harvesting the weedy trees and grafting onto tender coppiced sprouts. This technique could also be useful to upgrade to higher quality Peruvian stands after the harvest of trees for lumber. It would be most interesting to examine the performance of trees that combine scions of these high pod producing, fast growing trees with rootstocks that have been shown to be resistant to seawater salinity (Rhodes and Felker, 1987) or high pH 9.0 (Singh, 1995).

These clones should be of considerable value to the emerging industry in northern Peru using *Prosopis* pods in human food applications (Grados and Cruz, 1996) such as flavoring syrups, flour for baking specialty pastries and coffee substitutes. These products are currently made from pods collected from wild trees. Due to the high tree to tree variability in pod production and sensory characteristics, clonal *Prosopis* orchards should greatly facilitate the development of this human food based industry.

Furthermore in regions of Africa, India and Pakistan where existing *Prosopis* have bitter astringent pods, the harvest of the trees with non-palatable pods for lumber or fuel, and replacement with the clonal material described above would make human food based industries possible.

Due to the fact that *Prosopis* lumber compares favorably in color, hardness and shrinkage values to the world's finest timbers that also belong to the legume family such as padauk (*Pterocarpus soyauxii*), purpleheart (*Peltogyne* spp.), cocobolo (*Dalbergia retusa*), Indian rosewood (*Dalbergia latifolia*) and Brazilian cherry (*Hymenaea courbaril*) (Felker, 2000), logs from these clones should provide valuable timber for furniture and flooring. Given international prices of \$850 m^{-3} for equivalent fine timber and a *Prosopis pallida* wood density of 0.9 (Barriga, 1988), the sawn lumber could have a value of \$940 per metric ton which is much greater than other products from arid lands.

For more than 100 years, trees of the *Prosopis juliflora/P. pallida* complex have been widely introduced to the world's semi-arid regions including Hawaii, Senegal, Sudan, India, Pakistan and Australia. However, throughout these regions there have never been seedlots or clones available that resulted from genetic improvement trials.

In the last 10 years, comprehensive field trials in Cape Verde (Harris et al., 1996), Haiti (Wojtusik et al., 1993) and India (Harsh et al., 1996) have consistently demonstrated greater height growth and form for *Prosopis* of Peruvian origin than from all other regions. Given this superior performance it is important to evaluate these improved genetic strains in locations where *Prosopis* has previously shown to be adaptable and where lack of human and livestock food and firewood is especially critical. For example these strains should be evaluated in Somalia where Zollner (1986) demonstrated the superior performance of *Prosopis*, in the Sudan where Bristow (1996) reported on the use of *Prosopis* to control movement of sand dunes onto villages, in Senegal where Diagne (1996) reported that *Prosopis* was a major firewood species, in India where Varshney (1996) reported the Gujarat Forest Development Corporation collected 2,000 tons of pods used annually for livestock food and in Haiti where *Prosopis* is the major source of energy (Lea, 1996). In a review of CARE reforestation projects in Africa that used *Prosopis*, Butterfield (1996) stated that *P. juliflora* had excellent success but that the long thorns, wide crown and aggressive spread of seedlings were a serious deterrent to inclusion in future development projects. Thus use of clones with minimal thorn length, erect habit and abundant sweet pods should overcome the previous objections while continuing to provide high tree survival rates and vigorous growth.

There is an obvious danger in narrowing the germplasm base of any plant with clones. However, as evidenced by the spread of *P. juliflora* over nearly half of India in less than a century from very limited germplasm introductions, this species has great plasticity and adaptability to harsh environments. At this time we believe the greater problem with this species is the lack of uniformity in plantations, that precludes development of commercial plantations for pod production. Since single clones of fruit varieties (e.g. Ruby Red grapefruit, Jonathan delicious apples) have been planted over extensive areas for decades without catastrophic consequences, we believe these seven clones will serve as an excellent initial basis for commercialization of both the pod production and lumber industry in tropical arid regions. Obviously research must continue to identify additional clones with superior pod production, lumber production and resistance to pests, diseases and edaphic factors (e.g., salinity and high pH).

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