Utilization and Nitrogen Fixation of *Prosopis* in Senegal

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ABSTRACT

***Prosopis* is one of the best-known trees in Sénégal today. After the loss of several million hectares of forest due to the drought, *Prosopis* is in very high demand to recover the degraded soils. Two *Prosopis* species are widely used in Sénégal: the native *Prosopis africana* growing in the southern part of the country, and *Prosopis juliflora* introduced from Central America.**

The area of *Prosopis africana* extends from Sénégal to East Africa. It characterizes the dry forests, the fallows, and the sandy clay soils. It has an economic value due to its hard and durable wood. Thus, it is overexploited as timber and firewood especially. The pods and leaves of *Prosopis africana* are appreciated as forage. Almost all the tree parts are used in traditional medicine. Like most of the *Prosopis* species, *Prosopis africana* is not affected by pathogenic germs. However, its pods can be overrun by insects. Unfortunately, there is very little microbiological research on this species.

Although it has been introduced in Sénégal, *Prosopis juliflora* is better known and more demanded than *Prosopis africana*. Research activities are mainly conducted on *Prosopis juliflora*. In the Sahel *Prosopis juliflora* usually is found planted with *Acacia* species. In this area, it provides pods and leaves that are very useful to cattle during the dry season. *Prosopis juliflora* is also planted in bare areas as fences and windbreaks. Two of the major problems encountered with *P. juliflora* are lack of techniques to extract the seeds from the pods and to obtain homogenous growth from a lot of seedlings. Fortunately, micropropagation seems promising to multiply *Prosopis* more quickly.

Both species are multipurpose but their great interest is based on their nitrogen-fixing ability. Because of their symbiosis with *Rhizobium*, they can produce nitrogenous substances which play a major role in the poor soils. For this reason, the integration of *Prosopis* into the agroforestry systems is necessary. This benefits not only plant growth but also contributes to soil amelioration. Experiments conducted in the nursery have shown that *Prosopis juliflora* responds positively to inoculation with *Rhizobium*, increasing its biomass and nitrogen-fixing ability. However, the response to inoculation varied markedly according to the provenance of *Prosopis*. The potential of *Prosopis juliflora* to fix significant proportions of nitrogen has been established under seminatural conditions. The amount of N fixed by a tree was approximately 31 g.

INTRODUCTION

The rural populations of the Sahel are mainly farmers in the south or cattle raisers in the north. In the central part, agriculture is associated with sedentary cattle raising. Beside water, their principal needs consist of a wide range of products: firewood, timber, charcoal, food, and gum. However these natural resources are seriously degraded due to long periods of drought, which brought about a loss of several million hectares of forest.

Given the need to tackle this serious situation, several campaigns of reforestation have been launched using the multipurpose, fast-growing and nitrogen-fixing leguminous trees because of their many properties and characteristics. Among these trees, *Prosopis* species appear to be the most promising for the semiarid regions. The importance of *Prosopis* in Sénégal has been underlined previously (Diagne, 1992). The major part of this paper will be focused on the introduction of *Prosopis* species in Sénégal, their biomass production, seed germination, and micropropagation. *Prosopis* microorganisms associations should also be considered because of their role in soil amelioration.

Actually, the microbiological approach has been neglected in the past. The nitrogen fixation of several leguminous trees such as the *Acacia* has been widely studied. However little is known concerning the symbiosis with the *Prosopis* even though the nodulation and nitrogen fixation were proved (Felker and Clark, 1980; Shearer *et al*., 1983; Shoushtari and Pepper, 1985). In this paper, more attention will be given to the quantification of the nitrogen fixed by *Prosopis juliflora*.

UTILIZATION OF *Prosopis*

Biomass Production

Biomass production is very low in the semiarid regions because of the low rainfall. For instance, an experimental field in Bandia, Senegal, produces 1.5 m^3/ha of biomass per year (Freeman *et al*., 1983). According to FAO, more than 13 million people in the arid and semiarid regions and nearly 200 million people in the savanna are essentially dependant on firewood. According to a CILSS (Comité Inter-Etats de Lutte contre la Sécheresse au Sahel) report, the total consumption of firewood in the Sahel is estimated at 16 million m^3 per year, that is, 0.6 m^3 per inhabitant per year.

Given this important consumption of fuel, it is necessary to be interested in fast-growing, nitrogen-fixing trees. They can not only improve the basic economy of the rural populations, but also contribute to decreasing their imported sources of energy. Although the productivity of the Sahel woody lands is low, the nitrogen fixing trees contribute to a great extent to supplying firewood and charcoal to the cities and villages.

An estimation of the productivity in Bambey, Sénégal, has shown that a 17-year-old stand of *Prosopis juliflora* produced 4.2 m^3/ha/y of wood. This production is the best compared to *Acacia seyal* (native species) or to *Racosperma holocericeum* (introduced). When the total biomass harvested was harvested in the same stand at 0.10 m and 1.10 m above ground, 84% and 100% of the trees produced coppiced shoots at the respective cutting heights. A stand of an 8-year-old *Prosopis cineraria* produced 0.7 m^3/ha of wood at Bandia.

The nitrogen-fixing trees (NFT) are used in the craft and building industries. They can also supply timber and pods, whose export constitutes an inflow of currency. Sénégal imports 50,000 to 60,000 m^3 of wood each year from the forestry countries.

Food Production

Before the development of agriculture in the semiarid regions, leguminous trees had played a great role in human food, which was based partly on the gathering and the harvest of leaves and fruits from trees. The sale of the takings from the collection has brought about an improvement in the state of the economy of the populations.

In the Sahel, the leguminous trees are very important as source of food for the nomads who move to pastures with their cattle during the dry season looking for aerial forage, dry fruits and water. During that period, and unlike the annual crops, almost all the NFT keep their vegetative biomass alive and benefit from the underground water until the next rainy season. The aerial biomass is a factor of stability for the populations as it can be produced all year. Unfortunately this equilibrium is not always reached because of the instability of the rains and overgrazing. Thus, the density of the woody plants is tremendously decreasing from the south to the north of the Sahel (Breeman, 1982).

Contrary to the annual crops, the woody legumes have been scarcely studied at the food level. Findings have shown that NFT can play a fundamental role as forage for the cattle in semiarid regions by conditioning their survival in some cases during the long period of drought. In addition, the NFT can provide shade for the grasses. Therefore, it is necessary to associate cattle production with the culture of the NFT. The seeds and pods of some NFT contain an important proportion of proteins, lipids, vitamins, and soluble carbohydrates. The pods of *Prosopis juliflora* are highly edible for the cattle in West Africa because of their fleshy and sweet tissues. They contain 34% to 39% protein and 7% to 8% lipids (NAS, 1980). Leaves and bark of young *P. chilensis* constitute an appreciated food for hares.

Restoration of the Degraded Lands

In the past, the semiarid regions had benefited for a long time from the natural resources that protected the soil against erosion. The present vegetation hardly exists in many cases and the forest services of the concerned countries have launched several campaigns for reforestation. Such actions have faced up to difficulties linked to limited, heterogenous and poor soils. This has led to mass destruction of the natural vegetation to increase the area for mechanized plantings, generating other problems, such as deterioration in soil structure, decline in organic matter level and in biological activity, deterioration in water capacity, and a drop in soil pH (Siband, 1974).

The spreading of such phenomena added to the improper field clearings; the bush fires have been harmful to the ecosystem in the semiarid regions. This deteriorated the vegetation and made the soil sensitive to erosion. According to the World Bank (1978), the forest reserves of the developing countries (estimated at 1,200 million ha) are destroyed at a rate of 15 to 20 million ha per year.

Erosion can cause important losses of nitrogen equivalent to 3.5 kg/ha per year (Pieri, 1982). Thus *P. juliflora* is highly used as windbreak or to stabilize sand dunes (Green Belt in Nouakchott, Mauritania).

Other Utilizations

Even if we carry out a better planning and management of the natural resources, the problem of the degradation of the environment will remain due to the exponential increase of the population and their specific needs. For this reason, several forestry projects have initiated agroforestry trials in which

the tree is directly integrated into the activities of the farmer and the cattle raiser. This technique had been practiced in the past by the farmers who kept the NFT such as *A. albida* and *A. senegal* as fallow in their fields.

NFTs also have functioned as ornamental plants in the community groups and, therefore, have contributed to the amelioration of the surroundings of the populations. They form shade in villages or houses, windbreaks, and protective belts. *P. juliflora* has been introduced in most cities in the Sahel along roads and tracts (Figure 1).

Figure 1. *P. africana* near Saboya Village in Senegal (600 to 800 mm annual rainfall)

The curative properties of the leaves, roots, fruits, and bark of most of the NFT have been used in traditional medicine. For example, *P. africana* is frequently used as a medicinal plant (Diagne, unpubl. obs.).

SYLVICULTURE

Seed Treatment

P. juliflora produces an indehiscent sweet fruit which can contain up to 20 seeds or more. The seeds are protected by cellulosic and rigid tissues that make their extraction difficult. This may explain why *Prosopis* seeds are not attacked by pests.

Laboratory tests were carried out to germinate *Prosopis juliflora* seeds with their endocarps. Seeds were immersed in different concentrations of hydrochloric acid (N to 5N) for 60 minutes. Germination was very poor after four days (<20%). Other lots of seeds were immersed in 20% and 96% of sulfuric acid, respectively, for 10 and 30 minutes (Coulibaly, 1991). None of the treatments destroyed the endocarps entirely and the percentage of germination remained poor (<25%). After shaking for 5 hours in 96% sulfuric acid better results were achieved. The endocarps were destroyed by the acid and the seeds had 80% germination after one day. Although this last result seems encouraging, the chemical method of seed extraction is difficult to popularize. The quantity of concentrated acid to handle is enormous and expensive and its utilization is dangerous. For these reasons mechanical extraction of the seeds is most appropriate for our conditions.

The most traditional mechanical method of seed separation consists of crushing the dried pods into a mortar with a wooden pestle. This method is simple but time consuming and needs a lot of labor. For example 6 to 8 men are required to work for 8 hours to extract 1 kg of seeds (about 28,000 seeds). Electric-powered industrial equipment that includes an electric boiler and disintegrator are capable of extracting 100% of the seeds from the pods in 10 minutes, yielding seeds that have 90% germination in one day. Unfortunately, this equipment, produced by Rayneri, Department Industrie:

Melangeurs Industriels, Z.I., BP 9, 85601 Montagu, Cedex, France, is quite expensive (about US$80,000).

In vitro Germination

Seeds of *Prosopis juliflora* were surface sterilized with different concentrations of sodium hypochlorite (NaOCl) and calcium hypochlorite (CaOCl). The germinated seeds were then incubated in Murashige and Skoog (MS) (1962) medium enriched with 2.5 mg/l of naphthaleneacetic acid (NAA), 0.1 mg/l of 6-benzylaminopurine (BAP) and 20 g/l of sucrose (Seck, 1996).

All the seeds germinated 48 hours after using NaOCl and 72 hours after using CaOCl. These chemicals took effect according to their concentration and made soft the integument of the seeds, lessening the pressure needed for rooting. However, at least 50% of the seeds became infected with the low concentrations of CaOCl (100 to 150 g/l). The best result of disinfection (16% of infection) was obtained with 200 g/l of NaOCl for 15 minutes.

Micropropagation (Seck, 1996)

Microcutting for axillary shoot production

Microcuttings taken from *in vitro* plants were grown in three planting media: Murashige and Skoog (1962), Gamborg *et al.* (1968), and Woody Plant Medium (Lloyd and McCown, 1980). The result showed that the MS medium gave the highest percentage of survival (100% for *P. chilensis* and 87.5% for *P. juliflora*). It appears to be more favorable to the regeneration and elongation of the *Prosopis* buds compared to the other media (Table 1).

The addition of 5 mg/l of BAP allowed 100% survival for the two species and good multiplication. However, this concentration inhibited the extension of the buds. The addition of 1 mg/l of kinetin (6-furfurylaminopurine) to the MS medium did not modify the activity, the percentage of multiplication, or the number of buds of *P. chilensis*. In contrast, 100% survival was found in *P. juliflora* even though the multiplication was less important than the control plants (no Kinetin added).

The combination of 0.1 mg/l of BAP and 1 mg/l of NAA led to 100% of rooting for *P. chilensis* and 0% for *P. juliflora*.

Table 1. Effect of Three Media on the Survival of *P. chilensis* (P. c) and *P. juliflora* (P. j)

Media	N	Survival (%)		Bud Mean Number		Bud Mean Size (mm)		Mean Node Number		Multiplication Rate	
		P. c	P. j	P. c	P. j	P. c	P. j	P. c	P. j	P. c	P. j
MS	24	100	87	1.0a	1.1a	11.7a	6.8a	1.8a	0.9a	1	1.1
WPM	24	88	54	1.1a	0.7ac	2.5b	1.3b	0.4b	0.3b	1.1	0.7
B5	24	67	57	0.9a	0.5bc	2.5b	0.6b	0.4b	0b	0.9	0.6

Means within a column followed by a different letter are significantly different at $p<0.05$ by analysis of variance.
MS: Murashige and Skoog (1962)
WPM: Woody Plant Medium
B5: Gamborg *et al.* (1968)

Shoot production from cotyledons

Cotyledon explants were put in a growth-hormone-free MS medium. Two weeks after, the ratio of multiplication was high: 2 for *P. chilensis* and 2.2 for *P. juliflora*, corresponding to a mean of two active buds per explant. This medium allowed good elongation of buds: 9.56 mm after 30 days for *P. juliflora* and 1.69 mm for *P. chilensis*.

Hardening off of in vitro shoots

During the acclimation stage, about 54% of survival was obtained. After five months, height, number of leaves, and biomass were recorded. They varied according to the species. The length of the tap root reached 89 cm for *P. juliflora* and 68 cm for *P. chilensis*. The height, number of leaves, root length were, respectively, 33, 30, and 48 cm for *P. juliflora* and 52, 47, and 30 cm for *P. chilensis.*

Prosopis Introductions

Several *Prosopis* species were introduced in Bandia to test their adaptability to survive and perform in the Sahel conditions (Diagne, 1992).

P. alba introduced in 1982 seems to be well adapted since 90% of the plants survived after six years. Its scrubby trunk, thorny branches and poor forage palatability suggest its most appropriate utilization as a hedge. *P. chilensis* was introduced in Bandia in 1978. Seven years later, 86% of the plants survived the conditions. However, in the following year 22% mortality was observed. *P. chilensis* also appears to be most useful as a hedge because of its scrubby and very thorny branches. *P. cineraria* was introduced in 1977 in Bandia with a 4.5 m spacing between the trees.

In 1985, alternate rows of these trees were removed (leaving a 9-m spacing) to test their ability to coppice. Fourteen months after harvest all the trees produced coppice shoots. The production of biomass of the coppice can be important: after four months of rain, 1.4 t/ha of dry weight were obtained. Three years after this production reached 3.5 t/ha of fresh weight as against 2 t for the nonthinned trees. The soil structure seems to have an effect on the growth of *P. cineraria*. The best growth was recorded in the depressions (Table 2).

Table 2. Growth of *P. cineraria* (provenance Haryana, India)
According to Soil Characteristics of Bandia

Pedological Characteristics	Topography	Survival (%)	Height (cm)	Basal area per stem (cm^2)	Ground-based surface (m^2/ha)
Very compact clayey layer between 0.5 and 1.0 m	Subhorizontal	96	210	43	2.0
Deep soil and structure less compact	Depression liable to flooding	96	400	181	8.6
Cuirass at 1 m	Subhorizontal	96	270	74	3.6
Compact clayey layer at 1 m	Subhorizontal	100	290	90	4.4

P. juliflora and *P. cineraria* were tested for use in hedges with the following species: *Parkinsonia aculeata, Acacia mellifera* and *A. senegal*. The survival rate was recorded 6, 12, and 18 months after the plantations (Table 3) at Keur Mactar (central Senegal). Table 3 shows that *P. juliflora* and *P. cineraria* survived, in general, at the same rate (>60%) than the other tree species six months after transplantation into the field. After a year, their survival rate decreased rapidly and dropped to less than 40% in pure culture. *P. juliflora* benefited from its association with the *Acacia* species, contrary to *P. cineraria* with *P. aculeata.*

Table 3. Survival Rate of Tree Species
At Different Times After Plantation

Tree Species	Survival Percentage After 6 Months	Survival Percentage After 12 Months	Survival Percentage After 18 Months

P. juliflora (P. j)	85.0	20.0	19.0
P. cineraria (P. c)	65.7	50.5	38.0
Parkinsonia aculeata	100.0	99.5	34.0
Acacia mellifera	90.0	84.0	81.0
P. j + *Acacia senegal*	82.0 + 82.0	61.0 + 73.0	57.0 + 57.0
P. j + *Acacia mellifera*	96.0 + 92.0	83.0 + 92.0	73.0 + 82.0
P. c + *P. aculeata*	99.5 + 79.0	28.9 + 17.0	14.0 + 9.0

P. juliflora was tested as a windbreak in a sandy soil at Khayes (western Senegal). *P. juliflora* gave better results when grown with *Eucalyptus camaldulensis* than when grown with *Racosperma holocericeum*.

Provenance Trial

Seven provenances of *Prosopis sp*. from Ecuador and one provenance of *Prosopis juliflora* from Sénégal were grown at the nursery. Inoculated three-year-old seedlings were planted in Bandia in a randomized complete-block design with a space of 4 m between trees. The survival rate, height, and diameter of the plants were measured each month. The superiority of the local *P. juliflora* in terms of height at the nursery, disappeared three months after plantation in the field. No differences between the provenances have been noted for the other parameters. This suggests that the provenances from Ecuador are as well adapted as the Senegalese *P. juliflora* in the Sahel.

Although there was no difference between the provenances in terms of biomass and survival, large variability was noted in the number, size, color, and shape of the thorns; color of the foliage; and the shrubby shape. It would be useful to examine this variability as well as the characters of flowering and fruit formation in more detail.

SOIL AMELIORATION

Water is not the only limiting factor in the semiarid regions. Phosphorus and nitrogen are very important factors that need to be taken into account. The maintenance of the nutrients in the soil is often adversely affected by human actions. Actually, soil nitrogen balance is perpetually modified by farmers during cultivation. According to Jones and Wild (1975), the mean total soil nitrogen level decreases from the Guinean (0.104%) to the Sahelian zones (0.034%). In addition, only 4% of the total nitrogen is mineralized each year in the savanna soils for possible use by the plants (Singh and Balasubramania 1979).

Because of these factors, the degraded and poor soils upon which agrosylviculture is based, require fertilizer. Unfortunately mineral fertilizer is very expensive for the rural populations who obtain their main income from agriculture. Plant residues release mineral nitrogen to the soil, but the fallow is not long enough in the Sahel to substantially increase the soil-nitrogen pool. Due to the possibility of additional nitrogen contributions through nitrogen fixation, the integration of the nitrogen-fixing trees, such as the *Prosopis*, into the agrosylviculture systems could be very beneficial. Prosopis are able to produce nitrogenous substances from the nodules only with associated *Rhizobium*.

The effect of *Rhizobium* inoculation was tested on seven provenances of *Prosopis* sp. and one provenance of *Prosopis juliflora* (Diagne, 1994). The experiment was conducted in a nursery in unsterilized soil. The different responses to inoculation were estimated by comparing the inoculated with the uninoculated plants. After three months growth, plant height from inoculated plants was increased by 31% (provenance 2361) to 147% (provenance 2371), dry weight of shoots was increased by 15% (provenance 2363) to 213% (provenance 2371), and dry weight of roots was increased by

12% (provenance 1516) to 231% (provenance 2371). Although the growth of all the inoculated plants was increased, results indicate that responses to inoculation vary markedly with the provenance. The experiment suggests that given its excellent response to inoculation, provenance 2371 warrants further investigation as a promising semiarid agroforestry species.

Because of the great interest in *Prosopis* species and the need for increasing soil fertility through biological nitrogen fixation, it is important to quantify the amount of nitrogen *Prosopis juliflora* can fix. Despite the great number of research papers involving ^{15}N isotope-dilution estimation of biological N_2 fixation of other species (Chalk, 1985), there is no information on quantitative estimates of N_2 fixation by *Prosopis juliflora*. A few reports have measured nitrogen fixation by other *Prosopis* species using the acetylene-reduction method (Felker and Clark, 1980), micro-Kjeldahl techniques (Rundel *et al*., 1982), or the natural-^{15}N-abundance method (Virginia *et al*., 1984). A study was conducted to assess the nitrogen fixed by *P. juliflora* under seminatural conditions.

A ^{15}N-enrichment experiment was conducted to estimate the proportion of nitrogen derived from nitrogen fixation (pNdfa) for the symbiotic legume tree, *Prosopis juliflora* (Diagne and Baker, 1994). The nitrogen fixation was calculated from the N isotope abundance as described by Fried and Middleboe (1977). Seedlings of *P. juliflora* were inoculated with *Rhizobium* strain Pj-12 and grown in 1 m^3 concrete containers serving as individual microplots. Seedlings of *Eucalyptus camaldulensis* were used as a reference species. All microplots were provided with ^{15}N-enriched ammonium sulfate. Plants were harvested after one year of growth in the microplots. Yields of both *Prosopis* and *Eucalyptus* were good, although *Eucalyptus* had significantly higher total biomass.

In this experiment, the pNdfa for *Prosopis* was relatively low (13.5%) (Table 4). While this could be due to ineffective inoculation, it could also be due to a high level of available N in the soil. The latter explanation is more likely since the data show that *Prosopis* took up more N from the soil than *Eucalyptus*. In any case, the amount of N fixed by *Prosopis juliflora* was very low compared to other leguminous species such as *Leucaena leucocephala* and *Albizia lebbeck* (Högberg and Kvanström, 1982; Sanginga *et al*. 1985; Van Kessel and Nakao, 1986) or two other *Prosopis* species (Rundel *et al.* 1982; Virginia *et al*. 1984).

In a pure stand, *Prosopis juliflora* is usually planted with a 4x4-m spacing, which corresponds to a density of 650 trees/ha. Since the total nitrogen for *Prosopis juliflora* was 278.24 g, the amount of nitrogen fixed was 37.67 g per tree. By extrapolation, fixation would be about 25 kg N/year/ha. However the amount of N_2 fixed can vary according to the environmental conditions of the area where *P. juliflora* is growing. It also depends on the experimental design, and, especially, on the spacing between trees. The choice of a suitable spacing depends on the purpose of the *P. juliflora* plantation (Silva, 1988). For example, in agroforestry systems, *P. juliflora* can be planted with a spacing of 1 m between trees. It would be desirable to estimate the nitrogen fixed directly in the field and to take into account the fact that N_2 fixing activity can decrease with the progressive accumulation of N in the field (Gauthier *et al.*, 1985).

Table 4. Nitrogen Content of the Different Parts of Plants

Parameters	*Prosopis juliflora*		*Eucalyptus camaldulensis*	
	N atom (%)	^{15}N a. e. (%)	N atom (%)	^{15}N a. e. (%)
Leaves	2.49a	0.01c	1.11b	0.08d
Small stems (number <4mm)	0.99a	0.02c	0.33b	0.07d
Large stems (number >4mm)	0.70a	0.03c	0.23b	0.08d

Roots	1.23a	0.02c	0.22b	0.09d
Weighted average	1.35a	0.02c	0.47b	0.08d

Means within a line followed by a different letter are significantly different at $p<0.05$ by analysis of variance.

CONCLUSIONS

This work has permitted an assessment of the principal properties of the *Prosopis* in the African semiarid zones, especially in the Sahel. The numerous uses of a wide range of *Prosopis* products (wood, forage, fruits, gum, etc.) demonstrate the possibilities to improve the standard of living of the rural populations. Thanks to their symbiosis with the *Rhizobium*, *Prosopis* reforestation can also help in the fight to reclaim the desert and contribute to the amelioration of the soil fertility and soil stability.

Although it is difficult to extract seeds from Prosopis pods, the seeds germinate easily. Among the *Prosopis* introduced in Sénégal, *Prosopis juliflora* is the most promising and the most adopted by the populations. Successful micropropagation of *Prosopis* could be of a great benefit in avoiding some of the deleterious characters that occur as a result of high intra-specific variability in *Prosopis*.

Inoculation with appropriate *Rhizobium* strains can improve the potential of *Prosopis* to grow faster, produce substantial biomass and fix nitrogen. Trees also need mycorrhizae for their growth and functioning to take up nutrients from the soil more easily, especially phosphorus. If the environmental factors are matched closely to the correct provenance of the tree, much improvement in nitrogen fixation can occur. Further inoculation surveys are needed to combine the most appropriate *Rhizobium* strains and arbuscular mycorrhizal fungi to maximize the potential of *Prosopis* species.

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