Agroforestry Systems 19: 15–25, 1992.

Vegetative propagation studies of gum arabic trees. 2. The vegetative propagation of adult *Acacia senegal*

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Key words: *Acacia senegal*, -cutting, adult trees

Abstract. Vegetative propagation of *Acacia senegal* is possible from branch fragments taken from the crown of mature trees (13 years old), without causing ortet destruction.

The most responsive planting stock is taken from cuttings 15 cm long (with 12 to 15 nodes) and a diameter of 10 ± 6 mm (wood of 2 to 4 years old). With this material, the ablation of the leaves prior to planting is a factor which furthers development of the cutting's root system.

The development of the root system is also highly influenced by the time at which the cutting is taken: results vary from 10% during the dry season to more than 70% during the rainy season (June–October).

Cuttings can be taken from branches 50 cm long. It is thus possible to preserve the planting stock for 8 days in a continually humidified jute cloth and then to make the cuttings at the time of establishment in a nursery, without changing their rhizogenic aptitude.

The cuttings show characteristics of maturity (slow growth, plagiotropy, fructification). It is nevertheless possible to preserve the collected genotypes in the field, or better, in planting pots in the nursery, so as to further the process of physiologic rejuvenation with the aim of *in vitro* cloning.

Résumé

Le bouturage d'*Acacia senegal* est possible à partir de fragments de rameaux prélevés dans le houppier d'arbres adultes (13 ans), sans destruction de ceux-ci.

Le matériel végétal le plus réactif est constitué par des boutures de 15 cm de long (12 à 15 noeuds) et de 10 ± 6 mm de diamètre (bois de deux à quatre ans). L'ablation des feuilles avant mise en culture est un facteur favorable à l'enracinement des boutures.

Celui-ci est aussi très influencé par la date de prélèvement des boutures et il varie de 10% en saison sèche à plus de 70% en saison des pluies (juin-octobre).

La collecte de boutures peut se faire sous forme de fragments de rameaux de 50 cm de long. Il est alors possible de conserver ce matériel pendant huit jours dans une toile maintenue humide et d'y découper les boutures au moment de la mise en place en pépinière, sans altérer leur aptitude rhizogène.

Les boutures présentent des caractères de maturité (croissance lente, plagiotropie, fructification). Il est cependant possible de conserver les génotypes ainsi mobilisés au champ, ou mieux, dans des pots en pépinière, afin d'amplifier le processus de rajeunissement physiologique en vue de leur clonage *in vitro*.

1. Introduction

Acacia senegal (L.) Willd. is a shrub-like species growing in the Sahelian zone. It is adapted to an annual rainfall of 250 to 750 mm and eight consecutive months of drought [Giffard, 1966]. It fixes atmospheric nitrogen due to its symbiosis with *Rhizobium* [Dreyfus and Dommergues, 1981]. Some authors believe that it thus improves soil fertility [Gerakis and Tsangarakis, 1970]. It has an anti-erosive action, provides firewood, aerial fodder and most importantly, gum arabic [Giffard, 1966; Cheema and Qadir, 1973].

As a result of the recurrent droughts in the Sahel since 1970, human and animal pressure on this species have increased. The stands of gum trees are disappearing. Gum production is decreasing to a point where Senegalese exports which reached 9200 tons in 1971, are now at 800 tons [Mbaye, 1988].

To re-establish acceptable levels of production which are capable of generating foreign currency, and to reconstitute vegetative cover, reforesting with *A. senegal* should be promoted. The search for high-yielding and drought-resistant gum trees, which are also able to reproduce these characteristics therefore constitutes a program of fundamental economic and ecologic importance.

The selection of trees, based on gum yield characteristics, may be done only once they have attained maturity. However, these mature trees do not fully retain their ability to vegetatively reproduce. In particular, the ability of the cutting to root decreases with the age of the ortet from which it was taken [Franclet, 1981; Bonga, 1982]. The collection in proximity to the laboratory of the first vegetative specimens of selected trees (genotypes) and then their progressive rejuvenation thus constitutes a precondition for clonal multiplication.

The aim of the present study is to refine these methods. It follows up the work of Badji, Ndiaye, Danthu and Colonna [1991], taking into account the various material difficulties imposed by field conditions.

Since the stump shoot possesses juvenile characteristics which favor the attainment of our objectives [Franclet, 1981], it requires that the tree be thinned but this poses several constraints. One can note that: (i) selected trees must be cut while they are being exploited by the local population, (ii) difficult and uncertain growth of the stumps [Giffard, 1966], (iii) necessary protection of the stump from browsing livestock, (iv) the necessity of repeated site visits (prospection, thinning, surveillance, harvesting). One must thus focus on small branches which can be harvested at one time, without causing destruction to the ortet.

This study therefore focuses on planting stock production from lengths of small branches taken from the crowns of selected trees.

One should first determine the most responsive planting stock as well as the factors which will permit both the highest rates of survival and the establishment of the cutting's root system.

Given that the prospection of the best subjects must be conducted throughout the country and beyond, the time which the branches are in transit between the sampling site and the cutting nursery could be quite long. It would therefore be useful to determine if the selected planting stock can be harvested and conserved for several days prior to its planting, without losing its capacity to vegetatively propagate.

Finally, survival of rooted cuttings should be tested to improve the long-term possibilities for conservation of selected genotypes.

2. Materials and methods

Parts of branches were taken from the crowns of 13-year-old trees at eye level. The trees were located at the Bandia Research Station (17°01'W, 14°34'N) in Senegal. These samples, except as otherwise indicated in the text, were taken in May 1990. The tropical ferruginous soils found at the station are depleted and degraded. The average annual rainfall is approximately 450 mm. A substantial deficit was registered in 1990, when incident precipitation was only 299 mm.

The samples were taken from pieces of branches 50 cm long and conserved in a jute cloth which was kept humid. These branches were pruned to produce cuttings 15 cm long (except as otherwise indicated in the text) immediately prior to their placement in perforated polyethylene potting bags (12 × 25 cm, when flat) which contained a substratum composed of basalt chips (< 5 mm), sand from Mbao and soil rich in humus from Mbao, in volume proportions of 9/2/1. The cuttings were planted at a depth of 3 to 5 cm in the substratum and placed in a Gulfy cold frame [Grolleau, 1989], under an awning. The unregulated interior temperature varied from 20 °C to 38 °C according to the season, the time of day and the amount of shade. Atmospheric humidity in the cold frame was maintained at saturation point by a twice daily spraying with water. A weekly anti-fungal treatment was also applied: benlate (70 mg l^{-1}) and aliette (1 g l^{-1}) alternately.

Uniform hormonal treatments were applied before planting: the bases of the cuttings were coated with talcum powder containing 4% β-indolyl butyric acid (Rhizopon AA 4). This dosage was selected after trials covering a spectrum of concentrations ranging from 0 to 8%.

The purpose of the first series of experiments was to determine which planting stock was best adapted to the objectives enumerated above; the effects of the following five factors were studied:

- The age of the branches, as indicated from the diameter of the cuttings. Four classes were compared (< 4 mm, 4–9.9 mm, 10–15.9 mm and 16–25 mm) from samples taken during May from which the leaves had been removed.
- The length of the cutting. Three lengths were compared: 7, 15 and 23 cm.

The cuttings were taken in May, had diameters of 10 to 16 mm and their leaves had been removed at establishment time.

- The influence of leaves remaining on the cuttings. Cuttings (of 15 cm in length, diameters of 10–16 mm, taken in May) with four leaves remaining were compared with those which had been subjected to a complete denudation of the leaf system.
- The date of collection. Cuttings were taken at eight different times: January, March, April, May, July, September, October and December. The cuttings were 15 cm in length, had diameters of 10–16 mm and had their leaves removed at the time of establishment.
- From the planting stock defined above, conservation trials were conducted on pieces of branches which were maintained in a permanently humid jute cloth. The objective was to determine how long it was possible to preserve the cuttings before they lost their capacity to survive or produce roots. The duration of conservation was examined over six-time periods: 0, 2, 4, 6, and 8 days.

The objective of the final experiment was to study the behaviour and survival of rooted cuttings after transplanting. This was done in order to gain an idea of the possibilities for long-term conservation of the genotypes (head of clone) to be propagated. Two conservation methods were compared: (i) in 20 litre clay pots containing a mixture (1/2, v/v) of soil rich in humus and sand from Mbao which was watered daily, (ii) in the field, after plantation in small pit-holes (20 × 20 × 30 cm) at the Bandia Station, during the early part of the rainy season (August 14, 1990). These were planted after cumulative precipitation reached 80 mm. The plants thus received only natural precipitation (219 mm).

The parameters measured during the experiments were:

- The survival of the cuttings. A cutting was considered viable if it had at least one green leaf.
- The number of cuttings which sprouted at least one shoot.
- The number of cuttings which took root, i.e. having at least one root visible through the polyethylene bag.

The experimental design was full randomization, in each of two separate blocks, each of which contained 28 to 30 cuttings for each protocol described above. The analyses of variance and the comparisons of means were conducted after angular transformation of frequencies. In the tables (for each parameter) and figures, the values having the same index (a, b or c) belong to the same homogeneous group as established by the Newman-Keuls test at a 5% level of significance. The confidence interval of the means is 95%.

3. Results

3.1. *Definition of the most suitable planting stock*

Diameter of ramets

When the cuttings have a diameter of less than 4 mm (sprouted during the year of sampling), their survival rate after 90 days is zero (Table 1). Half of the large diameter cuttings (> 16 mm) are viable three months after establishment. A large majority of these cuttings produced at least one branch, however none of them produced roots.

Table 1. Survival, ramification and rooting of *Acacia senegal* cuttings, as a function of the diameter of the ramet, 90 days after planting (cuttings of 15 cm, taken in May from which the leaves had been removed at establishment).

Diameter of cuttings (mm)	Number of surviving cuttings (%)	Number of cuttings with new shoots (%)	Number of rooted cuttings (%)
< 4	0c	—	—
4–9.9	93a	30a	45a
10–15.9	68b	36a	39a
16–25	50b	43a	0b

For the two intermediate classes (4 to 9.9 mm and 10 to 16 mm), the survival rate is significantly lower for cuttings of larger diameters. However, in the two cases, approximately a third of the cuttings produced at least one root and one new shoot.

Length of cuttings

The study of the influence of cutting length, demonstrates the poor performance of long cuttings (23 cm) which dry out very rapidly and die; only 7% of them survived and had set roots after 90 days (Table 2). Cuttings of 7 cm show a good capacity to set roots but sprout few shoots. The best compromise is obtained with cuttings of 15 cm, as all those which established roots three months after planting (approximately 1/3) also produced new shoots.

Presence of leaves

Table 3 illustrates that the rooting and survival rates are significantly higher when the leaves are removed from the cuttings. After leaf removal, 68% of the cuttings survive and 39% set roots three months after establishment, compared with 39% and 11% respectively when four leaves are left on the stem.

Table 2. Survival, ramification and rooting of *Acacia senegal* cuttings, as a function of their length, 90 days after planting (cuttings 15 cm long, diameter 10—15.9 mm, taken in May from which the leaves had been removed at establishment).

Length of cuttings (cm)	Number of surviving cuttings (%)	Number of cuttings with new shoots (%)	Number of rooted cuttings (%)
7	57a	7b	47a
15	68a	36a	39a
23	7b	7b	7b

Table 3. Effect of ablation of leaves on the survival, ramification and rooting of *A. senegal* cuttings 90 days after planting (cuttings 15 cm long, diameter 10—15.9 mm, taken in May).

Condition of foliage at planting time	Number of surviving cuttings (%)	Number of cuttings with new shoots (%)	Number of rooted cuttings (%)
No leaves	68a	36a	39a
With leaves	39b	30a	11b

Period of collection

Cuttings were planted which were taken from the same tree at different times of the year. The eight dates selected represent four distinct climatic periods: the peak dry season (January–March), the peak rainy season (June–October) and two intermediary dates.

Rooting was optimal (more than 70%) when cuttings were taken during the rainy season (Fig. 1) and minimal but not zero (about 10%) during the dry season. During the month of May, the rooting rate was 30 to 40%, which is illustrated in the results contained in Tables 1, 2, and 3.

Three months after collection, regardless of the date on which the sample was taken, the cuttings manifest a root system having on average 25 $\pm$ 7 roots and a length of 7.2 $\pm$ 3.1 cm without callus (Photograph 1). Their tropism was very variable: the same cutting possesses horizontally oriented roots (angle in relation to the vertical of $\geqslant 60°$) and geotropic roots (angle $\leqslant 30°$); none of them had developed a vertical system.

The cuttings produced on average 1.5 $\pm$ 0.4 new shoots. Only one shoot out of six grew orthotropically (angle formed with the vertical of $\leqslant 30°$) and 50% were plagiotropic (angle $\geqslant 60°$) (Photograph 2).

About 20% of the cuttings produced inflorescence either from the primary

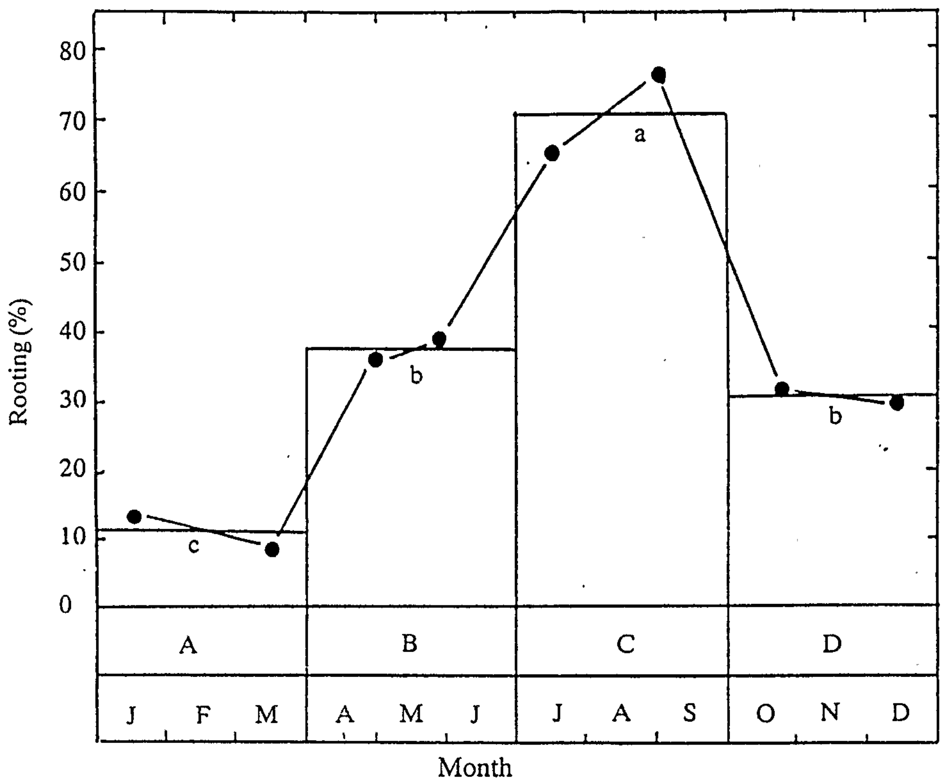


Fig. 1. Rooting success of *A. senegal* cuttings (15 cm long, diameter 10–15.9 mm) 90 days after planting as a function of the month or season in which the cuttings were collected (A: mid dry season, B: end dry season, C: rainy season, D: early dry season).

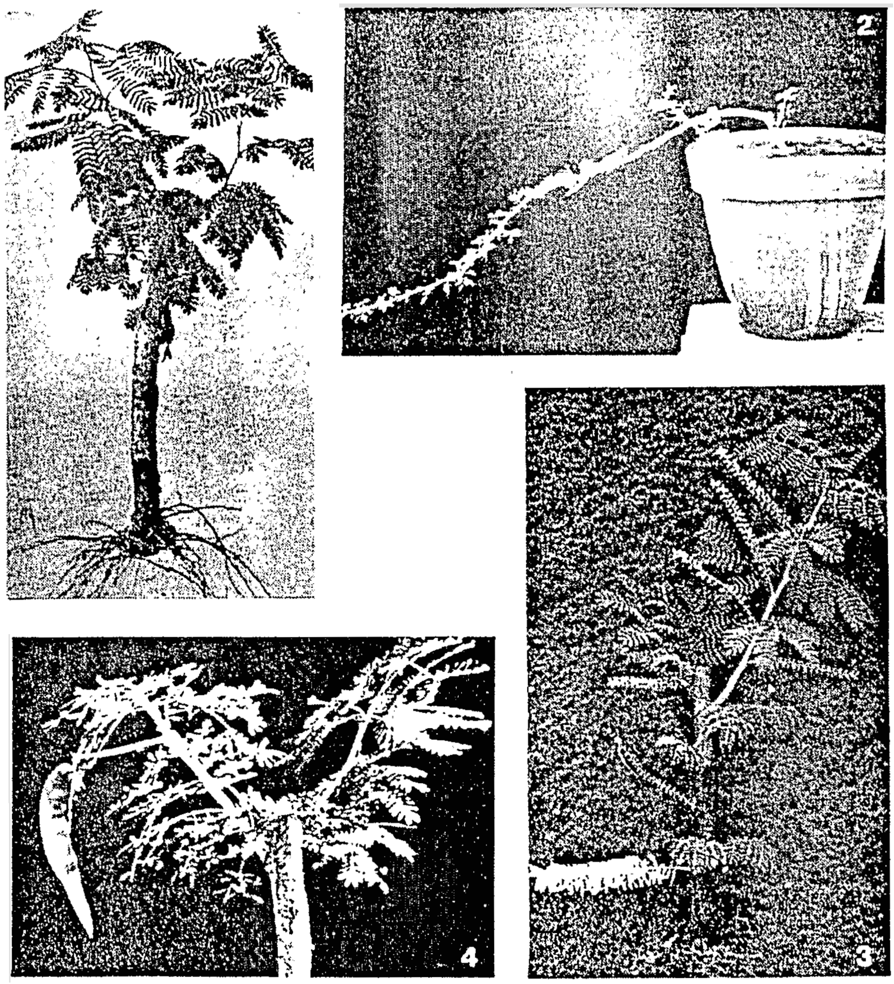
explant or from the newly formed shoots (Photograph 3). These may develop into pods containing seeds (Photograph 4).

Impact of storage time on sections of shoots taken from the field

From 35% to 46% of the cuttings made from sections of branchlings (diameter of 10–15.9 mm) kept in a humid cloth during 2, 4, 6, or 8 days rooted and produced new shoots branchlings after 90 days. These results do not show a significant difference from the rate of root establishment measured in the control group (cuttings established directly following sampling): 36%.

3.2. Conservation of clonal stock plant

Two conservation methods were employed for the cuttings sampled in May: in clay pots (Photograph 2) both in the nursery and in the field. After one year, at the end of the dry season, one third of the cuttings established in the



Photograph 1. Rooted *A. senegal* cutting showing new shoot growth, three months after planting (×0.3).
Photograph 2. One-year-old *A. senegal* cutting, conserved in a pot (×0.08).
Photograph 3. A flowering *A. senegal* cutting, three months after planting (×0.25).
Photograph 4. Fruiting *A. senegal* cutting, four months after planting (×0.3).

field remained alive (Table 4). They thus tolerated a period of seven months without water, in addition to a rainy season which was well below average (see 'Materials and methods'). Moreover, they were subjected to attacks by herbivores such as hares and myriapods. However, the cuttings which survived produced substantial growth, up to almost a meter in height. When the cuttings were kept in the nursery and watered daily, the survival rate after transplantation was 85% but their growth was less.

Table 4. Survival percentage of 1-year-old cuttings from *Acacia senegal* as a function of method of conservation (cuttings taken in May 1990).

Conservation method	Water source	Survival after 1 year (%)
In nursery	Daily watering	85a
In field	Rainfall	33b

4. Discussion and conclusion

This study shows that it is possible to propagate mature *A. senegal* (13 years of age when the life-span of this species doesn't excess 25 years [Aubreville, 1950]) from sections of ligneous shoots taken from the crowns of selected trees without provoking destruction of the ortet. It confirms the results obtained from four year old trees of the same species [Badji et al., 1991] and is similar to work conducted on adult *Faidherbia albida* [Danthu, 1991] branch fragments and roots.

The optimal planting stock is composed of sections of branchlings of 10 ± 6 mm in diameter and 15 cm in length (having 12 to 15 nodes).

The rooting success after 90 days of establishment was very dependent on the time at which the cuttings were taken. It varied between 10% for those cut during the dry season, to 70% for those cut during the rainy season, thus confirming the observations of Badji et al. [1991]. Two variables might explain these differences: the physiological state of the planting stock at the time the sample was harvested and the cultural conditions, in particular, the temperature inside the cold frame. However, it is not possible under our experimental protocol to disassociate these two factors.

The success of root establishment of our cuttings was significantly higher when their leaves were removed prior to planting. This result does not correspond with those of numerous other studies of ligneous propagation [Reuveni and Raviv, 1981; Leakey et al., 1982; Oduol and Akunda, 1988] nor is it in accord with those results reported by Badji et al. [1991] which treated small diameter cuttings (<4 mm) from *Acacia senegal*. After 60 days, these authors obtained: (i) a survival rate equal to 11.5% and a zero rate of root establishment for completely defoliated cuttings; (ii) a survival rate of 62% and a 12% rate of root establishment for cuttings which retained four leaves.

This contradiction may be less real than apparent. It is probable that small diameter cuttings contain insufficient reserves. Their metabolic functions cannot continue for two to three months without the contribution of photosynthesis. Moreover, one can see in Table 1 that no cutting with a diameter less than 4 mm was alive 90 days after planting. Only the presence of functioning leaves can facilitate both survival and formation of new roots among small diameter cuttings. In contrast, cuttings having diameters two to four times greater contain sufficient reserves to survive and also develop a root system due to the effect of rhizogenic substances.

One can also note that the works of Paton et al. [1971] showed that the influence of mature leaves of *Eucalyptus grandis* presented an impediment to root establishment. The results obtained here could also therefore be explained by the possible inhibiting influence of the mature leaves.

From a practical viewpoint, this capacity of large diameter cuttings to root in the absence of leaves allows their conservation in a humid cloth at least one week before use. During such a period of conservation, the leaves die and it is necessary to remove them before planting without altering the rhizogenic capacity of the cuttings. Thus, one has eight days to transfer the pieces of small branches from the sampling sites to the nursery.

Once rooted, the cuttings can be kept during a long period of time in the field or in pots in the nursery. The latter solution is preferable as plant survival is improved: it is possible to supplement natural precipitation and more effectively protect the plants from parasites and herbivores.

The growth of rooted cuttings is generally slow. They are often plagiotropic and show, in numerous cases, inflorescence. Both observations illustrate the state of physiological maturity of this planting stock [Franclet, 1981] and the necessity to initiate a process of rejuvenation in order to conduct *in vitro* cloning of this material. Studies in this area are presently being undertaken.

Acknowledgements

This study was financially supported by the C.R.D.I. (The Senegal Vegetative Multiplication Project, n° 3-P-87-0267), the CIRAD ("FAC-Binôme" n° 467–650), and by CEE (contract n° TSL* 0169). The authors would like to thank J. Roussel and the late A. Grolleau for their knowledgeable advice, A. Sarr and M. Sagna for their technical assistance and L. Livingston for the translation of this article.

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