## Vegetative propagation studies of gum arabic trees. 1. Propagation of *Acacia senegal* (L.) Willd. using lignified cuttings of small diameter with eight nodes

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Abstract. In order to study and monitor rates of rooting, callusing and survival, small diameter stem cuttings (less than 0.4 cm) with eight nodes (12–14 cm long) were taken from trees in the field at different times of the year in tests with small batches of cuttings. Among the four tested substrates, well drained crushed basalt powder placed on a bed of gravel was the best rooting medium. Rooting was generally poor or even non-existent with this type of cutting, but the presence of leaves both promoted rooting and significantly (using Chi square test) improved survival of the cutting. Four leaves were sufficient to assure the necessary metabolic functions. Only stem cuttings collected during the rainy season gave roots and then, only when hormonal treatments were applied to the cuttings. 8%-IBA resulted in significantly better rooting than did 2%-IBA, 0.2%-NAA and 1%-IAA. With 8%-IBA, the rate of rooting varied between 50 and 70% for leafy cuttings collected in the rainy season. Hormonal treatments had a significant effect on the survival rate of the stem cuttings during the two months observation period. Further investigations are being conducted with different types of cuttings.

**Resumé**. Des boutures de huit noeuds (12 à 14 cm de long) et de faible diamètre (inférieur à 0.4 cm) ont été prélevées au champ, sur des arbres adultes, à diverses périodes de l'année en vue d'étudier leurs taux d'enracinement, de formation de cals et de survie. La poudre de basalte concassée sur lit de gravier, avec un bon drainage, s'est révélée comme le meilleur substrat. Avec ce type de bouture, la présence de feuilles parait absolument indispensable pour obtenir un enracinement. Quatre feuilles sont suffisantes pour assurer le rôle trophique qui est probablement le leur. La néo-formation de racines n'est apparue que sur les boutures prélevées durant la saison des pluies. Sans traitement hormonal les boutures du type étudié n'ont donné aucun résultat. L'AIB-8% en poudre se révèle meilleur que l'AIB-2%, l'ANA-0.2% et l'AIA-1%. Avec l'AIB-8%, les taux d'enracinement vont de 50 à 70% si le substrat, l'hygrométrie, la présence de feuilles et la période de prélèvement sont convenables.

## 1. Introduction

Sahelian ecosystems have been severely damaged by the combined effects of extended drought and the excessive exploitation of natural resources by man and livestock. Leguminous woody perennials play a part in the soil conservation and enrichment, in the reconstitution of vegetative cover and in the establishment of agroforestry systems in this semi-arid region. Among these species, *Acacia senegal* (L.) Willd. produces fodder and firewood and has the additional advantage of exuding gum arabic, a product of economic value [ITC, 1983]. The species is well adapted to the edaphoclimatic conditions of central and northern Senegal [Giffard, 1975].

To assure success with this species, commercial ventures and reforestation projects will have to plant more drought resistant and high-yielding varieties of *Acacia senegal*. There is also a need for researchers to select both the partners, *Acacia senegal* and *Rhizobium*, of the nitrogen fixing symbiosis. Selection of Rhizobium bacteria is presently being conducted [Dreyfus et al., 1981; Badji et al., 1988]. As for the tree, it is necessary to obtain a reproductible method of propagation of the best individuals available. This calls for reproduction of individuals selected in nature for their abilities to produce large quantities of biomass and gum arabic, to resist drought and heat stress, and to have growth in poor soils.

This study was developed to determine if vegetative propagation by cuttings from adult *Acacia senegal* trees is possible and to give an initial idea of the conditions required for rooting.

#### 2. Materials and methods

The work was carried out in three experiments at the 'Direction des Recherches sur les Productions Forestières de l'ISRA' and at the 'Centre ORSTOM' in Dakar, Senegal. The preliminary experiment 1 concerned the nature of substrates. The preliminary experiment 2 concerned the presence of leaves, the period of collection and hormonal treatments. The third experiment used the results from the first two experiments.

Stem cuttings were made in the morning by pruning branches of tree saplings (4 years old) in the field. Selected branches were located in the upper part of the crown. The lignified cuttings were 12–14 cm long and had a mean diameter less than 0.4 cm. Each cutting contained eight nodes from the range of the seventh to the fifteenth as numbered from the tip of the twig.

Stem cuttings were soaked briefly in a 70 mg  $1^{-1}$  benlate solution as fungicide, then cut slantwise on the lower side and coated with powder containing phyto-hormones or rhizogenous substances according to the prescribed treatment. They were then immediately planted in the substrate.

Well-drained earthenware pots were used for the substrate experiment. In the other cases, wooden boxes  $(55 \times 55 \times 11 \text{ cm})$  with holes at the bottom to allow adequate drainage were used as propagators. All the propagators were put in a shaded cold greenhouse, under mist ten minutes per hour during a twelve hour day. The greenhouse temperature was not regulated and the relative humidity did not fall below 70%.

After two months, the number of cuttings that had given at least one root

(rooting), the number of cuttings that had callused without roots (callusing) and the number of cuttings surviving without roots and calluses (survival) was determined (Fig. 2). Evidently, the number of *all* (total) surviving cuttings is 'survival +callusing +rooting'.

## 2.1. Preliminary experiment 1: appropriate substrate

This experiment on substrates comprised four treatments: 1 - sterilizedDior type soil [Maignien, 1965], 2 - sterilized mixture (1-1, volume) of Dior soil and decomposed sawdust,  $3 - Fertiss^*$  turf, 4 - powder (granulometry of coarse-grained sand) of crushed basalt (layer 7 cm deep) on a bed of gravel, treated with benlate. Cuttings for this experiment were collected in early October; they had conserved one active leaf per cutting and were treated by IBA-2%. One hundred stem cuttings by treatment were observed. For any one treatment, there were five pots of twenty stem cuttings.

# 2.2. Preliminary combined experiment 2: presence of leaves, periods of collection, hormonal treatments

The effects of three factors were observed. These factors were:

- 'presence of leaves' with two modalities (L = leafy cuttings, NL = leafless cuttings);
- 'periods of collection' with 4 dates (November 2 = early dry season, March 6 = middle dry season, May 27 = early rainy season, July 29 = middle rainy season.);
- 'hormonal treatments' with 5 treatments (control without hormone, IBA 8% as Rhizopon AA8\*, IBA 2% as Rhizopon AA2\*, IAA 1% as Rhizopon A1\*, NAA 0.2% as Rhizopon B0, 2\*).

For each date of collection, there was one wooden box by hormonal treatment as specified in Fig. 1; each box contained a plot of 10 leafy cuttings (L) and a plot of 10 leafless cuttings (NL); the five wooden boxes (one by hormonal treatment) contained together 50 leafy cuttings and 50 leafless cuttings.

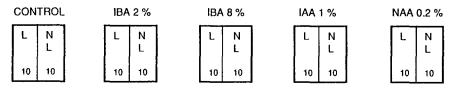
Consequently, for the four dates of collection, there were 400 cuttings (forty plots of ten) divided into 200 leafy cuttings and 200 leafless cuttings.

## 2.3. Experiment 3: confirmation test

The best of the preceding treatments were applied in a third test, one year later, in order to confirm whether the results could be reproduced. The stem cuttings were taken in August, immediately treated as outlined above

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#### HORMONAL TREATMENTS



*Fig. 1.* Experimental arrangement, with number of cuttings, for each of the four "Dates of Collection". L = leafy cuttings, NL = leafless cuttings.

and then planted in propagators containing crushed basalt on a bed of gravel. Propagators were put in the greenhouse under mist (see 'Methods'). Three treatments were compared: 1/ control without hormone; 2/ IBA-8%; 3/ NAA-0.2%. Each treatment was applied to sixty cuttings divided in three replicates.

### 3. Results and discussion

For the two first experiments, Chi square  $(\chi^2)$  test [Pearson, 1966] was employed with Yates' correction [Yates, 1951] if necessary  $(\chi^2 c)$ . The probability that the resulting distribution is due to random effects is generally less than 0.01 implying that the effects of treatments were significantly different from the control. For the third experiment, variance analysis (Newman-Keuls test at 5%) was used; angular transformation (y = 2 arc sinus  $\sqrt{x/n}$ ) was applied for frequency datas [Dagnélie, 1975].

## 3.1. Appropriate substrate

Among the tested substrates, the crushed basalt powder gave the highest level of rooting, but this was only 11% because the other conditions were not optimal. Differences with the other treatments were largely significant (Table 1). This substrate is the best drained one, and presents the best resistance to microorganisms development. On the contrary, many fungal mycelium appeared in *Fertiss* turf. This medium is too rich, it gave a strong microorganism proliferation. Such a proliferation was very detrimental to the survival of cuttings.

#### 3.2. Leaves, date of collection, hormone

The results are summarized in Fig. 2. Utilization of the Chi square test allows regrouping of the data corresponding to the levels of each factor to be analyzed [Schwartz, 1963].

Parameters	'Substrate' treatments				Chi square test		
	A	В	С	D	$\chi^2$	a	S
Initial number of cuttings	100	100	100	100	_	_	
Number of rooted cuttings	3	4	0	11	12.3	< 0.01	**

Table 1. Influence of 'substrate' on rooting of lignified, 8-node Acacia senegal cuttings of  $\leq 0.4$  cm diameter, with one leaf.

A. sterilized 'Dior' soil; B. sterilized mixture (1-1, v/v) of 'Dior' soil and decomposed sawdust; C. *Fertiss* turf; D. powder of crushed basalt; S. significance; \*\*. highly significant.

*Presence of leaves.* After two months, the 200 leafless stem cuttings didn't give any rooting or callusing, the number of total surviving cuttings was 23; for the 200 leafy cuttings, this number was 124; this result was very highly significant (Chi square = 109.7;  $\alpha < 0.001$ ). For 'rooting', the values were 0 for the 200 leafless cuttings and 24 for the 200 leafy cuttings; this result was so very highly significant (Chi square = 23.5;  $\alpha < 0.001$ ). For 'callusing', the results were 0 and 7, respectively, for leafless or leafy cuttings; this difference was just significant (Chi square = 5.2;  $\alpha < 0.05$ ).

So, the retention of some (4) active leaves seems to be necessary for these small diameter stem cuttings which do not have very large reserves. Comparable results are reported in the literature (Reuveni and Raviv).

*Periods or seasons of collection.* The results above had shown that cuttings of small diameter without leaves did not produce roots. Thus, it was only from leafy cuttings that the effects of period of collection could be established (Fig. 2).

Stem cuttings collected in the early rainy season (50 individuals) and in the middle rainy season (50 individuals) had given better 'rooting' rates (26% and 22%, respectively) than those collected in the early dry season (50 individuals) and in the middle dry season (50 individuals) which had shown 0% of 'rooting'. These differences were very highly significant (corrected Chi square = 23.2;  $\alpha < 0.001$ ). The period of collection was of great importance for these leafy type of cuttings of small diameter collected in the field. The composition of the hormonal pool in the tissues of the stem cuttings collected at rainy season was probably more favourable for 'rooting' than those of stem cuttings collected at dry season.

The 50 stem cuttings for each date of collection had given respectively, 38, 27, 32 and 27 total surviving stem cuttings after two months; these differences were not significant (Chi square =6.9;  $\alpha$  =0.1). So, the dates of collection had no influence on the total survival of stem cuttings of small diameter.

Hormonal treatments (Fig. 2). Leafless stem cuttings did not root with any



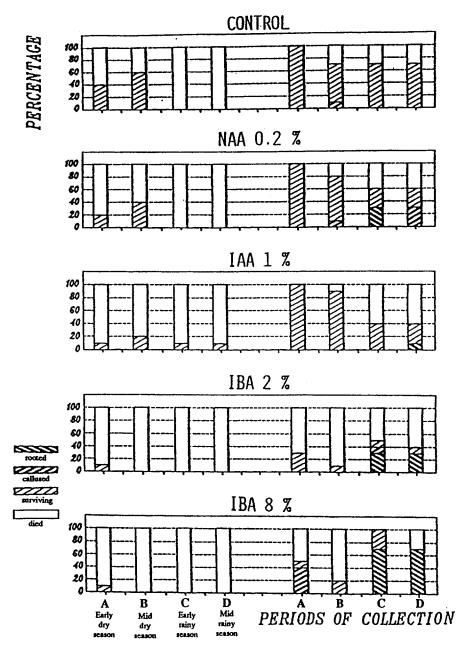


Fig. 2. Influence of presence of leaves, periods of collection and hormonal treatments on rooting, callusing, survival and death of 8-node lignified Acacia senegal cuttings.

of the hormonal treatments applied. It is the same for leafy stem cuttings collected during the dry season. Only leafy stem cuttings collected during the rainy season had produced roots under the hormonal treatments tested.

The 20 stem cuttings treated by IBA-8% had given 14 individuals with roots after two months (70 per cent); the rate of 'rooting' had risen 30 per cent for IBA-2%; 15 per cent for NAA-0.2%; 5 per cent for IAA-1% and 0 for the control without hormone. IBA-8% was the best rooting treatment. Differences between treatments were very highly significant (corrected Chi square = 29.3;  $\alpha < 0.001$ ) and reflected similar rates of rooting reported by others authors [Loreti and Hartmann, 1964; Lundquist and Torrey, 1984].

Results obtained for 'rooting + callusing' had shown similar responses (14, 7, 1, 6 and 0) to the same sequence of treatments from IBA-8% to control (corrected Chi square =26.2;  $\alpha < 0.001$ ).

With these stem cuttings collected at rainy season, the most effective treatment for 'rooting' had given the best 'total surviving' rate (17/20): hormonal treatments significantly effected the survival rate (corrected Chi square =26.2;  $\alpha < 0.001$ ).

#### 3.3. Confirmation experiment

Rooting (Fig. 3) occurred in:

- 50% of the cases after 8%-IBA powder treatment;
- 10% of the cases after 0.2%-NAA powder treatment and
- 0% for control without hormonal treatment.

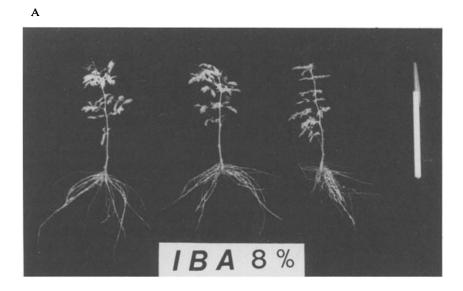
These significant (Newman-Keuls test) results (Table 2) clearly confirmed the previous results of 70%, 15% and 0, respectively, for the same treatments. The number of roots per rooted cuttings also confirmed that 8%-IBA powder was the best rhizogenous preparation for this type of *Acacia senegal* cutting.

#### 4. Conclusions

This study provides preliminary results concerning vegetative propagation of *Acacia senegal* by cuttings. The entire range of these experiments have shown that vegetative propagation of *Acacia senegal* by stem cutting is possible, in spite of a not very high rooting capacity of this species.

To give a neo-formation of roots on eight nodes lignified stem cuttings of small diameter, this species appears to need:

- a well drained rooting medium;
- retention of some active leaves on the cutting;
- collection of cuttings during the rainy season only;
- treatment with rooting hormone (IBA-8%).



В

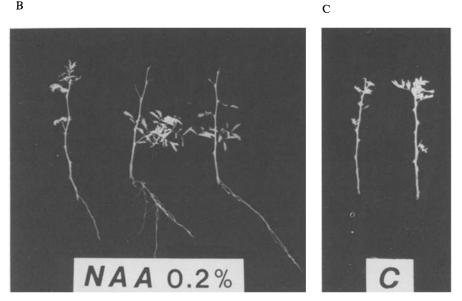


Fig. 3. Effects of hormonal treatments on rooting of eight nodes lignified cuttings of small diameter of Acacia senegal (confirmation test).

A - IBA 8% = hormonal treatment with 8% Indol butyric acid powder;

- B NAA 0.2% = hormonal treatment with 0.2% Naphtalen acetic acid powder;
- C C = control without hormonal treatment.

Treatments	After two months					
	Percentage of rooted cuttings	Number of roots per rooted cuttings				
Control (no hormone)	0% C	0 C				
8% – IBA powder	50% A	12.7 A				
0.2%—NAA powder	10% B	3.1 B				

Table 2. Results of trial  $n^{\circ}3$  testing the best conditions of rooting (see text). For each treatment there were 60 initial cuttings in three replicates of 20.

Data followed by different letters are significantly different (Newman-Keuls test at 5%).

If these conditions are respected, the rooting rate can rise 50 to 70%.

Further investigations are proceeding on the dynamics of different types of cuttings.

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