Meristem Micrografting of Adult *Faidherbia albida*

C. Detrez[1], S. Ndiaye[1], F. Kerbellec[1]
N. Dupuy[1], P. Danthu[2], and B. Dreyfus[1]

Abstract

Shoots isolated from vegetative buds (apical or axillary) on branches of adult Faidherbia albida, *were grafted in vitro on 2-day old seedlings. Grafts began elongating after only 10 days in culture. Shoots obtained by this method were used for second- and third-generation micrografting cycles. Micrografted seedlings developed well after acclimatization in the greenhouse. This technique resulted in rapid multiplication of selected ortets on root stock derived from seed. This method may facilitate rapid clonal increase of selected adult individuals.*

Introduction

Faidherbia albida is characterized by great genetic variability. Clonal propagation would be a useful tool to exploit and analyze this variability. Production of selected clones could result in genetic improvement in a relatively short period and would facilitate the study of symbiotic fungal and bacterial relationships.

It is known that the cloning potential of trees diminishes as the material ages (Bonga 1987; Franclet et al. 1987). *F. albida* shoots obtained in vitro from cotyledon buds are easy to propagate, and the individuals readily take root (Duhoux and Davies 1985). However, microcuttings obtained from root suckers of adult trees do not root easily (Gassama 1989).

The state of tissue maturity at the time of removal from the adult tree can be partially and progressively reversed by various cultural practices, termed 'rejuvenation' (Nozeran 1978). The return to juvenile traits, which facilitates clonal propagation, can be achieved by successive grafting cycles (Franclet 1977; Franclet et al. 1987). It is based on this principle that the original 'chain micrografting' technique presented in this paper was developed.

Materials and Methods

Treatment and Germination of Axenic Seedlings

Seeds of four *F. albida* provenances (Kagnobon, Mérina Dakhar, Bodé, and Ovadiour) collected in Senegal by the Direction de recherche des productions forestières (DRPF) of ISRA were scarified by immersion in concentrated H_2SO_4 for 1 h. They were then copiously rinsed in sterile water, immersed in 0.1% mercuric chloride for 30 seconds, and rinsed again in sterile water. Seeds were then soaked in sterile water for 3 to 4 h. The seeds were germinated on agar-agar water (0.8%) in the dark.

Choice of Ortets and Preparation of Grafts

Grafting stock was taken from three types of ortets: (1) axenic seedlings at the cotyledon stage; (2) 1- to 2-month old seedlings from the greenhouse; and (3) an adult tree (50 years, Bel-Air, Dakar). Ramets (leafy, soft-tissue branches, 5-7 cm long) from ortet types (2) and (3) were collected and sterilized in 0.1%

1. Institut français de recherche scientifique pour le développement en coopération (ORSTOM), B.P. 1386, Dakar, Senegal.
Institut sénégalais de recherches agricoles (ISRA), B.P. 2312. Dakar. Senegal.

Detrez, C., Ndiaye, S., Kerbellec, F., Dupuy, N., Danthu, P., and Dreyfus, B. 1992. Meristem micrografting of adult *Faidherbia albida*. *Pages* 91–95 *in* *Faidherbia albida* in the West African semi-arid tropics: proceedings of a workshop, 22-26 Apr 1991, Niamey, Niger (Vandenbeldt, RJ., ed.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics: and Nairobi, Kenya: International Centre for Research in Agroforestry.

mercuric chloride for 20 seconds. From these ramets, meristems, composed of meristematic domes and their bases trimmed to a bevelled edge, and protected by 1-4 young chlorophyllous leaves, were selected for grafting.

Root Stock and Micrografting Techniques

Two micrografting techniques were compared using 2-day old axenic seedlings as root stock: (1) top- or cleft-micrografting which consists of inserting the graft into an incision made in the terminal part of the hypocotyl after removal of the epicotyl and the cotyledons, and (2) lateral micropraftinp onto the hypocotyl whose cotyledons and meristem were left in place during the first 12 days following the implantation of the graft, after which they were decapitated to suppress apical dominance of the root stock.

Successive Micrografting Cycles-Adult Ortet

Shoots obtained after the first micrografting operation and the cauline elongation phase of the micrografted meristems provided a second generation of meristems for another cycle which used the top micrografting technique. This second micrografting was followed by a third, done under the same conditions.

TheCultureEnvironment

After micrografting, the plants were grown in culture tubes (25 x 150 mm) containing 10 mL of nutritive medium. The culture medium was composed of the minera1 base and vitamin mix of Murashige and Skoog (MS) (1962). with saccharose added (20 g L-l). The pH was adjusted to 5.8, and the agar (Bacto Difco 0.8%) was incorporated. Media were sterilized by autoclaving for 20 min at 110°C.

Following each micrografting cycle and after a period of elongation, the grafts were separated from the root stock and their rooting ability was tested on the MS medium, with additions of either 60 g L^{-1} of sucrose (Duhoux and Davies 1985) or 20 g L^{-1} of sucrose and indole butyric acid (5 mg L^{-1}) (Gassama 1989).

Cultures were placed in a controlled-climate chamber maintained at 28 ±2°C and were continuously exposed to 3200 lux of artificial light. The grafted seedlings were acclimatized on a substratum (Bel Air soil:vermiculite; 3:1, v:v) in a grecnhouse under humid conditions.

Results

Reactivation and Elongation of the Graft

For the three types of ortets studied, callus formation of the grafts occurred 7-10 days after insertion into the hypocotyl. The lateral clcft grafting technique (Figs. l and 2) and top-grafting technique (Fig. 3) were successful with both the juvenile and adult ortets. In the case of lateral grafting, it was necessary to

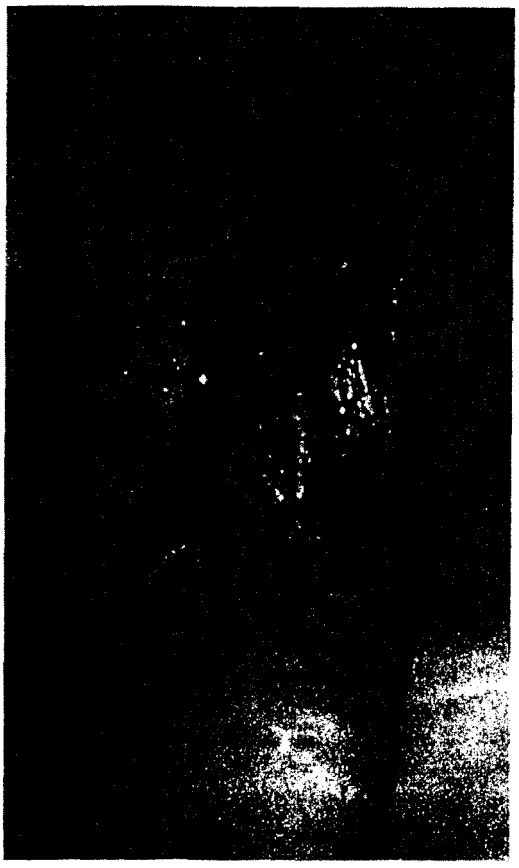

Figure L Insertion of a *Faidherbia albida* micrograft by the lateral-cleft technique. The graft (G), consists of a meristem removed from an axillary bud of a branch of an adult tree, trimmed basically to a bevelled edge, and inserted into an incision (I) made in the hypocotyl (H) of a 2-day-old seedling. (Bar = 1 mm.)

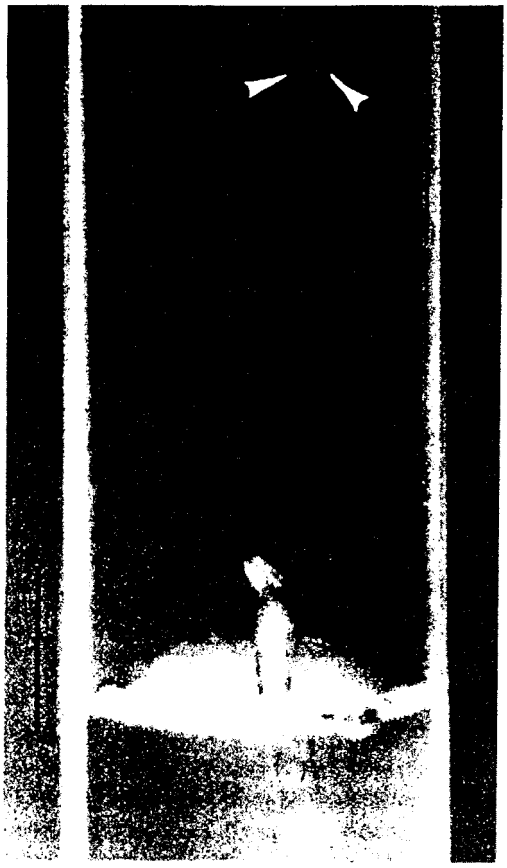

Figure 2. Elongation of a meristem removed from an adult tree and micrografted onto the hypocotyl using the lateral-cleft technique (3 weeks of growth). The meristem and the cotyledons of the root stock, kept in place for the first 15 days following the graft, were selected (see arrow) after the callus formation (C). (Bar = 1 cm.)

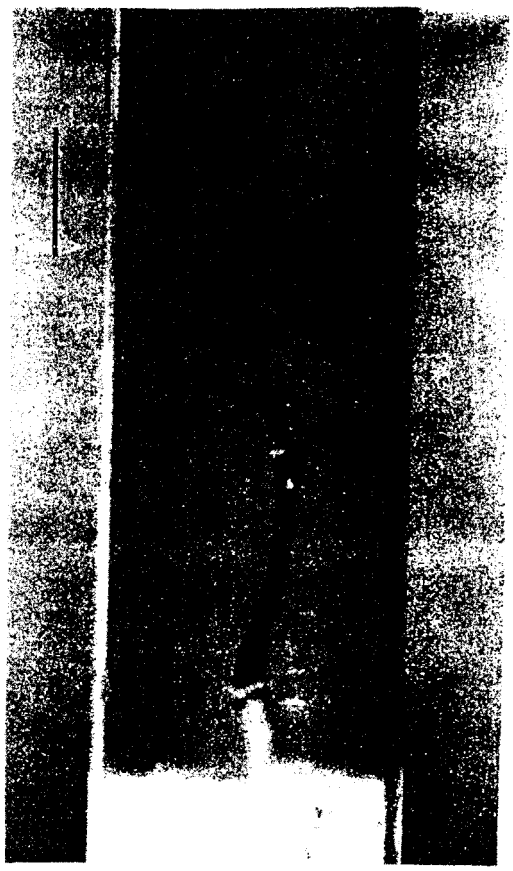

Figure 3. Elongation of a meristem taken from an adult tree, then micrografted using the top-grafting technique after removal of the cotyledons and the epicotyl of the root stock (6 weeks of growth). (Bar = 1 cm.)

remove the terminal bud of the root stock when callus formation began. This additional manipulation did not appreciably improve the degree of rooting of the graft. Therefore, the lateral grafting was discontinued and only top-grafting used.

On the juvenile ortets, callus formation was immediately followed by a foliar growth and the onset of cauline elongation after 10-12 days of growth. For the 2-day old ortets, graft elongation occurred for 23 of the 24 grafted plants. For the 1- to 2-month old ortets, elongation occurred on 22 of the 24 grafts.

In the case of the adult ortet, successful grafting appeared to be correlated with the date of ramet collection (Table 1). The best results were obtained with ramets collected at the beginning of the foliating season (Nov/Dec, Dakar region)–19 of 24 grafts observed showed cauline elongation. The placement of

Table 1. Frequency of elongation of grafts taken from an adult ortet of *Faidherbia albida* as a function of sampling period, Dakar, Senegal, 1990.

Sampling period	Phenological stage of ortet	Phenological state of ramets	Success frequency
Nov/Dec	Vegetative	Foliating	19/24
Jan/Feb	Foliating	Foliated	2/36
Mar/Apr	End foliation	Fqliated	1/48

Figure 4. Growth and development of a micrografted individual after transplanting in the greenhouse (15 weeks after the micrografting operations). (Bar = 1 cm.)

the grafts, whether axillary or terminal on the branches of the ortet, made no difference in the rate of elongation. Starting in January, the ramets became highly infected with a fungus, despite treatment of the ramets with a fungicide (Benlate®, 70 mg L^{-1}) 2 days before collection. Treatment with $HgCl_2$ failed to completely control this problem.

Micrografted individuals were maintained in a greenhouse (Fig. 4). For the first 3 months of this acclimatization period, the plants had weak stems. This was supplanted by a second, more vigorous stem originating from a basal axillary bud.

Chain Micrografting of Adult *albida*

The frequency of graft elongation increased during chain micrografting (Table 2). Likewise, the graft coefficient of multiplication (i.e., the average number of nodes produced by the foliated shoots at the end of each of the three successive micrografting cycles) increased appreciably during the second and third micrografting cycles, maximizing at a value of 10.

Table 2. **Frequency of elongation and coefficient of multiplication of grafts taken from adult *Faidherbia albida*,** following three successive micrografting cycles (G1, G2, G3), Dakar, Sengal, 1990.

	Micrografting Cycle		
Parameter	GI'	G2	G3
Frequency of graft elongation	19/24	6/8	10/10
Coefficient of multiplication*	5 M . 2 4	8.5±0.32	10.2±0.27

1. Micrografting cycle started with meristem harvests done in November and December.
2. The average number of nodes produced by two foliated shoots after 6 weeks of culture (±SE).

Discussion

It is possible to graft in vitro meristems of *F. albida* on root stock obtained from seed. Both juvenile (3 month) and adult ortets can be used. Successful grafting with adult ortets allows immediate procurement of selected individuals grafted onto seedlings, which can be readily used in field trials. This micrografting technique is advantageous as any type of bud from the ramet can be used, and two grafting locations on the hypocotyl (top or lateral cleft) are suitable. The optimal collection period determined by this study is in accordance with observations made by Danthu (1992) for horticultural cutting establishment of *F. albida*.

Chain micrografting by the multiplication of axillary buds produces an abundance of newly formed shoots and shows a high coefficient of multiplication by 6 weeks. Extrapolating this result, 6 months of chain micrografting from one graft could result in 10 000 identical grafted individuals.

Although shoots have been propagated on root stock, a suitable technique for root formation has yet to be developed. We hope to achieve rooting through tissue rejuvenation during chain grafting. Investigations currently under way in the laboratory are aimed at obtaining rooted clones, a necessary condition for a stand improvement project of *F. albida*.

Acknowledgment. The authors thank M.Y. Dommergues for his critical reading of the manuscript.

References

Bonga, J.M. 1987. Clonal propagation of mature trees: problems and possible solutions. Pages 249-271 *in* Cell and Tissue Culture in Forestry, Vol.1 (J.M. Bonga and Don J. Durzan, eds.). Martinus Nijhoff, Dordrecht, Netherlands.

Danthu, P. 1992. Vegetative propagation of adult *Faidherbia albida* by branch and root cuttings. These proceedings.

Duhoux, E., and Davies, D.W. 1985. Caulogenèse à partir des bourgeons cotylédonaires d'Acacia *alhida* et influence du saccharose sur la rhizogenèse. Journal of Plant Physiology 121:175-180.

Franclet, A. 1977. Manipulation des pieds-mères et amélioration de la qualité des boutures. Etudes et Recherches 8. 20 pp. France: Afocel.

Franclet, A., Boulay, M., Bekkaoui, F., Fouret, Y., Verschoore-Martouzet, B., and Walker, N. 1987. Rejuvenation. Pages 232-248 *in* Cell and Tissue Culture in Forestry, Volume 1. General Principles and Biotechnology (Bonga, J.M., and Durzan, D.J., eds.) Martinus Nijhoff, Dordrecht, Netherlands.

Gassama, Y.K. 1989. Culture in vitro et amélioration symbiotique chez *Acacia alhida* (Leguminosae) adulte. Pages 286-290 *in* Trees for development in sub-Sahara Africa: proceedings of a regional seminar held by the IFS, Nairobi, Kenya, 20-25 February. Kenya: International Foundation for Science.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Plant Physiology 15:473-497.

Nozeran, R. 1978. Réflexions sur les enchaînenments de fonctionnement au cours du cycle des végétaux supérieurs. Bulletin de la Societé Botanique Française 125:263-280.