African Journal of Biotechnology Vol. 9(50), pp. 8613-8618, 13 December, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.895
ISSN 1684–5315 ©2010 Academic Journals

Full Length Research Paper

In vitro micrografting of Sterculia setigera Del.

Dame Niang[1]*, Yaye Kène Gassama[1], Aliou Ndiaye[1], Maurice Sagna[1], Samba Arona Ndiaye Samba[2] and Mamoudou Abdoul Toure[2]

[1]Laboratoire Campus de Biotechnologies Végétales, Département de Biologie Végétale, Faculté des Sciences & Techniques, Université Cheikh Anta Diop de Dakar, BP 5005, Dakar-Fann, Sénégal.
[2]Laboratoire National de Recherches sur les Productions Végétales-Institut Sénégalais de Recherches Agricoles (LNRPV -ISRA), Route des Hydrocarbures, Bel Air, B.P. 3120, Dakar, Sénégal.

Accepted 18 August, 2010

An *in vitro* procedure micrografting of adult scions of Sterculia setigera was developed to overcome low rooting in adult shoot. Axenic micro shoots of 0.5 cm length taken from adult trees as scions were grafted on seedlings rootstocks cultured on MS medium. 100% success was obtained with micrografts using adult apex as scions. Upon three cycles of *in vitro* micrografting, rejuvenation capacities of S. setigera was recovered as shown by vigour, length and rooting of shoots grown from grafts cultured on MS medium compared to seedlings. Successful micrografts were transferred to plastic pots containing soil under mist house conditions before they were finally exposed to an external environment. 80% of the plantlets survived in the nursery.

Key words: Sterculia setigera, micrografting, rejuvenation, rooting.

INTRODUCTION

Sterculia setigera is a forest woody tree belonging to the Sterculiaceae with more than one thousand species (Diallo, 1997). The species grows under different ecological and soil conditions (Von Maydell, 1983). In Senegal, S. setigera known as karaya tree is specially growing in southern part of the country characterized by a Soudano-Guinean climate where the natural populations are estimated to be 20 million trees (Dieng, 1995). The gum produced by exudation is used in human nutrition, medicine preparations, cosmetics and soft drinks (Lô, 1996). Because of its commercialization, the gum contributes also to increase women's' income in the rural areas. Senegal is a major producer of gum karaya (1500 tons per year) in the world after India (3500 tons/year) (Ba et al., 1999; Jonhson et al., 2005). Beside its tremendous importance, S. setigera trees are threatened by population reduction due to gum overexploitation inducing intensive mortality and luck of natural regeneration.

*Corresponding author. E-mail: niangd@ucad.sn.

To overcome these problems, the implementation of a program for identifying and popularizing elite trees gum producer by biotechnology methods become a need for genetic resource conservations. Unfortunately, many woody plants which have economical importance for timber and/or fruit production are often difficult to generate by micropagation techniques (Damiano and Monticelli, 1998).

A suitable microgafting method is used as solution when confronted with difficult or limited rooting, characteristics of old tissues and explants (Amiri, 2005). Successful experiments of *in vitro* micrografting of tropical woody trees have been already reported on Khaya senegalensis (Sanogo, 1995), Acacia tortilis (Detrez, 1994), Acacia senegal, Faidherbia albida, Balanites aegyptiaca (Hane, 2000), Ziziphus mauritiana (Danthu et al., 2002b), Pistachia Vera (Onay et al., 2002), Prunus avium (Amiri, 2005) and A. senegal (Khalafalla and Daffalla, 2008). *In vitro* micropropagation of Sterculia urens from seedling plants has been developed by Sunnichan et al., 1998, Hussain et al., 2007 and Hussain et al., 2008. With S. setigera, some studies have already

been done related especially to its economic role (Lô, 1996) and agroforestry where Bakhoum et al. (1999) had demonstrated a depressive effect of S. setigera on crop yield. A first step of *in vitro* culture of S. setigera from seedling material was achieved by Diallo (1997) and its seed physiology germination and vegetative propagation was then studied by Touré et al. (2009).

This paper is the first report related to *in vitro* micrografting of S. setigera adult material. This paper aims to propose a simple and reproducible method to regenerate clones via *in vitro* micrografting of mature and elite trees.

MATERIALS AND METHODS

In vitro seeds germination and culture of rootstocks

Seeds were collected in 2004 from S. setigera natural populations of Maleme Niani (13° 57' N, 14° 18' W) located in the south-eastern part of Senegal. The scarification process is performed using concentrated H_2SO_4 (95%) for 100 min. Germination was carried out under dark condition for one week on humidified cotton. Germinated seeds were transferred in liquid medium of Murashige and Skoog (MS) (1962) free hormone and seedling were cultivated on a support of absorbent sponge. After one week in darkness, rootstocks were transferred to a lightened room (25°C under 16/8 h photoperiod pounded by white fluorescent tubes) for their growth for another week and ready to receive the scions (Figure 2A).

In vitro culture of adult material

Adult material was harvested in the same site of Maleme Niani from mature trees of approximately 20 years old. A horticultural growth of cuttings was first made in greenhouse in Dakar to allow rejuvenation before *in vitro* culture. Adult shoots taken from adult trees were cultured in pots with a mixture soil + compost then covered with transparent polyethylene to prevent shoots from desiccation. Cultures were kept in a greenhouse and watered every two days.

The plant material used for *in vitro* introduction was excised in the morning from the new shoots of one-year-old cuttings cultivated in greenhouse. Each shoot contains at least 3 to 4 nodes. They were washed in soapy water and rinsed in tap water. Double disinfection using 1% mercuric chloride ($HgCl_2$) for 8 min combined with NaOCl (8%) for 15 min was carried out. To avoid cultures from browning due to presence of polyphenols, shoots were pretreated with a solution of 150 mg/l citric acid + 150 mg/l ascorbic acid, followed by an anti-fungal treatment (5 g/l Mancozebe). Stems were cut into 1.5 - 2 cm length nodal explants and cultured in a MS medium supplemented with 1.5 mg/l BAP for *in vitro* propagation. After 30 days culture, old newly formed shoots were excised and used as microscions.

Scion source

We used scions from young material (15 days old seedlings) and scions excised from adult shoots cultivated *in vitro* during 30 days.

Grafting method

Using shoots and rootstocks compatible in diameter, cleft grafting method was realized by inserting scion (0.5 cm length) into the split of rootstock so that cambium tissues of the two plants became aligned. Grafting was stabilized by a ligature with sterile parafilm and then replaced in the growth MS medium.

After four weeks, new scions were excised from first apex grafts (G1) and grafted again on new rootstocks; the subsequent second generation (G2) is cultivated on the same medium and after three weeks, was used for G3 grafting cycle.

Rooting and acclimatization

Two steps were used for root shoots taken from elongated scions of micrografting cultures. Shoots were first induced in solutions supplemented with 20 mg/l IBA for a short period of 24 h and then transferred to a strength MS medium solidified with 8 g/l agar for root expression. Rate of rooting and root system morphology were noticed after one month.

Regarding acclimatization, successful micrografts were initially put into cups filled with a mixture of sand + soil. Each cup containing a plant is covered with a transparent plastic pocket. The cups are then maintained in shade for one month before being transferred in natural air in pots from larger diameter for their natural development. Percentages of survival were evaluated after three months of explants transfer to soil.

Observation and presentation of results

Each treatment was made with 24 grafts. After 15 and 30 days cultivation period, records were made on grafting success (%), length (cm) of scion and number of rooted shoots. A graft is considered as successful when union of rootstock and scion is followed by growth and opening of leaves. Means of shoots length and roots were analyzed using ANOVA test at 5% level.

RESULTS AND DISCUSSION

In vitro propagation of adult material

In vitro culture of S. setigera adult material seems to be difficult due to their low propagation with latency periods and long exposition to contaminations. Sterile explants obtained from disinfection were transferred to MS supplemented with 1.5 mg/l BAP for multiplication. Only 1.33 of 24 explants cultured gave shoots newly formed with a mean of elongation of about 4.58 cm. In addition to the low capacity of gum trees to react in *in vitro* conditions, its rooting is not easy like numerous woody plants (Table 1). Best percentage of rooting was obtained in MS medium and supplemented with 20 mg/l IBA during 30 days.

Micrografting of adult explants on young rootstocks can provide the best way to propagate S. setigera.

In vitro micrografting

Effect of origin of scion

Results of Table 2 show that scions from shoot-tips of

Table 1. Root formation of *S. setigera* adult shoots after 30 days cultivation on MS medium supplemented with various IBA concentrations.

IBA concentration (mg/l)	Number of shoots per media	Number of rooted shoots	Rooting (%)
15	24	0 a	0
20	24	5 b	20.83
25	24	2 c	8.33
30	24	2 c	8.33

Means followed by the same letter are not significantly different (p = 0.05) according to ANOVA test.

Table 2. Effect of scion origin on grafts success and growth.

Scion	Number of micrografts	Gourmands (%)	Grafting success (%)	Shoot length (cm)
Seedlings	24	16.66	83.33	4.90 a
Adult shoots	24	0	100	1.62 b

Means followed by the same letter are not significantly different (p = 0.05) according to ANOVA test.

seedling plantlets and shoots derived from old S. setigera propagated *in vitro* realized good percentage of grafting success. The highest percentage (100%) of successful grafts was obtained with apex of adult material (Figures 2B and 2C), while only 83.33% of success grafts were noticed with young apex scion. The high level of production of gourmands (16.66%) and their extreme vigour, competing for medium nutrients and water with grafts, affect grafting success. After one week of cultivation in MS medium, a callus development in junction of rootstock and scion was noticed. This callus was observed on all successful micrografts and can be considered as an important step in the micrografting process (Tourina and Noureddine, 2009). According to Moore (1984) and Hartmann et al. (1997), four general steps characterize a compatible graft union formation: formation of the union, development of a necrotic layer and proliferation of callus bridge at the graft interface, differentiation of new vascular and restoration of the continuity of new vascular tissue. The results of Celik (2000), suggests that the grade of callus formation at the graft union is the main factor for good compatibility between stock and scion. Another factor related to the method of grafting can influence its success.

Higher elongation was also noticed with juvenile scions than apex of adult material after four weeks of cultivation in MS medium (Table 2). These results are linked to the best capacities of young scions to grow faster when their vascular connection is effective with rootstock than adult scions. Similar results had already been obtained on Acacia mangium by Monteuuis (1995). With this species, the author mentioned that grafts with young scion elongated faster than adult scion. Different factors influence micrografting success of numerous ligneous. With Pistacia Vera L cv. “Siirt”, Onay et al. (2002) reported that success of *in vitro* grafting decreased with scions resulting from microcuttings of 1, 5, 10 and 30 years old. According to Fraga et al. (2001), in addition to scion age, grafting method, scions location harvested from trees or cuttings and their developmental stage appear to play a crucial role in establishment of Pinus radiata micrografting. Best rates of grafting were obtained by using scions of 11 months old taken in January from terminal shoots. Touré et al. (2009) reported that an application of five horticultural grafting techniques of adult scions on juvenile material has demonstrated that S. setigera grafting potential could reach 83% success with the “terminal cleft” technique compared to the others ones. Similar results were observed with S. setigera micrografting where ability of the graft unions to form successfully varied with scion type used for grafting.

Rejuvenation

Rejuvenation capacities of S. setigera were tested by comparing elongation of scions taken from different grafting cycles G1; G2 and G3 (Table 3 and Figure 1). The propagation behavior of shoots derived from these explants was compared with shoots established via bud culture from the same donor trees. Shoot explants from micrografts grow more rapidly than shoots explants from the original adult donor trees. Comparison of their length has caused an increase from G1 to G3 compared to control. G3 micrografts elongation (4.80 cm) was not significantly different from seedling shoots length (4.87 cm) and indicates a rejuvenation of adult material with re-acquisition of their growth capabilities (Figure 1).

Table 3. Influence of graft cycles on rooting performance of elongated scions during 30 days cultivation.

IBA concentration	Origin of shoots	Number of rooted shoots	Rooting (%)
20 mg/l	Seedlings	6 a	25
	Adult shoots	5 b	20.83
	G1	5 b	20.83
	G2	6 a	25
	G3	7 c	29.16

Means followed by the same letter are not significantly different (p = 0.05) according to ANOVA test.

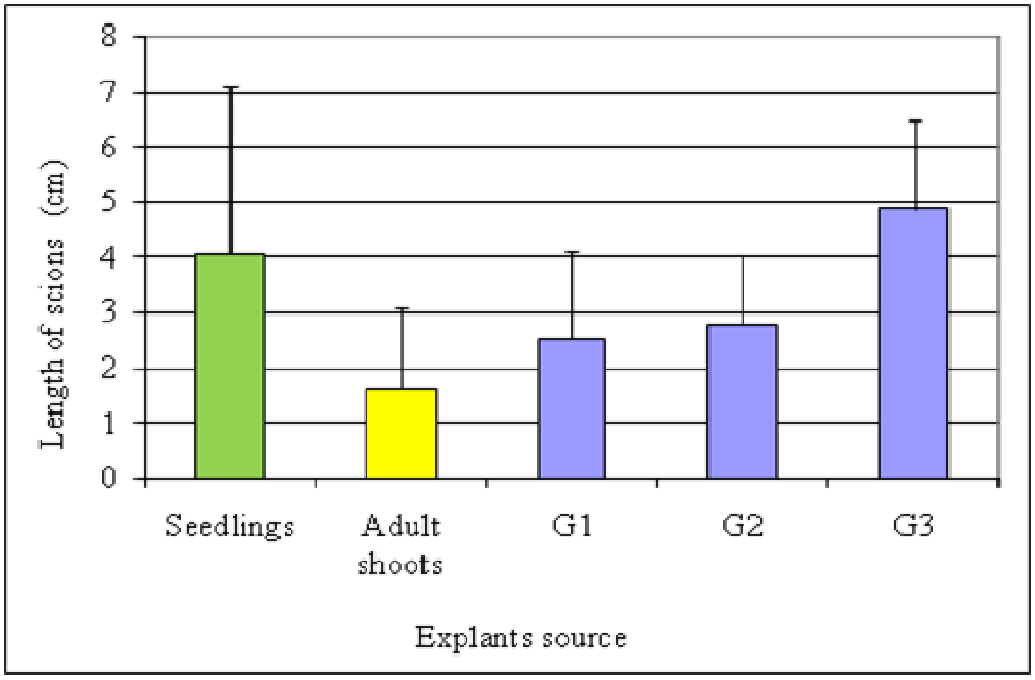

Figure 1. Influence of successive grafting on scions elongation after 30 days cultivation on MS medium.

Micrografting have been applied for rejuvenation of many species (Sanogo, 1995; Revilla et al., 1996; Estrada-Luna et al., 2002). Similar results were obtained with Larix decidua with which rooting was used as rejuvenation criteria (Ewald and Kretzschmar, 1996). Rooting experiments with cuttings from six micrografted clones resulted in 49.9 ± 11.9% rooting. Cuttings from donor trees invariably showed no root formation. The results confirm a rejuvenating influence of micrografting in larch.

Rooting aptitude of elongated shoots resulting from successive micrografting was used as rejuvenation criteria of S. setigera. Results of rooting parameters are shown in Table 3. Root induction was done in 20 mg/l IBA solution for 24 h, and then shoots were transferred to a strength MS medium for root elongation. Results have shown that successive grafts increase root formation of shoots taken on cycle G1 to G3 compared to adult shoots rooting (control). Rooting rate of juvenile shoots (25%) was achieved with shoots taken from G2 micrografting step. With G3, shoots produced higher percentage of roots (29.16%) than seedling explants. About root system aspect, the same morphology was noticed in all rooting cultures which characterize S. setigera with a pivoting rooting system.

These results indicated that successive grafting restore rooting aptitude of S. setigera adult material. Rejuvenated shoots produced roots more quickly and can be cloned with an appropriate method of propagation (Kwapata, 2003).

Micrografting of S. setigera shoots overcomes rooting difficulties of adult trees material cultured *in vitro.* Similar results have been obtained by Danthu et al. (2002a) with Faidherbia albida. The authors mentioned that as early as the third cycle of micrografting, a significant increase

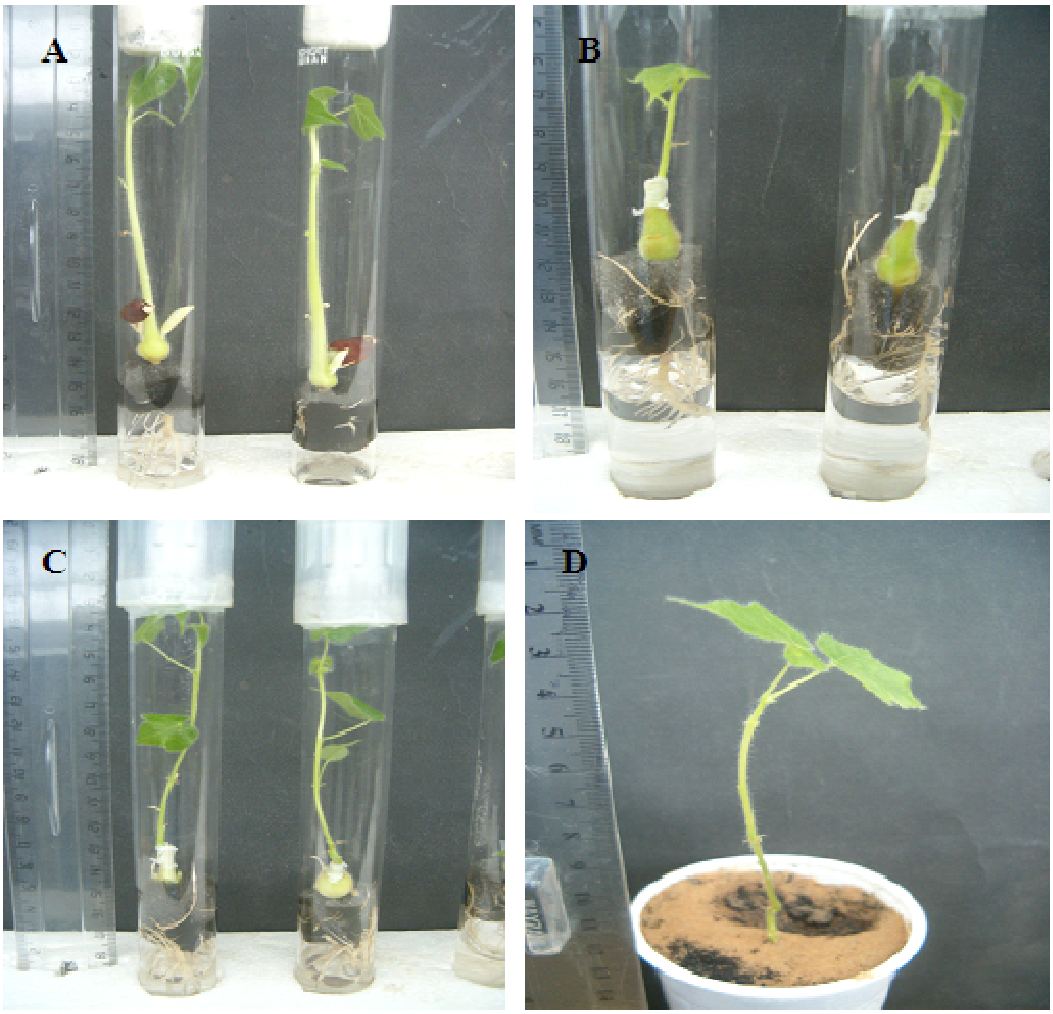

Figure 2. *In vitro* micrografting of *S. setigera*. A: Rootstocks of 2 weeks old cultured in MS medium. B: Micrografts after one week cultivation. C: Growth of scion of micrografting *S. setigera* for four weeks. D: Micrograft transferred to soil for acclimatization.

in reactivity was gained. This is determined by growth of the scion and the rooting competence of microcuttings severed from the scions after each micrografting cycle. At that point, the rooting competence is completely restored. This technique presents several advantages and could offer great opportunities of rapid mass propagation for healthy plant materials selected from elites trees.

Acclimatizing

A gradual transfer procedure was found imperative to prevent plants from dehydration due to immediate atmospheric conditions. Vigorous grafts with rootstock were primarily transferred to pots covered with polyethylene to maintain high humidity and were kept in growth chamber for two weeks. Covers were removed progressively upon growth of new leaves appearance. Survival rate were about 80% after three weeks cultivation in natural conditions (Figure 2D).

REFERENCES

Amiri ME (2005). *In vitro* techniques to study the shoot-tip grafting of Prunus avium L. (cherry) var. J. Food, Agric. Environ. 4(1): 151-154.

Ba B, Badiane S, Badji A, Dieng A, Faye A, Ndione C (1999). Ressources sauvages de la région de Tambacounda: un diagnostic participatif. I.S.R.A./BAME, p. 52.

Bakhoum C, Samba SAN, Ndour B (1999). Sterculia setigera Del. Effet sur les cultures. Ann. For. Sci. 58 : 207-215.

Celik H (2000). The effects of different grafting methods applied by manual grafting units on grafting success in grapevines. Turk. J. Agric. For. 24: 499-504.

Damiano C, Monticelli S (1998). *In vitro* fruit trees rooting by Agrobacterium rhizogenes wild type infection. Elect. J. Biotechnol.

1(2): 0717-345.
Danthu P, Hane B, Sagna P, Gassama YK (2002a). Restoration of rooting competence in mature Faidherbia albida, a Sahelian leguminous tree, through serial root sucker micrografting. New For. 24: 239-244.
Danthu P, Soloviev P, Toure MA, Gaye A (2002b). Propagation végétative d'une variété améliorée de jujubier introduite au Sénégal. Revue Bois et Forêts des Tropiques N° 272 (2).
Detrez C (1994). Shoot production through cutting culture and micrografting from mature tree explants in *Acacia tortilis* (Forsk.) *Hayne* subsp. raddiana (Savi.) Brenan. Agrofor. Syst. 25(3): 171-179.
Diallo B (1997). Recherches des conditions optimales pour la micropropagation de *Sterculia setigera*. Mémoire de DEA, biologie Végétale, UCAD, Dakar-Sénégal, p. 55.
Dieng O (1995). Etude sur le prélèvement et l'exploitation de la gomme de *Sterculia setigera* à Koumpentoum au Sénégal. Mémoire de Master of Sciences, Université de Laval, Québec, Canada.
Estrada-Luna AA, Lopez-Peralta C, Cardenas-Soriano E (2002). *In vitro* micrografting and the histology of graft union formation of selected species of prickly pear cactus (*Opuntia* spp.). Sci. Horticult. 92: 317-327.
Ewald D, Kretzschmar U (1996). The influence of micrografting *in vitro* on tissue culture behavior and vegetative propagation of old European larch trees. Plant Cell, Tissue Organ Cult. 44: 249-252.
Fraga MF, Cañal MJ, Aragonés A, Rodríguez R (2001). Factors involved in Pinus radiata D. Don. Micrografting. Ann. For. Sci. 59(2002): 155-161.
Hane B (2000). Utilisation des vitrométhodes (microbouturage, microgreffage) pour le rajeunissement et le clonage de trois essences ligneuses des zones tropicales sèches: *Acacia senegal* (L.) Wild, *Balanites aegyptiaca* (L.). Del et Faidherbia Albida (Del) Chev. Mémoire de DEA de Biologie Végétale-FST-Université Cheikh Anta Diop.
Hartmann HT, Kester DE, Davies FT, Geneve Jr. RL (1997). Plant Propagation: Principles and Practices. 6th edition. Prentice-Hall, Inc., Upper Saddle River, NJ., p. 770.
Hussain TM, Chandrasekhar T, Gopal GR (2007). High frequency shoot regeneration of Sterculia urens Roxb. an endangered tree species through cotyledonary node cultures. Afr. J. Biotechnol. 6(14) : 1643-1649.
Hussain TM, Chandrasekhar T, Gopal GR (2008). Micropropagation of Sterculia urens Roxb., an endangered tree species from intact seedlings. Afr. J. Biotechnol. 7(2): 95-101.
Jonhson AD, Sy MS, Gaye M (2005). Etude de cas sur les produits naturels: le lallo mbepp au Sénégal. USAID/EGAT, IRG, p. 66.
Khalafalla MM, Daffalla HM (2008). *In vitro* micropropagation and micrografting of gum Arabic tree [*Acacia Senegal* (L.) Wild]. Int. J. Sustain. Crop Prod. 3(1): 19-27.
Kwapata M (2003). Micropropagation: principe et technique. In la multiplication végétative des ligneux en agroforesterie. Manuel de formation et bibliographie. World agroforestry Centre, ICRAFT, p. 142.
Lô M (1996). Contribution à l'étude botanique et physico-chimique des gommes de Sterculia L. (*Sterculiaceae*): Valorisation de la production de *Sterculia setigera* Del. et incidences socio-économiques au Sénégal.», Thèse de doctorat Es-sciences pharmaceutiques, Université Cheikh Anta Diop, Faculté de Médecine et de Pharmacie, Dakar, p. 319.
Monteuuis O (1995). *In vivo* grafting and *in vitro* micrografting of *Acacia mangium*: impact of ortet age. Silvae genetica. 44(4): 190-193.
Moore R (1984). A model for graft compatibility-incompatibility in higher Plants. Am. J. Bot. 7(5): 752-758.
Onay A, Pirinc V, Adiyaman F, Isikalan C, Tilkat E, Basaran D (2002). *In vivo* and *in vitro* micrografting of Pistachio, *Pistacia vera* L. cv. Siirt. Turk J. Biol. 27: 95-100.
Revilla MA, Jose P, Abelardo C, Roberto R (1996). *In vitro* reinvigoration of mature oil tree (*Olea europaea* L.) through micrografting. *In vitro* Cell. Dev. Biology-Plant, 32: 257-261.
Sanogo D (1995). Micropropagation et rajeunissement *in vitro* de Khaya senegalensis (Desr.) Juss. Mémoire de DEA de Biologie Végétale, Département de Biologie Végétale, FST, UCAD. p. 65.
Sunnichan VG, Shivanna KR, Mohan Ram HK (1998). Micropropagation of gum karaya (*Sterculia urens*) by adventitious shoot formation and somatic embryogenesis. Plant Cell Rep. 17: 951-956.
Touré M, Samba ANS, Dramé A, Wade M, Gaye A, Niang D, Gassama YK (2009). *Sterculia setigera* Del: Germination and vegetative propagation. J. Sci. Technol. 8(1): 35-44.
Tourina H, Noureddine EM (2009). *In Vitro* Micrografting of Mature Carob Tree (*Ceratonia siliqua* L.). The Open Horticult. J. 2: 44-48.
Von Maydell HJ (1983). Arbres et Arbustes du Sahel. Leurs caractéristiques et leurs utilisations. GTZ, Verlag JM (ed.). Scientifics Books, p. 523.