Advances in Environmental Biology, 6(10): 2749-2757, 2012
ISSN 1995-0756

This is a refereed journal and all articles are professionally screened and reviewed **ORIGINAL ARTICLE**

Propagation Of African Baobab (*Adansonia Digitata* L., *Bombacoideae, Malvaceae*) Germplasm Through *In Vitro* Cloning

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N'DOYE Amadou Lamine, SAMBE Mame Abdou Nahr and SY Mame Ourèye: Propagation Of African Baobab (Adansonia Digitata L., Bombacoideae, Malvaceae) Germplasm Through In Vitro Cloning

ABSTRACT

Baobab tree, one of the most useful species in Sahel, is now threatened with depletion and worse instinction. In many localities, the overexploitation of the species and land hinder natural population renewal. Some agricultural practices and use of all parts of the baobab tree in pharmacopoeia and crafts also endanger the natural survival and regeneration of this phytogenetic and emblematic resource. The case of the baobab forest of Nguékokh located in Senegal reflects the situation alarmingly. Studies on morphogenetic potential and ability to *in vitro* cloning of this African genetic resource of great economic value have been undertaken for its preservation and use in reforestation programs. The *in vitro* propagation was carried out from different types of explants taken from twenty-day aged sterile seedlings such as cotyledonary nodes, axillary nodes and terminal apex. In presence of 0.5 mg. L^{-1} BAP, a multiplication rate of 2.31 was obtained for apex explants; 1.88 for axillary nodes and 2.0 for cotyledonary nodes. In presence of 2 g. L^{-1} activated charcoal, BAP at 5 mg. L^{-1} promotes the development of different cultured explants compared to Kinetin at the same concentration. After 72 h of an induction period with 5 mg. L^{-1} of NAA and transfer into the expression medium without hormones, 50 % of the apex and 75 % of cotyledonary nodes rooted. In contrast, 57.14 % of axillary nodes took root after an induction with NAA at 2.5 mg. L^{-1}. After 30 days of acclimatization, on a substrate consisting of a mixture of sand/compost (v/v) in a mini-greenhouse with an adjustable opening, survival rates were respectively 77.77 % for miniplants from apex, 72.72 % for those from axillary nodes and 57.14 % for those from cotyledonary nodes.

Key words: Baobab, Micropropagation, Rhizogenesis, Acclimatization, Conservation, Phytogenetic resource.

Introduction

The baobabs are a stem-succulent tree native to the dry regions of tropical Africa, Australia and Madagascar but dispersed widely by human activities. The Baobab is a majestic tree of the Sahelian savannah, generally associated to Senegal as its national symbol. The members of the genus *Adansonia* are united by several characters that serve to discriminate them from other *Bombacaceae*, including a characteristic indehiscent fruit with reniform seeds and a powdery whitish pulp [1]. Baobabs are long-lived, small to large trees with broad, sometimes bottle-shapped trunks and relatively compact crowns [2]. Firstly assigned to *Malvaceae* family by [3], a systematic revision was made by [1] on the basis of morphology. *A. digitata* was classified as the only species of section Adansonia [1], In fact, *Adansonia digitata* is the largest, the oldest known tropical angiosperm species with reliable carbon dating results [4] and the best known of the eight species of *Adansonia*. The genus belongs to *Bombacoideae*, a subfamily of *Malvaceae* [5]. *Adansonia digitata* is an autotetraploid species issued from a reduced aneuploid chromosomic type such as 4x = 176 [6]. A phylogeographical reconstruct of the species made to identify its centre of origin, after many decades of controversy, revelaed by pcr-rlfp of DNA Chloroplast fragments, that *A. digitata* probably originated from west Africa and migrated subsequently throughout the tropical parts of that continent and beyond, by natural and human-mediated terrestrial and overseas dispersal [7]. This recent study on chloroplast DNA has shown that there are genetic differences between baobab populations from western and south-eastern Africa.

Due to the presence of equatorial rain forest and the Mega-Chad Lake in the Quaternary, these

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populations have been isolated from one another for a long period of time which might indicate that both genetic clades have developed different mechanisms to cope with drought [7]. *Adansonia digitata* best known as the African baobab is one of the most useful species in the Sahel as it represents significant nutritional adjuncts [8]. Indeed, the seed is a good source of energy (445 to 760.41 kcal), protein (21.42 to 41.6 g) and fats (12.0 to 31.5 g). The pulp of the baobab fruit is tasty and nutritious and is very rich in potassium, tartaric acid and vitamin C [9]. The seed and pulp contain substantial amounts of calcium (Whole seed: 238 to 300 mg; pulp: 221 to 2750 mg), phosphorus (Whole seed: 1494 to 1540 mg; pulp: 82 to 196 mg) and iron (Whole Seed: 12 to 13.9 mg; pulp: 0.0 to 7.4 mg), but also glutamic acid, aspartic acid, oleic, linoleic and palmitic acids [10]. Due to the high content of lipids in baobab seeds (28 %) which are surrounded by a very tough and thick testa [9], it is recommended to store them less than 6 months before use to avoid low performances of seedlings after germination. Its leaves are rich in vitamins (especially A and C), iron and also contain mucilage (10 % dry matter). Young leaves can be eaten as a vegetable but are frequently dried out and powdered [8]. In addition, the uses of baobab are many and varied: pharmacopoeia, rope [11] or as a vegetable plant in Mali [12]. More recently, the baobabs have become important ecotourism values as the *A. digitata* forest of Bandia and the one of Nguekokh in Senegal.

Baobab tree can be propagated by seeds and vegetatively as well. Conventional techniques such as cuttings, air layering and grafting are applicable to the species and are economically less costly but success rates are low. The success rate of cuttings does not exceed 30% in presence of IBA and is equivalent to 2% in absence of hormone [13].

Nowadays, the observation of land degradation is well established [14]. The latter is due to the persistence of unfavorable climate conditions [15] and the increasing population pressure in these regions [16] which further accentuates the imbalance between the demand and productivity of the ecosystem. In Sahelian area, seeking a balance, between conservation of natural resources and their exploitation, constitutes one of the major development challenges. The increasing population pressure in these regions increases the imbalance between demand and productivity of the ecosystem. In Senegal, the persistent drought cycles seriously affects the floristic diversity and causes depletion of forest resources with the disappearance of valuable tree species. The African and Madagascarian baobabs are threatened with depletion or extinction for some species. In many localities, the overexploitation of the species and the land prevents the renewal of the population [17]. In Senegal, the natural regeneration of the baobab tree is low and the populations are old. The harvesting of non timber forest products (NTFPs) affects baobab [18]. The populations of threatened baobabs are not replaced due to lack of regeneration. The abuse of seeds, the pasture and the bush fires reduce considerably the stock of seeds in soil [19].

To face this threat, a study of the optimal conditions that would make possible the *in vitro* mass propagation of Baobab tree was undertaken. The major aim was to develop an efficient germination and an *in vitro* regeneration system for *A. digitata* L. *(Bombacoideae, Malvaceae)* to constantly layout homogeneous vitroplants capable of being distributed to producers or foresters after weaning. It would also serve to renew the settlements for reforestation programs and its conservation in african ecosystems, particularly in Senegal where the emblematic *A. digitata* is the only existing species of the genus.

Material and Methods

Plant Material:

The seeds for *in vitro* germination were ripe and were harvested from individuals of the same origin *i.e.* Bandia forest and without apparent diseases. Freshly harvested pulped seeds were used for the experiments. In order to sort particle size, which may reflect the density, the seeds were soaked in a basin of water and immerged for 30 minutes. The non viable and floating ones were then discarded and the viable seeds were left to dry on paper at ambient air for 48 hours and then stored at 5 °C until their use.

Scarification And Disinfection:

The seeds of *Adansonia digitata* were collected as a result of density test underwent chemical scarification with concentrate sulfuric acid (H_2SO_4 at 95%) as suggested by Danthu *et al*. [20] for 12 hours and then rinsed thoroughly with sterile distilled water. The seeds were soaked for 10 min in sterile distilled water added with few drops of Tween 20, then they were surface disinfected with bleach (NaOCl, chlorometric 8°) for 5 minutes. Finally, they underwent four successive rinses in sterile distilled water.

In Vitro Germination:

The pretreated seeds were also maintained under aseptic conditions. Lots of treated seeds were sown in the basal mineral medium of Murashige and Skoog [21] without hormones. The macronutrients in the nutrient medium were diluted by half MS/2 (0). The medium adjusted to pH 5.7 was solidified with agar at 8 g. L^{-1}. It was then, distributed in 660 ml capacity jars, with 50 ml per jar. The jars were then autoclaved at 110 °C for 20 min. 10 seeds were sown aseptically per jar. The jars were subsequently placed

in the dark in an incubator where the temperature was set at 27 ± 1 °C. The breakthrough of the radicle from the seed coat was used as the criterion for germination [22] [23]. The seeds were scored daily for germination. As soon as the seeds have germinated, the jars were transferred in a growth chamber at 27 ±1 °C under a photoperiod of 16 h of daylight provided by fluorescent tubes which emit a light intensity equivalent to 101.4 µmoles. m^{-2}. s^{-1}.

Tissue Culture Initiation:

For *in vitro* micropropagation, three types of explants were tested: cotyledonary nodes, axillary nodes and terminal apex. These explants, measuring 1 to 2 cm long, were taken from sterile seedlings, 20 days of aged. These different types of explants were transferred separately and individually in culture tubes containing 20 ml of MS solidified medium [21] mixed with or without growth regulators.

Culture Media And Experimental Design:

The basic nutrient medium used was that of Murashige and Skoog [21]. This medium adjusted to pH 5.7 and solidified with agar at 8 g. L^{-1} was supplemented or not with growth regulators. Some media contained or not activated charcoal (AC) at 2 g. L^{-1}.

A completely random design was set up. The explants factor (cotyledonary nodes, axillary nodes and terminal apex) was combined with the levels of the growth regulators factor (cytokinins like BAP at concentrations of 0.5, 1, 5 mg. L^{-1} and kinetin at 5 mg.L^{-1} combined or not with NAA at concentrations of 0.1 mg. L^{-1} and 0.2 mg. L^{-1}) to test the hormonal influence on the morphogenesis of *Adansonia digitata* explants. A total of 24 treatments was ultimately generated with 24 replications. The media composition is described in Table 1.

Table 1: Hormonal Composition of the different Media tested on different types of *A. digitata* explants.

Media	Composition
M1	MS (0)
M2	MS + BAP 0.5 mg. L^{-1}
M3	MS + BAP 1 mg. L^{-1}
M4	MS + BAP 1 mg. L^{-1} + NAA 0.1 mg. L^{-1}
M5	MS + BAP 1 mg. L^{-1} + NAA 0.2 mg. L^{-1}
M6	MS (0) + AC 2 g. L^{-1}
M7	MS + BAP 5 mg. L^{-1} + AC 2 g. L^{-1}
M8	MS + Kin 5 mg. L^{-1} + AC 2 g. L^{-1}

Each type of media was dispensed into culture tubes (20 ml per tube) and then autoclaved at 110 °C for 20 min. For each type of explants, a group of 24 per medium was defined. For each type of medium tested, 3 subcultures were conducted. The culture tubes were then incubated in a growth chamber at 27 ± 1 °C under a photoperiod of 16 h light / 8 h night with an incident light of 101.4 µmoles. M^{-2}. S^{-1}. Measurements were performed after 15 and 30 days of incubation, which corresponded to two measures for each type of explant and each type of medium. The measured parameter concerned the presence or absence of activity recovery, the number and length of shoots newly formed, the number of formed nodes. Then, the averages were calculated, the coefficients or multiplication rate determined and the best media identified.

Rooting:

A completely random design was also set up for rooting protocol. The explant factor (cotyledonary nodes, axillary nodes and terminal apex) was combined with the levels of the growth regulators factor (NAA or IBA at concentrations of 1, 2.5 and 5 mg. L^{-1}) in the induction media.

Shoots of the third generation, from three successive subcultures over a period of 30 days each, were induced to root in the dark in the MS/2 solid medium supplemented with NAA or IBA at concentrations of 1, 2.5 and 5 mg. L^{-1}.

The rooting induction took three days before the transfer of the explants to the light in the expression medium MS (0)/2 without growth regulator. For each type of explant and each treatment, a group of 12 explants was used per duration and for induction medium (Table 2). A batch of 12 explants, maintained in MS media (0)/2 without growth regulator, served as a control group. Measurements were performed every 15 and 30 days to determine rooting parameters such as the rate of rooting, the number of newly formed roots per explant and root length for each treatment.

Table 2: Rooting media composition for the different types of *A. digitata* explant.

Rooting Media	Composition
R1	MS/2 (0)
R2	MS/2 + NAA 1 mg. L^{-1}
R3	MS/2 + NAA 2.5 mg. L^{-1}
R4	MS/2 + NAA 5 mg. L^{-1}
R5	MS/2 + IBA 1 mg. L^{-1}
R6	MS/2 + IBA 2.5 mg. L^{-1}
R7	MS/2 + IBA 5 mg. L^{-1}

Acclimatization:

After 30 days of incubation in the expression medium, *Adansonia digitata* plantlets that had well rooted were submitted to weaning conditions. The vitroplants were removed from the jars and freed of agar by rinsing the root system with distilled water. The roots had also been cut to 1 cm above the apical tips. The plants produced *in vitro* were then transplanted into pots containing a substrate composed of a mixture of sand and compost (v/v) previously sterilized (121 °C, 1 hour) and to which one adds carbofuran and lannate 90. The substrate was packaged in plastic cups pierced by a hole in the base in which plants were individually transferred. These pots were placed in a mini greenhouse hermetically closed to maintain them in an atmosphere with relative high moisture or in mini-greenhouse with an adjustable opening. Watering was made with tap water and the mini greenhouse was gradually opened 3 h per day for 7 days.

The number of plants that survived after 15 days and 30 days of acclimatization were counted, to determine the survival or recovery rate after transplantation.

Statistical Analysis :

After an Analysis of Variance of the treatments, Student, Newman and Keuls test was performed to compare means at 5 % using SPSS 10.1 package software.

Results:

In Vitro Germination And Micropropagation:

After chemical scarification and disinfection, previously pulped baobab seeds offer a germination rate of 90 % after 20 days of culture (Plate 1A).

The influence of various growth regulators was carried out on *in vitro* morphogenetic potential of *Adansonia digitata*. The results obtained after 30 days of storage in a growth chamber were presented as followed: Cotyledonary nodes, axillary nodes and terminal apex of seedlings from *in vitro* germination were sowed on different culture media containing various hormonal combinations and described below in Material and Methods. The results obtained are shown in Table 3.

- *For the apex*: an average number of 1.25 shoots corresponding to a multiplication factor of 2.31 was obtained in M1 (MS + BAP 0.5 mg. L^{-1}), with an average of shoot length of 2.25 cm. The comparison reveals a significant difference of this medium on the average number of node compared with control medium M1 (MS (0); Plate 1B), M3 (MS + BAP 1 mg. L^{-1}), M4 (MS + BAP 1 mg. L^{-1} + NAA 0.1 mg. L^{-1}) and M5 (MS + BAP 1 mg. L^{-1} + NAA 0.2 mg. L^{-1}).

An average number of shoots of 1.12; an average shoot length of 3.45 cm and an average number of node of 2.25 were recorded in the M6 medium (MS + AC 2 g.L^{-1}), whereas in media M7 (MS + BAP 5 mg. L^{-1} + AC 2 g. L^{-1}) and M8 (MS + Kin 5 mg. L^{-1} + AC 2 g. L^{-1}) values obtained for the apex are lower while slightly higher in M7 (MS + BAP 5 mg. L^{-1} + AC 2 g. L^{-1}) than in M8 (MS + Kin 5 mg. L^{-1} + AC 2 g. L^{-1}).

- *For axillary nodes*: the average number of shoots of 1.33 corresponding to a multiplication factor of 1.88 is obtained in the M2 medium (MS + BAP 0.5 mg. L^{-1}), with an average length of shoots of 1.27 cm (Plate 1C).

The average number of nodes of 1.75 and the average shoot length of 2.37 cm were obtained in the M7 medium (MS + BAP 5 mg. L^{-1} + AC 2 g. L^{-1}).

Statistical analysis showed that the average number of nodes in M7 medium was significantly different from that in the M8 medium (MS + Kin 5 mg. L^{-1} + AC 2 g. L^{-1}). An average number of nodes of 1.75 is obtained in the control medium M6. However, this value was not significantly different from that of 1.75 obtained in M7 medium.

- *For cotyledonary nodes*: an average number of shoots of 1.47 was obtained in M3 medium, and a multiplication factor of 2 in the M2 medium, an average shoot length of 1.05 cm is obtained in the M1 control medium (Plate 1D). M7 medium gave an average shoot number of 1.20, not significantly different from the others, an average shoot length of 1.12 cm, non-significantly different compared to other values and an average number of nodes of 1.2.

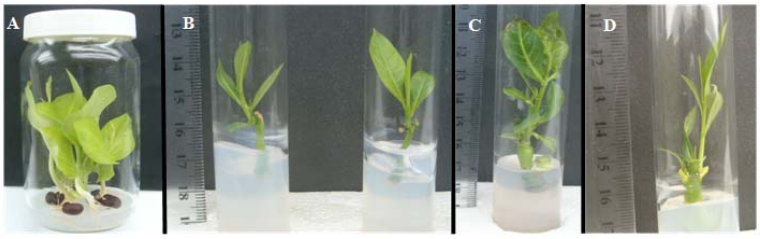

Plate 1: Sterile seedlings of 20 days old and Growth of different types of explant in M2 medium after 30 days of culture. A. Sterile seedlings of *A. digitata*; B. Apex (APX); C. Axillary nodes (AN); D. Cotyledonary node (CN) neoformed.

Adv. Environ. Biol., 6(10): 2749-2757, 2012

Table 3: Influence of different hormonal combinations on the *in vitro* morphogenetic expression of *Adansonia digitata* explants after 30 days of culture.

Media	Explant type	Mean number of shoots	Mean lenght of shoots (cm)	Mean number of nodes
M1	APX	1.16a	1.93ab	1.72a
	AN	1.22ab	0.77a	1.16a
	CN	1.27ab	1.05a	1.66ab
M2	APX	1.25a	2.25a	2.31b
	AN	1.33a	1.27b	1.88b
	CN	1.22ab	1.02a	2.00a
M3	APX	1.15a	1.66b	1.31ac
	AN	1.05b	0.55ac	1.05a
	CN	1.47a	0.68ab	1.70ab
M4	APX	1.10a	1.62b	1.21c
	AN	1.05b	0.42c	1.10a
	CN	1.11b	0.47b	1.16b
M5	APX	1.05b	1.63b	1.26ac
	AN	1.00b	0.33c	1.00a
	CN	1.26ab	0.65ab	1.47ab
M6	APX	1.12a	3.45a	2.25a
	AN	1.00a	2.05ab	1.75a
	CN	1.00a	1.78a	1.57a
M7	APX	1.00a	3.30a	1.57b
	AN	1.00a	2.37b	1.75a
	CN	1.20a	1.12a	1.20a
M8	APX	1.00a	3.00a	1.28b
	AN	1.00a	0.97c	1.12b
	CN	1.00a	1.00a	1.00a

APX: apex AN: axillary node CN: cotyledonary node

In the same column and for the same type of explant, figures followed by the same letter are not significantly different at the 5 % level by Student Newman-Keuls test.

Rooting:

The results obtained after 30 days of culture are listed in Table 4 according to the induction media in Table 2.

- *For the apex* (APX), the most significant values were obtained with the medium enriched with NAA at 5 mg. L^{-1} (R4) giving a rooting rate of 50 % after 3 days of induction (Plate 2A), an average number of roots of 2, with an average length of 9.63 cm. A rooting rate of 12.5 % was obtained for the IBA 2.5 mg. L^{-1} (R6) and 5 mg. L^{-1} (R7), while there was no rooting for the IBA 1 mg. L^{-1} (R5). An average number of roots of 1 having an average length of 7 cm was obtained for IBA 2.5 mg. L^{-1} (R6) and 5 mg. L^{-1} (R7).

- *For axillary nodes* (AN), a rooting rate of 57.14 % was recorded for R3 medium (Plate 2B). An average roots number of 1 for NAA 1, 2.5 and 5 mg. L^{-1} and an average root length of 6.76 cm for R4 medium were also recorded. A rooting rate of 14.28 % with an average of 1 root, an average length of 6.50 cm was obtained for the medium R6. This average number of roots did not differ significantly from that of other environments.

- *For cotyledonary nodes* (CN), a rooting rate of 75 % was obtained with the NAA 5 mg L^{-1} (R4 medium; Plate 2C), and a mean length of roots of 8.10 cm, whereas an average number of roots of 2 was recorded for the medium R3. The medium R5 provided a rooting rate of 25 % with an average number of roots of 1 and an average length of roots of 4.60 cm. No root was recorded after induction in the R6 and R7 media.

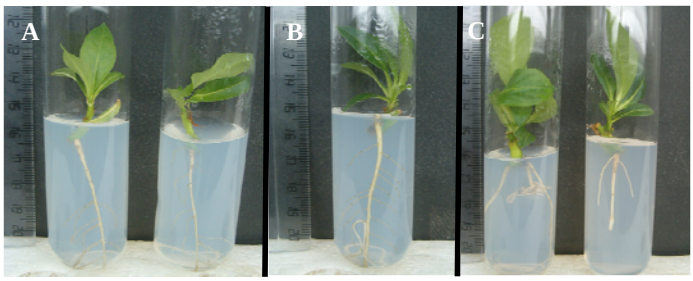

Plate 2: Rooting of regenerated shoots from different type of explants after 30 days; according to the induction medium R4 and R3.
A. Rooted Apex induced in MS / 2 + NAA at 5 mg. L^{-1} (R4).
B. Rooted Axillary node induced in MS / 2 + NAA at 2.5 mg. L^{-1} (R3).
C. Rooted Cotyledonary node induced in MS / 2 + NAA at 5 mg. L^{-1} (R4).

Table 4: Effects of the hormonal induction on the rooting of plantlets after 30 days of culture.

Media	Induction period (days)	Explants type	Rooting rate (%)	Mean root number	Mean root lenght (cm)
R1	3	APX	0	0 a	0 a
		AN	0	0 a	0 a
		CN	0	0 a	0 a
R2	3	APX	37.5	1.33 c	9.17 c
		AN	14.28	1 b	5.50 bc
		CN	0	0 a	0 a
R3	3	APX	37.5	1 b	6.83 b
		AN	57.14	1 b	5.90 bc
		CN	25	2 c	6 b
R4	3	APX	50	2 d	9.63 c
		AN	28.57	1 b	6.76 c
		CN	75	1.67 c	8.10 c
R5	3	APX	0	0 a	0 a
		AN	14.28	1 b	5 bc
		CN	25	1 b	4.60 b
R6	3	APX	12.5	1 b	7 b
		AN	14.28	1 b	6.50 c
		CN	0	0 a	0 a
R7	3	APX	12.5	1 b	7 b
		AN	14.28	1 b	4 b
		CN	0	0 a	0 a

APX: apex; AN: axillary node; CN: cotyledonary node

In the same column and for the same type of explants, figures followed by the same letter are not significantly different at the 5 % level by Student Newman-Keuls test.

Acclimatization:

To keep the plants produced *in vitro* in an atmosphere of high relative humidity, the pots were placed in a mini-greenhouse under a plastic dome with adjustable opening. According to Skolmen and Mapes [24], this procedure was common and can reduce sweating and prevent dehydration. The acclimatization test results were obtained after a gradual opening of the mini-greenhouse. The different types of explants were transplanted into cups containing a sterile sand-compost mixture and then kept in a mini-greenhouse completely closed for one week. Watering was carried out with tap water. Beyond the seventh day, the shutter was half-opened to avoid prolonged confinement that can lead to the decay of plants. The shutter was opened on the eighth day, although the explants were always kept in the shade for three days. Thus, after 30 day of acclimatization, survival rates were respectively 77.77 % for plants formed from the apex (Plate 3) and 72.72 % for those stemming from the axillary nodes. However, the plants formed from cotyledonary nodes showed a 57.14 % of survival rate.

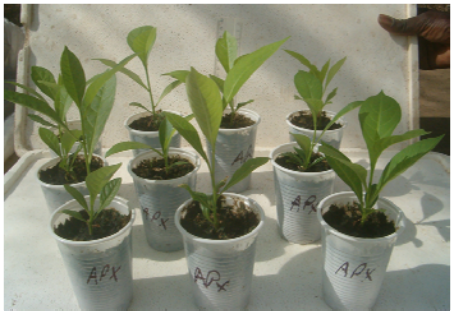

Plate 3: Acclimatization of Plantlets from shoot tips of *Adansonia digitata* and well rooted after 30 days of weaning.

Discussion:

The objective of this work was to study the optimal conditions for *in vitro* propagation of the baobab tree that would allow for a mass production of plants for their subsequent introduction into the planting areas.

The reactivity rate is 100% for apex, cotyledonary and axillary nodes cultured in various media. The number of newly formed shoots depends

on the type of explant but also the nature and concentration of the hormone. It is the same for the average shoot length of and mean number of newly formed nodes.

Following this study, our results showed that for micropropagation:

- The MS [21] medium supplemented or not with phytohormones is efficient for the *in vitro* reactivity of *A. digitata* explants, regardless of their types. These results corroborate those of Margara [25]. The MS medium [21] proved largely effective for induction of organogenesis in woody species, especially for the formation of new shoots. The effect of this culture medium would come from the interactions of different chemical elements that compose it. They stimulate positive morphogenetic processes, namely the bud or bud neoformation. In particular, nitrogen as nitrate or ammonium would be beneficial in controlling the biosynthesis of endogenous hormones responsible for organogenesis.

- The *A. digitata* explants react spontaneously in MS culture media containing or not growth regulators. After 15 days of culture, draft formed shoots appear at the nodes of the explants and are more prevalent in media supplemented with hormones. The rate of reactivity is 100% for apex, cotyledonary nodes and axillary cultured in various media. The number of newly formed shoots depends on the type of explant but also the nature and concentration of the hormone. It is the same for the average length of shoots and mean number of neoformed nodes. The favorable effect of growth regulators on the formation of new shoots of *Adansonia digitata* has also been reported by Mroginski *et al.* [26] who observed a bud development in stems of *Arachis hypogaea* in MS medium containing NAA at 0.01 mg. L^{-1} and BAP 1 mg. L^{-1},. Giang & Hong [27] had observed on papaya a better rate of proliferation of shoots in MS medium enriched with BAP 0.5 mg. L^{-1} + NAA 0.1 mg. L^{-1}. Similar results were also obtained by Fraternale *et al.* [28] on *Bupleurum fruticosum*, Casado *et al.* [29] on *Santolina canescens*, Ndoye *et al.* [30] on *Balanites aegyptiaca*, and Sambe *et al.* [31] on *Parkia biglobosa*.

- Thirty days after planting, the best medium for the growth of *A. digitata* explants is the medium MS + BAP 0.5 mg. L^{-1}. Comparing the growth rate of explants cultured in MS media containing different cytokinins like BAP and Kinetin has allowed us to determined that the BAP is more effective than kinetin for the formation of new shoots whatever the type of explant tested. So, in the absence of any polyphenolic interactions, which could interact on morphogenetic capacities of baobab explants, due to the power of activated charcoal adsorbent on baobab explants, the BAP is more organogenic at the same concentration than Kinetin.

- The stage of the rooting of tissue culture plants is a critical stage that determines the success of the acclimatization of plants and their subsequent transfer to the field. The rooting tests applied during experiments showed that rooting induction in the presence of auxin is essential to promote root formation from in vitro plantlets from explants of *A. digitata* because no root was formed in the control medium MS (0). Moreover, the auxin NAA is more effective than IBA in inducing rooting of plantlets. During this phase, the use of the NAA at 5 mg. L^{-1} in rooting induction for 3 days was effective to stimulate growth and development of root during the expression phase, with best rooting rate obtained on the plantlets from cotyledonary nodes. This favorable effect of NAA on plantlets rooting of was also observed by Badji *et al*. [32] on *Acacia Senegal*; Basbaa *et al.* [33] on *Gleditsia triacanthos* and Laberche *et al*. [34] on *Lotus alpinus*.

- The substrate constituted of sand supplemented with compost is the best support for weaning and development of *A. digitata ex vitro* plantlets.

The goal with the complete closure of the mini-greenhouse for the first 7 days of transplantation was to maintain an ambient atmosphere saturated with moisture. This yielded plants whose vegetative part is stronger. Taking into account that unnecessary confinement accompanied by a high temperature in an atmosphere saturated with water may suffocate microplants and, indeed be conducive to the development of pathogenic micro-organisms, we conducted a gradual opening of the mini-greenhouse for 2 or 3 hours per day beyond the seventh day to avoid necrosis and microbial contamination of plants. However, this exposes them to drying with a significant decrease in relative humidity. Indeed, according to Mapes *et al.* (1981), cited by Ndoye [35], plants from *in vitro* culture generally have a thinner cuticle than the parent plants, causing their rapid drying when relative humidity is low. This fragility of tissue culture is the main difficulty during the transplantation phase. Thus, 43 % of plants of *Prosopis juliflora* and *Prosopis chilensis* are necrotic during this stage [36]; [37]. On the 30th day of weaning, the combination of all these factors has resulted in a best survival rate after gradual opening of the mini-greenhouse for miniplants stemming from apex (77.77 %).

Acknowledgments

The authors are grateful to the World Federation of Scientists (WFS) for a fellowship and to Dr. Agbangba E. C. for critical reading of the manuscript. Our thanks also go to the lab technician Sagna M. for technical support.

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