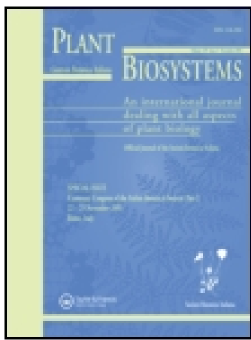

Taylor & Francis
Taylor & Francis Group

Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology
Official Journal of the Societa Botanica Italiana

ISSN: 1126-3504 (Print) 1724-5575 (Online) Journal homepage: http://www.tandfonline.com/loi/tplb20

An optimized method for in vitro propagation of African baobab (Adansonia digitata L.) using two-node segments

E. Rolli, F. Brunoni & R. Bruni

To cite this article: E. Rolli, F. Brunoni & R. Bruni (2016) An optimized method for in vitro propagation of African baobab (Adansonia digitata L.) using two-node segments, Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology, 150:4, 750-756, DOI: 10.1080/11263504.2014.991362

To link to this article: http://dx.doi.org/10.1080/11263504.2014.991362

Accepted author version posted online: 13 Dec 2014.
Published online: 17 Dec 2014.

Submit your article to this journal

Article views: 94

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=tplb20

Download by: [Umeå University Library] **Date:** 27 September 2017, At: 04:39

Plant Biosystems, 2016
Vol. 150, No. 4, 750–756, http://dx.doi.org/10.1080/11263504.2014.991362

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

An optimized method for *in vitro* propagation of African baobab (*Adansonia digitata* L.) using two-node segments

E. ROLLI[1], F. BRUNONI[1], & R. BRUNI[2]

[1]*Dipartimento di Bioscienze, Parco Area delle Scienze 11/A, 43124 Parma, Italy and* [2]*Dipartimento di Scienze degli Alimenti, Parco Area delle Scienze 95/A, 43124 Parma, Italy*

Downloaded by [Umeå University Library] at 04:39 27 September 2017

Abstract

Adansonia digitata L. (African baobab), is an important multi-purpose tree, whose distribution is at present limited to wild or semi-domesticated individuals widespread in Africa. Its distribution is threatened by seedling clearance for other land use and potentially by overharvesting induced by growing commercial use of baobab fruit. Recently, efforts have been made to establish baobab domestication and conservation strategies, with mixed results due to the low germinability of baobab seeds, a factor that hinders the possibility of developing commercial *A. digitata* plantations. Here, micropropagation was tested as a method for clonal propagation of explants from *in vivo*-grown seedlings. *In vitro* shoot multiplication was achieved by enhanced axillary bud proliferation of sterilized two-node segments. Bud break was dependent on cytokinin supply, but the combination of 1.0 or 10.0 μM zeatin riboside and 10.0 μM indole-3-butyric acid (IBA) increased the formation of microshoots after 8 weeks of culture. Regenerated microshoots rooted successfully in *in vitro* nutrient medium containing 10.0 μM IBA and normally grew in a greenhouse after acclimatization.

Keywords: Adansonia digitata *L, two-node explants, micropropagation, cytokinins*

Abbreviations: BAP, 6-benzyl aminopurine; IBA, indole-3-butyric acid; MS, Murashige & Skoog; 2iP, N6-(2-isopentenyl)adenine; HF, hormone-free; ZR, zeatin riboside

Introduction

The iconic baobab tree (*Adansonia digitata* L., Malvaceae subfam. Bombacoideae) is a slow-growing tree endemic to African savannas of most sub-Saharan countries (Sidibé & Williams 2002). Because baobab leaves and fruits are an important source of proteins, vitamins, minerals and sugars (Lockett et al. 2000; Besco et al. 2007), this species is relevant for its high nutritional value and medicinal uses (Kamatou et al. 2011). Moreover, as a consequence of its peculiar phytochemical composition and of the increased use by the food industry, the commercial value of baobab has vastly accrued during the past decade, spurring a mounting interest in its cultivation and sustainable management in the wild (Cuni Sanchez et al. 2011). However, natural reproduction and regeneration cycles of baobab are threatened due to damage caused by domestic animals and seedlings clearance for other land use (Assogbadjo et al. 2009). Furthermore, the increased harvesting of fruits from wild or semi-domesticated individuals for commercial purposes is suspected of potentially reducing seeds available for the spontaneous generation of new plants (Munthali et al. 2012). Domestication and cultivation programmes are thus urgently needed to preserve genetic diversity, maintain multiple specimens and promote the cultivation of varieties suitable for commercial cultivation (Assogbadjo et al. 2011; Cuni Sanchez et al. 2011). To improve the basic knowledge for *A. digitata* domestication and preservation, African baobab has been included in several EU-supported research projects, such as DADOBAT, SAFRUIT and NuTree and various studies have analysed phenological, phytochemical and genetic variation between different populations of *A. digitata* (Leakey 1999; Assogbadjo et al. 2008, 2009, 2010, 2011; Tsy et al. 2009; Cuni Sanchez et al. 2010, 2011; Jensen et al. 2011). Given the growing role of the baobab tree in sustainable development of rural economies in Africa,

Correspondence author: E. Rolli, Dipartimento di Bioscienze, Parco Area delle Scienze 11/A, 43124 Parma, Italy. Phone: +39 521 905576. Fax: +39 521 905403. Email: enrico.rolli@unipr.it

© 2014 Società Botanica Italiana

Downloaded by [Umeå University Library] at 04:39 27 September 2017

it is advisable to start long-term programmes to improve conservation of selected plant material. Among basic plant conservation strategies, *ex situ* conservation is deemed particularly appropriate for fruit trees and their wild relatives (Engelmann & Engels 2002; Freeman et al. 2014; Rossi et al. 2014). Specific techniques of germplasm preservation include seed banks, pollen and tissue storage, vegetative propagation and maintaining of planted individuals and semi-domesticated orchards (Volis & Blecher 2010). Nevertheless, the recourse to baobab seed banks has a limited viability due to both environmental and genetic factors and because seeds have a low germination rate and require scarification, with erratic results (Assogbadjo et al. 2011). As an alternative off-site collection, multiplication and storage of baobab germplasm can be achieved by means of vegetative propagation methods (Jensen et al. 2011). *In vitro* propagation techniques have been developed for a wide range of species and offer major advantages over conventional propagation strategies, allowing rapid multiplication under controlled conditions (Engelmann & Engels 2002).

However, this biotechnological approach has been rarely applied to baobab (Jensen et al. 2011). Ishii and Kambou (2007) first performed *in vitro* culture of *A. digitata*, testing the effect of different media and 6-benzyl aminopurine (BAP) concentrations on different explants from 1-month-old *in-vitro* grown seedlings. On the other hand, Singh et al. (2010) focused their attention on *in vitro* seed germination by examining embryo development in mature and immature seeds. These authors reported that seed germination was significantly affected by the age of fruits/seeds and that mature seeds failed to germinate. In our previous study, aseptic cultures were obtained after 3 days of *in vitro* culture of *A. digitata* seeds pre-treated with sulphuric acid (Rolli et al. 2012). However, the seed germination was asynchronous, requiring from 3 to 30 days for radicle protrusion and due to insufficient availability of same-aged *in vitro* seedlings, it was not possible to establish any micropropagation protocol. In their work with baobab, Ishii and Kambou (2007) obtained *in vitro* culture by exposing nodal segments from *in vitro*-germinated seedlings to increasing concentrations of BAP, but with poor results. On the other hand, Singh et al. (2010) focused on *in vitro* seed germination but not on *in vitro* shoot multiplication. N'Doyle et al. (2012) described a solid protocol for the proliferation of various types of baobab explants using two cytokinins, claiming that BAP is more effective than kinetin for the formation of new shoots. However, in these previous works, little attention has been given to the use of different cytokinins, at different concentrations, alone or in combination with auxins on the *in vitro* culture of baobab.

Therefore, a reliable and reproducible method to initiate microprogation of *A. digitata* through shoot proliferation was developed. Aseptic cultures were established using *in vivo*-grown seedlings as source of axillary buds. To induce *in vitro* shoot multiplication, the effects of three different cytokinins alone or in combination with auxin were tested.

Materials and methods

Plant growth regulators

BAP, 2iP and IBA were dissolved in a minimum volume of 0.1 M NaOH before being prepared as appropriate aqueous stock solutions. Zeatin riboside (ZR) was dissolved in 70% ethanol. All plant growth regulators were then sterilized by filtration with 0.2 μm sterile disposable filter units.

Plant material

Seeds of *A. digitata* L. harvested in the outskirts of Tambacounda, Senegal, were kindly provided by Baobab Fruit Company Senegal (via Mondadori, 15 Poggio Rusco MN, Italy).

In vivo *sowing*

Seeds were soaked in sulphuric acid (H_2SO_4, 98% v/v) for 24 h, then washed in running water and placed into 100 mM $NaHCO_3$ solution for 30 min to neutralize the remaining acid. Afterwards, seeds were rinsed, incubated in deionized water for 24 h at 37°C and sown on sterilized vermiculite moistened with distilled water in a plastic box. They were covered with 2 cm of the same substrate and then incubated for 4 weeks in a climatic chamber maintained at 22 ± 2°C with a light regime of 8 h darkness and 16 h light provided by cool-white fluorescent lights (27 $\mu mol\ m^{-2}\ s^{-1}$).

Surface-sterilization procedures and in vitro *shoot multiplication*

For explant sterilization, 4-week-old *in vivo*-grown seedlings were collected and cut to obtain two-node segments (approximately 3 cm in length, without basal leaf), surface-sterilized in 70% (v/v) ethanol solution for 30 s, and then vacuum-infiltrated for 5 or 10 min with 1 or 2% (v/v) sodium hypochlorite and finally rinsed three times (5 min each) in sterile distilled water. The sterilized two-node explants were placed vertically and cultured individually in glass culture tubes containing about 15 ml of full-strength MS medium (Murashige & Skoog 1962). The medium was solidified with 0.8% (w/v) phyto agar. pH was adjusted to 5.8 with 0.1 M NaOH before

autoclaving at 121°C for 20 min. To evaluate the effect of three different cytokinins on baobab shoot multiplication, MS medium was supplemented with 0.1, 1.0 or 10.0 μM BAP, 2-iP or ZR, alone or in combination with 1.0 or 10.0 μM IBA and the sterilized explants were randomized to receive the treatment with phytohormones for a total duration of 8 weeks. Explants cultured on MS medium lacking plant growth regulator (hormone-free; HF) were used as control. Cultures were grown at 26 ± 1°C, under 16-h day length, with light provided by cool-white fluorescent lights at a photon flux of 27 μmol m^{-2} s^{-1}. After 8 weeks of culture, occurrence of contamination and the number of contaminated cultures were discarded. The percentage of cultures showing bud break, the mean number of shoots per explant, the mean number of nodes per shoot and the mean shoot length were calculated only for the healthy cultures. Ten explants were used for each concentration/combination of the evaluated phytohormones. The experiment was replicated three times.

Rooting and acclimatization

For *in vitro* root induction, newly formed 8-week-old shoot-tips (3–4 cm in length), obtained from explants cultured on 10.0 μM ZR plus 10.0 μM IBA, were excised and transferred in MS medium supplemented with 1.0 or 10.0 μM IBA. Shoots cultured on HF medium were used as control. Cultures were grown at 26 ± 1°C, under 16-h day length, with light provided by cool-white fluorescent lights at a photon flux of 27 μmol m^{-2} s^{-1}. Eight explants were used for each treatment, and the experiments were repeated three times. After 8 weeks of culture, the number of rooted shoots and the number of roots per rooted shoot were counted. Acclimatization of the *in vitro*-rooted plantlets was carried out in a growth chamber at a light intensity of 27 μmol m^{-2} s^{-1} under fluorescent tubes with a 16-h photoperiod. Day/night temperatures were 25°C and 20°C, respectively. Rooted plantlets were gently washed free of agar, after which each plantlet was transferred to a vessel containing a 1:1 (v:v) mixture of garden soil:vermiculite previously autoclaved. During the first week of acclimatization, the vessels were covered with transparent plastic film, which was gradually removed to promote hardening. After 15 days, all the plantlets were fully acclimatized and transferred to greenhouse conditions.

Data analysis

The effect of exogenously applied phytohormones on shoot regeneration was evaluated by the following variables: percentage of regenerating explants, mean number of shoots formed per explant, mean number of nodes per shoot and mean shoot length. Experiments were carried out with a completely randomized design by analysing three factors: type of cytokinin, concentration of cytokinin and auxin. Data were subjected to analysis of variance and pairwise comparisons were evaluated by Dunnett's multiple range test. Data were examined for homoscedasticity with Levene's test. To evaluate the rooting ability by shoots in the presence of different auxin concentrations, the mean number of roots per rooted shoot, the rooting percentage and the mean rooting time were recorded. Data were analysed using ANOVA and Tukey's test for multiple pairwise comparisons. Percentage values (percentage of regeneration and rooting) were arcsine-transformed before statistical analysis. All statistical analyses were performed with SPSS 19 for Windows software (SPSS Inc).

Results

Sterilization of in vivo-*germinated explants*

A 65% germination rate was observed in seeds subjected to chemical scarification. All the *in vivo*-germinated seedlings developed into healthy plantlets. Two-node segments of 4-week-old seedlings grown in non-aseptic condition were surface sterilized by soaking in 1 or 2% (v/v) sodium hypochlorite (NaOCl) for 5 min or 10 min. As shown in Figure 1, 83.4% of the explants were successfully sterilized using 2% NaOCl for a period of 10 min. For plant material, 5 min soaking in 1% NaOCl resulted in 63.8% of the nodal explants being sterilized. Because the stronger sterilization (2% NaOCl for 10 min) offered a higher percentage of recovery from *in vivo* explants and did not noticeably affect tissues (except for 1-mm basal stem portion,

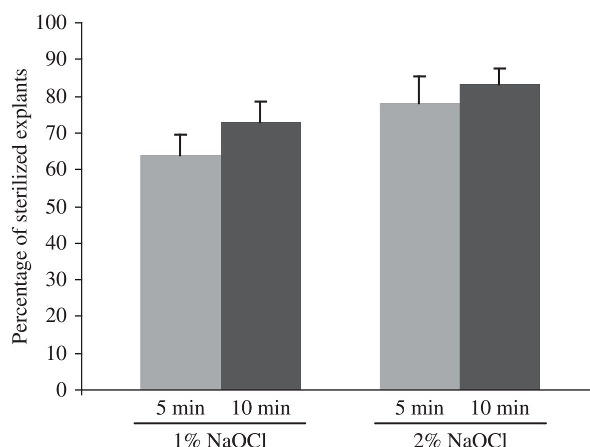

Figure 1. Percentage of two-node explants sterilized after 5-min or 10-min treatment with 1% or 2% NaOCl.

Downloaded by [Umeå University Library] at 04:39 27 September 2017

Table I. Effect of BA, ZR and 2iP alone or in combination with IBA on shoot regeneration from sterilized two-node segments after 8 weeks of culture.

Treatment							
	Cytokinin (μM)	Auxin (μM)	Regeneration (%)	Mean shoot number per explant	Mean node number per shoot	Mean shoot length (mm)	Callus
Control	0.0	0.0	0	1.0 ± 0.00 a	1.9 ± 0.19 a	6.7 ± 0.94 a	
BA	0.1	0.0	8.3	1.1 ± 0.10 a	2 ± 0.43 a	8.2 ± 2.58 a	
	1.0	0.0	7.3	1.1 ± 0.06 a	3.3 ± 0.49 b	9.2 ± 1.90 a	
	10.0	0.0	0	1.0 ± 0.00 a	3.1 ± 0.37 b	7 ± 0.83 a	++
ZR	0.1	0.0	4.2	1.1 ± 0.08 a	2.7 ± 0.34 b	7.2 ± 1.37 a	
	1.0	0.0	15.8	1.1 ± 0.05 a	4.1 ± 0.60 c	16.1 ± 2.37 b	+
	10.0	0.0	52.1	1.7 ± 0.33 a	4.9 ± 0.49 c	20.1 ± 2.63 b	+++
2-IP	0.1	0.0	0	1.0 ± 0.00 a	2.0 ± 0.27 a	5.9 ± 0.97 a	
	1.0	0.0	0	1.0 ± 0.00 a	3.7 ± 0.47 b	13.3 ± 2.57 b	
	10.0	0.0	11.2	1.1 ± 0.07 a	3.8 ± 0.30 b	19.4 ± 2.04 c	+
IBA	0.0	1.0	0	1.1 ± 0.08 a	3.45 ± 0.70 c	15 ± 2.69 b	+
BA	0.1	1.0	5	1.1 ± 0.08 a	3 ± 0.46 c	9.6 ± 2.38 b	
	1.0	1.0	6.7	1.1 ± 0.08 a	2.8 ± 0.45 c	12.7 ± 2.61 b	
	10.0	1.0	29.1	1.3 ± 0.17 a	3.1 ± 0.35 c	7 ± 1.02 a	
ZR	0.1	1.0	7.3	1.1 ± 0.04 a	3.4 ± 0.41 c	12.3 ± 1.86 b	
	1.0	1.0	16.2	1.2 ± 0.09 a	4.4 ± 0.43 c	22.6 ± 3.3 c	++
	10.0	1.0	38.3	1.5 ± 0.22 a	4.6 ± 0.60 c	17.9 ± 2.67 b	+++
2-IP	0.1	1.0	0	1.0 ± 0.00 a	3.6 ± 0.40 c	11.8 ± 2.21 b	
	1.0	1.0	0	1.0 ± 0.00 a	3.9 ± 0.48 c	16.5 ± 3.82 b	
	10.0	1.0	0	1.0 ± 0.00 a	4.7 ± 0.65 c	19.3 ± 3.59 c	+
IBA	0.0	10.0	0	1.0 ± 0.00 a	4 ± 0.79 c	15.3 ± 5.06 b	+
BA	0.1	10.0	0	1.0 ± 0.00 a	3.0 ± 0.51 c	12.9 ± 4.17 b	
	1.0	10.0	0	1.0 ± 0.00 a	3.6 ± 0.46 c	12.3 ± 2.64 b	
	10.0	10.0	6.2	1.1 ± 0.08 a	2.9 ± 0.34 c	7 ± 1.01 a	+
ZR	0.1	10.0	14.5	1.1 ± 0.08 a	5.1 ± 0.51 c	20.8 ± 4.42 b	
	1.0	10.0	62.5	1.8 ± 0.25 b	5.2 ± 0.63 c	15.6 ± 2.08 b	+
	10.0	10.0	76.7	2.1 ± 0.63 b	4.4 ± 0.34 c	19.1 ± 2.93 b	++
2-IP	0.1	10.0	0	1.0 ± 0.00 a	3.3 ± 0.63 c	20.4 ± 6.84 b	
	1.0	10.0	6.2	1.1 ± 0.06 a	4.3 ± 0.39 cd	18.3 ± 3.09 b	
	10.0	10.0	0	1.0 ± 0.00 a	5.3 ± 0.74 d	18.2 ± 2.49 b	++

Notes: +, 40–59%; ++, 60–70%; +++, 80–100%. In each column means (± SE) followed by the same letter are not significantly different ($p \leq 0.05$) according to Kruskal–Wallis one-way analysis of variance on ranks and Dunnett's multiple range test.

Downloaded by [Umeå University Library] at 04:39 27 September 2017

which had been removed), it was therefore adopted in all micropropagation tests.

In vitro shoot multiplication

For *in vitro* shoot multiplication, two-node sterilized explants were cultured for 60 days in MS medium containing various concentrations (0.0, 0.1, 1.0 and 10.0 μM) of three different cytokinins (BAP, 2iP and ZR) alone or in combination with different concentrations (0.0, 1.0 and 10.0 μM) of auxin IBA (Table I). Two-node segments cultured on MS without growth regulator supplement (HF) developed a single shoot with an average length of 6.7 mm and with an average of 1.9 nodes. Therefore, only explants with more than one regenerated shoot were considered in estimating the adventitious shoot regeneration rate. The type and the concentration of cytokinin influenced (Table II) shoot formation, shoot length and the number of nodes when applied alone. Indeed, 10 μM ZR was the most effective for inducing *de novo* shoot regeneration, either in terms of number of shoots generated per explant (1.7 shoots) or percentage of regenerating explants (52%) (Table I). In addition, either 1.0 or 10.0 μM ZR were capable of inducing a significantly higher number of nodes per shoot (4.1 and 4.9 nodes per shoot, respectively) than other cytokinins (2iP and BAP). Significant difference was also observed in shoot length among treatments. Indeed, newly formed shoots displayed a greater elongation in response to either 1.0 and 10.0 μM 2iP (13.3 and 19.4 mm, respectively) or ZR (16.1 and 20.1 mm, respectively) than that in response to the other treatments and HF (Table I). However, the efficiency of shoot regeneration was enhanced when two-node explants were cultured in the simultaneous presence of cytokinin and auxin. Indeed, among the tested combinations of auxin and cytokinin, 1.0 or 10.0 μM ZR plus 10.0 μM IBA induced the highest number of shoots per explant (1.8 and 2.1, respectively) and percentage of regenerating explants (62.5 and 76.7%,

Table II. Analysis of variance for effect of growth regulators on micropropagation of two-node explants of *A. digitata*.

	Regeneration (%)	Number of shoots per explant (average ± SE)	Number of node per explant (average ± SE)	Length of shoots (mm)
Cytokinin type (A)	***	***	***	***
Cytokinin level (B)	***	***	***	*
Auxin level (C)	NS	NS	***	***
A × B interaction	***	***	NS	NS
A × C interaction	**	*	NS	NS
B × C interaction	NS	NS	*	**
A × B × C interaction	NS	NS	NS	NS

Notes: NS, non-significant; *significant at $p \leq 0.05$; **significant at $p \leq 0.01$; ***significant at $p \leq 0.001$.

respectively). Moreover, shoots developed following exposure to these treatments showed limited callus formation. The combination of 10.0 μM 2iP plus 10.0 μM IBA was most effective in inducing node development in newly formed shoots (5.3 nodes). Shoot elongation was significantly enhanced in the presence of either 10.0 μM 2iP plus 1.0 μM IBA or 10.0 μM ZR plus 1.0 μM IBA (19.3 and 22.6 mm, respectively) compared with control (1.0 μM IBA alone). Conversely, shoot elongation was significantly reduced in the presence of either 10.0 μM BAP and 1.0 μM IBA or 10.0 μM BAP and 10.0 μM IBA. In these experiments, when applied alone, IBA enhanced either the shoot elongation or the number of nodes per shoot compared with HF, in a concentration-independent manner. Because the combination of 10.0 μM ZR plus 10.0 μM IBA induced the greatest shoot proliferation response and well-developed shoots were obtained (Figure 2A and 2B), the further *in vitro* rooting was accomplished by using the shoots formed in the presence of this treatment.

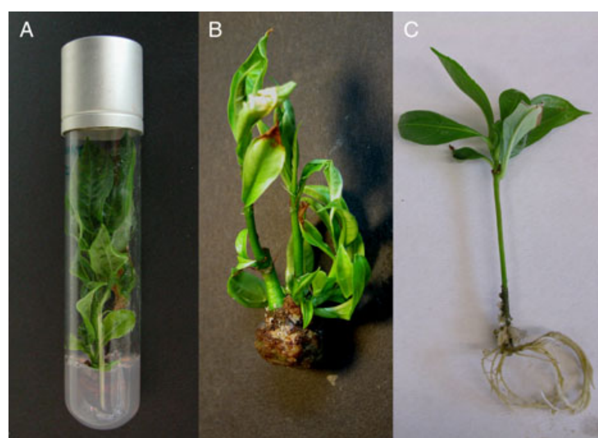

Figure 2. Micropropagation of *A. digitata*. (A) shoot induction from two-node explant after 8 weeks of culture. (B) multiple shoot obtained from explants cultured on 10.0 μM ZR + 10.0 μM IBA. (C) well-developed root system in micrografting cultured on 10.0 μM IBA.

Rooting and acclimatization

To induce *in vitro* rooting, microshoots derived from proliferating cultures were excised and placed in MS media with 1.0 or 10.0 μM IBA. When no auxin was used (HF), 13.0% of explants rooted (Table III). However, rooting frequency was enhanced when the shoots were cultured in the presence of IBA. Indeed, at 10 μM, IBA induced significantly higher rooting percentage (45.0%) than HF (Table III). There was no significant difference either in roots number or in rooting time among treatments (Table III). All the rooted plantlets survived during acclimatization and further transplantation to the greenhouse.

Discussion

Partially successful attempts to micropropagate *A. digitata* from *in vitro*-germinated explants were obtained by Ishii and Kambou (2007), Singh et al. (2010) and N'Doyle et al. (2012). These authors obtained high multiplication rates by culturing apex, cotyledonary and axillary nodes. However, in our previous work, we observed that abnormal seedling growth or shoot-tip necrosis from *in vitro*-germinated seeds could occur, making it difficult to obtain reliable cultures to initiate *A. digitata* micropropaga-

Table III. Effect of auxin treatment (IBA) on *in vitro* root formation after 8 weeks.

	Rooting (%)	Mean root number per rooted cutting	Timing of root emergence	Acclimatization (%)
HF	13 ± 2.3 a	1.6 ± 0.3 a	32.9 ± 2.2 a	100
IBA1.0 μM	23.5 ± 5.2 ab	1.9 ± 2.6 a	29.7 ± 1.2 a	100
IBA10.0 μM	45.0 ± 3.5 b	2.1 ± 0.2 a	25.8 ± 1.0 a	100

Note: Values followed by the same letter are not significantly different ($p \leq 0.05$) according to ANOVA and Tukey's HSD mean separation test.

Downloaded by [Umeå University Library] at 04:39 27 September 2017

Downloaded by [Umeå University Library] at 04:39 27 September 2017

tion (Rolli et al. 2012). To overcome these problems, we decided to establish *in vitro* culture protocol of *A. digitata* from *in vivo*-germinated seedlings. A high germination rate of baobab seeds (more than 65.0%) and well-developed seedlings were obtained by *in vivo* germination. Aseptic cultures were successfully established by sterilization with sodium hypochlorite of two-node segments from 4-week-old baobab *in vivo*-grown seedlings. In a previous work, it was observed that two-nodal segments of *Hyssopus officinalis* were capable of producing a greater number of shoots if compared with mono-nodal explants (Rolli et al. 2011). Therefore, in order to improve shoots formation of micropropagated baobab, we opted for the use of two-nodal explants.

The best results for shoot multiplication were obtained with MS medium supplemented with 10 μM ZR alone or with combinations of 1.0 or 10.0 μM ZR and 10.0 μM IBA. In the presence of these proliferative conditions, the highest percentage of regenerating explants was obtained, callus formation was limited to the basal part of the two-node segment and completely absent in the regenerated shoots. In addition, the shoots obtained were well developed, both in terms of mean number of nodes and shoot length. Therefore, two-node explants from these adventitious shoots were used for six successive subcultures in the same medium, and they maintained their regenerative potential (data not shown). The effect of cytokinin on shoot multiplication and development was ascribable to cytokinin type and concentration. Indeed, our results showed a positive relationship between the type and the concentration of exogenously applied cytokinin with regard to the percentage of regeneration and the number of shoots per explant. The data obtained indicated that exogenous application of auxin alone also promoted shoot growth. In addition, when auxin was simultaneously applied with cytokinin, there was a positive relationship between the concentration of auxin and cytokinin with regard to the number of nodes per shoot and the shoot length (Table II).

Our data showed that auxin treatment with 10 μM IBA considerably increased rooting percentage over the *hormone-free* condition. No difference among treatments in terms of mean root number per rooted microcutting and mean rooting time was detected. Acclimatization was achieved with 100% success for all the *in-vitro* rooted plantlets.

We obtained a theoretical number of plants similar to that reported by N'Doye et al. (2011), although these authors used mono-nodal explants as starting plant material for *in vitro* culture.

In culture of two-node explants, one or a few shoots were obtained from the development of the upper bud, whereas treatment with cytokinins was able to promote the development of shoots also from basal buds. This observation can be explained by setting up of apical dominance in two-nodal segments, removed by high doses of cytokinin in the culture medium.

Nonetheless, results presented here clearly show the yield of vigorous and well-developed adventitious shoots without the occurrence of shoot-tip necrosis or abnormal seedling growth. Hence, the proposed multiplication procedure can be considered as a starting point for developing *in vitro* baobab micropropagation schemes from juvenile specimens, which allows an improved formation of new shoots by enhancing the multiplication factor. In addition, the newly formed shoots were able to develop a strong and healthy root system, which ensured the plant acclimatization and survival to greenhouse conditions. The procedure adopted in this work might represent a suitable and reproducible protocol for cloning plant material aimed at establishing commercial cultivations of baobab, starting from germplasm endowed with optimal fruit productivity, phytochemical composition and agronomic traits. Further studies are necessary to ensure the effectiveness of this approach as a routine micropropagation method, and the genetic stability of long-term micropropagated shoots should be considered, in an attempt to assess the genetic integrity of the regenerants before transfer to soil.

Taken together, our data suggest that two-node segments from *in vivo*-germinated seedlings represent good starting material for *A. digitata in vitro* cultures because plant tissues in vegetative phase were resilient to sterilization with sodium hypochlorite. Moreover, we demonstrated that further *in vitro* shoot multiplication of nodal segments is influenced by the type and the concentration of cytokinins and by the simultaneous presence of auxin in the medium. Of the three tested cytokinins, the superiority of ZR to BAP and 2iP on shoot development was clear at all tested concentrations. The resulting microshoots represented an ideal source for further subculturing and for rooting.

References

Assogbadjo AE, Glèlè Kakaï R, Chadare FJ, Thomson L, Kyndt T, Sinsin B, et al. 2008. Folk classification, perception and preferences of baobab products in West Africa: Consequences for species conservation and improvement. Econ Bot 62: 74–84.

Assogbadjo AE, Glèlè Kakaï R, Edon S, Kyndt T, Sinsin B. 2011. Natural variation in fruit characteristics, seed germination and seedling growth of *Adansonia digitata* L. in Benin. New Forests 41: 113–125.

Assogbadjo AE, Glèlè Kakaï R, Kyndt T, Sinsin B. 2010. Conservation genetics of baobab (*Adansonia digitata* L.) in the parklands agroforestry system of Benin (West Africa). Not Bot Hortic Agrobot Cluj 38(2): 136–140.

Assogbadjo AE, Kyndt T, Chadare FJ, Sinsin B, Gheysen G, Eyog-Matig O, et al. 2009. Genetic fingerprinting using AFLP cannot distinguish traditionally classified baobab morphotypes. Agrof Syst 15: 157–165.

Besco E, Braccioli E, Vertuani S, Ziosi P, Brazzo F, Bruni R, et al. 2007. The use of photochemiluminescence for the measurement of the integral antioxidant capacity of baobab products. Food Chem 102: 1352–1356.

Cuni Sanchez A, De Smedt S, Haq N, Samson R. 2011. Variation in baobab seedling morphology and its implications for selecting superior planting material. Sci Hortic 130: 109–117.

Cuni Sanchez A, Haq N, Assogbadjo AE. 2010. Variation in baobab (*Adansonia digitata* L.) leaf morphology and its relation to drought tolerance. Genet Resour Crop Evol 57: 17–25.

Engelmann F, Engels JMM. 2002. Chapter 9 Technologies and Strategies for *ex situ* conservation. In: Engels JMM, Ramanatha Rao V, Brown ADH, Jackson MT, editors. Managing Plant Genetic Diversity. Oxford: CABI Publishing. pp. 89–104.

Freeman KLM, Bollen A, Solofoniaina FJF, Andriamiarinoro H, Porton I, Birkinshaw CR. 2014. The Madagascar Fauna and Flora Group as an example of how a consortium is enabling diverse zoological and botanical gardens tocontribute to biodiversity conservation in Madagascar. Plant Biosyst 148: 570–580.

Ishii K, Kambou S. 2007. *In vitro* culture of an African multipurpose species *Adansonia digitata* L. Propag Ornam Plants 7: 62–67.

Jensen JS, Bayala J, Sanou H, Korbo A, Ræbild A, Kambou S, et al. 2011. A research approach supporting domestication of Baobab (*Adansonia digitata* L.) in West Africa. New Forests 41: 317–335.

Kamatou GPP, Vermaak I, Viljoen AM. 2011. An updated review of Adansonia digitata: A commercially important African tree. South Afr J Bot 77: 908–919.

Leakey RRB. 1999. Potential for novel food products from agroforestry trees: A review. Food Chem 66: 1–14.

Lockett CT, Calvert CC, Grivetti LE. 2000. Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, Northeastern Nigeria. Int J Food Sci Nutr 51: 195–208.

Munthali CRY, Chirwa PW, Akinnifesi FK. 2012. Genetic variation among and within provenances of *Adansonia digitata* L. (Baobab) in seed germination and seedling growth from selected natural populations in Malawi. Agrof Syst 86: 419–431.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15: 473–497.

N’Doye AL, Sambe MAN, Sy MO. 2012. Propagation of African Baobab (*Adansonia Digitata* L., Bombacoideae, Malvaceae) Germplasm through *in vitro* cloning. Adv Env Biol 6: 2749–2757.

Rolli E, Ricci A, Bianchi A, Bruni R. 2011. Optimisation of in vitro propagation of *Hyssopus officinalis* L. using two-node explants and N-phenyl-N(-benzothiazol-6-yl-urea (PBU), a new urea-type cytokinin. J Hortic Sci Biotech 86: 141–145.

Rolli E, Bigliardi MV, Ricci A. 2012. Micropropagation of *Adansonia digitata* L. Acta Italus Hortus 6: 68–72.

Rossi G, Orsenigo S, Dhital D, Shrestha S, Shrestha BB, Maharjan SR, et al. 2014. *Ex situ* plant conservation initiative in developing country: Nepal as a case study. Plant Biosyst 148: 565–569.

Sidibé M, Williams JT. 2002. Baobab. *Adansonia digitata* L. Southampton: International Centre for Underutilised Crops.

Singh S, Rai S, Khan S. 2010. *In vitro* seed germination of *Adansonia digitata* L.: An endangered medicinal tree. Nanobiotech Univer 1: 107–112.

Tsy JMLP, Lumaret R, Mayne D, Vall AOM, Abutaba YIM, Sagna M, et al. 2009. Chloroplast DNA phylogeography suggests a West African centre of origin for the baobab. *Adansonia digitata* L. (Bombacoideae, Malvaceae). Mol Ecol 18: 1707–1715.

Volis S, Blecher M. 2010. *Quasi in situ*: A bridge between *ex situ* and *in situ* conservation of plants. Biodivers Conserv 19: 2441–2454.

Downloaded by [Umeå University Library] at 04:39 27 September 2017