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Full Length Research Paper

In vitro regeneration of adult trees of *Bambusa vulgaris*

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We developed procedures for the regeneration of *Bambusa vulgaris* using nodal segments from adult plants. Optimal shoot growth was after 16 days cultivation on modified Murashige and Skoog (MMS) medium supplemented with 2 mg/l of BAP. Elongated shoots of *B. vulgaris* rooted (45.85%) when cultured in MMS + 20 mg/l IBA. The rooted and acclimated shoots were successfully transferred into the field with 100% of plantlets survival.

Key words: *Bambusa vulgaris,* micropropagation, adult.

INTRODUCTION

Bambusa vulgaris is a rhizomatous plant. The genus of bamboo (*Arundinaria, Oxytenanthera, Oreobambos* and *Bambos)* are present throughout the continent under rainfall ranging from 700 to 1500 mm (Sene, 1998). Because of the monocarpic character, episodic flowering, over-exploitation and bush fires, the bamboo is extinct in the region of Fatick and strongly fragmented and threatened in Kaolack (both in Senegal) (Sene, 1998). Thanks to its very easy adaptation to certain unfavourable ecological conditions, bamboo is often used to fight against water and wind erosions. Bamboo is a multipurpose species and is utilized in various handicraft, building, food and medicine.

In Senegal, 500 millions CFA per annum is derived from the taxes paid to produce the bamboo panels in hundred thousands piece annual quota (Sene, 1998). The Bassari and Coniagui people of Eastern Senegal use bamboo to make mats, beds, basketry, etc. Thatches of bamboo are part of house making for 500 millions people in the world (Sastry, 2000). Now, vigorous bamboo forest can be found only in protected areas and mostly in Niokolo Koba National park (Senegal).

In spite of all the problems encountered, the regeneration of bamboo in Senegal has not been undertaken in a significant way. Vegetative multiplication is an important way to restore bamboo formations. The vegetative multiplication with nodal fragments of twigs permits to obtain clones in order to maintain the genetic homogeneity of species offering important genetic characters. Vegetative multiplication has been practised on young *Bambusa ventricosa*, but the growth of the stem from the axillary bud is slow and the cultivation causes necrosis and the plant finally dies after 6 months (Dekkers et al., 1987). Similar results were found by Rao et al. (1985) on stems of young *Dendrocalamus strictus*. Because of the problems in the vegetative multiplication by nodal explants as well as the poor rooting, somatic embryogenesis ought to be used for bamboo *in vitro* cultivation. The *B. vulgaris* species investigated in this work is a woody graminaceae introduced into Tropical Africa (Berhaut, 1981). This paper describes a method of clonal multiplication from nodal fragments collected from adult trees of *B. vulgaris*.

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Abreviations: IAA, Indol-3-acetic acid ; **IBA** : Indol-3-butyric acid. **NAA** : α-naphthalene acetic acid. **BAP** : 6-benzylaminopurine. **MS** : Murashige and Skoog. **MMS** : modified Murashige and Skoog.

MATERIALS AND METHODS

Plant material and explant sterilization

One year old twigs collected from 20 years old *B. vulgaris* were used for *in vitro* regeneration. The *B. vulgaris* trees were located in the Botanical garden of the science faculty of Cheikh Anta Diop University of Dakar/Senegal. 1 to 2 cm long nodal fragments were abundantly washed in running tap water. Their surface disinfected by dipping them into $HgCl_2$ (0.1%) for 20 min followed by 4 times rinces in sterile distilled water.

Table 1. Effect of media on shoot induction from nodal segments of *Bambusa vulgaris* after 16 days cultivation on media.

Media	% of regeneration	Number of buds	Number of elongating shoots	Length of shoot
MS	87.5	3a	2a	32a
MMS	100	3a	2a	38a
B_5	91.66	3a	1b	27a
WPM	91.66	3a	1b	33a

Means within a column followed by the same letter were not significantly different (p=0.05) according to Fisher's test.

Table 2. Effect of different concentration of BAP and kinetin on shoot induction from nodal segments of *Bambusa vulgaris* after 16 days cultivation on MMS medium.

Supplement (mg/l)	Number of shoots	Number of elongating shoots	Length of shoots
MMS_0	3c	2a	36b
$BAP_{0.5}$	4b	2a	47a
BAP1	5a	1b	48a
BAP_2	5a	2a	56a
Kin_2	3c	2a	41b

Means within a column followed by the same letter were not significantly different (p=0.05) according to Fisher's test.
MMS: Murashige and Skoog medium modified.
$BAP_{0.5}$: 0.5 mg/l of BAP
1: 1 mg/l of BAP
2: 2 mg/l of BAP
2: 2 mg/l of kinetin

Culture media and conditions

Four basal media were compared: Murashig and Skoog (MS) medium (1962); Gamborg et al. medium (1960); Lloyd and Crown medium (1980); Murashig and Skoog medium modified (MMS) (Mathur et al., 1995). The basal media added with 30 g/l sucrose. Different concentrations of phytohormones (0.5, 1, and 2 mg/l of BAP and 2 mg/l of kinetin) were used. Media were solidified with gelrite (3.5 g/l); pH was adjusted to 5.5 – 5.6 before autoclaving (120°C for 20 min). After placing bamboo nodes on media, the cultures were incubated at 25°C under 16 h/8 h photoperiod pounded by white fluorescent tubes.

Induction of rooting and acclimatization

After 20 to 30 days cultivation, newly formed shoots were induced to MMS rooting medium (Figure 1) with different combinations of NAA and IBA (5 – 20 mg/l) and BAP (0 – 0.1 mg/l). After 7 days in root induction medium, shoots presenting root primordia were transferred to MMS medium without hormone. Acclimatization of rooted shoots was performed in pots containing sterile perlite/peat (1 volume/2 volumes) placed in greenhouse.

Observation of culture and presentation of results

24 explants were used per treatment. After 16 days of cultivation in the different media, the variables measured were: the rate of regeneration, the number of buds, the elongating shoots, the length of shoots and the percentage of rooting. Statistical analysis was performed using ANOVA (Fisher's test) at 95%.

RESULTS AND DISCUSSION

Culture media

The 4 media tested did not show significative difference in the number of shoot newly formed. But MMS medium shows also the highest rate of regeneration (100%) followed by WPM and B5 (91.66%) and MS (87.5%) (Table 1). The same observations were made by Sadio (2000) on *Hibiscus sabdarifa*. In general, BAP and kinetin stimulate formation and development of the buds. The gibberellins effects are rarely noticed (Margara, 1969).

Phytohormones

The number of shoots newly formed increased with the concentration of BAP with 5 shoots using MMS with BAP 2 mg/l. Kinetin concentration (2 mg/l) used was not found to have significant influence on shoot multiplication. But all the buds formed did not elongate, only one or two buds grew in the culture medium (Table 2). The same medium was optimal for shoot elongation with 56 mm after 16 days culture compared to MMS with BAP 1 mg/l (48 mm) and MMS without hormones (36 mm). According to Sabapathy and Nair (1992), the high concentration of BAP stimulates the buds development.

The highest number of shoots per explant was obtained when the medium was supplemented with 0.5 µM BAP,

Figure 1. *In vitro* regenerated plants of *Bambusa vulgaris*. A: 15 days old plants; B: rooted plants; C: acclimatized plants after 30 days in greenhouse; and D: 40 days old acclimatized plants.

Table 3. Effect of media supplemented with different concentrations and combinations of IBA, NAA and BAP on rooting.

Media	Shoots rooted %
MMS+IBA_5	8.33
MMS+NAA_5	8.33
MMS+IBA_5+$BAP_{0.1}$	4.16
MMS+NAA_5+$BAP_{0.1}$	8.33
MMS+IBA_{20}	45.83

MMS: Murashige and Skoog medium modified 5: 5 mg/l of IBA 20: 20 mg/l of IBA 5: 5 mg/l of NAA 0.1: 0.1mg/l of BAP Total number of shoots per media = 24.

and the greatest shoot length was obtained when the medium was supplemented with 0.5 µM BAP (Gomez and Segura, 1995). According to Ndoye et al. (2003), MS medium supplemented with 5 mg/l BAP induced a mean of 4.30 shoots per node explant of *Balanites aegyptiaca*. Explants from adult trees present difficulties for rooting; addition of 5 mg/l of auxin did not induce rooting which was achieved after using high concentration (20 mg/l) of IBA. The optimal rate of rooting obtained was 45.83% in *B. vulgaris* (Table 3). The high concentration of IBA (20 mg/l) added to the MMS medium could be explained by the lack or the feeble synthesis of endogenous auxin by the explants. For the control (without hormone), the rooting was 0% after 30 to 40 days.

According to El Nour et al. (1991), the IBA hormone did not improve rooting significantly, which is contrary to our results. The root induction *in vitro* culture is generally preceded by the formation of basal cal (Margara, 1984). Our results are contrary to the observations of Margara (1984). The roots appear after the eighth day without basal cal formation. After taking roots, generally, the initial explant necroses and is replaced by one or two basal rejects. There were no significant differences between the auxins (IBA, NAA) on the nature of the roots.

Acclimatization constitutes a very important tricky stage *in vitro* cultivation for some vegetal species. Rooted shoots after acclimatization showed 100% survival, and grew well in greenhouse before planting in the fields. These satisfactory results in the transfer and acclimatization could be related to the easy adaptation of the bamboo in marginal ecological conditions (Crouzet, 1981). Wei and Tien (1995) have successfully transferred *in vitro* regenerated plants of *Bambusa beecheyana* Munro var *beecheyana* into the field.

The present results showed that the MMS medium with hormones enhanced axillary buds formation and shoots

development.

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