



IN VITRO PLANTLET REGENERATION FROM NODAL EXPLANTS OF FIELD GROWN CULMS, EVALUATION OF GENETIC FIDELITY USING RAPD MARKERS AND FIELD PERFORMANCE OF BAMBUSA NUTANS

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ABSTRACT: *Bambusa nutans* Wall. is an industrially important bamboo species used in construction, furniture, paper and pulp industry. An efficient and reproducible protocol has developed for in vitro propagation of *B. nutans* through axillary shoot proliferation. Nodal explants obtained from ten years old field grown clumps, produced multiple shoots (5-6 shoots/explant) on Murashige and Skoog (MS) liquid medium supplemented with additives viz; ascorbic acid (50 mg/l) + citric acid (25 mg/l) + L-cysteine (25 mg/l) + NAA 0.25 mg/l + BAP 5.0 mg/l. Shoot multiplication were carried out with different concentration of 6-benzylamino purine (BAP), kinetin (Kn) and thidiazuron (TDZ) with NAA in MS liquid medium with additives and different types of nutrient media (MS, B₅, SH, WPM and Heller) for high frequency shoot multiplication. Highest rate of shoot multiplication (6-7 folds) was obtained on MS liquid medium incorporated with additives and NAA 0.25 mg/l + TDZ 0.25 mg/l within 4 weeks period at 25 ± 2°C temperature and 37.5 μ mol m⁻² s⁻¹ intensity of light for 12 h photoperiod. In vitro shoot propagule (2-3 shoots/clump) of 3-4 cm in length exhibited high frequency rooting (100%) on MS/3 basal salts medium supplemented with 2.0 mg/l IBA, within 4 weeks period. Ex vitro rooting (95%) was achieved in 4 weeks by pulse treating the in vitro produced shoots with IBA (1000 ppm) for 20 min and planted in sand and soilrite (1:1 v/v, ratio) medium in trays in polytunnel in green house. Based on the protocol developed, 2000 plants were produced and transferred to field in Shimoga (Karnataka), Eluru (Andhra Pradesh) and Agalote (Goa) in India. Survival rate was more than 90 % in all the three sites after six months. Genetic fidelity studies using RAPD markers revealed no genetic variation in the micropropagated plants raised through axillary shoot proliferation from the 18 months old shoot multiplication cultures. This is the first report on in vitro propagation from field grown mature and superior candidate plus clump of *B. nutans* evaluation of genetic fidelity and field performance.

Key Words: *Bambusa nutans*, mature clump, in vitro regeneration, in vitro and ex vitro rooting, RAPD markers, field performance.

Abbreviations: BAP, N⁶-benzylaminopurine; IAA, indole 3-acetic acid; IBA, indole 3-butyric acid; Kn, 6-furfurylaminopurine; MS, Murashige and Skoog medium; NAA, -naphthaleneacetic acid; NOA, naphthoxyacetic acid; TDZ, thidiazuron; B₅, Gamborg et al. medium; HE, Heller medium; RAPD, Random Amplified Polymorphic DNA; SH, Schenk and Hildebrandt medium; WPM, Woody Plant Medium.

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Bamboo constitutes single most important forest produce, which contributes to a great

extent to the economy of rural communities in developing countries, particularly in the Asia Pacific region. Out of 75 genera and 1250 species of bamboo recorded from all over the world (Soderstrom and Ellis 1987), about 125 species belonging to 23 genera occur in India with a wide range of distribution, covering an estimated area of 8.96 million hectares, which constitutes 11.71% of the forest area in India (Rai and Chauhan 1998). Demand of bamboo is estimated to 26.6 million tons/year, whereas supply is of 13.7 million

tons/year and there is a wide gape in demand and supply (Anonymous, 2003). In order to meet the increasing demand of bamboo, plantation of bamboo in forests and outside forestland is a viable alternative.

Bambusa nutans Wall., is an economically and industrially important bamboo species of the family Poaceae (Gramineae). It grows the best at altitudes of 500-1500 m. *B. nutans* thrives well on moist hill slopes, flat uplands and well-drained sandy loam to clay loam soils. In India, commonly found in the North- Eastern states, Orissa and Bengal. The culm attains height up to 20 m, and dark green, loosely clumped, straight and smooth. Due to fast growth and spine less branches, *B. nutans* is an important agro-forestry species. During