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Declaration by the Candidate

The work described in this thesis was carried out by me in the Plant Biotechnology Project of Institute of Fundamental Studies, Kandy under the supervision of Ms. S. M. S. D. Ramanayake, Senior Research Fellow, Institute of Fundamental Studies, Kandy and Prof. (Mrs.) Hemanthi Ranasinghe, Head, Department of Forestry & Environmental Science, University of Sri Jayawardenepura and a report on this has not been submitted in whole or in part to any University for another Degree/Diploma.

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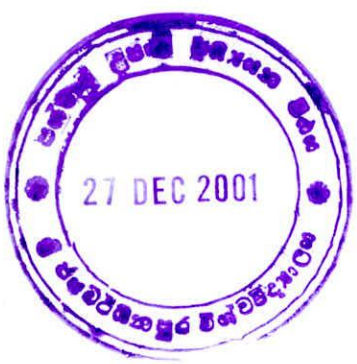

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In-vitro responses of a mature clump of giant bamboo (*Dendrocalamus giganteus* Wall. ex Munro) towards micropropagation, callus cultures and somatic embryogenesis

By

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Thesis submitted to the University of Sri Jayawardenepura for the award of the degree of Master of Philosophy

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(*Dendrocalamus giganteus* Wall. ex Munro)

towards

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W. A. V. R. Wanniarachchi

ABSTRACT

In-vitro responses of a mature clump of giant bamboo (*Dendrocalamus giganteus* Wall. ex Munro) towards micropropagation, callus cultures and somatic embryogenesis were studied over a period of two and half years (from Sept. 1996 to May 1999), in the Plant Biotechnology Project of the Institute of Fundamental Studies, Kandy.

Single nodal segments from a mature clump of giant bamboo, reported to be over 70-year old, were used as the starting material. Low rooting and survival of plantlets is the most serious bottleneck in the development of complete protocols of propagule production in this species as well as in most other species of bamboo. Therefore efforts at improving rooting in this species were made. Different growth regulators and other additives such as coconut water and sucrose were incorporated in the shoot proliferation medium to study their effects on shoot proliferation and subsequent rooting.

The sampling of nodal segments was carried out from Sept. 1996 to Feb. 1997. Bud break ranged from 0 - 32.5% with a mean of 14.58% and contamination was 42.5 - 100%. Axenic cultures were established during September 1996, when microbial contamination was the

lowest. These axillary shoots proliferated for over two and half years in a liquid culture medium (MS with 6.0 mg l^{-1} BAP, 0.1 mg l^{-1} kinetin and 2% sucrose). Shoots showed 1.6 - fold increase in shoot number during subculture cycles of 10 days at culture initiation. Rate of shoot proliferation was 1.5, 1.9, 3.2 and 1.7 - fold after six and twelve months of culture initiation, just before flowering and during *in-vitro* flowering respectively.

The responses of shoot cultures to different sucrose levels were not consistent. Two and half months after culture initiation the shoots were not able to survive on increasing the sucrose level to 4%. After one and half years they proliferated well in 4% sucrose with a significantly high shoot number compared to 2 or 3 % sucrose, indicating a possible change in the physiological status of cells with prolonged culture. Elimination of coconut water from the shoot proliferation medium increased shoot proliferation. Lowering the level of BAP (3.0 mg l^{-1}) or increasing it to 8.0 or 12.0 mg l^{-1} reduced shoot proliferation significantly while shoots did not survive in the absence of BAP. The replacement of BAP (6.0 mg l^{-1}) with Thidiazuron (TDZ) (1.5 mg l^{-1} and 3.0 mg l^{-1}), another cytokinin, brought about rapid shoot proliferation. However, TDZ caused browning and stunting of shoots within a few days.

Root induction was difficult due to browning of shoots on transfer to a rooting medium (half MS with 3.0 mg l^{-1} IBA and 10.0 mg l^{-1} coumarin), when only 20% shoots rooted. Supplementing the shoot proliferation medium with coumarin and phloroglucinol, before transferring shoots into the rooting medium improved rooting to 73.3%. But browning of shoots could not be overcome. Shoots pretreated with lower level of BAP (3.0 mg l^{-1}) and IBA (3.0 mg l^{-1}) for four subculture cycles in the shoot proliferation medium resulted in 40% root induction in the rooting medium. However, shoots turned brown and there was no

new shoot development. On transfer of these rooted shoots to the rooting medium further supplemented with 1.0 mg l^{-1} kinetin brought about the formation of new shoots resulting in survival of 50% of rooted shoots. These were acclimatized to soil in the nursery.

In-vitro flowering took place after 29 months. *In-vitro* flowering was not the expression of a species-specific mechanism believed to occur during gregarious flowering, as the mother clump did not flower. The development of axillary meristems into vegetative or generative shoots (spikelets) depended on the level of BAP in the medium. The rate of shoot proliferation increased to 3.2 - fold just before flowering and decreased to 1.7 after flowering was induced. Floral reversion also took place. The possible role of BAP and changes in the rate of shoot proliferation leading to build up and release of stress in relation to flowering and its reversion are discussed. The similarity of *in-vitro* florets to *in-vivo* florets, from a non-related clump of the species, indicated possible applications of this technique in taxonomic studies. However, the florets did not mature and anthesis did not occur.

In-vitro shoots and roots were used in induction and proliferation of callus in a MS medium with 2,4-D (7.5 mg l^{-1}), 4% sucrose and agar. Cell suspension cultures were also established in this medium without agar. Attempts at somatic embryogenesis in cell suspensions, resulted in the formation of pro-embryos that did not develop further, in the presence of 2,4-D (7.5 mg l^{-1}) and NAA (3.0 mg l^{-1}). A few plantlets were also regenerated from callus in the same treatment solidified with agar. Organogenesis/embryogenesis is common in seedling-derived tissues but not in adults such as the 70-year old bamboo used in the present investigation. Further investigations leading to consistent plantlet regeneration is recommended to exploit this potential.

Chapter 1

INTRODUCTION

Bamboo belongs to the sub family of Bambusoideae of the family Poaceae. Unlike other members of the family, they are arborescent, woody-stemmed giant grasses.

There are as many as 75 genera and 1200 species of bamboo. Most are confined to the tropical parts of South-East Asia, which can be regarded as the center of diversity of bamboo (Mandal and Subramanian, 1992).

Sri Lanka has ten native species and a number of introduced species. A remarkable feature of the native species is the high degree of endemism (80%) with one genus (*Davidsea*) and eight species being reported as unique to the country (Zoyza *et al.*, 1988). Among the introduced species, seven are cultivated, with *Bambusa vulgaris* being the most widely cultivated. *Dendrocalamus giganteus*, *D. strictus*, *D. asper* and *D. membranaceus* are cultivated on a smaller scale. *B. multiplex* and *Thyrsostachys sidmensis* are cultivated as ornamentals while the remaining introduced species are restricted to the three botanic gardens (Zoyza *et al.*, 1988).

Bamboos differ greatly in size and form. Some species are tall and grow up to a height of over 30 - 40 m while others are shrubs, stragglers or climbers. The individual stem, known as a culm, has many jointed cylindrical nodes and internodes. These culms arise from underground rhizomes and reach their full height within a few months (Rao *et al.*, 1992b).

Another characteristic is that the plant spreads laterally by the outward growth of the rhizome forming a bush known as a clump, which quickly colonizes the land. Thus, bamboos are regarded as a fast growing group of plants. The rhizome also has jointed nodes and internodes with tufts of strong roots at each node. These roots form an interlocked mat like structure. This feature together with the rhizome that generally grows within the top 50 cm of the soil, serves to prevent erosion of precious topsoil.

The woody culm is flexible and easy to split due to the vascular bundles that remain separate along the internodes. This makes it amenable to handicrafts. Another important feature in bamboo is the long fiber length and the relatively low proportion of lignin in it. This makes bamboo pulp eminently suited in the paper industry (Rao *et al.*, 1992b).

Some species of bamboo are monocarpic and flower only once and die at the end of the first fruiting season (Nadgauda *et al.*, 1990). This is intriguing not only in that it occurs after lapses of many years, but also because it is gregarious (semelparous) (McClure, 1966; Janzen, 1976; Nadgauda *et al.*, 1990). Causes of rare flowering in bamboo are under speculation. Janzen (1976) believed that it is caused by an internal mechanism of a biological clock.

Species of bamboo have a flowering cycle ranging from 2 to 120 years (McClure, 1966; Mandal and Subramanian, 1992). In *Thyrostachys oliveri* the flowering cycle is about 48 years. The cycle ranged from 20 - 65 years in *D. strictus* and 30 - 45 years in *B. arundinacea* and 20 years in *D. hamiltonii* (Janzen, 1976). According to the reports of

Janzen (1976), the flowering cycle of D. *giganteus* is 76 years. In Sri Lanka this species has flowered occasionally (Macmillian, 1907; Ramanayake and Yakandawala, 1998).

Bamboos have been cultured since time immemorial and have been the most important renewable plant resource that grows naturally. They are grown as ornamentals, hedges and in landscape gardening. They are also grown as a windbreak and are widely grown to control soil erosion, especially along the edges of water bodies.

Bamboo has been extensively associated with human civilization due to the variety of its uses. It has continued to contribute to human welfare. In modern society a number of new products that are turned out on a commercial scale has been innovated. These include bamboo chipboards, bamboo parquet tiles, modern furniture and housing. The most extensive use of bamboo as a raw material is in the paper manufacture. It is estimated that nearly two thirds of the annual bamboo production in India is consumed by the paper industry (Saxena and Dhawan, 1994). In addition, bamboo is traditionally used in building constructions, basket making, and manufacturing of agricultural implements, containers and also in the handicrafts sector. Processing of edible bamboo shoots is thriving industry in Thailand (Visuphaka, 1985).

The high demand for the resource with increased utilization has resulted in the over exploitation and mismanagement of natural stocks of bamboo. Gregarious flowering followed by death and seed predation are also responsible for the depletion of bamboo. Therefore, large scale replanting and developing bamboo plantations is necessary. This would require a continuous supply of large numbers of planting material.