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IN VITRO MICROPROPAGATION OF *DENDROCALAMUS STRICTUS* (SOLID BAMBOO)

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ABSTRACT

Multiples shoots were induced from *in vitro* seedlings of *Dendrocalamus strictus* on Murashige and Shoog's medium supplemented with B.A and kinetin. About 15-25 shoots were obtained within 20-25 days from nodal explants of seedling in the primary culture. The seedling derived cultures were separated into groups of 5-7 and transferred to fresh subculture medium. Rooting of the shoots was achieved under *in vitro* and *ex vitro* conditions. 85 -90% of rooting was achieved by the *ex vitro* method using IBA. A rapid and highly effective method for Micropropagation of *Dendrocalamus strictus* was established. Seeds of *Dendrocalamus strictus* were inculcated on MS medium containing 4.5gm/lit agar and 3% commercial sugar and different combinations of 6-Benzyladenine (BA) with Kinetin (KN). 100% of seeds were germinated on the medium without any growth hormone compared to the medium containing BA (0.5-1.0mg/lit). Nodal segments were proved to be the best region comparison to the shoot tips having high rate of shoot induction and more number of shoots multiplication. Maximum rate of multiplication were observed in the medium containing BA (1.0-3.0mg/lit).

KEYWORDS: GH/GR-(Growth Hormone/ Growth Regulators), DW- (Distilled Water), AC- (Activated Charcoal), CW/CM-(Coconut Water/ Coconut Milk).

INTRODUCTION: - General Introduction

The application of modern biotechnology techniques like plant tissue culture has proven to be a powerful tool in forest plant improvement programs. Scientists hit upon a technique where by not only can these plants be preserved from being lost but are also able to develop a complete plant from a small plant part. Basically plant tissue culture is the technique of growing plant cells, tissues and organs in an artificial, prepared nutrient medium, static or liquid, under aseptic conditions. These explants divide and gradually develop into an give rise to shoots or embryos. Plant tissue culture is now the major component of technologies which are applied in plant biotechnology. Advances made in genetic engineering and molecular biology can be made manifest in plant through the application of various techniques developed in the field of plant tissue culture. Many of the crop plants regarded as recalcitrant are now amenable to regeneration *in vitro* using cultured protoplasts, cells or call, thus each of them can be used as a tool in plant genetic manipulation programs. Considerable progress has been made with regard to the development of media or techniques as well as in understanding the basic aspects, such as cell culture, cellular totipotency, somatic embryogenesis and the development of embryogenic culture. Development of plant tissue culture is closely linked to improvement of techniques of protoplast, cell and tissue and organ culture followed by the success achieved in regenerating whole plants from culture plant materials. Knowledge of plant tissue culture has contributed greatly to our understanding of the factor responsible for growth, metabolism, differentiation and morphogenesis of plant cells. Plant tissue culture is presently of great interest to molecular biologists, plant breeders and industrialist. Tissue culture methods have been used as a tool for the propagation of genetically manipulated superior clones and for ex-situ conservation of valuable germplasm. The progress in use of cell or tissue culture in producing pathogen free plant as well as in the synthesis of many important secondary compounds (including pharmaceuticals) has been very significant. Recently FAO/IAEA established a coordinated research programme to harness the advantages offered by tissue culture in agricultural and general plant biotechnology. Through a considerable progress has been made in tissue culture of plant species, the method is not widely applicable in its present state for cloning, improvement, soma clonal variation, disease resistance, protoplast culture and genetic engineering of trees of and regions therefore, basic information generated will be useful on these lines of work for specific and selected cases for developing clones for fodder, fuel and various types of resistance. The forest constitutes one of the most complex natural ecosystems and the dependency of human society on forest is as old as human evolution itself, because of its

multifunctional role in the viability of life support system. The preservation of forestry species in sufficient number is essential for the ecological health of this planet. The accelerated degradation of forest would over have become a matter of global concern. Large-scale removal of forest cover passes serious anthropogenic interference in the national ecological balance and also destruction of biodiversity, which constitute source of genes for the highly successful breeding program. Locally adopted ancient varieties and their wild relatives have been the traditional sources of breeding materials, changes in the natural habitat and day by day depletion of the ecological system has resulted in losses of locally adopted population of the wild ancestral species. It is increasingly recognized throughout the world that the conservation of biological diversity is most important and this has raise the urgency for preserving natural strains for future. In the forest of M.P species being used for a forestation of wastelands and other areas under natural regeneration schemes, some species are of great importance, for their ecological and economic importance. Some of them are Tectona grandis (teak), azadiracta indica, delbergiasisso, bamboo species (*Dendrocalamus strictus*, bambusa arundinaceae) pongamia pinnata etc. *Dendrocalamus strictus* a bamboo is one of the most important natural and renewable sources in the tropical and sub-tropical regions of the world. Historically woody bamboos have been used for different applications but rather recently interest from paper and wood industries has increased, both for tropical and temperate wood bamboos. Temperate bamboos of the northern hemisphere are used for agro forestry, mainly in China, and as ornamental plants in Europe and the U.S., although some preliminary trials to use bamboo as source of biomass are ongoing in these regions (it was Thomas Jefferson who said that the best contribution one could give to a culture, was to add a valuable crop to its agriculture). Bamboo is often advocated as an ideal renewable resource for biomass in industry. Positive arguments this also include ecological arguments; indeed in the future forests and agriculture water conservation and carbon cycling will become very important criteria. However, the classical economic criteria (profit and added value) will remain very important moreover, if bamboo is to be used as source of biomass, it will have to complete with other plants no to speak about competitions with industrial powers. This will certainly impose much pressure on bamboo, e.g. regarding selection of elite genotypes, Silvicultural methods, new approaches for harvesting and the production of quality biomass. So the time horizon of mass scale bamboo utilization may be quite far beyond what the advocates of bamboo fore see (or hope for) at present. One of the main problems with *Dendrocalamus strictus* is that it has been regarded as a resource which is simply there to take as has been done for thousands of years by people in rural economies.

However, in industrial economies such practice leads to considerable overexploitation and rapid depletion of bamboo resources in the vicinity of the paper mills and factors. Up to the point that transportation costs have become too high for bamboo to be economical (indeed transportation of culms is a lot of air). To cope with this forecasted shortage some large or mass scale bamboo planting has been planned/carried out such a Green Sawn in Thailand to projected reforestation in India. Several pilot plantations or larger scale plantings are planned or carried out throughout the world. It is clear that bamboo propagation and silviculture are the pivotal upstream technologies in the whole process. If something fails there it will have consequences down the line (in the worst case interest in bamboo may fade instead of increase). The whole downstream process of bamboo production and transformation consists of many different steps. Each of these steps is important and as in any successful industrial enterprise or chain of processes optimization in each step as well as integration of steps and feedback is important. Because as a general rule it will be the consumer who has to be satisfied and the whole production scheme should be aiming at the market. At the production side propagation is crucial for mass scale utilization of bamboo. *Dendrocalamus strictus (Roxb.)* Nees are a group of woody Perennial Evergreen Plants in the True Grass family Bambusoideae, tribe Bambuseae. Some of its members are giants forming by far the largest members of the grass family. *Dendrocalamus strictus* is a deciduous densely tufted bamboo. Culms 8-16m high, 2-5-8cm diameter, pale blue green when young dull green or yellow on maturity much curved above half of its height nodes somewhat swollen basal nodes often rooting lower nodes often with branches internodes 30-45cm long thick walled. The steams are jointed with regular nodes each node bears one side bud. These buds do not necessarily develop (especially in lower portions of the Culm of tall bamboos) but are present. Buds that do develop ramify quickly with very short basal internodes into a cluster of several shoots which usually develop into branches and occasionally into adventitious rhizomes. Culm-sheaths: variable lower ones shorter 8-30cm long with golden brown stiff hairs on the back sometimes glabrous in dry localities striate rounded at the top margin hairy ligule 2-3mm high toothed auricles small blade triangular awl-shaped hairy on both ;sides. Leaves: Linear-lanceolate small in dry localities, up to 25cm long and 3cm broad in most areas rounded at the base into a short petiole tip sharply acuminate with twisted point rough and often hairy above softly hairy beneath ligule very short. Inflorescence: A large panicle of large dense globular heads 4-5cm apart rachis rounded smooth. Spikelet's spinescent usually hairy the fertile intermixed with many sterile smaller ones 7-5-12mm long and 2.5-5.0mm broad with 2 or 3 fertile flowers empty glumes 2 or more ovate spinescent many nerved flowering glumes

ovate ending in a sharp spine surrounded by ciliate tufts of hair palea ovate or obovate emarginated lower ones 2-kelled ciliate on the keels and 2-nerved between them uppermost not keeled often nearly glabrous 6 to 8 nerved. Stamens long-exerted turbinate stalked hairy above and surmounted by long style ending in a purple feathery stigma. Caryopsis: Brown, shining, ovoid to sub-globose, ca. 7.5mm long, hairy above, beaked with persistent base of the style, pericarp coriaceous. Different chromosome numbers are reported as 2n=72, 70 and 56 hexaploid (Sobita Devi and Sharma, 1993). Flowering and Fruiting Gregarious flowering cycle varies from 25-45 years. This does not mean that all the clumps of a tract flower at the same time. It commences with intensive sporadic flowering for 2-3 years, increasing progressively resulting in the flowering of all the clumps in a period of five years. Sporadic flowering is seen almost every year. Gregarious flowering is related to injury nutrition, climatic conditions and soil factors. Management practices and biotic interference influences flowering of *Dendrocalamus strictus*. When proper Silvicultural practices are adopted flowering is reported to be delayed by 3-5 years. Flowers appear from November to February and fruits are seen from February to April. Freak flowering of 1-3 year old seedlings in nurseries and natural forests are occasionally reported. Studies have shown that precocious flowering was induced by tissue culture and mutants through gamma irradiation of seeds. Gregarious flowering has been reported from different parts of the country, Kalagarh Forest Division of Uttar Pradesh during 1950-56 and in Kalahandi and Rayagada Division of Orissa State in 1967, Mandal Division of Madhya Pradesh during 1961-63varous localities of Maharashtra during 1940- 1942 -1948 -1949 -1957-1958-1961- 1962 -1978 -1980 Blatter (1930) listed the flowering years of this species from various parts of India and adjacent regions for the period 1865-1914 Ahmed (1969) Uppin (1978) and Kadambi (1949) reported that non-production of new culms in the preceding years could be an important event which signifies the approach of flowering in this species but Bank (1981) observed that all the clumps produced new culms in the preceding years, some in the first year of flowering and no new Culm production in the second year of flowering. Studies on floral biology and breeding behavior showed that the species is dichogamous and protogynous. The gynoecium matures 3 to 4 days before the androecium. Flower opens between 6 to 13 hours and opening depends on atmospheric temperature. The species is anemophilous. The insects feed on pollen but not pollen vectors. Parthenocarpy and apomixes do not occur. Pollen fertility is about 98 percent (Nadgauda et al, 1993). Fruit is a glans, fusiform with obtuse or aristate rostrum at the apex covered with white pubescence. The length of rostrum varies from 2 to 4mm the fruit is covered with three persistent glumes. Surface is smooth with hard seed coat. Average length

and width of seed varies from 7 to 7.3mm and 2.98 to 3.33mm respectively. Fruit navel and ventral suture are absent and pericarp is crustaceous (Appasamy, 1993).

Distribution and Ecology

This species occupies 53 percent of total bamboo area in India. This is one of the predominant species of bamboo in Uttar Pradesh, Madhya Pradesh, Orissa, and Western Ghats. Widely distributed in India in semi dry and dry zone along plains and hilly tracts usually up to an altitude of 1000m, also commonly cultivated throughout the plains and foot hills. *Dendrocalamus strictus* is widely adaptable to temperatures as low as -5^{o}C and as high as 45^{o}C. This species is mainly found in drier open deciduous forests in hill slopes, ravines and alluvial plains. It prefers well-drained, poor, coarse, grained and stony soils. It occurs naturally in tracts receiving as low as 750mm of rainfall and also in extensive gregarious patches or as an under storey in mixed forests and teak plantations.

Chemistry

Proximate chemical analysis showed cold water solubility 6.6 percent, hot water solubility 7.6 percent, 1 percent NaOH solubility 22 percent, alcohol benzene solubility 6.9 percent, holocellulose 70.9 percent, Klason lignin 27.6 percent, pentosan 17.1 percent, ash 4.6 percent (Maheswari and Satpathy, 1990). The pulping characteristics vary with age, locality and position of Culm. Pulping properties of the species show moisture 10 percent, screened pulp yield 45.2 percent, total pulp yield 46.8 percent, kappa No 285 viscosity 30.2 (Cp) at 25^{o}C lignin in bamboo 26 percent, lignin in pulp 3 percent pentosan in bamboo 23.2 percent in pulp 15.3 percent pulp yield unscreened 57 percent screened 50.9 percent (Singh et al 1976). Yield and chemical analysis of holocellulose and hemicelluloses is also reported by Rita Dhawan and Singh (1982). Spectral absorbance of cellulose 0.354, lignin 0.296 (Sekar and BAL Subramanian personal communication). The hemicellulose of bamboo is similar to hemicellulose of hardwoods. The sugar composition of hemicellulose consists of xylose, arabinose and glucose. Glucuronic acid is also present in small amounts with xylose as the main constituent. The nodal portion has lower holocellulose content, but pentosan, lignin and ash are higher compared to inter-nodal region (Maheswari and Satpathy, 1988). Studies on the effect of pH on prehydrolysis of *Dendrocalamus strictus* indicate that pulp yield decrease at higher ph. Analysis of seeds showed 73mg starch, 8mg total sugars, 1mg phenol, 4mg lipid and 14mg protein per 100mg of sample (Appasamy1993).

Seed Storage

The seeds if not sown immediately after collection, may be stored in bags. If it is to sown after one year, it should be dried and stored in sealed tins. The seeds can be stored for longer duration by keeping over silica get or anhydrous calcium chloride in desiccators at 3^{o}C to 5^{o}C after reducing the moisture content to 8 percent (Gupta and Sood, 1978). Hydration and dehydration treatments also reduce loss of viability (Sur et al. 1989). Abnormalities observed in seeds during storage were such as coagulated ball embryo, concentrated sporulation of storage fungi, embryo detachment along the epithelial layer, black encrustation of inner walls, discontinues black spots, embryo less endosperm, jelly like embryo fragments, shrunken embryo, shredded endosperm and pitly air space (Karivaratharajuet al, 1978).

Natural Regeneration

After gregarious or sporadic flowering under natural conditions the seeds germinate soon after the first monsoon showers. It is observed that large number of seedlings survive particularly on newly exposed soils.

Vegetative propagation

Different methods like offset planting rhizome planting, rooting of Culm cuttings and tissue culture are used. One year old culms are cut through with a slanting cut about 90 or 120cm from the ground and the rhizomes to which they are attached are dug up with roots intact and cut off to a length sufficient to include a well-developed bud. Planting is done before the onset of the rainy season. Rhizomes are separated from the mother plant during the onset of monsoon and planted in pits of 45x45cm. Culm cuttings can be used for propagation when seeds are not available. About 40to 70 percent of rooting can be obtained in Culm cuttings depending on the period of collection, age of Culm and treatment with growth regulating substance. Cuttings treated with NAA 100ppm during February to March gave maximum rooting response (Surendran and Seethalakshmi, 1985). Horizontal planting in the nursery beds was better than the vertical and oblique planting methods. Seasonal variation in rooting response is reported and it is attributed to the variation in nutrient contents in the Culm (Gupta and Pattanath, 1976). Observations on fertilizer and spacing trials in the nursery of less than two years, indicated that closer spacing is better and the application of NPK enhance the biomass production by three times (Patil and Patil 1990). Considerable work has been done on the tissue culture of this species. The various explants used are node seed seedling shoot excised embryo and other methods like multiple shoot production rooting and *in vitro.*

As compared to teak, bamboo has in general higher basic strength. A comparative study with mild steel has shown that the average ultimate tensile strength of *Dendrocalamus strictus* is nearly equal to the strength of mild steel. The specific ultimate tensile strength of bamboo specimen is nearly six times the specific ultimate tensile strength of mild steel. The notch impact strength of bamboo specimens is only about 15-20 percent of the impact strength of mild steel. But by taking into account the densities of mild steel and bamboo, the specific impact strength of bamboo specimens is 50 percent grater and specific impact strength of bamboo specimens soaked in araldite is 100 percent greater than the specific impact strength of mild steel. Bamboo specimens have poor torsional shear strengthen comparison to the torsional shear strength of mild steel. Bamboos have maximum stiffness along the fibers and minimum stiffness transverse to the fibers. The variation of modulus ratio (E/E2) for bamboo specimen is similar to the variation of modulus ratio of fibers reinforced. This species is one of the two most important bamboos in India. It is found suitable for reclamation of ravine land. It is extensively used as raw material in paper mills and also for a variety of purpose such as construction, agricultural implements, musical instruments, furniture etc. young shoots are commonly used as food. Decoction of leaves and nodes and siliceous matter is used in the traditional medicine.

Importance of Bamboo

Bamboos important can be gauged from the varieties of used to which it is put to. (i) Used as pulp for manufacture of paper. (ii) Used as fodder. (iii) Used as timber. Long bamboos are used in construction of houses. (iv)Used in making agricultural implements, arrows, baskets, walking sticks, furniture, etc. (v) Useful for soil conservation. Bamboo is also widely carved for decorative artwork. Modern companies are attempting to popularize Bamboo Flooring made of bamboo pieces steamed, flattened, glued together, finished, and cut. However bamboo wood is easily infested by wood-boring insects unless treated with wood preservatives or kept very dry. Bamboo canes are normally round in cross-section but square canes can be produced by forcing the new young culms to grow through a tube of square cross- section slightly smaller than the Culm's natural diameter. There by constricting the growth to the shape of the tube. Every few days the tube is removed and replaced higher up the fast-growing Culm. The fiber of bamboo has been used to make paper in China since early times. A high quality handmade paper is still produced in small quantities. Coarse bamboo paper is still used to make spirit money in many Chinese communities. Bamboo's long life makes it a Chinese symbol of longevity, while in India it is a symbol of friendship.

However, its rare *blossoming* has led to the flowers being regarded as a sing of impending famine. This may be due to rats feeding upon the profusion of flowers then multiplying and destroying a large part of the local food supply. The most recent flowering began in May 2006. Bamboo is said to bloom in this manner only about every 50 years. Perhaps the environmental crises at hand have not yet touched your life, but the time is shortly to come. Recent NASA reports of a 60% loss of ozone over the arctic provide an explanation for increased severity in the world's weather patterns which has only begun to affect us whether directly or indirectly. The social, political and economic implications are difficult to imagine as our ozone layer continues to thin, forests disappear and desertization is occurring at an alarming rate. Bamboo is the fastest growing canopy for the regreening of degraded lands and its stands release 35% more oxygen than equivalent stands of trees. Some bamboo even sequester up to 12 tons of carbon dioxide from the air per hectare. Bamboo can also lower light intensity and protects against ultraviolet rays. Traditional belief holds that being in a bamboo grove-the favorite dwelling place of Buddha restores calmness to emotions and stimulates creativity. A peerless erosion control agent, its net like root system creates an effective mechanism for watershed protection, stitching the soil together along fragile river bank, deforested areas and in place prone to earthquakes and mud slides. Because of their wide spreading root system, uniquely shaped leaves, and dense litter on the forest floor, the sum of stem flow rate and canopy intercept of bamboo is 25% which means that bamboo greatly reduces rain run off preventing massive soil erosion and keeping up to twice as much water in the watershed. Bamboo is a pioneering plant and can be grow in soil damaged by overgrazing and poor agricultural techniques. Unlike with most trees proper harvesting does not kill the bamboo plant so topsoil is held in place. Bamboo is a high yield renewable resource "Ply boo" is now being used for wall paneling and floor tiles, bamboo pulp for paper making, briquettes for fuel, raw material for housing construction, and rebar for reinforced concrete beams. There are 1500 species of bamboo on the earth. This diversity makes bamboo adaptable to many environments. It can be harvested in 3-5 years versus 10-20 years for most softwood. Bamboo tolerates extremes of precipitation, from 30-250 inches of annual rainfall. Bamboo related industries already provide income, food, and housing to over 2.2 billion people worldwide. There is a 3-5 year return on investment for a new bamboo plantation versus 8-10 years for rattan. The governments of India and China, with 15 million hectares of bamboo reserves collectively, are poised to focus attention on the economic factors of bamboo and its protection. In Limon, Costa Rica, only bamboo houses from the national Bamboo Project stood after their violent earthquake in 1992. Flexible and

lightweight, bamboo enables structures to "dance" in earthquakes. Bamboo shoots provide nutrition for millions of people worldwide. In Japan, the antioxidant properties of pulverized bamboo skin can prevent bacterial growth, and it is used as a natural food preservative. Bamboo litter fodder for animas and food for fish. Taiwan alone consumes 80,000 tons of bamboo shoots annually, constituting a $50 million industry. Bamboo has for centuries been used in Ayurvedic medicine and Chinese herbal medicine. Tabasheer, the powdered, hardenened secretion from bamboo is used internally to treat asthma, coughs and can be used as an aphrodisiac. In China, ingredients from the root of the black bamboo help treat kidney disease. Roots and leaves have also been used to treat venereal disease and cancer. Sap is said to reduce fever, and ash will cure prickly heat. A village in Indonesia reports that the water from within the Culm is used to treat broken bones effectively and that the Tabasheer is used to promote fertility in their cows. Current research points to bamboo's potential in a number of medicinal uses. Bamboo is an exquisite component of landscape design. For the human environment bamboo provides shade, wind break, acoustical barriers, and aesthetic beauty. 'The Bamboo Forest is an ecological wastewater utilization system that essentially grows away, waste, producing a marketable crop in the process. Comprised of a subsurface evaporation-transpiration bed planted with bamboo and other rapid-growing, non-invasive plants, the system is engineered to provide an aerobic rhizosphere (the home of living orgasms the root system), in which damaging polluting components are transformed into plant nutrients" Go to the Discover magazine article on Bamboo used to treat waste water.

Micropropagation of Bamboo

Dendrocalamus strictus is commonly called as solid or lathy bamboo. The species is widely distributed in dry deciduous forests and grows rapidly in all climatic conditions. It grows better in the drier parts and on sandstone, granite and coarse grained soils with low moisture-retaining capacity and soils with pH 5.5-7.6 it grows more than 8 feet in 6-months. It is rightly called as 'Poor man's timber'. The major problem in the propagation of *Dendrocalamus strictus* is the erratic flowering and non-availability of seeds on regular basis, besides low viability of seeds. Moreover the seeds have to be stored in 3 to 5$^{\square}$C after reducing the moisture (8%) or stored in desiccators with anhydrous calcium chloride. Under these circumstances, other techniques of propagation become important. Development of novel methods of propagation by tissue culture techniques, micro-propagation and clonal propagation is desirable for large-scale application. In recent years vegetative propagation techniques are standardized and adopted to improve self-incompatible bamboos with poor

seed set. Attempts to develop low-cost planting stocks of bamboo using Culm-cuttings and *in vitro* techniques are of paramount importance in bamboo improvement. We describe here an efficient technique of clonal propagation of bamboo. Indeed, the order of magnitude of the demand for bamboo planting materials indicates that micro-propagation will inevitably be necessary for mass scale propagation (Subramanlam, 1994, Gielis, 1995). Classical techniques alone can never solve this problem. It is also important to point out that the total world production of all tissue cultured plants in 1995, was estimated at 600 million (Debergh, pers. Comm.), or less than the total projected needs for bamboo planting material. Biotechnological techniques, including tissue culture, *in vitro* hybridization, molecular markers and genetic transformation are crucial for the future to bamboo. Micro-propagation of bamboos has allowed developing a new type of ornamental bamboo that can be produced year round with a high quality/ price ration and distributed far more widely than classically propagated ornamental bamboos. Molecular markers are used in quality control procedures. Glowering of bamboos is still one of the greatest mysteries in botany, and breeding systems are non-existent. However, flowering can be induced reproducibly in tissue culture, both in seedlings and in adult bamboos, providing the only method for hybridization, the flowering structures that are used are pseudo spikelet's, morphological features unique to the subfamily of Bambusoideae. These special propagules can be used for propagation, long term storage, for hybridization and for genetic transformation. While flowering can be induced, controlled and reversed in tissue culture, a more fundamental approach to unravel the mechanisms of flowering include studies of cell division patterns and profiles of volatile component. At one time the supply of bamboo was thought tube perpetual. Bamboo was often viewed by foresters in many countries as a weed species and a nuisance due to its rapid growth, and therefore its mass-utilization in the paper industries was welcome. This impression soon proved to be a mirage. The rapidly increasing population with associated demands for fuel and farmland resulted in a significant decrease of land area under forest in Asia (including that under bamboo). Between 1960-80 one half of the increase in food or plantation production in Southeast Asia was achieved by extending plantation areas under cultivation by clearing forest land. In addition to the decrease in forest cover, overexploitation, bad management, lack of adequate state control over natural forest stands and vested interest have resulted in a situation where the forest stock of bamboo and the total annual net growth has decreased and will continue to decrease significantly in Asia (Barney,1980). Principally, however, it is the mass utilization of bamboo by the paper and pulp industry that is quickly taking the plant out of reach of the common man such that it no longer remains the poor

growing bamboos for shoots has proved to be more profitable than rice cultivation. These facts war rant reconsideration of the classification of bamboos as a minor forest produce in some countries and in others as 'non-commercial species' (Sharma, 1987). This in itself would serve to bring a new focus on the bamboos and help in their conservation and replantation. As of now, many areas in countries where bamboos grew in dense thickets a decade ago now lay barren, save for a few isolated clumps, or the land has been reserved for other purposes after clearing bamboos. Principally, however, it is the mass-utilization of bamboo by the paper and pulp industry that is quickly taking the plant out of reach of the common man such that it no longer remains the poor man's timber. On the other hand, it is fast becoming a high-value crop. In certain countries growing bamboos for shoots has proved to be more profitable than rice cultivation. These facts war-rant reconsideration of the classification of bamboos as a 'minor forest produce' in some countries and in others as 'non-commercial species' (Sharma, 1987). This in itself would serve to bring a new focus on the bamboos and help in their conservation and replantation. As of now, many areas in countries where bamboos grew in dense thickets a decade ago now lay barren, save for a few isolated clumps, or the land has been reserved for other purposes after clearing bamboos. The conventional method of seed propagation is unreliable due to loss of seed viability, infection etc. multiplication by vegetative methods is rather slow, and several studies have been carried out on propagation of *Dendrocalamus strictus* using conventional and tissue culture method. In the present study an improved protocol has been developed for obtaining mass multiplication of *Dendrocalamus strictus* through tissue culture.

Material Methods:-General Methodology

The technique involves the isolation, inoculation and regeneration of plant cells tissues organs under controlled conditions in culture vials, containing synthetic nutrient medium. Both the chemical compositions of the medium and the controlled environmental conditions (light, temperature, humidity, aeration etc.) effectively control the expression of any genotype or phenotype potential in the explant.

A Tissue Culture Laboratory should be Equipped to Facilitate the Following Procedures

(1) Sufficient space for washing sterilization of glass wares and other equipment's. (2) Preparation, sterilization and storage of nutrient medium. (3) Aseptic transfer conditions. (4)

Biochemical analysis of the material, microscopic studies of cell and tissue, etc. (5) Growing cultures in incubation.

Equipment's required for Plant Tissue Culture

(1) Gas, water and electricity supply. (2) Hot plate and magnetic stirrer. (3) Sensitive balance. (4) Ph. Meter, Distillation apparatus. (5) Autoclave or pressure cooker. (6) Refrigerator. (7) Laminar air flow cabinet. (8) Culture racks. (9) Required glassware's. (10) Chemicals.

Methods Involves

(i) Sterilization of room. (ii) Washing and sterilization of glassware's. (iii) Preparation and sterilization of medium. (iv) Selection and isolation of explants. (v) Sterilization and inoculation of explants. (vi) Incubation and observation. (vii) Extraction of photochemical. (viii) Identification through HPLC method.

Sterilization of Room

Maintenance of highly aseptic condition is vital factor for successful tissue culture laboratory. Thus the room of such laboratory should be washed with disinfectant followed by wiping with 2% sodium hypochlorite solution or 95% ethyl alcohol, commercially available disinfectants like extran, Lysol, zephiran and roccal are effective disinfectants. (Razdim 1993).The final sterilization should be done either by organo mercury lamps of low pressure or by UV radiations or more recently by ozone generating system which give 90 to 100% disinfection in transfer area can be used eight minutes after sterilization is over (Kumar 1998).

Washing and Sterilization of Glassware's

Borosil glass tubes (150mm x25mm) Erlenmeyer flasks (250ml and 100ml) jam bottles and laxbro disposable Petriplates (55mm) were used in most of the experiments. The newly purchased glassware's were acid-bathed in 30% nitric acid before washing with distilled water, used tubes or flasks containing old agar medium were first autoclaved to remove contaminants and liquefied agar medium was discarded. The glassware's then dipped in 2% acid and detergent solution for at least 2 to 8 hours, followed by washing under running tap water. They were then rinsed with distilled water and dried at room temperature. The tubes and flasks were plugged with non-absorbent cotton, the culture bottles were capped with autoclavable polyproropylene caps, and were autoclaved at $120^{O}C$, and 15 pounds pressure per square inch (Ib. psi) for 40 -60 minutes. The other accessories required for medium,

explant preparation, pipettes, Petriplates, beakers, forceps, scalpels, etc. were wrapped individually with wrapping paper or aluminum foil. All the glassware's and instruments were autoclaved at 120^0C 15 Ib psi for one hour.

Preparation Sterilization and Storage of Nutrient Medium

Murashige and Skoog's 1962 nutrient medium was used throughout the experiment. In addition, media were supplemented with growth regulators, other additional vitamins, organic supplements and carbon sources. Inorganic salts used are of Asnalar grade (BOH, E. Merck India Ltd. Bombay) Vitamins, hormones and other organic additives were obtained from Sigma Chemicals Co, USA, or Loba Chemicals, Mumbai, India Gelling agent used was agar from Qualigens, India. Inorganic macronutrients include Nitrogen (N), Phosphorus (P), Sulphur (S), Potassium (K) Calcium (Ca) and Magnesium (Mg). These are needed in large quantities and their concentration in dry weight is in mg/lit.

Growth Hormones

Growth hormones used in plant tissue culture medium are. **Auxins:-**Indole 3- acetic acid IAA Napthalene acetic acid NAA and 2-4 Dichloro phenoxy acetic acid 2-4D being thermos table were added to the medium prior to autoclaving while IAA being thermos table had to be filter sterilized. **Cytokinins:-**6-benzyl amino purine (BAP) kinetin (KN) and Thidiazuron (TDZ) were used. **Organic Supplements:-**Casein hydrolyste (CH) and Coconut water (CW) were used. **Carbon Sources:-**Varying concentration of sucrose glucose, maltose was used as carbon source. The composition of nutrient medium is the governing factor for successful growth and morphogenesis of plant tissue in culture (Narayan swami 1994). The composition of MS is presented in table. Preparation of stock solution of nutrients, phytolhormones, vitamins and their storage and sterilization is also described in table. For the preparation of all the culture media, glass distilled water was used. All the major and minor salts and vitamins were added the growth regulators additional organic sources the ph. was adjusted by adding 1N HCl or 1N NaOH. **Preparation of stock solutions:-**Stock solutions or concentrated solution of media components individually or in groups prepared well in advance and used subsequently to prepare several batches of media. Stock solution should be prepared before experimentation because dissolution of different components during media preparation is time consuming. It is necessary to dissolve each constituent in double distilled water completely before adding another, otherwise precipitations may occur. After preparation stock solutions should be placed in autoclaved amber bottles and stored in refrigerator. Life

of different stock solutions varies. The stock solutions of Murashige and Skoog (MS) medium is given in table.

Stock solution of Phytohormone

The concentration of phytohormones in plant tissue culture medium is usually represented in milligrams (mg) parts per million (ppm) or micromoles (um). The procedure for the preparation of stock solution of hormones is given in table. The preparation of one liter MS media involves following steps:- (i) Take 500ml of distilled water in sterile flask. (ii) Take 20 or 30gm of sucrose (w/v) and shake to dissolve. (iii) Add 100 ml of macro-salts, 5ml of micro-salts, 5ml of vitamins, 5ml of myo-inositol, and 5ml of iron stock solution. (iv) Add required amount of any growth regulators. (v) Make the volume of the medium to 1000ml by adding distilled water. (vi) Adjust pH of the medium 5.4-5.8 by adding 1N HCl or 1N NaOH. (vii) Add 4.5gm/lit agar. (viii) Boil the medium to dissolve the agar and then dispense in culture bottles or tubes. (ix) Finally the culture vials with medium were capped and autoclaved at 15 1bs, 121^0C for 15 to 20 minutes. (x) For sterilization of media the minimum time required depends upon the volume of the media in the vessel. Prolonged autoclaving may result in breaking and de-maturation of media ingredients.

Autoclavings

The nutrient is generally sterilized by autoclaving at 120^0C for 20 minutes. The minimum times required for sterilization depend upon the volume of the medium in the vessel. Prolonged autoclaving may result in breaking and denaturation of media in small aliquots.

Selection and Isolation of Explant

The explants like seeds, stems, apical and axillary nodes, leaves, etc. were selected from healthy, identified elite variety of required plant species from field.

Sterilization and inoculation of explants

After dehusking the mature seeds are washed in 2% Teepol solution (Shell, India) on a magnetic stirrer for 5 minute the Teepol solution is removed by washing in running tap water for 15.20 minute followed by a rinse in distilled water. (Rao et al 1985) For tissue culture studies of bamboo the required used explants where seeds, washed with tap water from several times to remove soil or dust deposits on the surface. For surface sterilization explants were treated with 79% alcohol and followed by rinsing with sterilized distilled water for 3

times. Further it was treated with 0.1% mercuric chloride solution and finally rinsed with sterilized distilled water for 4 to 5 times to remove all the traces of surfactant.

***Invitro* Seed Germination**

The sterilized seeds were inoculated on the prepared MS medium with or without growth hormones in the laminar air flow chamber. Inoculated cultures were incubated in the culture or growth room at controlled environment of the temperature of 25 ± 4^{0}C of 16 hours photoperiod of 2000 lux and dark condition. The seeds were incubated in dark condition for 3-4 days, after seed germination the cultures were shifted in light.

Induction of Shoot

For shoot induction *invitro* raised seedlings were isolated and the sterile buds from the nodes were inoculated on MS basal medium with vitamins, supplement with cytokinins like BAP, KN (0.5-3.0mg/lit) alone or in combination with other cytokinins of each containing sucrose 30 grams and gelled with agar 4 grams per liter. In addition of Auxins like NNA 0.1-1.0 mg/lit were used with cytokinins for promoting the shoot initiation. Numbers of experiments were carried out to maximize the initiation of shoots from axillary nodes. The measurement of growth was taken by the parentage of buds showing response, number of shoots initiated per explants, shoots length and callus formation according to the method described.

Experiment for Multiplication

After three to five days of initiating experiment the buds start responding by bud break and sprouting from the lower end of the seedling. After 20 days of culture the initiated grown shoots start multiplying. Subcultures proceeds further multiplication into shoot proliferation medium containing coconut water. Number of experiments was carried out to maximize the rapid multiplication of shoot. The measurement was taken on the basis of percentage if shoot response, number of multiple shoots developed, shoot length and callus formed from each ten replicates.

Experiments for Root Induction

Regenerated multiple shoot of *Dendrocalamus strictus* were separated, each strong and elongated shoots were inoculated on the rooting medium containing Auxin IBA and additive like activated charcoal.

RESULTS AND OBSERVATIONS

The seeds were remained 70-80% sterile when treated with 0.1% mercuric chloride solution for 15-20 minutes. After 5-10 days the seeds start germinating into seedlings. The highest percentages of seed germination were recorded in the half strength MS medium. About 70-90% of seeds were germinated within 15 days in this medium as compared to the medium containing any growth regulators. Initiation of shoots from seedling derived nodal explants occurred within 7 to 10 days in all medium containing BAP alone. The highest percentages of shoot buds initiation from the lower node of the Culm were observed in the medium containing 0.5-3.0mg/lit BAP. About 2-3 shoots were initiated with in 10days culture which is of 1-2cm in length. The cultures were observed at the intervals of one week. The measurement of the growth was taken by the percentage of buds showing response, number of shoots initiated, number of multiple shoots formed, shoot length and callus formed. After 10-20 days of induction the induced shoots were subcultured in the multiplication medium containing higher concentration of BAP (3.0-5.0) maximum numbers of shoots multiplied were reported in the medium containing BAP (3.0-5.0mg/lit). About 10-15 shoots were developed with 10 days of incubation. Addition of Auxins does not show any favors in the multiplication of shoots while kinetin containing medium produces callus at the lower end of shoots. The growths of shoots developed in the BAP containing medium are green, healthy and vigorous. Repetitive multiplication was achieved when groups of shoots were separated and transfer to medium containing coconut milk. Among all the explants used nodal parts at the base of the seedling were more responsive and multiples more shoot at short time. Cultures containing CW in liquid medium gave better response showed increase in shoot number and shoot length. After rapid multiplication the multiplied shoots were separated and generated roots within 10 -15 days in the medium containing MS supplement with Auxins like IBA (0.5mg/lit) and Activated Charcoal (200mg/lit). About 2-5cm were developed which increases at with the age of culture. Clonally propagation through matured stem cuttings will provide definite advantage in immediate age transfer and will save time in propagation compared to seedlings. But the seedling explants were more responsive added advantage is that the superior clones can be directly propagated. This technique can be adopted for large scale plantation of wastelands and saline soils to meet the growing needs of *Dendrocalamus strictus*.

Table;-1. Composition of media used for *in vitro* culture (Bajaj, 1998).

Constituents	Murashige and Skoog (MS, 1962) Mg/lit
Inorganic	
(1) Macronutrients	
$MgSO_4.7H_2O$	370
KH_2PO_4	170
$NaH_2PO_4.H_3O$	…..
KNO_3	1900
NH_4NO_3	1650
$CaCl_2.\ 2H_2O$	440
$NH_4H_2PO_4$	…..
(2) Micronutrients	
H_3BO_3	6.2
$MnSO_4.\ 4H_2O$	22.3
$ZnSO_4.\ 7H_2O$	8.6
$Na_2MoO_4.\ 2H_2O$	0.25
$CuSO_4.\ 5H_2O$	0.025
$COCl_2.\ 6H_20$	0.025
KI	0.83
$FeSO_4.7H_2O$	27.8
Na_2EDTA	37.3
Organic	
Thiamine HCl	0.1
Pyrrdoxine HCl	0.5
Nicotinic Acid	0.5
Myo-inositol	100
Glycine	2.0
Sucrose	30

Table:-2. Stock of solution of MS 1962 Macro salts (*10).

Constituents	Amount (mg/lit) present in original medium	Amount (gm/lit)to be taken for stock solution (*100)	Final volume of stock solution (ml)	Amount to be used/liter (ml)
NH_4NO_3	1650	16.5	1000	100
KNO_3	1900	19.0	1000	100
$CaCI.\ 2H_2O$	440	4.4	1000	100
$MgSO_4.7H_2O$	370	3.7	1000	100
KH_2PO_4	170	1.7	1000	100

Table:- 3. Stock of solution of MS 1962 Macro salts (*100).

Constituents	Amount (mg/lit) present in original medium	Amount (gm/lit) to be taken for stock solution (*100)	Final volume of stock solution (ml)	Amount to be used/liter (ml)
$MnSO_4.\ 4H_2O$	22.3	2230		
$ZnSO_4.4H_2O$	8.6	860		
H_3BO_3	6.2	620		
KI	0.83	83	500	5

$Na_2MoO_4.2H_2O$	0.25	25		
$CuSO_4.5H_2O$	0.025	2.5		
$CoCl_2.6H_2O$	0.025	2.5		

Table:-4. Stock of solution of MS 1962 Vitamins (*100).

Constituents	Amount (mg/lit) present in original medium	Amount (gm/lit) to be taken for stock solution (*100)	Final volume of stock solution (ml)	Amount to be used/liter (ml)
Glycine	2.0	200		
Nicotinic acid	0.5	50		
Pyriodxine HCl	0.5	50	500	5
Thiamine HCl	0.1	10		

Table:-5. Stock of solution of MS 1962 Inositol (*50).

Constituents	Amount (mg/lit) present in original medium	Amount (gm/lit) to be taken for stock solution (*100)	Final volume of stock solution (ml)	Amount to be used/liter (ml)
Myo- Inositol	100	5	250	5

Table:-6. Stock of solution of MS 1962 Iron Source (*100).

Constituents	Amount (mg/lit) present in original medium	Amount (gm/lit) to be taken for stock solution (*100)	Final volume of stock solution (ml)	Amount to be used/liter (ml)
$FeSO_4.7H_2O$	27.8	2.78		
$Na_2EDTA.2H_2O$	37.3	3.73	500	5

Table:-7. Stock of solution of Phytohormones

Phyto hormones	Molecular weight	Required amount of stock solution	Amount of solvent required to be dissolved	Amount of water to be added (ml)	Final volume of stock solution	Final concentration (mg/lit)
Auxin						
2, 4D	221.04	10	1ml (0.1) NaOH	99	10	0.1
IAA	175.18	10	1ml (0.1) NaOH	99	10	0.1
NAA	186.20	10	1ml (0.1) NaOH	99	10	0.1
IBA	203.23	10	1ml (0.1) NaOH	99	10	0.1
NAA	202.30	10	1ml (0.1) NaOH	99	10	0.1
Cytokinin						
BAP	225.20	10	HCl (0.1ml) 1ml	99	10	0.1
Kinetin	215.21	10	HCl (0.1ml) 1ml	99	10	0.1
Zeatin	219.20	10	HCl (0.1ml) 1ml	99	10	0.1
21 IP	203.30	10	HCl (0.1ml) 1ml	99	10	0.1
Giberlin						
GA_3	346.36	10	1ml (0.1N) NaOH	99	10	0.1

Table:-8. Different media used for seed Germination of Bamboo.

Medium	% of seed germination	Length of seedling
WH Full	40%	1 cm
B5 Full	45%	1 cm
MS Plain Full	80	1-2 cm
MS Plain Half	80-90%	2-3 cm

Table:-9. Effect of growth regulation on shoot induction of Dendrocalamus strictus.

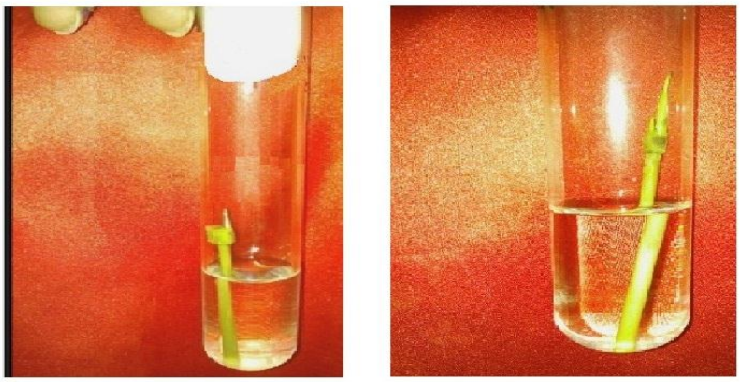
S No.	Medium + growth hormones mg/lit	Percentage of shoot induction	Number of shoots per culture	Average shoot length in cm	Callusing
1	MS+0.5 BAP	70%	1-2	1-2	-
2	MS+1.0 BAP	75%	1-3	2 cm	-
3	MS +2.0 BAP	70%	1-2	2 cm	-
4	MS +3.0 BAP	60%	1-2	1-2	-
5	MS+0.5 KN	40%	1	1-2	+
6	MS+1.0 KN	42%	1	1-2	-++
7	MS+1.0 PAP+0.5 KN	45%	1	1	++
8	MS+2.0 BAP +0.5 KN	45%	1-2	1	+

Table:-10. Effect of growth regulation on multiplication of shoots in *Dendrocalamus strictus*.

S No.	Medium + growth hormones mg/lit	% age of response	Average number of shoots	Average shoot length in cm	Callusing
1	MS+0.5 BAP	30	15	2-3	-
2	MS+1.0 BAP	50	15-20	2-3	-
3	MS +1.0 BAP+0.1 KN	50	8-10	2-5	+
4	MS +2.0 BAP	65	10-15	3-5	-
5	MS+0.2BAP+1.0 KN	50	8-10	1-2	+
6	MS+3.0 BPA +0.5 KN	70	14-15	1-2	++
7	MS+3.0BAP+0.5NAA	75	20-25	3-5	-
8	MS+4.0BAP+0.1 NAA	78	20-22	3-5	-
9	MS+3.0BAP+CW20%	80	4-5	1-2	+
10	MS +4.0+CW 30%	80	4-5	1-2	+

Photographs

(a) *In vitro* raised plants in sterilised vermiculite (b) *In vitro* raised plant in pot
Fig 5. IIardening and Acclimatization



(a) In 0.25(mg/l) TDZ+ 4.0(mg/l) BAP **(b).** In 5.0(mg/l) BAP

Fig. 1 Bud Induction

Fig. (a &b) subculturing after 3-4 weeks of bud proliferation

Fig. 4 (a &b) rooting in D. strictus using 5mg/1 IAA

(a) In 5.0(mg/l) BAP **(b)** In 0.25(mg/l) TDZ+ 4.0(mg/l) BAP

Fig 2. Shoot proliferation

CONCLUSION

We have often been asked about the utility of raising polyclonal populations of Bamboos through tissue culture as the initial explants largely remains the zygotic embryo (in the seed) or seedling parts. It has also been argued that seeds are always available through sporadic flowering and that the problems of limited viability can be overcome by establishing seed-rhizome banks in the field. The latter are estimated to have a useful like of 8-10 years during which they can be dug up and planted out. however, the problems of bulkiness, transportation, expense and labor involved in maintaining and working of the seed-rhizome banks as also the poor efficiency, productivity and output of the system cannot be wished away (Stapleton,1987). Seed supplies also remain short and seed set from sporadic flowering

remains very low and unreliable. The problem of sifting large quantities of empty florets and recovering the few well-formed seeds in sporadic flowering remains. Large quantities of viable seeds are obtained only when gregarious flowering takes place. Also bamboo seeds remain viable only for short periods. Seed storage methods are also currently primitive and result in further loss of seed viability. To overcome this foresters raise seedling nurseries from which seedlings emerging from the few viable seeds are picked up and transplanted into polyethylene bags for further growth. At present in a situation where there is severe paucity of planting materials plantlets regenerated through somatic embryogenesis from juvenile tissues can satisfy this requirement to an extent identical to that obtainable with seeds. The use of seeds or seedlings only in the initial step of culture establishment with subsequent autonomy being attained in the generation of plants (using somatic embryo-raised plantlets for culture establishment) makes this method very attractive. Embryogenic cultures are also very rapid in growth and facilitate the production of large numbers of plantlets fairly easily. It needs to be remembered that conventionally one seed ordinarily gives rise to only one plant whereas numerous plantlets can be through the method of somatic embryogenesis. By this method, one seed can "plant" a whole and perhaps several hectares. If the plants prove to be of good quality, a whole hectare of monoclonal, superior plants will be available for further propagation by conventional methods besides; the original cultures can be maintained and can give rise to several more plants. Tissue culturing of bamboos would also lead to the isolation of superior soma clonal variants, induction of in vitro flowering and an understanding of rhizome physiology. Above all it will lead to the restoration of our bamboo forests to their earlier green glory. Micropropagation technology of *Dendrocalamus strictus* has been briefly analyzed. During propagation of *Dendrocalamus strictus* the protocol efficiency has revealed about 80% plant recovery. Pilot plant propagation system however need to be launched for the scaling up of production of million available superior planting material to make the cultivation of imported forests plant in India as economically viable venture, and contribute to sustainability of economy. Mankind is facing today a number of problems in areas of agriculture and forestry that require urgent solution. The conservation and management of natural resources is one method of maintaining the quality of the environment. Such problem can be circumvented to some extent by increasing the productivity or by rapid replanting of superior quality planting material. In addition to conventional technique like plant breeding, biotechnology plays an important role in solving some of these problems via improved agriculture, time conservation and prompt production.

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