Chapter II

Review of Literature
2.0 Review of literature

Bamboos are tall perennial, arborescent, fast growing grass of family Poaceae (subfamily Bambusoidae). It embraces a considerable diversity of grasses that are growing few inches in height with vigorous rhizome root system, that can be attained over 100 feet with trunk like stem (http://www.bamboo.html). Bamboo is a large group of over 1200 species found from the tropical to temperate region.

Bamboos are valued for more than fifteen hundred documented applications. It is native to all continents around the world except Europe and Antarctica. Surprisingly, the widest diversity exists in Central and South America (Saxena and Dhavan, 2004). The bamboo is an ornamental grass. The tender shoots are popular items in China, Japan and North-Eastern India. Shoots are used for their high nutritive contents. However some species are used as an ornamental grass in the garden and now a days some are used as indoor ornamental plants.

Bamboos are used for making medical instruments such as acupuncture needles as well as artificial limbs. In remote villages, the umbilical cord of the newborn is served with a piece of bamboo. It is a valuable ingredient of many ayurvedic medicines. Chemical analysis of bamboo have revealed the presence of terpenoids viz. sterol like betasito sterol, stigma sterol etc. and constituents such as miliacins, cylindrin, crusgallin, cynogenetic glycosides etc. The extracts of leaves are considered as useful remedy for gout, piles, cough and asthma and roots are effective against oedema (Ghosh, 2003).
Bamboo offer:

- Rapid growth. It is drought and pollution tolerant.
- It is tough and durable.
- It grows creating rhizome root system, providing erosion control on slopes and clearing.
- It creates a groove creating woodland, attractive to many types of birds and other animal for nesting, enhancing wildlife habitat.
- It has tremendous ability to recycle carbon dioxide (12 tones/hectors) clearing the air around us (35% more oxygen than equivalence stands of trees).
- It is an agro forestry crop.
- Bamboo display beautiful ornamental characteristics with culm of amazing colors with enormous leaves or tiny delicate leaves.
- Other than its traditional uses, its application is also for preparation of handicraft and novelty items application (http://www.bamboo.html).
- It is used from 'cradle to grave'.

2.1 Bamboo raw material for Phytosterol

Occurrence of various ingredients of nutritional significance in succulents bamboo shoots has been described by many workers. Its medicinal importance has been studied. The waste material of sugar industries, press mud, has already been explored as a nonconventional source of sterol to be used for steroidal drug after its microbial conversion. Extensive study on metabolic changes during fermentation of edible bamboo shoots has been made including, estimation of a number of metabolites like sugar, organic acids, alcoholic nitrogenous compounds, vitamins (Srivastava,
The increasing demand for steroidal drug has resulted in the depletion of various natural resources such as *Dioscoria* and *Solanum*. Hence an alternative source for a starting material is imperative phytosterol, which are used for the production of steroidal drugs. The succulent bamboo shoots are proposed as an alternate source of phytosterol.

Bamboo cultivation is practiced in many tropical countries. In India bamboo is grown as a cash crop in Northeastern part. The fermented preparation of bamboo shoots slices called ‘Sobium’ is a highly precised vegetable item. The sobium is manufactured by thin slices of fresh succulent and soft bamboo shoots in specialized container or chamber for 2 or 3 months. The fermentation chambers are made up of either of bamboo plants or of roasted earthen pots. The phytosterol extracted from fresh succulent shoots and also from the fermented products can obtained from market. The steroid was isolated from oven dried fresh and fermented material. The concentration of total phytosterol was 1.6-2.8% in the dried fermented bamboo shoot samples obtained from different manufactures (Srivastava, 1990).

2.2 Edible bamboo
Succulent bamboo shoots are edible and are used in food industries for vegetable pickles, soup etc. *Dendrocalamus asper* and *Dendrocalamus tilda* (an edible species of bamboo) are important for their edible tender shoots which are sold as food all over the World. Bamboo shoots can be eaten fresh after boiling. Bamboo shoots are seasonal and therefore preservation is necessary for storage. There are
many methods of preservation, both cottage industrial size and large industrial setup. The protein content of bamboo is 2.4%. It contains 17 different amino acids. It is low in fat, high edible fiber and rich in mineral elements. The amino acids like Lysine, Glutamine and Arg etc. are found in bamboo shoots. There are 10 kinds of mineral elements in bamboo shoots such as Cr, Zn, Mn, Mg, Ni, Co, Cu, etc. In bamboo shoots the total sugar content is 2.5%, and fat is 0.05% (http://www.bamboo.html).

Bamboo vinegar is the bye product of bamboo charcoal production. The steam coming out from bamboo charcoal production is condensed to produce bamboo vinegar. China produces beer from bamboo, which is testy and quite acceptable. Medicine is produced from bamboo extracts. Shoots contains element of selenium, which is called Miracle life element' (http://www.bamboo.html).

India is the second largest resource of bamboo after China with about 23 genera and 136 species (Rao et al., 1988). Precious germplasm of bamboo is getting lost due to indiscriminate cutting of culms. Therefore, conservation of germplasm approach is required urgently. In vitro conservation of these precious germplasm in laboratory will ensure that, they are not lost due to natural calamities. In addition mother culture of these elite accessions are supplied to various tissue culture laboratories on demand for mass multiplication (Saxena and Dhavan, 1999).

The propagated plants are generally identical to the mother plants. Therefore a lot of time and energy is used to select elite genotypes.
The selection of mother plant by micropropagation is a critical process. Because micropropagation via axillary branching mimic the natural growth of bamboos.

Using tissue culture methods in vitro, flowering of somatic embryo has been achieved in *Dendrocalamus strictus* and *Bambusa arundinacea* within 8-10 weeks (John et al., 1995). A method of clonal propagation has been initiated, by which tissue culture clones proved superior in the field can be selectively mass propagated.

### 2.3 Tissue culture

Tissue culture is a generic term encompassing several in vitro aseptic techniques that enable any part or tissue or cells of a plant to be cultivated artificially on nutrient medium for differentiation of cells and regeneration of plants. The media formulated to permit diverse growth pattern including cell multiplication, organ formation and plant regeneration (Razdan, 1994). Steward et al., (1972) reported that plant regeneration was genotype dependent and the frequency of responses varies considerably from one genotype to another. It is now well accepted that genetic factors contribute to the response of plant tissues in vitro for differentiation and regeneration.

Morel and Martin (1952) applied culture technique of elimination of viral infection in plant. They cultured meristem tips excised from infected Dahilia and obtained disease free plants. There are number of evidences of meristem culture through which the diseases are eliminated.
in various plants and now a days the commercial production of disease free plants has become more popular. The somaclonal variation in plant derived from tissue culture is accepted to provide a new source of variability (Heintz and Mee, 1977; Larkin et al., 1984). A high level of auxin substances generally favored cell culture of monocotyledonous plants (Schenk and Haberlandt, 1971). In the present investigation all the different auxin and cytokinin have been tested with various concentrations and combinations to initiate the cell differentiation in various forms like callus induction, embryogenesis and regeneration.

2.4 Research on in vitro Bamboo propagation
In bamboo different technique are available, such as seed propagation, clump division, rhizome and culm cutting (Banik, 1994) for mass scale propagation. Classical techniques are largely insufficient and inefficient and tissue culture is a viable method. In India major research focused on the clonal propagation of elite genotype either juvenile or adult (INBAR 1991; Zamora, 1994). The number of research papers about this subject however is much less, and this is solely due to lack of success.

Other laboratories focusing on a bamboo tissue culture include West Wind Technology, USA and Bamboo Wood Australia (Cusack, 2000). Several other laboratories such as Piccoplant Germany and Microflor Belgium produce a more limited number of bamboo species as a part of their tissue culture operation. In agriculture prospective it is now possible to produced the same genotype for large scale plantation.
Embryo, shoot tip can produce several shoots. This strategy was adapted for *D. strictus* (Nadgir *et al.*, 1984). Dekkers (1989) also obtained multiple shoots from the embryo. However only few shoot could be rooted on subsequent transfer to a rooting medium. Multiple shoots can also be obtained from axillary bud (Banik, 1987) and branch bud (Prutongse and Gavinletravarta, 1987). Dekkers (1989) cultured nodal explant of *Bambusa ventricosa* where sprouting of the axillary shoot buds and multiple shoots formation took place but continued growth of the shoots and roots were not obtained.

The application of cell suspension culture system of bamboos was only reported by (Hung, 1988). Dekker (1989) attempted to raised suspension culture but was not successful. Protoplasts from *Bambusa sp.* were obtained by Tseng *et al.*, 1975. The protoplast sources was non embryogenic callus induced with 2,4-D (Dekker, 1989) was able to obtained low yield of protoplast from stem tissue of *Bambusa ventricosa*, and *Schizostachyum branchycladum*, however culture of protoplast collapsed after 3 to 11 days.

In general the explant type and its stages of development can be crucial to the success of embryonic callus initiation. The availability and presence of active meristematic tissue have been primary consideration for the selection of explant material. The explant which have been used for the callus induction in a bamboo are mature embryo, inter nodal section, leaves, leaf bases, shoot tip and regenerated roots. In most grasses, callus could be induced in bamboos with the auxins, picloram and NAA and with less success of IAA and IBA (Hung and Murashige, 1983). Three types of callus formation from inflorescence segments of *B. beecheyana* has also been reported (Rao *et al.*, 1990).
Micropropagation is the method to produce large quantities of plants in very short time. This is an excellent method to propagate a new introduction or new selections very rapidly. Moreover, these plants are small but have a vigorous growth, free from diseases or pest (Murashige, 1978; Razdan, 1994; Morel, 1960).

A major consideration in commercial micropropagation is the final cost of the plant produced, which is generally higher than commercially propagated plant. In developed countries such as USA, UK labor accounted for 60% to 80% of the production cost of micropropagated plants (Anderson et al., 1977, Donnara, 1986). The contrast in developing countries such as India the labor charges are significantly lower (Prakash, 1990) and cost of the medium ingredients and power charge for air conditioners and illumination are the major contributing factor to the cost of tissue culture plants.

Protoplast has been successfully isolated from juvenile and embryogenic tissue of Dendrocalamus strictus. Protoplasts are capable of forming whole plants. This opens up the possibility of successfully obtaining newer variant and somatic hybrids. Somaclonal variations have also been isolated and are being assessed as a source of desired characters (Rao et al., 1990).

2.5 Callus induction
Street (1996) described that callus is nothing but unorganized mass of cells, which is formed following wounding of plant organ leaving parenchyma cell adjacent to the wound frequently became meristematic and form a mass of undifferentiated cells. Callus on a wounded part or on a culture is made of an amorphous aggregate of loose
parenchyma cells that proliferate from the mother cells. Callus is either homogenous parenchymatous mass or treachery elements or sieve elements or summarized cells or the trichomes. Callus formation has been found in angiosperm, gymnosperm, pteridophytes and bryophytes. Callus contains no organized meristem but is some what abnormal tissue which potentially produce normal roots and embryos and in turn it develops into plantlets. Callus may be hard, compact, friable or brittle and sometimes soft. Colors of callus also varies depending upon the culture conditions, type of tissues and metabolite contents.

Ogita Shinjiro (2005) reported that, the maximum callus formation in Phyllostachys nigra observed on MS medium containing 2,4-D, Picloram and BA using bamboo shoots as an explant. The initial sign of callus formation begins approximately 2-3 week after culture. Whitish yellow calli proliferate on the surface of the explant. Abundant callus formation could be found from cut pieces of the shoots in the same media containing 3-30 µM of 2,4-D and 3-10 µM of Picloram. Where as BA containing media has a negative effect on callus induction. In order to investigate morphological and histochemical characteristics of the cells a liquid suspension cultures are established. Approximately 500 mg fresh weight of callus tissue transferred in 50 ml liquid half strength MS medium containing 3 µM 2,4-D. Suspension is initially generated within 3-6 week after inoculating the culture.
Rao et al., (1990) reported that B5 basal medium with 2% sucrose and 0.8% agar supplemented with 2,4-D at 10 and 30 μM concentration, the callusing start soon after germination of the zygotic embryo which developed into compact and friable callus. The compact callus was whitish to cream in color. When mature nodes were used, similar compact callus was induced. Callus origin was tested to the vascular bundle. The general appearance of the callus at this point of time varies from white to cream with green areas where the embryos were matured and became chlorophyllus. Prior to the differentiation of embryo, the embryogenic compact callus can be maintained by subculturing on 2,4-D medium.

Generally bamboo callus could be induced with auxin, picloram and NAA and with less success IAA and IBA (Huang and Murashige, 1983). However, the most commonly used auxin 2,4-D has proved to induce the embryogenic callus with concentration ranging from 1 to 25 mg/l and more frequently 2-3 mg/l. Huang and Murashige (1983) reported that the BAP, 2 iso pentyl adenine, 6 furfuril amino purine (Kinetin) and zeatin were repressive alternatively with low concentration.

Godbole and Sood (2002) proved that when new sprout bud segment of D. hamiltonii were cultured on MS medium supplemented with different concentrations of 2,4-D and NAA, callus formation started at the cut end, within 8-10 days and the frequency of its induction varied with concentration of 2,4-D. However, lower concentration of 2,4-
D (0.5 mg/l), callus initiations took place around 30 days and grew slowly. The callus remained compact, nodular and cream white and differentiated into distinct white color embryo on a maturation medium. The best results were obtained in half strength MS 2 mg/l of BA, 2,4-D and NAA each.

2.6 Somatic embryogenesis

In tissue culture, organogenesis is the development of adventitious organs from undifferentiated cell mass. Mostly a balance between auxin and cytokinin controls it. A relative high ratio of auxin: cytokinin induces root formation in callus tissues, whereas low ratio induces shoot formation. Callus formation is a type of organogenesis by which only adventitious shoot bud initiation takes place. When it is applicable for root, it is known as rhizogenesis. Anomalous structure when developed during organogenesis is called organoids. The localized meristematic cell on a callus, which gives rise to shoots and/or is termed as meristemoids (Razdan, 1994; Dixon and Gonsales, 1994).

On account of the very long vegetative phase, in most of the species breeding for improved varieties, generation of hybrids and maintaining perennial supply of seeds are almost impossible. Somatic embryogenesis and regeneration of bamboo plants in culture were achieved some years ago. *In vitro* flowering of bamboo reported for the first time by Usha and Ramanuja Rao (1986). Rao et al., (1986) reported that somatic embryo developed from vegetative tissue as an
embryo of Dendrocalamus strictus, Bambusa arundinacea and Dendrocalamus brandissi.

The work of Rao et al., (1986) differ from that of Nadgauda et al., (1999) in two important aspects in the former instances, somatic embryo differentiated in tissue culture was to flower. In the latter case the nodal explant in vitro gave flowering of bamboo, which has important implication for genetic improvement of bamboo. It offers possibility of interspecies and intergeneric hybridization within the laboratory in a short time frame.

Somatic embryogenesis has a potential application in plant improvement. The role of genotype in conferring regeneration capacity is further supported by studies on zygotic embryo, cloning of wheat, rice, and maize (Razdan, 1994). Diallel analysis of various cultivars demonstrated that the regeneration capacity to undergo repetitive somatic embryogenesis can be back crossed to elite line in order to transform the later with capacity for high regeneration of somatic embryos. Such a transformation could play an important role in plant breeding. Since through in vitro technique high quality somatic embryos have been produced in 80 species of tropical crop (Redenbergh, 1990).

There is considerable Worldwide interest in the development of method for encapsulation of somatic embryos to enable them to sow under field condition as synthetic seeds or artificial seeds. Artificial seeds consisting of a somatic embryo enclosed in a protective coating have been proposed as a low cost high volume
propagation system. However, the somatic embryos after encapsulation can be stored at very low temperature for long period of time and it can be used in off-season as an artificial seed for propagation (Chikhale et al., 2005).

Embryogenic callus, suspension culture and somatic embryos have been employed as a source of protoplast isolation for a range of species. Mutation during adventitious embryogenesis may give rise to a mutant embryo, which on germination would form a new strain of plant (Bhojwani and Razdan, 1984).

There is active research going on in India, China, Taiwan and the Asian countries on experimental methods of in vitro planted protection of bamboo. Micropropagation is being attempted using nodal cuttings from the minor branches of bamboo. This method has the distinct advantages through which an identical bamboo plant with superior characteristics can be a rapidly and clonally propagated using nodal cutting. The plants identical to the parent, elite plant can be produced on large scale and used for plantation.

The studies of Saxena and Dhavan (1999) showed that the addition of 250 mg/l PVP to a medium promoted the growth of somatic embryos in Dendrocalamus strictus and was effective in overcoming the browning of the culture medium.

2.7 Nutritional requirements
The success of plant tissue culture is mostly depending on largely governed by the composition of the culture media. The principal components of the most plant tissue culture media are inorganic and organic nutrients, carbon source,
growth regulators and vitamins. For most of the culture it is essential to supplement the medium with vitamins, amino acids and growth regulators. Hormone plays a very important role for initiating and promoting growth. Skoog and Miller (1957) proposed the concept of hormonal control of organ formation. Their classical experiment on tobacco showed that a balance between auxin and cytokinin conditioned root and bud initiation. High concentration of auxin promotes rooting, whereas proportionally more cytokinin initiate bud or shoot formation.

Auxin is a class of growth hormone, which causes cell elongation, apical dominance and root initiation. The most frequently used auxin is 2,4-D and IAA. Of this IAA occurs naturally in plant. Cytokinin promotes cell division and proliferation of tissue. The most widely used cytokinin in tissue culture is Kinetin and BAP (Murashige, 1978; Razdan, 1994).

The medium used for bamboo tissue culture has mostly been the Murashige and Skoog medium (1962). Embryo culture was induced on other media such as B5 (Gamborg et al., 1968). Huang and Murashige (1983) provided a detailed study of the organic requirements of the bamboo callus tissue. Inositol (100 mg/l), thiamine HCl (1 mg/l) and nicotinic acid (0.5 mg/l) were found to be beneficial for callus growth. Sucrose could be replaced with glucose but it doesn't improve the growth of cultures (Huang and Murashige, 1983). The optimum concentration of sucrose that is commonly used for growth is 20-30 gm/l. Other
authors have reported much higher level of sucrose i.e upto 50 gm/l (Mehta et al., 1982) or 60 gm/l (Yeh and Chang, 1986 a, b).

Morel (1948) observed that NAA and 2,4-D was the most effective auxin for induction of callus. Presence of medium is generally essential for somatic embryo initiation. Mostly 2,4-D has been induced somatic embryogenesis. In some system, other auxins have also been used for this purpose. The fact that auxin rich culture of carrot tissue produce ethylene than auxin free cultures. The 2,4-D induced embryo suppression may be mediated through ethylene production is being attempted using nodal cuttings from the minor branches of bamboo. This method has the distinct advantage. In that an identical bamboo plant with superior characteristics can be rapidly and clonally propagated using nodal cuttings so that desired responses to achieve the set goal can be attained (Razdan, 1994). Carbohydrates are the essential source for supply of carbon and energy to the culture. Galactose, mannitol, and sorbitol were the best carbon source for many tissues. In tissue culture, one has to make use of plant growth regulators to obtain the minerals, vitamins and sugar. Plant cells and tissues in the culture medium lack autotrophic ability and therefore need external carbon for energy. Addition of an external carbon sources to the medium enhances proliferation of cells and regeneration of green shoots (Bhojwani and Razdan, 1984).

2.8 In vitro propagation of Dendrocalamus species
Murashige (1974) has developed standard method of propagation \textit{in vitro} of species ranged from fern to foliage flowers and fruit plants. This new method of vegetative propagation is exploited extensively of many monocotyledons, dicotyledons and gymnosperm plants. Arya \textit{et al.}, (1996) developed the protocol for rapid micropropagation of edible bamboo, \textit{Dendrocalamus asper}. They produce multiple shoots through axillary bud activation within 2-8 weeks on Murashige and Skoog’s medium supplemented with 0.1 to 15 mg/l Benzyl adenine. \textit{In vitro} generated shoots were excised and subcultured on MS medium supplemented with 3.0 mg/l BAP for further shoot multiplication.

Godbole and Sood (2002) worked on somatic embryogenesis in a multipurpose bamboo \textit{Dendrocalamus hamiltonii} and they observed when MS medium containing BA and 2,4-D (1 mg/l) in addition to GA$_3$ (0.5 mg/l) induced shoot bud differentiation and as a consequence well developed shoots were formed in 4-5 weeks. Mehta \textit{et al.}, (1982) reported somatic embryogenesis in bamboo for the first time in \textit{Bambusa arundinacea}.

In 1997 Maity and Ghosh suggested that the phytohormone 2,4-D had negative effect on shoot regeneration. They proved that lower level of GA (0.5-1.5 mg/l) gets the shootlets. The number of shootlets decreased gradually as GA concentration increased from 2.0- 3.0 mg/l. GA at lower concentrations had significant shoot regeneration potentially
but higher concentration became toxic to usual shootlets development.

2.9 *In vitro* rooting in Bamboo

The suitability of culm and culm branch cutting for adventitious rooting under the treatments of various auxins was investigated in view of the problem of inadequate adventitious rhizogenesis in *Bamboo nutans*. However, culm branch cutting exhibited markedly better adventitious root formation and growth. The cutting response more positively to exogenous auxin treatment enhanced adventitious rooting (Singh, *et al.*, 2002).

In addition there is huge variability in responsiveness in a tissue culture (Saxena and Dhavan 1994). When using the adult bamboo main problem are endogenous contamination hyper hybridity, and the instability of multiplication rate and many problems with rooting in bamboo. Rooting percentage for adult bamboos range from very low percentage of 10% for *Bambusa vulgaris* to 73% from adult *Dendrocalamus longispathus* (Saxena and Dhawan 1994). A rooting percentage of 77% was obtained for adult *Dendrocalamus giganteus* in 3 to 4 weeks (Ramanayabae and Yakandawala, 1997). Low rooting frequencies are the major problem to developing commercially viable protocol (Saxena, 1993).

Arya and *et al.*, (1996) observed that the multiple shoots were cultured to auxin rich MS medium supplemented with NAA (3 mg/l) or IBA (10 mg/l) for rooting in which 95- 98% rooting was achieved in MS medium within 15 days. The *in vitro* plants were ready for transfer after 30-35 days.
2.10 Hardening of *in vitro* seedlings of bamboo

The most important thing is to transfer the *in vitro* seedling in field for plantation. As the *in vitro* seedlings are highly delicate systems and sensitive to tolerate the outer environment, normally the mortality rates seem to be very high in majority of plant systems. Therefore, it is prerequisite need to improve and develop the standard protocol to reduce the mortality while transplantation and increase the survival of *in vitro* plants in field condition. Many researchers have studied the hardening techniques in various plants. However in bamboo, *D. hamiltonii*, Sood *et al.*, (1991) reported that the plants grown in agar medium were first transferred to a low sucrose medium and after 10-12 days, plants were directly transferred to the soil. This later improved hardening and proved to be more useful.

Arya *et al.*, (1996) observed that *D. asper* plants when kept in 80% relative humidity in half strength MS medium, after 20-25 days when the plants were transferred to 1:1:1 mixture of soil: sand: organic manure and when these plants were kept in high density agro net shed, they were ready in one month for field transfer.

Gradual acclimatization is necessary for these plants to survive transition from culture to the greenhouse or field. In most of the species investigated *in vitro* developed leaves show poor mesophyll differentiation and week vasculature. The palisade tissue is lacking or is poorly developed and the mesophyll tissue mainly composed of spongy parenchyma with large intercellular spaces. The chloroplasts were poorly developed with low chlorophyll and protein content.

The literature cited for this project is very scanty on *Dendrocalamus asper* and *Dendrocalamus tulda*. It seems that these most valuable edible species of bamboo are neglected by scientific community for research. Looking after the various importances of these bamboo species this work will help in rendering the guidelines to the researcher for *in vitro* multiplication through embryogenesis.