Chapter 2
REVIEW OF LITERATURE

2.1 History of bamboo research

Bamboo is the common term applied to a broad group of large woody grasses comprising of about 1575 species distributed under 111 genera of Bambusoideae sub family (poaceae) (http://www. guaduabamboo. com/ bamboo-genera.html). Bamboo has been in use of mankind since ages. Use of bamboo as mats and baskets can be traced back at the Younger Stone Age (3,300-2,800 BC) (Ding, 1996). Besides the traditional applications, modern processing techniques opened new horizons for its utilization. It was only in second half of the nineteenth century the research on bamboo was initiated by scientists from Europe like v. Mohl (1845), Munro (1868), Schwendener (1874), de Bary (1877), Camus (1913). Asian botanists like Brandis (1874), Kurz (1876), Gamble (1888), Riviere 1879, Shibata (1900), Takenouchi (1931) along with Botanists from United States *viz*. Ueda, Fairchild and McClure also provided substantial contribution in the initial stage of bamboo research worldwide. Though bamboo research was initiated round the globe in bamboo growing countries, but the outcomes were restricted to contributors only because there was no facility to make the findings available in the public domain. Thus there was a need either to make data available in public domain or create a platform so that researchers working on bamboo from different parts of the world could meet and share their valuable findings. This need was fulfilled by the setting up of International organizations like International Development Research Center (IDRC), Ottawa, Canada, International Union for Forestry Research Organization (IUFRO), Vienna, Austria which strengthened the bamboo research. It was only at the 16th IUFRO world congress hosted in Oslo in 1976, the bamboo researchers

from round the globe shared a platform to exchange their knowledge for the betterment of this wonderful natural resource (Liese, 1987). This was further strengthened by the establishment of "Japan society of bamboo development and protection" in 1977. Since then workshops and conferences are organized by International organizations like IDRC and IUFRO on regular basis on various topics where hundreds of scientists and researcher present their work on various aspects of bamboo (Liese, 1999). Besides these bamboo societies have been founded and also journals purely dealing with bamboo articles have sprouted in many countries like China, India, Japan, United States etc.

Today because ofits growing awareness with innumerable excellent properties research on bamboo has increased many folds with an overflow of information in the internet.

In India, the research on bamboo is being carried out in large scale in states like Assam, Arunachal Pradesh, Kerela, Tamil Nadu, Uttarakhand etc.

2.2 Bamboo taxonomy

2.2.1 Traditional system of bamboo taxonomy

Traditionally, like all other plant species bamboo alsoinvolves morphological features like rhizomes, bud, leaf, branching patterns, inflorescence, flowers and fruits for any documentation or taxonomic treatment (fig 2.1). Bamboos are also gifted with some exceptional morphological features like the culm sheath and well developed branching complements that are generally absent in the grasses and thus can play a key role in proper identification of the bamboo and systematic grouping. However it has its limitations, since many of these characters are not uniformly applicable to all the species and thus there is absolute necessity to have detailed descriptors which can be followed to identify the species (Williams and Rao, 1994) that can be utilized by different group of people dealing with bamboo. Apart from this there is an urgent requirement to refine and better understand the taxonomic techniques and classify the taxonomic diversity in bamboos. As per conventionalmethodthe morphological characters were used for taxonomic identification, especially the flowers. This has been problematic in case of bamboo since the number of characters is limited and there is the scarcity of flowering material because of the intriguing flowering behaviour

Figure 2.1: (a) Newly emerging shoot; (b) Culm Sheath; (c) Branching pattern; (d) Clumping type rhizome; (e) Running type rhizome and (f) Inflorescence.

of the bamboos. Flowering in bamboo remains one of the great mysteries in botany as flower of bamboo is unusual and the period may vary between 15-120years (Janzen, 1976). Incongruity stemmed out among taxonomists due to different interpretations of morphological features and the terminology used for different parts of the plant materials. Thus it the very first step required is to refine the characters that are used currently and also try to find some unique new characters that can prove to be helpful in the long run. Identification and classification of bamboo using the anatomical features didn‟t prove to be successful as at first hoped. The credit for revealing the importance of branch and buds characteristics in bamboo taxonomy goes to Usui (1957). Following this McClure (1966, 1973) studied the morphology of the rhizome, branching patterns, culm sheath and the inflorescence. Apart from this (Soderstrom and Ellis, 1988) also considered the anatomical characters of leaf for bamboo classification at the subfamilies and tribes level however they failed to apply so at the generic level (Soderstrom and Ellis, 1982; Ding and Zhao, 1994). Stapleton (1994a) revealed that branching pattern of inflorescence can also be applied in bamboo taxonomy. Two new characters (prophyll keel and branch replication) were detected by Stapleton (1994b) while studying the Himalayan bamboo and he inferred that these characters can aid in identification of bamboo at the generic level.

2.2.2 Molecular taxonomy of bamboo

The taxonomy of bamboo is in a state of flux and molecular studies are required to help resolve systematic issues. With the advent of molecular biology, the taxonomy of different plants has been revolutionized including bamboo. The use of molecular markers has been increasing at an exponential state in all the fields of biology. The application of molecular marker in classifying bamboo where the basic biology is so little understood can prove to be a landmark. Though the use of molecular markers is innumerable, in bamboo these can be employed for dual function, firstly for precise identification of bamboo genotypes and secondly assessment of genetic variation within species irrespective of the geographic location or other factors responsible to phenotypic variability. Extensive progress has been achieved in bamboo by the implementation of

molecular markers. The present study aimed at reviewing the different molecular tools that have been applied to date.

In recent years, a number of assays have been proposed to detect DNA polymorphism, which has become increasingly precise. The methods employ the use of restriction enzymes or polymerase chain reaction (PCR) or combination of both.

Nuclear restriction fragment length polymorphisms (RFLP) is based on the differences in the restriction enzymes recognition site between genome sequences. Friar and Kochert (1991, 1994) were the first to use restriction fragment length polymorphisms for bamboo identification of 61 accessions and 20 species of *Phyllostachys*. The study supported the earlier observations of the presence of two distinct sections (Phyllostachys and Heteroclada) in *Phyllostachys* species pool. However, they disagreed to place *Phyllostachys nigra* under the section Heteroclada and thus contradicted a previous study (Wang *et al*., 1980). The regular use of RFLP in plant genotyping as well as bamboo has been limited mainly due to the requirements of large amount of DNA along with the use of radioactive isotopes.

The big boom of molecular markers came with PCR-technology i.e. Random Amplified Polymorphic DNA (RAPD) developed by Williams *et al.* (1990), where a single and short arbitrary primer is used. Since its discovery this technique has been successfully employed in the evaluation of genetic relationships in bamboos and other plant species. In bamboo, RAPD analysis has been successfully employed to study the population genetic structure of *Yushnia niitakayamensis* (Hsiao and Rieseberg, 1994) and also to study the genetic relationships within *Phyllostachys* (Gielis *et al.*, 1997; Lai and Hsiao, 1997; Ding, 1998). Nayak and his co-researchers (Nayak *et al*., 2003) had used this technique to study the genetic variations among 12 species of tropical bamboo. Using the RAPD based neighbour-joining tree, Sun and his co-workers (Sun *et al*., 2006) segregated a thorny core *Bambusa* cluster from a cluster of *Dendrocalamus* species with more capitate inflorescences. Bhattacharya *et al*. (2006) developed a RAPD fingerprint profile for a single bamboo species, *Bambusa tulda*. Das and his group (Das *et al*., 2007) used two independent parameters viz. 32 key morphological descriptors and 120

polymorphic loci in the genomic DNA to assess the phylogenetic relationships between 15 tropical bamboo species. Genetic diversity and relationship among nine species of bamboo belonging to four genera was studied by Ramanayake *et al*. (2007) using RAPD analysis. To access the genetic similarity among the 20 different accessions of *Melocanna baccifera*, Lalhruaitluanga and Prasad (2011) used 40 arbitrary RAPD primers. Zhang *et al*. (2011) performed the RAPD of the chloroplast DNA of 22 bamboo species to assess the polymorphism, similarities and relationships among them.

Developed by Vos *et al.* (1995), Amplified fragment length polymorphism (AFLP) is a method described as a combination of restriction digestion and PCR amplification. The use of AFLP for identification as well as determining genetic and relationships among bamboo species was first attempted by Loh *et al.* (2000). They conducted AFLP analysis on 15 species belonging to four genera using eight primer combinations. Unique banding pattern were observed in 13 out of the 15 species experimented. AFLP markers were also used to study the phylogenetic relationships among *Phyllostachys* (Hodkinson *et al.*, 2000) and clonal structure in *Sasa senanensis* (Suyama *et al.*, 2001). Marulanda *et al*. (2002), reported distinct genetic differentiations among the American wood bamboos employing this technique. In 2011, Sen Mandi *et al.*, conducted AFLP analysis on 12 bamboo species belonging to five different genera using six pairs of primer combinations to study the genetic various among them. From the phylogenetic tree it was revealed that all the five species under the genera *Bambusa* were included in one cluster, while the four species under the genera *Dendrocalamus* formed a discrete cluster. However both these clusters had the same origin, while the genus *Melocanna*, *Chimonobambusa*, *Schizostachyum* segregated out as independent clusters. Ghosh and his co-workers (Ghosh *et al*., 2012) applied AFLP markers to better understanding of the taxonomic grouping of nine bamboo species belonging to four genera found in Manipur using six primer pairs. The dendrogram showed that all the *Bambusa* species (except *Bambusa balcooa*) clustered together and the species under *Dendrocalamus* genera also segregated into a major

cluster. Both the genera *Bambusa* and *Dendrocalamus* shared a common origin. *Bambusa balcooa* clustered separately with *Thyrostachys siamensis* exhibiting more genetic similarity. *Melocanna baccifera* though shared the common root with *Bambusa* and *Dendrocalamus* but revealed separate existing suggesting independent evolution.

Paran and Michelmore (1993) developed the Sequence-Characterized Amplified Regions (SCARs) markers which is nothing but the conversion of RAPD markers to overcome the reproducibility problems encountered in the RAPD technique. Das *et al.* (2005) are the sole authority till date to develop the SCARs for bamboo species. They were successful in developing two species-specific SCAR markers, „Balco836" for *Bambusa balcooa* and „Tuldo609" for *B. tulda*.

Inter-simple sequence repeat (ISSR) is a molecular marker which has been used for identification of genetic diversity of many plants including the bamboos. The use of ISSR markers is however limited in case of bamboos. Lin *et al*. (2009) used the ISSR markers to study the genetic diversity of different cultivars of *Phyllostachys pubescens*. Using ISSR markers, Lin *et al.* (2010) succeeded in identifying the bamboo hybrids (formed by crossbreeding) from the parents. Twenty five ISSR markers were used by Mukherjee *et al*. (2010) to investigate the genetic diversity among 22 taxa of bamboos of which 12 resulted in reproducible and scorable bands. Lin and his team (Lin *et al*., 2011) also used the ISSR markers to study the genetic diversity of different cultivars of *Phyllostachys violascens*.

With the advent of time ESTs have become valuable and first-hand source of *in silico* mining of simple sequence repeats (SSR)markers providing insight into the organisms genetic diversity. Twenty-five EST-SSR markers derived from maize, wheat, sorghum, and rice were used by Barkley *et al.* (2005) to assess the genetic diversity of 92 bamboo accessions classified under 11 genera and 44 species. Polymorphic EST–SSR markers obtained from major cereal crops have also been analyzed by Sharma *et al.* (2009) to assess phylogenetic and genetic diversity of twenty five different species of Bambusoideae. Twelve EST-SSR markers were used by Mukherjee *et al.* (2010) to investigate the genetic diversity among 22 taxa of bamboos of

which 4 resulted in reproducible and scorable bands. Dong *et al*. (2011) report the use of Bambusa expressed sequence tags (ESTs) to develop and validate additional microsatellite markers, determine their cross-species transferability and use them to identify bamboo interspecies hybrids. Markers BOM01 and BOM02 transferred successfully to most of the caespitose bamboo species showed r ich polymorphism, and are therefore potentially valuable as species-specific alleles for the identification of caespitose bamboo interspecies hybrids.

Transposable elements are mobile genetic elements broadly classified into two classes (Retrotransposons or Class I and DNA Transposons or Class II) based on their mechanism of transpositions (Feschotte *et al*., 2002). Transoposons occupy considerable proportions of many eukaryotic genomes (SanMiguel and Bennetzen, 1998). In 1995, Huttley and his co-workers reported the presence of Ac-like transposable element in *Bambusa multiplex* while Gielis (1998) also found the presence of Ac-like transposable element in *Bambusa vulgaris, Sasa veitchii and Phyllostachys edulis*. Applying PCR Keukeleire *et al.* (2004) detected hAT group-related sequences in *Bambusa vulgaris* (hATbrna1). Zhou *et al*. (2010a & b) performed molecular phylogenetic analysis of 82 *mariner*-like elements (MLE) transposase gene fragments in 44 bamboo species and PIF-like (P instability factor) elements in the Bambusoideae family. Zhong and his co-workers (2010) initiated the comprehensive characterization and analysis of *Pong*-like superfamily of transposases in 6 subtribes including 44 species in 38 genera under Bambusoideae subfamily. Two transposable elements Ty1-*copia* and Ty3-*gypsy* are reported in *Phyllostachys pubescens* (Zhou *et al*., 2011).

Several studies have shown the use of DNA sequence based methods for phylogenetic study of grasses and bamboos. The chloroplast genome has been used to assess the phylogenetics of the grasses since the birth of plant molecular systematic. In 1994, Nadot and his coworkers used the chloroplast gene rps4 to study the phlogentics of 28 poaceae species including bamboo. They succeeded in resolving the position of Bambusoids in relationships with other groups and also showed how closely the rice and bamboo are

associated. On the basis of the rbcL gene, Barker *et al*. (1995) revealed relationships between monophyletic bamboos and Pooideae. Clark *et al*. (1995) sequenced the chloroplast gene ndhF to address the phylogenetic relationships among the 47 grass sequences including two outgroup sequences. Their study resolved the Streptochaeteae and Anomochloeae (tribes of the neotropical herbaceous bamboos) as the most basal clade within the family. The trnL-F has been attempted by a few researchers (Hodkinson *et al* . 2002 ; Nı´ Chonghaile 2002; Yang *et al*. 2007). In 2005, Qiang and his co-workers performed the preliminary analysis of the genera *Arundinaria* in comparison with other closely related genera like *Pleioblastus, Pseudosasa, Bashania, Clavinodum* and *Oligostachyum* to screen the phylogenetic relationships among them using trnL-F region of the cpDNA. The trnL-F based sequencing method has also been attempted by Yang *et al*. (2008) to establish a phylogenetic of major gropus of Paleotrophical Woody Bamboos (Liang and Hilu, 1996; Hilu *et al*., 1999). The atpB-rbcL and rps16 regions have not previously been used to study bamboo phylogenetics. However, rps16 has proven useful for plant molecular systematics both for dicots, for example Caryophyllaceae (Oxelmann *et al*., 1997), and for monocots, for example Palmae (Asmussen *et al*., 2000) and Marantaceae (Andersson and Chase 2008). Combined analyses of plastid DNA regions are often useful for improving phylogenetic resolution and support (Reeves *et al*., 2001; Hodkinson *et al*., 2007). Sungkaew and his coworkers (Sungkaew *et al*., 2009) performed the combined analysis of five different plastid DNA regions viz. trnL intron, trnL-F intergenic spacer, atpB-rbcL intergenic spacer, rsp16 intron and matK to access the phylogentic relationships among 60 taxa including all the subtribes of Bambusae and related non-bambusoid grasses. Their study resolved the non-monophyly of the woody Bamboos. They further emphasized that the classification of Bambuseae needs to be revised to have a clear picture of the different genera of bamboos.

In contrast to the vast majority of studies done to date on bamboo taxonomy and systematics, investigations on genetic diversity at the population level are in its infancy. This review presents precisely how the

molecular marker helps in sorting out the problems related to genotype identification in general and bamboo taxonomy in particular. It provides a clear picture of the application of various molecular techniques in the population studies especially in bamboo. Though the progress in this field is encouraging, yet these methods should not be considered appropriate for phylogenetic studies above the species level. These markers are undoubtedly useful tools to address the population genetics but for phylogeny reconstruction and taxonomy these might be problematic and misleading, so they must be used with caution. Molecular genetics is a fast-moving field and new techniques are likely to be developed in the near future which will have their own strengths and limitations. Thus it is necessary that these concerns motivate bamboo researchers to a wise and well consider ed imple mentatio n of molecular markers as tools for complementing other techniques.

2.3 Propagation of bamboo

2.3.1 Propagation using traditional methods

Traditionally bamboo is propagated either through sexual or asexual methods. The sexual method involves the use of seed, but this is not considered to be reliable method because of unavailability of seed of bamboo due to its peculiar flowering behavior and even seeds are produce they remain viable only for a short span of time. The asexual method involves the use of vegetative parts including the rhizome, clump division, culm, culm cutting, offset, branch cutting, clump division etc. Asexual propagation can be attempted thorough out the year but has some limitations (Banik, 1985).

2.3.2 Propagation using in vitro techniques

Historically the development of cell and the subsequent proposal of the cell theory paved the path of the plant tissue culture. The basis of plant tissue culture is the concept of „Totipotency" which in turn is an inherent part of the cell theory of Schleiden (1838) and Schwann (1839). *In vitro* technique dates back to 1902, when Haberlandt predicted the totipotency of plant cells. Two major events that revolutionized plant tissue culture were the discovery of plant growth regulators auxins and cytokinins and the formulation of nutrient media i.e., Murashige and Skoog (1962). According to Murashige (1974) there are three possible methods

available for micropropagation.

- Enhanced release of axillary buds
- Production of advantageous shoots through organogenesis.
- Somatic embryogenesis.

In shoot tips, nodal and axillary bud cultures, clonal fidelity is conserved to a greater extent. However in case of callus mediated organogenesis and somatic embryogenesis there is a risk of producing aberrant and thus is not recommended for clonal propagation. Though limited to a few species *in vitro* somatic embryogenesis, tends to be the most effective and rapid method of plant regeneration (Evans *et al.*, 1981). Currently, *in vitro* micropropagation has been adopted for a number of economically and medicinally important plant species.

In bamboo breeding is seriously handicapped because of its long vegetative phase and monocarpic flowering behaviour and poor seed set. Moreover, since it is near impossible that two desirable plants will flower simultaneously, therefore conventional breeding also seems to be difficult. Thus for meeting the raw material demand the best possible way to manage the bamboo forest is through scientific management. Major limitation to bamboo production has been overcome by propagation methods. *In vitro* culture offers a method for producing variations and exploring the resultant variations for crop improvement. *In vitro* culture techniques provide an alternative means of plant propagation and a tool for crop improvement (Rahman, *et al.*, 2004). *In vitro* regenerated plants are superior to conventionally propagated plants in respect of productivity and disease resistance.

The main aim here is to successfully and aseptically transfer the explant into culture medium and then provide *in vitro* environment for growth and differentiation. The important aspects of this are explant disinfection, explant selection and culture medium (Hartmann *et al.*, 1997). The success of establishment of culture *in vitro* depends on the selection of explant, sterilization of explant, composition of the culture media and finally on culture conditions provided for growth and development. The credit for heralding the start of tissue culture in bamboo goes to Alexander and Rao (1968), reported the aseptic germination of bamboo seeds. Since then, micropropagation through axillary bud proliferation where no intermediary

callus formation occurs has been largely attempted.

The use of tissue culture as a tool for plant propagation could be particularly relevant for vegetatively propagated crop plants that resist conventional asexual propagation (Hackett, 1966) or when mass propagation of single plant is required in short period of time. The different explants such as axillary bud, shoot tips, meristem tips, root tips are commonly used. Various explant like emergig rhizome buds, rhizome pieces, axillary bud, shoot tip, leaves are used.

In tissue culture of bamboos different plant parts are used as explant. The most common explants used for bamboo micropropagation are young branch node, immature embryos, mature embryos, mesocotyl, leaf sheath, leaf and root of the young seedling.

The main objective behind explants disinfection is to get rid of the bacterial and fungal contamination with hampering the biological activity of the explants. The commonly used sterilants are bleach, ethanol, sodium hypochlorite, mercuric chloride. The type of sterilant used, concentration and time depends on the nature of explant and species (Razdan, 2003). The list of various disinfectants used in the tissue culture of bamboo is given in table 2.1.

Media plays a vital role in the successful growth and differentiation of excised plant tissues and organs. The artificially prepared nutrient medium is called culture medium. The culture media is composed of several components like inorganic salts, vitamins, aminoacids, sugars, growth regulators (phytohormones), agar or gelrite. The minerals present in the plant tissue culture medium are used by the plant cell as building blocks for the synthesis of organic molecules or as catalysts. The ions of different salts play an important role in transportation or osmotic regulation and in maintaining the electrochemical potential of the plant.

The nutrient requirement varies not only among different plants but also for different parts of the same plants. Therefore, a single media is not suitable for optimum growth of all plant tissues. To overcome this, different nutrient solutions were proposed by different authors from time to time like MS medium (Murashige and Skoog, 1962), B5 medium (Gamborg *et al*, 1968), Nitsch medium (Nitsch and Nitsch, 1969), White‟s medium (White, 1943),

Woody plant medium (Lloyd and McCown, 1980) etc. Consequently the most suitable medium for a particular tissue must be determined by trial and error.

Murashige and Skoog‟s (MS) (1962) medium was used extensively in the regeneration of bamboos. In cultures of *Dendrocalamus strictus,* Nadgir and his co-workers (Nadgir *et al.*, 1984) used Whites basal medium for rapid multiplication. Tsay and his co-workers (Tsay *et al*., 1990) used N_6 medium along with MS medium for the regeneration of *Sinocalamus latiflora* while Ndiaye *et al*. (2009) used three different types of media for the rapid proliferation of *Bambusa vulgaris* viz. MS medium, Gamborg medium and Lloyd and Crown medium.

The sugar is supplied in the form of sucrose. Sucrose was added as the source of carbon at a concentration of 3% (w/v) in almost all the experiments. In the culture of *Bambusa glaucescens* (Banik, 1985) 4% sucrose was used for shooting from dormant culm bud whereas Nadgir *et al*. (1984) used only 2% sucrose. Yeh and Chang (1986) supplemented the media with 3-6% sucrose while Tsay and his co-workers (Tsay *et al*., 1990) used different concentration of sucrose as 3,6,9 and 12% for their experiment. Cheah and Chaille (2011) used 0-6% sucrose for *Bambusa ventricosa* and similar concentration was also experimented by Bejoy and his co-workers (Bejoy *et al*., 2012) in case of *Ochlandra wightii*.

The range of acidity or alkalinity is an important factor that determines the quality of regenerated plantlets from a tissue culture media. The optimum pH for regeneration varies with the type of explant used. In the cultures of the different bamboo species pH of 5.8 were generally maintained in most of the cultures. However pH of 5.7 has also been considered for the successful regeneration of bamboo through tissue culture techniques (Yeh and Chang, 1986; Tsay *et al*., 1990; Devi and Sharma, 2009; Bejoy *et al*.,2012) while as per Ndiaye *et al*. (2009), pH in the range of 5.5-5.6 was suitable.

Three types of media are mainly used in plant tissue culture viz. solid, semisolid and liquid. A media becomes solid or semisolid depending upon the concentration of the solidifying agents used. Agar-agar (obtained from algae like Gelladium or Gracilaria) and gelrite (naturally-derived gelling polymer) are most commonly used as soldifying agents.

Table 2.1:Various disinfecting chemicals were used in the culture of bamboos

Plant species	Sterilant used	References
Bambusa glaucescens	20-30% Javex and 5-6% Sodium hypochlorite	RL Banik, 1985
Bambusa oldhamii	0.01% antiseptal for 3 h, 75% ethanol for 1 min and 2% sodium hypochlorite solution for 15 min	Yeh and Chang, 1986
Sinocalamus latiflora	70% alcohol for 30 sec and 1% sodium hypochlorite solution for 10 min	Tsay *et al*., 1990
Bamboo (54 species)	70% alcohol as spray and 1% sodium hypochlorite solution for 10 min	Prutpongse and Gavinlertvatana, 1992
Bambusa vulgaris	1 g dm^{-3} (m/v) Bavistin for 45 min and 0.2% (m/v) mercuric chloride solution for 30 min	Rout and Das, 1997
Dendrocalamus asper	4% sodium hypochlorite solution for 20 min	Arya *et al*., 1999
Bambusa wamin	Wiped with 70% alcohol followed by 0.2% mercuric chloride for 5-20 min.	Arshad *et al*., 2005
Bambusa vulgaris	0.1% mercuric chloride for 20 min	Ndiaye *et al*., 2009
Guadua angustifolia	Extran (0.05% w/v) for 10 min, combination of Agri-mycin and Benomyl (@$2gl^{-1}$) for 10 min, sodium hypochlorite (1.0 or 1.5% w/v) for 10 min, or with calcium hypochlorite (10% w/v) for 40 min supplemented with a drop of Tween 80 per 100 ml	Jimenez *et al*., 2006
Bambusa glaucescens	70% alcohol followed by 5-10 min in 1% w/v Cetrimide and finally in 0.1% mercuric chloride for 10 min.	Shirin and Rana, 2007
Dendrocalamus asper	5% cetavelon for 15 min followed by 0.1% mercuric chloride for 7-10 min	Arya *et al*., 2001
Arundinaria callosa	0.1% mercuric chloride for 10 min	Devi and Sharma, 2009
Bambusa nutans	Tween 20, bavistin (0.1%) and streptomycin sulfate (0.05%) for 20 -25 min, 70% ethanol for 1-2 min. Finally (0.04%) mercuric chloride with 1-2 drop of liquid detergent for 5-6 min	Mehta *et al*., 2011
Bambusa ventricosa	70% alcohol for 5 min followed by 10% Clorox bleach for 40 min	Cheah and Chaille, 2011
Dendrocalamus farinosus	70% ethanol for 30 s followed by 0.1% $HgCl_2$ for 30 min	Hu *et al*., 2011
Ochlandra wightii	1% commercial bleach and 0.2% labolin for 30 min	Bejoy *et al*., 2012
Dendrocalamus giganteus	20% sodium hypochlorite for 20 min	Devi *et al*., 2012

The media was solidified with agar 0.8% (w/v) in all the cultures (Nadgir *et al*., 1984; Tsay *et al*., 1990; Rout and Das, 1997; Mehta *et al*., 2011) with a few exceptions. 0.75 % agar was used in the cultures of *Bambusa wamin* (Arshad *et al*., 2005). In cultures of *Bambusa oldhamii* (Yeh and Chang, 1986), *Bambusa glaucescens* (Shirin and Rana, 2007), *Ochlandra wightii* (Bejoy *et al*., 2012), media were gelled with 0.7% agar agar. Lower concentrations of agar like 0.6% agar were also reported (Prutpongse and Gavinlertvatana, 1992) and 0.42% in *Bambusa vulgaris straita* (Ramanayake *et al*., 2006) was used prior to their cultures. Gelrite at a concentration of 2g/l, 2.2g/l, 3g/l and 3.5g/l was used as a gelling agent in *Bambusa balcooa* (Negi and Saxena, 2011), *Bambusa edulis* (Lin *et al*., 2004), *Bambusa venticosa* (Cheah and Chaille, 2011) and *Bambusa vulgaris* (Ndiaye *et al*.,2006) respectively while Ogita (2005) used 3g/l gellan gum for *Phyllostachys nigra* and Jimenez and his co-researchers (Jimenez *et al*., 2006) used phytagel (0.2%) for *Guadua anguistifolia* culture.

Many researchers prefer to call plant hormones as plant growth substances or plant growth regulators. Plant hormones added to plant tissue culture media are taken up and increase the level within the tissue. Most of the increase is however, transient because plant hormones are rapidly inactivated after uptake. Usually only very small amounts of the applied hormones remain in the free form. It has been seen that, for auxins, equilibrium exists between the free and conjugated form, of which only less than 1% being present in the freeform. The effect of hormones not only depends on the rate of uptake from the medium, or on the stability in the medium and in the tissue, but also on the sensitivity of the target tissue.

The main plant growth regulators used in tissue culture are auxins (indole-3-acetic acid, indole-3-buturic acid, 1-napthaleneacetic acid, 2, 4-dichlorophenoxyacetic acid, piloram etc); cytokinins (zeatin, 6-benzylamino purine, kinetin, thidiazuron etc); gibberellins (GA_3, GA_4, GA_1, GA_7 etc); abscisic acid; ethylene etc. A list of the bamboo species and the plant growth regulator used for its regeneration are provided in table 2.2. In the regeneration of bamboos plant growth regulators like TDZ, BA, Kn, NAA, IAA, 2, 4-D etc were extensively used. Organic additives

like coconut water were used as a supplement in some media for the regeneration of the bamboos (Nadgir *et al*., 1984; Prutpongse and Gavinlertvatana, 1992; Cheah and Chaille, 2011)

Incubation conditions play a vital role in plant tissue culture after aseptic inoculation of the explants. An optimum temperature is required for obtaining desirable clone since high temperature may lead to dissociation of the culture media and tissue damage while at very low temperature tissue growth is seriously restricted. Moreover some tissue grows in dark while other prefers light conditions. The amount of light also has substantial effect on the regeneration. The incubation conditions attempted for bamboos by different workers are shown in table 2.3.

Callus is defined as an unorganized and undifferentiated proliferated mass of cells produced from isolated plant cells, tissues or organs under controlled conditions *in* vitro. It is formed due to the cell expansion and cell division of the cells of the explants.

In plant tissue culture different juvenile parts of the experimental plant like leaf, stem, roots, nodes etc containing meristematic cells are used for the initiation of callus culture since meristematic cells has a pre-existing growth momentum. Once the explant is cultured in the nutrient media, it absorbs the exogenously supplied nutrients and growth regulators; divide asynchronously to form the unorganized mass of tissue. During the initial growth phase the cells enlarge or swell to rupture. This indicates the response of tissue to the medium for callus formation. As the cells rupture some endogenous growth substances ooze out which in turn stimulates the cell division along with the penetration of the exogenously supplied hormone and nutrients. The unorganized callus tissue gradually increases in size and ultimately the whole part of the explants starts to divide.

In order to obtain embryogenic callus in *Bambusa edulis*, Lin and his co-workers (Li *et al*., 2004) used MS medium supplemented with 9.2 µM kinetin (KN), 13.6 µM 2,4 dichlorophenoxyacetic acid (2,4-D), 0.1% (v/v) coconut milk in addition to 0.046 µM thidiazuron (TDZ). A protocol for callus induction from the shoots of *Phyllostachys nigra* was developed by Ogita (2005). The cultures produced callus in half strength MS medium supplemented

with 3µM 2,4-D.

In *Bambusa vulgaris* a protocol for producing friable callus was achieved by using *in vitro* sprouted shoots as explants. MS medium supplemented with 2.2 µM BAP, 9.04 µM 2,4-D and 14.76 µM IBA was used for callus initiation (Rout and Das, 1997).

Mehta and her co-workers (Mehta *et al*., 2011) in their study on *Bambusa nutans* reported the formation of embryogenic calli from *in vitro* sprouted buds in MS medium containing 5 mg/l 2,4-D. Similarly, Cheah and Chaille (2011) in their research on *Bambusa ventricosa* also reported the formation of embryogenic

Table 2.2: List of bamboo species and the plant growth regulators used for its regeneration

Plant species	Plant growth regulator	Organic additives	References
Dendrocalamus strictus	BAP, Kn, IAA	Coconut water	Nadgir *et al*., 1984
Bambusa glaucescens	BA and NAA	-	RL Banik, 1987
Bambusa oldhamii	2,4-D and Kn	-	Yeh and Chang, 1986
Sinocalamus latiflora	2,4-D, BA and NAA	Coconut water	Tsay *et al*., 1990
Bamboo (54 species)	2,4-D, BA and NAA		Prutpongse and Gavinlertvatana, 1992
Bambusa vulgaris	BA, Kn, 2,4-D, IBA		Rout and Das, 1997
Dendrocalamus asper	BA, NAA and IBA		Arya *et al*., 1999
Bambusa wamin	BAP, BA, Kn and IBA		Arshad *et al*., 2005
Bambusa vulgaris	IBA, NAA and BAP		Ndiaye *et al*., 2006
Guadua angustifolia	BAP		Jimenez *et al*., 2006
Bambusa glaucescens	BA and Kn		Shirin and Rana, 2007
Dendrocalamus asper	BA, NAA and IBA		Arya *et al*., 2008
Arundinaria callosa	BAP and IBA		Devi and Sharma, 2009
Bambusa nutans	BAP, NAA and Kn		Mehta *et al*., 2011
Bambusa ventricosa	BAP, Kn, IAA, IBA and NAA		Cheah and Chaille, 2011
Dendrocalamus farinosus	2,4-D, 2,4,5-T, Kn, IAA and IBA		Hu *et al*., 2011
Ochlandra wightii	BAP, Kn and TDZ		Bejoy *et al*., 2012
Dendrocalamus giganteus	BAP, Kn, IBA, NAA, 2,4-D and GA_3		Devi *et al*., 2012

calli from *in vitro* developed shoots in MS medium containing 3 mg/l 2,4-D, 2 mg/l kinetin.

In their study on callus induction and plant regeneration of *Dendrocalamus farinosus*, Hu and his co researchers (Hu *et al*., 2000) employed two different types of explants i.e. mature seeds and young shoots. MS medium in combination with 2 mg/l 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2 mg/l Kn and 0.4 mg/l IBA gave good

Table 2.3:Incubation conditions required by bamboo species

Plant species	Temperature	Light	References
Dendrocalamus strictus	25ºC	16 h photoperiod of 1500 lux light intensity	Nadgir *et al*., 1984
Bambusa glaucescens	28ºC	14 hour photoperiod	RL Banik, 1987
Bambusa oldhamii	26±1ºC	16/8h day and night regime (15-40 µE/m^2s)	Yeh and Chang, 1986
Sinocalamus latiflora	25±1ºC	16/8h light/dark cycle with 135 µE/m^2s	Tsay *et al*., 1990
Bamboo (54 species)	25ºC	250 µmol·m^{-2}·s^{-1} cool-white fluorescent illumination for 16 h·	Prutpongse and Gavinlertvatana, 1992
Bambusa vulgaris	25±2ºC	16 h photoperiod (55 µmol·m^{-2}·s^{-1})	Rout and Das, 1997
Dendrocalamus asper	25±1ºC	16 h photoperiod (30 µmol·m^{-2}·s^{-1})	Arya *et al*., 1999
Bambusa wamin	25±2ºC	16 h photoperiod (30 µmol·m^{-2}·s^{-1})	Arshad *et al*., 2005
Bambusa vulgaris	25ºC	16 h/8 h photoperiod	Ndiaye *et al*., 2006
Guadua angustifolia	26ºC	In dark	Jimenez *et al*., 2006
Bambusa glaucescens	25±2ºC	16 h photoperiod (35 ± 10 µmol m^{-2} S^{-1}).	Shirin and Rana, 2007
Dendrocalamus asper	26ºC	16 h photoperiod (3000 µE·m^{-2}·s^{-1})	Arya *et al*., 2008
Arundinaria callosa	25±2ºC		Devi and Sharma, 2009
Bambusa nutans		14/10h day and night regime (70 ± 5 µmol·m^{-2}·s^{-1})	Mehta *et al*., 2011
Bambusa ventricosa	25±2ºC	16-h photoperiod with a light intensity of 47.29 µmol·m^{-2}·s^{-1}	Cheah and Chaille, 2011
Dendrocalamus farinosus	25±1ºC	12 h photoperiod (80 µmol·m^{-2}·s^{-1})	Hu *et al*., 2011
Ochlandra wightii	24±2ºC	16h/8h photoperiod	Bejoy *et al*., 2012
Dendrocalamus giganteus	25±2ºC	16-h photoperiod with a light intensity of 90-95 µmol·m^{-2}·s^{-1}	Devi *et al*., 2012

results when mature seeds was used as explants. Callus induction frequency was found to be 95% for mature seeds and 21% to 29.7% in case of young shoots.

Recently in 2012, Devi and her team developed a protocol for callus induction and proliferation in edible bamboo *Dendrocalamus giganteus*. MS medium in addition to 3 mg/l 2,4-D and 0.5 mg/l Kn was found to be best suited for callusing in *D. giganteus*.

The discovery of growth regulators like auxins, gibberlins, cytokinins and abscicins along with other organic compounds led to new vistas in plant tissue culture. The role of growth regulators and their concentration should be carefully chosen for obtaining desired responses in tissue culture.

In *Dendrocalamus strictus* the best shoot multiplication and growth was observed in MS medium containing 2 mg/l BAP and 5% Coconut milk, where a maximum of 8-10 shoots were observed per flask in liquid culture within 6-7 weeks. Regular sub-culturing was practised and in every sub-culture 6-7 shoots were obtained from each shoot (Nadgir *et al*., 1984).

The axillary bud of *Bambusa vulagris* „Straita" when cultured in MS medium having 4mg/l BA resulted in highest mean shoot number. Other than this BA at the concentration of 6mg/l and TDZ at 0.1mg/l also showed produced same number of shoots (Ramnayake *et al*., 2006).

Shirin and Rana (2007) observed similar shoot multiplication in *Bambusa glaucescens* in MS medium supplemented with 5µM BAP and 15µM Kn either alone or in combination.

In case of *Bambusa balcooa*, of different hormones tried for obtaining shoots MS medium supplemented with BAP was found to be most suitable. BAP at a concentration of 1mg/l resulted in 20 shoots while at 5mg/l produced 29 shoots. The sub-culture was regularly done every 3-4 weeks to get the desired number of plantlets (Mudoi and Borthakur, 2009).

In *Ochlandra wightii*, Bejoy *et al*. (2012) showed that combined action of two cytokinins i.e. BAP and TDZ at a concentration of 0.5 mg/l each could enhance shoot multiplication upto 9.8 shoots in two months following regular subculture.

Additional plant growth regulator may or may not be required for *in vitro*

rooting in bamboo tissue culture.

Nadgir *et al*. (1984) found that the shoots of *Dendrocalamus strictus* when transferred to MS medium free of plant growth regulator though produced roots but the percentage was as low as 40%, but when the shoots were treated with IBA prior to culturing in MS medium the percentage was elevated to 80%.

Embryogenic calli of *Sinocalamus latifora* when cultured for a long time in MS medium or subcultured in auxin free medium rooted well (Tsay *et al*., 1990). Simialr type of result was also obtained in case of *Bambusa beecheyana* (Chang and Lan, 1995).

Prutpongse and Gavinlertvatana (1992) reported that depending upon the species of bamboo used, NAA at the concentration between 2.7 to 5.4 µM was found to be optimal for rooting.

In 1997, Rout and Das found that the isolated shoots of *Bambusa vulgaris* rooted well in half strength MS medium supplemented with IBA. Similar observations were also made by Arshad and his co-workers (Arshad *et al*., 2005) in *Bambusa wamin*, Shirin and Rana (2007) in *Bambusa glaucescens.*

Spontaneous rooting of shoots of *Dendrocalamus asper* occurred in MS medium supplemented with IBA (10 mg/l) and NAA (3 mg/l) (Arya *et al*., 1999; Arya *et al*., 2008).

Jimenez and his co-workers (Jimenez *et al*., 2006) noted spontaneous rooting of *Guadua angustifolia* in plant growth regulator free MS medium. In the same year Ramanayake *et al*.(2006) and Ndiyae *et al*.(2006) reported that rooting can be enhanced by addition of TDZ (0.1 mg/l) and IBA (20 mg/l) in case of *Bambusa vulgaris* „Striata‟ and *Bambusa vulgaris* respectively.

In *Arundinaria callosa* the number of roots formed from the *in vitro* shoots was significantly higher in ½ MS medium supplemented with 25 µM IBA along with 0.05 µM BAP. A maximum of 3.8±0.6 healthy roots were regenerated from the *in vitro* shoots (Devi and Sharma, 2009).

In *Bambusa balcooa* three different auxins (IBA, NAA and IAA) were experimented to see their effect on rooting. It was found that NAA (6.71 µM) was suitable compared to other two. However, when half strength MS medium was supplemented with different concentrations and combinations of auxins (5.71 µM IAA, 4.9 µM IBA, 5.37 µM NAA) resulted in maximum rooting (Negi and Saxena, 2011). The effect of combinations of

auxin (0.4 mg/l IBA and 0.25 mg/l IAA) in rooting was also reported in *Dendrocalamus farinosus* by Hu and his co-workers (Hu *et al*., 2000).

Recently in 2012, Devi and her co-researchers and Bejoy and his co-workers found that IBA at the concentration of 5 mg/l and 0.5 mg/l was optimum for rooting in *Dendrocalamus giganteus* and *Ochlandra wightii*.

Once the plant is well established *in vitro* the major obstacles that develops is the successful transfer of plantlets from the laboratory to the field (Wardle *et al.*, 1983). This difficulty probably appears due to the drastic change in the environmental conditions *in vitro* and outside. Under *in vitro* condition there is low light intensity, high humidity and poor root growth in contrast to field and/or greenhouse conditions where there is higher light intensity, low humidity along with different microflora (Desjardins *et al*., 1987). Several protocols have been developed by different tissue culturist to overcome some of these constraints.

In hardening of regenerated tissue cultured plantlets of the bamboo different hardening materials like river sand and charcoal, sterilized potting soil, vermiculite, soil and sand, soil and manure etc. have been used (Table 2.4). The success rate of hardening depends upon the hardening material and the condition of the regenerated plantlet. High rate of survival of regenerated plantlets have been achieved in field.

Bamboo has been the subject of men‟s curiosity since ages. In bamboo the traditional breeding is seriously handicapped because of the peculiar flowering and production of poor seed sets and thus *in vitro* regeneration is the best alternative. From the available literature it is though impossible to trace out the exact mechanism behind the *in vitro* regeneration of bamboo, but still published information gives some idea about the different factors which play cumulative role. Form the literature it is also clear that *in vitro* regenerated plants are superior to conventionally propagated plants in respect of productivity and disease resistance.

2.4 Bamboo and human health

In the process of economic development, with the increase in income, human society tends to care more about their health. Therefore, demand for healthy herbal organic foods developed from various plants has also increased. Production of more

efficient and productive food items by the researchers are on demand. One such plant with multiple qualities is bamboo.

Bamboo has been used over centuries by the humans both in daily life and for medicinal purpose in China and other Asian countries. The earliest scientific evidence of use of bamboo in traditional medicine dates back to 1963 (Sakai *et al*., 1963). This marked the beginning of the use of bamboo as medicine which was followed by series of research carried out by different workers since then (Okabe *et al*, 1975; Otani *et al*, 1990; Tsunoda *et al*, 1998; Hu *et al*, 2000; Kweon *et al*, 2001; Kim *et al*, 2003; Ren *et al*, 2004; Kurokawa *et al*, 2006; Lu *et al*, 2005; Lu *et al*, 2006; Park *et al*, 2007; Seki *et al*.,2008; Seki and Maeda, 2010). Bamboo has attracted attention world over due to its high antioxidant content and therapeutic effects on inflammation, fat igue, cancer, hyperlipidemia, diabetes, aging and

Table 2.4: Hardening materials used and survival rate of regenerated bamboos

Plant species	Potting mixture	Survival rate	References
Dendrocalamus strictus	Sterile soil: sand (1:1)	70-80%	Nadgir *et al*., 1984
Bambusa glaucescens	Moist sterile soil		Banik, 1987
Bambusa vulgaris	Soil:Manure:Sand (1:1:1)	90%	Rout and Das, 1997
Dendrocalamus asper	Soil	95%	Arya *et al*., 1999
Bambusa wamin	Vermiculite	80-85%	Arshad *et al*., 2005
Bambusa vulgaris		100%	Ndiaye *et al*., 2006
Guadua angustifolia	Soil:Sand:Rice hulls (1:1:1)	>85%	Jimenez *et al*., 2006
Bambusa glaucescens	Soilrite with half strength MS medium (organic free)	80%	Shirin and Rana, 2007
Dendrocalamus asper	Soilrite	95%	Arya *et al*., 2008
Arundinaria callosa	Soil mixture	60-70%	Devi and Sharma, 2009
Bambusa nutans	Soil and sand (1:1)	90%	Mehta *et al*., 2010
Bambusa ventricosa	Pro-mix/black cinder:potting mixture (1:1)		Cheah and Chaille, 2011
Dendrocalamus farinosus	Peat moss, vermiculite and garden soil (2:1:1)	90.1%	Hu *et al*., 2011
Ochlandra wightii	River sand: coarse charcoal (3:1)	80%	Bejoy *et al*., 2012
Dendrocalamus giganteus	Sand and soil (1:1)	80-90%	Devi *et al*., 2012

hypertension.

Free radicals might occur either by the accidents of chemistry or due to specific metabolic purpose in the body. The free radicals produced by either way have different reactivity with some leading to damage to biomolecules such as DNA, lipids and proteins (Halliwell, 1994). Antioxidants can react with free radicals during the oxidation process by acting as a reactive species scavenger and liberating catalysts, so antioxidants can be used to reduce the oxidative process (Gulcin *et al.*, 2005) but they are not 100% effective. Mere large doses of diet-derived antibody was thought to be important to stay healthier for long time, but with the passage of time and development of science and technology the supply of „pro-oxidants" is thought to be a better option (Halliwell, 2012). Bioactive compounds like ascorbic acid, carotenoids, tocochromanols and phenols are antioxidants.

The bamboo leaf extract (BLE) is thought to be good source of natural antioxidants and also have great pharmaceutical potential. BLE is mainly composed of flavonoids, lactones and phenolic acid. The flavonoids are represented mainly by the flavones C- glycosides which include homoorientin, isovitexin, orientin and vitexin. Apart from this quercetin, luteolin, rutin, caffeic acid, *p* -coumaric acid, chlorogenic acid and tricin are also present (Zhang *et al.*, 2002b) (table 2.5). The flavonoid content was recorded to be 3.44% in different bamboo leaves species (Singhal *et al.*, 2011).

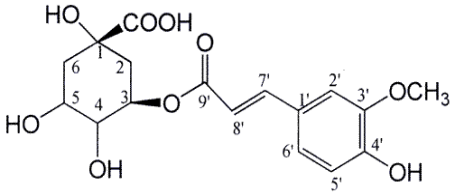
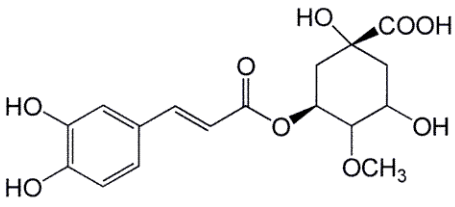
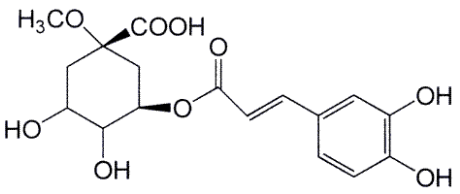
Diabetes Mellitus (DM) is prevalent among almost 200 million people worldwide, which is thought to increase exponentially to 300 million in the next two decades, type 2 being common (Alberti, 2002).

In the study conducted by Ding and his coworkers (Ding *et al.*, 2007) with moso bamboo leaves on 50 diabetic rats, they evaluated that different doses of polysaccharide were found to possess good hypoglycemic effect. Hyun and Hyeon-Skoog (2009) in their experiment with *Sasa borealis* leaf extract found that when substituted for meat in patty the leaf extract significantly lowered plasma glucose indicating anti diabetic activity of BLE. The anti diabetic activity of *Sasa borealis* leaf extract was also studied by Choi and his co workers (Choi *et al.*, 2008). The inhibitory effect of the leaves of *Pseudosasa japonica* was

evaluated on high fat diet induced obesity and diabetes in C57BL/6J mice. All the mice had access to high fat diet for a week and then switched over to either the bamboo extract diet or control diet. The mice were regularly monitored for their daily intake of food and weight gained. Though the food intake of mice assigned to bamboo extract was found to be slightly higher than the control, but the weight gain was however restricted in mice on bamboo extract compared to control (Panee, 2008).

With modernization and industrialization the number of death and disability due to chronic heart diseases such as cardiovascular disease, diabetes etc has surpassed the death and disability due to nutritional deficiencies and infectious diseases (Yusuf *et al*., 2001). Fu and his co researchers (Fu *et al*., 2005a) experimentally proved that when the high cholesterol mice were treated with different concentrations of BLE, there was great reduction in the serum cholesterol. *Phyllostachys pubescens* leaves proved to have protective effect against palmitic acid induced lipo apoptosis (Panee *et al*., 2008). Experiments conducted on rats showed that flavonoids rich bamboo beer could significantly lower the blood triglycerides and cholesterol. Apart from this the beer could elevate HDL-

Table 2.5: List of chemical compounds and their structure isolated from different bamboo species

Plant name	Chemical compound	Chemical structure	References
Phyllostachys edulis	3-*O*-(3‟‟-methylcaffeoyl) quinic acid		Kweon *et al*., 2001
	5-*O*-caffeoyl-4-methylquinic acid		
	3-*O*-caffeoyl-1-methylquinic acid		

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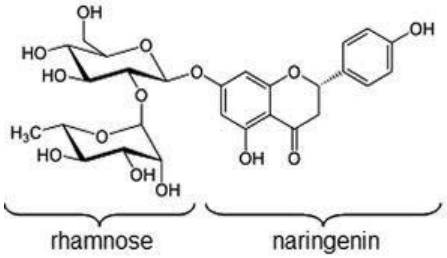
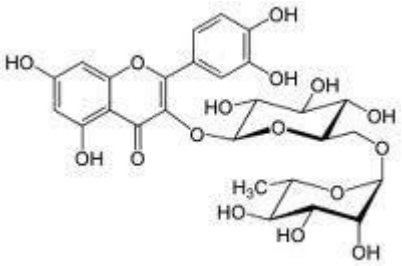
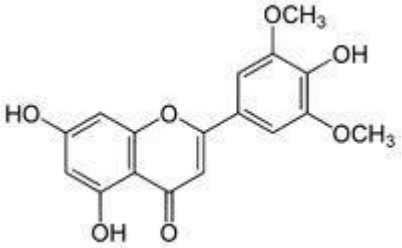
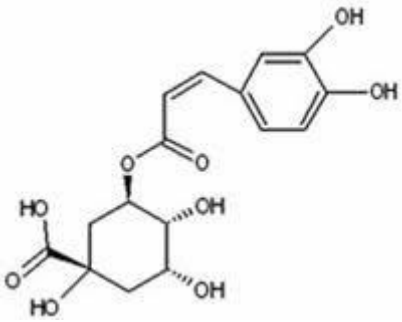
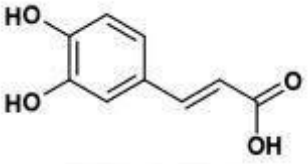
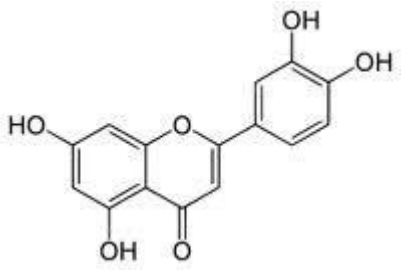
Plant name	Chemical compound	Chemical structure	References
Phyllostachys nigra var. henonis	naringin-7-rhamnoglucoside		Lu *et al*., 2005
	Rutin		
	Tricin		
	Chlorogenic acid		
	Caffeic acid		
	Luteolin		

Table 2.5 continued to next page

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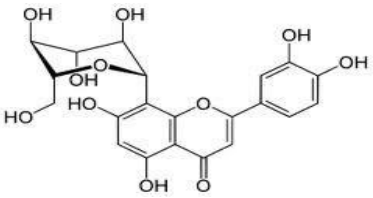
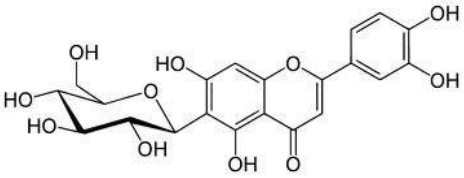
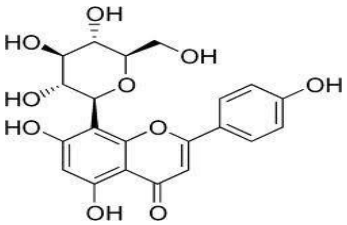
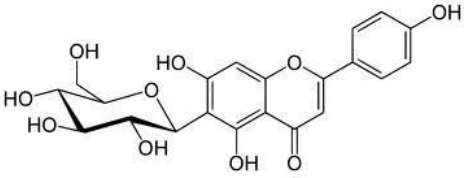
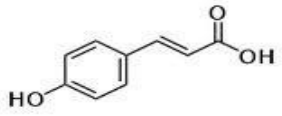
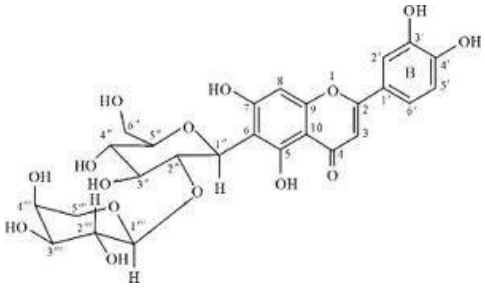
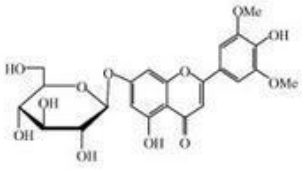
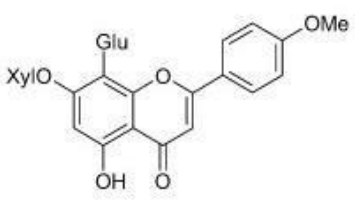
Plant name	Chemical compound	Chemical structure	Reference
Phyllostachys nigra var. henonis	Orientin		Lu *et al*., 2005; Zhang *et al*., 2007; Zhang *et al*., 2008
	Homoorientin		
	Vitexin		
	Isovitexin		
Phyllostachys nigra var. henonis	p-coumaric acid		Zhang *et al*., 2007
Sasa borealis	isoorientin 2"-O-c~-L-rhamnoside		Park *et al*., 2007
	tricin 7-O-13-D-glucopyranoside		
	apigenin 6-C-13-D-xylopyranosyl -8-C-13-D-glucopyranoside		

cholesterol and reduce LDL-cholesterol in a dose dependant manner (Ying *et al.*, 2000). The cardioprotective potential of flavone C-glucosides i.e. Orientin obtained from the leaves of *Phyllostachys nigra* has been proved by Fu and his co workers (Fu *et al*., 2006). They also stated that it could also inhibit apoptosis by blocking the mitochondrial apoptotic pathway.

The leaves of *Sasa senanensis* (popularly known as Kumaizasa) have been used in Eastern Asia as a potential source of natural drug since hundreds of years. The alkaline extract prepared from the leaves (in hot water at 100ºC) of *S. senanensis* is popularly known as ".Sasa health". Tsunoda *et al.* (1998) from their experiment on mammary tumor strain of SHN virgin mice proved that oral administration of Sasa health for 12 days could significantly inhibit both the development and growth of mammary tumor in experimental models. In 2008, Seki and his team also made an attempt to prove the anti-tumor activity of Sasa health. They used three different temperatures (100ºC, 121ºC and 196ºC) to prepare the Sasa health to evaluate the anti-tumor potential in three mouse tumor models (S-180, C38 and Meth-A). Oral administration of the extract a concentration of 0.05% or more was found to be effective in suppressing tumor growth in mouse models S-180 and C38. The extract also accelerated immunostimulating activity, which in turn activated the macrophages and human natural killer (NK) cells in tumor models and thus suppress the tumor. Panee (2008) conducted experiment to test the effect of leaves of *Pseudosasa japonica* on the development of DMBA (7,12-Dimethylbenz[a]anthracene) induce breast cancer in SD (Sprague-Dawley) rats. He found that oral administration of bamboo extract for 3 weeks prior to DMBA injection could delay the onset of breast cancer by one week as compared to the control. Moreover, the bamboo extract also showed the potential of decreasing the incidence of occurrence of tumor by 44% and restricting the growth rate of the tumor by 67% after 11weeks of DMBA treatment.

Leaf extract of *Phyllosatchys nigra* var henonis have been reported to enhance the anti-fatigue capacity in mice (Zhang and Tang, 1997). You and his coworkers (You *et al*., 2006) found that oral administration of 80% ethanol extract of *Pseudosasa japonica* leaf for

18 days could drastically increase the swimming time in experimental mice up to one and half folds and simultaneously reduce the blood lactate and elevate the removal of lactate suggesting its potential to reduce fatigue compared to the control group.

Methanol extract of the leaves of *Bambusa vulgaris* have been shown to possess anti-inflammatory activity against the various anti-inflammatory tests performed which includes formaldehyde induced rat paw edema, acetic acid induced vascular permeability test, carrageenan induced peritonitis and cotton pellet granuloma in albino rats (Carey *et al.*, 2009).

Lin *et al.*, (2008) showed that the ethyl acetate fraction of *Sasa quelpaertensis* leaf which is mainly rich in lipid soluble compounds could significantly reduce lipopolysaccharide induce TNF-α, IL-1β and IL-6 mRNA levels. This finding indicates that the ethyl acetate fraction exhibited anti-inflammatory activity by inhibiting the production of anti-inflammatory mediators and thus exerts health benefits.

Leaves of different species of bamboo have been in use since long time not only as medicine but also as fodder. A number of studies have been done on animal models to judge the potentiality of bamboo leaf extract not only as food additive but also as medicine. The scientific validation and experiments clearly reveals that bamboo leaf is not only safe as food additive but also exhibit potential as raw materials to the pharmaceutical and nutraceutical industries. But a lot needs to be explored because the reports available are confined to some selected species of bamboo of the thousands that exists in nature.

2.5 Databases

Since the days of yore plants has been indispensible to mankind because of their multiple utility stating from food, construction to medicine. In most part of the world, the medicinal properties of the plants which are traditional used to treat various ailments has been handed over by the means of folklore from generation to generation which may disappear with the passage of time. Thus there is a need of systematic documentary, to preserve the traditional knowledge. Today with the advent of computers the documentation of different plants and their products in the form of databases seems to be not only easy but also accessible by everyone and everywhere. Through this database the knowledge of a particular plant is globalized so that

people from all over the world gets the maximum benefits out of it. Several databases have been designed in the recent years with different attributes. Some includes all the relevant information regarding a single plant like the database named PlantGM which gives information related to genetic markers in rice (*Oryza sativa*) and Chinese cabbage (*Brassica rapa*) (Kim *et al*., 2008), TNAURice which provides detailed information regarding the quantitative and qualitative descriptors of rice along with parental origin (Ramalingam *et al*., 2010), CIMAN, which is a compilation of *Citrus* bioresources of Manipur (Sanabam *et al*., 2012), while there are some databases which maintains the records of plants that are effective against various diseases like asthma (Kasiranjan *et al*., 2007) and diabetes (Singh *et al*., 2009; Middha *et al*., 2009). Apart from these there are some databases which particularly deals with folklore medicines like NeMedPlant which describes traditional formulations of plants from North-east region of India (Meetei *et al*., 2012) and MEDDB is a storehouse of over hundreds of plants which finds their uses in treatment of various ailments in and around Madurai, Tamil Nadu (Mary *et al*., 2012). Like many other plants bamboo also has innumerable exceptional properties which are confined to bamboo growing regions. Though there are a few databases based on this "Green gold" but they merely give information regarding bamboo trade and trade development. So emphasis must be given to develop scientific database based on various aspects of bamboo plants including the taxonomy, morphology, medicinal properties (if any), whether the shoot is edible or not etc. which will surely benefit the scientific community in general and bamboo lovers in particular.