Chapter-2
Review of Literature
2.1 Bamboo forest cover and species richness

Bamboo is a group of fast growing, giant, perennial and woody grasses of subfamily Bambusoideae and family Poaceae. There are around 1575 bamboo species belonging to 111 different genera distributed worldwide. However, only 50 species of them are routinely cultivated for commercial utilization (Hunter 2003). Bamboo bioresources are estimated to cover 37 million hectares forest land (1%), globally (FRA 2005). Its distribution varies from a minimum of 2.8 million hectares in five countries of African sub-continent to maximum of 24 million hectares in 16 different countries of Asia. However, ten Latin American countries are expected to have over 10 million hectares of bamboo genetic resources (FRA 2005). India, although has maximum bamboo forest cover still ranks the second largest in terms of bamboo biomass production and species richness after China (Bhatt et al. 2003; Bystriakova 2003). The total bamboo cover in India is estimated to be 11.4 million hectares (16.8 % of total forest area) which accounts for roughly half of the total area under bamboo cover reported for Asia.

India with 136 bamboo species has very rich bamboo biodiversity. North-eastern region of India with occurrence of 58 species representing 10 genera is considered to be the hot spot for bamboo biodiversity among the other bamboo cultivation zones in India. Of the 22 genera found in India, 19 are indigenous and 3 are reported as exotic. In general, based on growth pattern, bamboo species can be categorized as sympodial (clump forming) and monopodial (non-clump forming). Most of the Indian forest land is under sympodial bamboo cultivation (67 %) and remaining 33 % forest land is covered by monopodial bamboo species (FAO 2006). Among different bamboo genera, *Bambusa* and *Dendrocalamus* (sympodial bamboos) are widely cultivated for commercial utilization (Tewari 1992). Of these, *Dendrocalamus strictus* contribute a maximum of 45 % followed by, *Bambusa bambos* (13 %), *D. hamiltonii* (7 %), *B. tulda* (5 %) and *B. pallida* (4 %) to the species wise distribution to growing stock. However, another bamboo species namely *Melocanna baccafera*, a monopodial bamboo species accounts for 20 %. All other species contribute only 6 %. Details of economically important bamboo genera are given in Table 2.1.
The world bamboo distribution ranges between 46 degree N Latitude and 47 degree S Latitude. Although, altitudinal occurrence of bamboo begins just above sea level (asl) to 4000 m asl. However, elevation of 770 m- 1,080 m asl are found to be most suitable range for their occurrence. Among the various vegetation zones, uneven bamboo distribution is confined to almost all regions of the world, whereas, moist deciduous, semi-evergreen, tropical, and subtropical forest regimes are found to be most conducive. While, bamboo naturally occurs abundantly in tropical and subtropical belts, some species (*Arundinaria gigantea, A. tecta*) also grow in sub-temperate zones in Europe and North America. In India bamboo species are distributed throughout the country except Jammu & Kashmir. However, due to diverse climatic zones across the country, the tropical, sub-tropical and temperate regions of the country with annual rainfall range of 1,200 mm to 4,000 mm and temperature variation between 16°C and 38°C are considered to be the most suitable zones for natural bamboo distribution in India. The north-eastern region of India fulfilling most of these climatic criteria has greatest species diversity as compared to other regions, hence,
considered home of bamboo diversity. The major bamboo distribution and diversity centres with occurrence of genera and species are shown in figure 2.1.

![Bamboo diversity hot spots in India](image)

**Figure 2.1: Major bamboo diversity centres with occurrence of their genera and species in India.**

2.2 Bamboo Cytogenetics

2.2.1 Polyploidy in bamboo

Polyploidy is more common among plants than in animals. More than 35% of flowering plants are polyploid (Stebbins 1971; Heywood 1995). Highest percentage of polyploids is recorded in perennial herbs and bamboo species. It is suggested that the rhizomatous perennial habit of bamboo drains the photosynthates to build up the vegetative biomass suppressing or postponing the event of flowering until the end of vegetative growth period as seen in many of the monocarpic plants including bananas and some palms. The period recorded for completion of vegetative growth before flowering is the longest in bamboo among all the angiosperms. However, flowering period also varies among different species from a few decades (*Dendrocalamus strictus*) to more than a century (*Fargesia nitida*). In polyploids, the sexual reproduction system is modified or
upset by involving apomictic and parthenocarpic tendencies substituting sexual reproduction by asexual means, as reported in most of grass species. Loss of fertility and seed production is another common phenomenon among polyploids, which is usually noticed in certain bamboo species. Chromosome segregation during mitosis or meiosis is not studied among bamboo species. The autopolyploids and allopolyploids are generally identified through cytological studies of metaphase chromosomes, which is yet to be studied in bamboo species. Majority of bamboo species are polyploids where as diploids are rarest of rare in bamboo, however, two diploid species each belonging to genus *Phyllostachys* and *Arundinaria* were reported from China by Hsu (1967, 1972).

### 2.2.1.1 Chromosome Number

In general, the somatic chromosome number of various bamboo species varies between 12-72. On the contrary, woody and herbaceous bamboo species differ in their basic chromosome numbers, as reported 11 chromosomes in case of herbaceous and 12 chromosomes for woody bamboo species (Gaut 2002; GPWG 2001). Further, cytological studies categorize woody bamboo species in two sections namely tropical and temperate woody bamboo species. All the tropical woody bamboo species are hexaploids with 72 chromosomes (2n= 6x= 72), while temperate woody bamboos with 48 chromosomes (2n= 4x= 48) have been classified as tetraploids (Clark et al. 1995; Ghorai and Sharma 1980; Kellogg and Watson 1993). Further, karyotypic studies revealed that the tropical bamboo species have smaller chromosomes whereas; chromosomes are very complicated in temperate bamboo (Kondo 1964).

#### 2.2.1.2 Genome size and Flow Cytometric studies

Flow Cytometry is a technique by which the DNA content of a nucleus can be estimated and therefore is a very important tool for estimation of genome size of a plant species. Genome size estimation inferences are helpful in studying evolutionary and adaptation mechanisms. Further, this information is pre-requisite for genome sequencing and genome analysis projects. However, only 1 % of angiosperms have been explored for DNA content estimations (Bennett and Leitch 1995). Moreover, such information is restricted to only few species of bamboo. Flow cytometric studies revealed genome size variation among temperate and tropical bamboo species which ranged from 2.04 Gb - 2.6 Gb in temperate and 1.14 Gb – 1.6 Gb in tropical bamboo species (Gielis et al. 1997). These inferences also suggest that polyploidy is the important driving force in the evolution of woody bamboos. Recently, two independent flow cytometric studies on 37
bamboo species (Kumar et al. 2011) and a tetraploid *Phyllostachys pubescens* (Gui et al. 2007) showed that genome size in different bamboo species ranges from 1.2 Gb to 2.9 Gb, which is slightly higher in range as compared to previous studies by Gielis et al. (1997). Further, these estimates revealed that the genome sizes in bamboo species are more than three to seven folds larger than the genome sizes of *Nipponbare* (*Japonica* rice) and 10–24 times larger than *Arabidopsis thaliana* genome size.

### 2.3 Bamboo utilization

"Their strength, lightness, smoothness, straightness, roundness, and hollowness, the facility and regularity with which they can be split, their many different sizes, the varying length of their joints, the ease with which they can be cut and with which holes can be made through them, their hardness outside, their freedom from any pronounced taste or smell, their great abundance, and the rapidity of their growth and increase, are all qualities which render them useful for a hundred different purposes, to serve with other materials which require much more labour and preparation, the bamboo is one of the most wonderful and most beautiful product of the tropics" (Wallace 1869). Various useful properties associated with this renewable bioresource makes bamboo as an exceptional commercial commodity for more than 1500 documented uses, ranging from simple domestic items such as various kinds of utensils, baskets, toys, storage drums etc. to highly sophisticated materials such as parts of aircrafts and medicines (Shukla and Das 1981; Liese 1985; Tewary 1992; Salam 2008). Many bamboo species are used as source of low quality timbers, for pulp and paper mills, scaffolding in construction purposes, food and fodder. Among the south-east Asian countries, India leads in the utilization of bamboo germplasm in paper manufacturing. About 2 million tons of raw bamboo (over 40 % of annual production) is utilized for making the pulp annually which fulfils 70 % need of the pulp utilized for paper manufacturing in India (Soderstrom and Calderon 1979).

Due to multiple properties such as smoothness, brightness, stability, high resistance, flexibility and insulation qualities, bamboo biosource is emerging globally as source of high quality flooring. Annual production of bamboo flooring in China was estimated around 17.5 million square metres in 2004. Bamboo charcoal with better calorific value and absorption capacity is becoming most simple, economic, popular and alternative energy source, other than woody charcoal (FAO 2005).
2.3.1 Edible bamboo species

There are 200 species of bamboo used worldwide for their food products. Due to higher fiber content, bamboo shoots are becoming popular source of various palatable products (Hunter 2003; Seethalakshami and Kumar 1998; Bhatt et al. 2003). The major edible bamboo species belongs to genus Phyllostachy, Bambusa and Dendrocalamus worldwide (Table 2.2).

Table 2.2: Details of globally most popular edible bamboo species

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidosasa</td>
<td>A. edulis</td>
</tr>
<tr>
<td>Bambusa</td>
<td>B. rigida, B. pervariabilis</td>
</tr>
<tr>
<td>Chimonobambusa</td>
<td>C. quadrangularis</td>
</tr>
<tr>
<td>Qiongzhuea</td>
<td>Q. tumidinoda</td>
</tr>
<tr>
<td>Phyllostachys</td>
<td>P. heterocycla var. pubescens, P. praecox, P. dulcis, P. iridescens, P. makinoi, P. muda, P. prominens, P. sulphurea cv. viridis, P. vivax,</td>
</tr>
<tr>
<td>Pleioblastus</td>
<td>P. amarus</td>
</tr>
<tr>
<td>Schizostachyum</td>
<td>S. funghomii</td>
</tr>
</tbody>
</table>

In India, Dendrocalamus hamiltonii, D. longispatus, D. brandisii, B. balcooa, B. polymorpha, B. pallida, M. baccifera, A. aristata, A. hirsuta, B. bambos, B. glaucescens, B. longispiculata, B. vulgaris, Cephalostachyum capitatum, C. fuchshianum, D. hookeri and Oxytenanthera albociliata are among the most popular edible bamboo species (Bhatt et al. 2004; Shanmughavel 2004).

2.3.1.1 Pharmaceutical applications

Edible bamboo species are also being harnessed for the preparation of medicines and flavouring commodities. Bamboo vinegar, a most common fermented bioproduct of edible bamboo is used for multiple purposes such as traditional medicine for stomach disorders, as biofertilizer and bioinsecticide (ERG 2003). Fermented shoots extracts with successive incubation of about 50-60 days can be used as flavouring materials for vegetables (Sharma and Borthakur 2008). Further, bamboo shoots are also rich in vitamins, cellulose, amino acids and trace elements and are shown to be effective against cancer (Tripathi 1998). Different types of leaf extracts of B. vulgaris are shown to be as effective anti-diabetic, with abortifacient potentials and hypotensive effects. (Senthilkumar 2011; Musa 2009; Nguessan Koffi 2009).

2.3.2 Bamboo Housing

Bamboo housing is another major area where bamboo with the aid of new technologies is being utilized for housing and schemes like “Global Bamboo Housing
Programme” were executed by International Network on Bamboo and Rattan (INBAR), National Mission on Bamboo Application (NMBA) and other collaborating companies at the world level for training the people. Other miscellaneous uses of bamboo include furniture, fuel, transport, packaging, ladders, staff, mats and other wood working industries. The consumption pattern of bamboo in India is shown in Table 2.3

Table 2.3: Consumption pattern of bamboos in India. Source: Tewary 1992

<table>
<thead>
<tr>
<th>Uses</th>
<th>Percentage consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulp</td>
<td>35.00</td>
</tr>
<tr>
<td>Housing</td>
<td>20.00</td>
</tr>
<tr>
<td>Non-residential</td>
<td>5.00</td>
</tr>
<tr>
<td>Rural uses</td>
<td>20.00</td>
</tr>
<tr>
<td>Fuel</td>
<td>8.50</td>
</tr>
<tr>
<td>Packing, including Baskets</td>
<td>5.00</td>
</tr>
<tr>
<td>Transport</td>
<td>1.50</td>
</tr>
<tr>
<td>Furniture</td>
<td>1.00</td>
</tr>
<tr>
<td>Other wood working industries</td>
<td>1.00</td>
</tr>
<tr>
<td>Others, including ladders, staff, mats etc.</td>
<td>3.00</td>
</tr>
</tbody>
</table>

2.4 Bamboo Economics and market statistics

Bamboo based products are first choice among various household items which have been well established in global market. While exact estimate of domestic as well as international bamboo trade is not available, due to lack in records of local consumption and problems in identification of composite products (Hunter 2003), different authors have given divergent estimations of bamboo trade in global market. According to Hunter (2003), the current estimated value of bamboo trade is somewhere between 2- 5 billion US Dollar, which is comparable to banana export in international market. Among the various exporters, China with 75 % - 90 % of the total export remains the major exporter (Hunter 2003; Parker 2005; VanderLugt 2005) while USA is the major importer. Xuhe (2003) concluded that bamboo industry contributes significantly in providing food, housing and livelihood to the 2.2 billion people across the world. Around half of the world population is involved in the use and trade of bamboo products. Being eco-friendly, bamboo based products are mounting tremendously in global market and are estimated approximately of worth 20 billion US Dollar by 2015, which is just double as compared to current figure of 10 billion US Dollar.

Bamboo is in high demand throughout Asia with a commercial value of at least US$7 billion per year (Stevens 1995). Bulkiness of bamboo culms makes its transportation
somewhat difficult to far flung areas therefore; the radius of economical transport is limited to the adjoining areas from centres of its production. Even after transportation difficulties, Taiwan alone exported bamboo products to more than 80 countries earning US$116 million in 1979 (Liese 1985).

In India, bamboo provides livelihood to many people. It generates 60–72 million workdays before primary processing and 120 million workdays for weaving works (Janssen 2000). As per the report of planning commission of India, the estimated size of Indian bamboo industry has grown up to rupees 2,040 crore in 2003. However, potential of domestic market was estimated to be rupees 4,463 crore. Therefore, there is a scope to increase the size of the bamboo industry by at least 2.2 times. Further, considering 15-20 % growth rate, the projected value of bamboo industry is of worth rupees 26,000 crore by 2015. According to Pacific Bamboo Resources, estimated size of the domestic bamboo industry is rupees 6,505 crores.

Table 2.4: Bamboo products and their value in India, statistics for the year 2005

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity (1000 tonnes)</th>
<th>Value (million US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood</td>
<td>14 615</td>
<td>409</td>
</tr>
<tr>
<td>Fuel wood</td>
<td>1 145</td>
<td>-</td>
</tr>
<tr>
<td>Bamboo shoots</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Utensils</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Other plant products</td>
<td>4</td>
<td>13</td>
</tr>
</tbody>
</table>

2.5 Bamboo Taxonomy

Peculiar flowering is the most prominent feature of majority of bamboo species. Due to its adaptation to wide range of environments, the internast period of different bamboo species ranged from 3 -120 years (Brandis 1899; Janzen 1976; Jeffrey 1995; Tewari 1992). Many of the bamboo species are monocarpic having long vegetative cycles and their reproductive phase varies from a decade to even a century (Janzen 1976; Cambell 1985; Dransfield and Widjaja 1995). These species are often recorded with an onset of synchronized reproductive phase. The species flower simultaneously and die in a larger area (Taylor and Quin 1988; Makita 1992; Dwivedi 1990). On the other hand, few polycarpic bamboo species which show sporadic flowering with comparatively shorter flowering intervals were also recorded (Janzen 1976; Soderstrom and Caldeeron 1979; Campbell 1985). Owing to unusual flowering behaviour, bamboo species posed many problems in their nomenclature. Consequently, existing taxonomical classification of
bamboo is largely based on complex and limited vegetative characters such as culm sheath, ligule, branching pattern and stem characters, which are prone to vary with changing environmental conditions (Wu 1962). Therefore, classification of bamboo, solely based on vegetative characteristics remained inconsistent. *Bonia*, primarily classified as an independent genus by Balansa, was categorized as a synonym of *Bambusa* by Baillon and eventually considered as subgenus of *Bambusa* by Camus (Ye Sun *et al.* 2006). However, both floral and vegetative characteristics are also used in combination for correcting inferences of bamboo classification, which otherwise is solely based on vegetative characteristics (Holltum 1956; Gilliland 1971; Tewari 1992). With the advent of molecular markers in the year 1980, Inter-Transcribed Sequence (ITS) data is being routinely used for phylogenetic studies in bamboo. An ITS based phylogenetic study in *Thamnocalamus* and its allies groups revealed inconsistency of bamboo classification which was based on morphological parameters (Gua *et al.* 2002).

Owing to peculiar flowering habit, bamboo classification has always remained extremely challenging. Taxonomists however proposed various classification systems that eventually helped bamboo researchers in establishing genetic relationship of different bamboo species. For the first time, such a bamboo classification was proposed by Rumphinus (1750) in his publication titled “Herbarium Ambionense”. After more than three decades, Schreber (1789) described *Bambusa arundinacea* from India. Blanco (1837) gave description of some bamboo species and based on vegetative characteristics classified them under a common genus *Bambusa*. Soon after, Ruprecht (1839) published a monograph on bamboo with complete description of 18 species from Indo-Malayan region. Later on, based on flowering parts and fruit structures, this monograph was extensively strengthened by Munro (1868) with inclusion of 170 species representing 21 genera. It was in the year 1876, when Kurz for the first time recognized the importance of vegetative characters to study taxonomy of living bamboo specimens in the field; however he did not give any formal classification. Bentham (1883) followed Munro’s criteria for bamboo classification and described 18 genera from Indo-Malayan region. Further, based on Kurz's recommendations, Gamble in 1896 developed a comprehensive classification system and categorized 115 species in 15 different genera. Gamble's classification system remained a fundamental framework in Indian Bamboo systematics. Stapf (1897) classified bamboo species into five subtribes, while Hooker divided bamboo species in four subtribes later in 1897. In 20th century, Camus (1913) described bamboo species belonging to Indo-China region in his book “Les Bambusees”. His classification broadly followed the criteria
proposed by Munro and Gamble and he described 490 species representing 33 genera. Based on floral and vegetative characters, Brandis (1921) has given a description of 14 bamboo genera; which was jointly strengthened by the efforts of two popular taxonomists namely Backer (1924) and Holttum (1956) and they added some additional description of bamboo species from various regions. McClure (1961), for the first time described woody bamboo species, this work was later on strengthened by adding the herbaceous members by Parodi. Soderstrom and Ellis (1987) classified all the bamboo species into 11 tribes under subfamily Bambusodeae. Of these, 5 tribes were considered as monophyletic and identified as ‘core’ Bambusodeae while other remaining six were recognized as ‘peripheral’ tribes. Later on, Clayton and Renvoize (1986) and Renvoize and Clayton (1992), grouped ‘core’ and ‘peripheral’ tribes together. Two research groups namely Kellog & Campbell (1987) and Kellog & Watson (1993) classified bamboo species as monophyletic or polyphyletic and also revised the work of Soderstrom and Ellis (1987). Dransfield and Widjaja (1995) in their classification described 69 woody bamboo genera.

Stapleton (1997) based on floral and vegetative characters, extended the Dransfield and Widjaja's efforts and gave an account of 78 woody bamboo genera. In the beginning of 21st century, Grass Phylogeny Working Group (GPWS 2001) attempted a most extensive effort in which representative members of all the bamboo research groups around the globe worked together to establish phylogenetic relationships in grass family. Based on vegetative and molecular studies, grass family Poaceae is classified as monophyletic family in which Bambusoideae formed a clade with Pooideae and Ehrhartioideae. The Bamboo Phylogeny Working group (BPWG 2001), concluded that Bambusoideae is monophyletic and bamboo species are not the most primitive grasses as speculated by many earlier workers.

2.6 Molecular markers development and applications

The advent of molecular marker techniques provided scientific community with the tools for genetic analysis of the genomes without sequencing and has led to a great advancement in the knowledge of structural and functional genomics of various plant genomes. DNA markers, which reveal variable sites in DNA are the most widely used marker types predominantly due to their abundance, precision and reproducibility irrespective to changing environment and the developmental stage of the plant (Jones et al. 1997; Winter and Kahl 1995). These variations arise from different types of mutations at the DNA level, which include point mutations, insertions or deletions and errors in replication of tandemly repeated DNA regions (Paterson 1996). Considering multiple
advantages, molecular markers are preferred as compared to morphological and biochemical markers (Winter and Kahl 1995) and are widely used for evaluation of genetic diversity, construction of linkage maps, cultivar identification, quantitative trait loci (QTLs) analysis and many other purposes in molecular breeding and conservation studies (Baird et al. 1997; Henry 1997; Jahufer et al. 2003; Weising et al. 1995; Winter and Kahl 1995). Restriction Fragment Length Polymorphism (RFLP), a hybridization based DNA marker, was the first DNA marker technology developed simultaneously with the advent of recombinant DNA technology. RFLP difference between samples of homologous DNA molecules is the result of difference in locations of restriction enzyme sites (Jeffrey et al. 1985). Polymerase Chain Reaction (PCR), developed by Kary Mullis (1983) revolutionized the various medical and biological researches as it was suitable for a variety of applications including identification of genetic variations among the different DNA samples. Random Amplified Polymorphic DNA (RAPD) is based on amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence and is among the first PCR based techniques (William et al. 1990). However, due to low stringency in PCR reactions coupled with short primers length (8-10 bp), RAPD has often led to non-reproducibility in repeated experiments. However, later on limitations of RAPD have been taken care of by development of molecular marker technique named Amplified Fragment Length Polymorphism (AFLP) by Vos et al. (1995). AFLPs are differences in restriction fragment lengths caused by SNPs or INDELs that create or abolish restriction endonuclease recognition sites. This technique generates genome wide marker data and uses advantage of PCR for speed and recombinant DNA technology for genome wide coverage. Microsatellites, also known as Simple Sequence Repeats (SSRs) or short tandem repeats (STRs), are tandemly repeated sequences of 2-6 base pairs of DNA. Primers designed flanking to these repeated regions represent one of the best co-dominant marker systems and are exploited in genome diversity, genome mapping and conservation studies in crops. Bi-allelic single nucleotide polymorphisms (SNPs) are among the most abundant marker systems and utilized in diversity, phylogenetic and genome wide association studies in many crops (Kump et al. 2011; Kilian and Graner 2012; Jones et al. 1997; Joshi et al. 1999; Winter and Kahl 1995). An account of various molecular marker techniques is given in Table 2.5. DNA markers are particularly useful if they reveal differences between individuals of the same or different species. These markers are called polymorphic markers, whereas markers that do not discriminate between genotypes are called monomorphic markers.
<table>
<thead>
<tr>
<th>Marker system</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First-generation markers (Based on recombinant Technology)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restriction fragment length polymorphism (RFLP)</td>
<td>Co-dominant; highly reproducible</td>
<td>Low multiplex ratio*, high on time/labour</td>
</tr>
<tr>
<td><strong>Second-generation markers (Based on PCR Technology)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleavage amplification polymorphism (CAP)</td>
<td>No requirement for radioactivity, Produces</td>
<td>Insensitive to DNA methylation</td>
</tr>
<tr>
<td></td>
<td>informative PCR products</td>
<td></td>
</tr>
<tr>
<td>Random amplified polymorphic DNA (RAPD).</td>
<td>Low on time/labour; medium multiplex ratio*</td>
<td>Dominant; low reproducibility</td>
</tr>
<tr>
<td>Amplified fragment length polymorphism (AFLP)</td>
<td>High reproducibility; high multiplex ratio*</td>
<td>Dominant; moderate time/labour</td>
</tr>
<tr>
<td>Sequence-specific amplification polymorphism (S-SAP)</td>
<td>Applicable for targeting any gene, transposon</td>
<td>Sequence must be known to enable design of element</td>
</tr>
<tr>
<td></td>
<td>or sequence of interest</td>
<td>specific PCR primers</td>
</tr>
<tr>
<td>Simple sequence repeat (microsatellite) (SSR)</td>
<td>Co-dominant; highly reproducible; low on time</td>
<td>High cost of development; low multiplex ratio*</td>
</tr>
<tr>
<td></td>
<td>and labour</td>
<td></td>
</tr>
<tr>
<td>Inter-simple sequence repeat (ISSR)</td>
<td>Technically simple; no prior genomic information</td>
<td>Dominant markers; band staining can be weak</td>
</tr>
<tr>
<td></td>
<td>needed to reveal both inter- and intraspecific</td>
<td></td>
</tr>
<tr>
<td></td>
<td>variation</td>
<td></td>
</tr>
<tr>
<td>Variable number tandem repeat (minisatellite) (VNTR)</td>
<td>Numerous multiallelic loci</td>
<td>Low-resolution fingerprints in plants</td>
</tr>
<tr>
<td>Sequence tagged sites (STS)</td>
<td>Co-dominant; useful for mapping</td>
<td>Reproducibility; based on some degree of sequence</td>
</tr>
<tr>
<td>Sequence characterised amplification region (SCAR)</td>
<td>May be dominant or co-dominant; better</td>
<td>More difficult to reproduce than</td>
</tr>
<tr>
<td>Method</td>
<td>Advantages</td>
<td>Disadvantages</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Sequence amplification of microsatellite polymorphic loci (SAMPL)</td>
<td>High multiplexing*; co-dominant markers; extensive polymorphism</td>
<td>Some blurred banding; stutter bands</td>
</tr>
<tr>
<td><strong>Third-generation markers based on DNA sequencing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single nucleotide polymorphism (SNP)</td>
<td>Common; evenly distributed; detection easily automated; high throughput; low assay cost; useful for association studies; potentially high multiplex ratio*</td>
<td>Usually only two alleles present</td>
</tr>
<tr>
<td><strong>Genome scanning for expressed genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expressed sequence tag (EST)</td>
<td>Easy to collect and sequence; reveals novel transcripts; good representation of transcripts</td>
<td>Error-prone; isolation of mRNA may be difficult</td>
</tr>
<tr>
<td>Sequence-related amplified polymorphism (SRAP)</td>
<td>Simplicity; high throughput; numerous co-dominant markers; high reproducibility; targets coding sequences; detects multiple loci without previous knowledge of sequence information; PCR products directly sequenced.</td>
<td>Detects co-dominant and dominant markers, which can lead to complexity; null alleles detected directly</td>
</tr>
<tr>
<td>Target recognition amplification protocol (TRAP)</td>
<td>Simple to use; highly informative; produces numerous markers by using existing public EST databases; uses markers targeted to a specific gene</td>
<td>Requires cDNA or EST sequence information for primer development</td>
</tr>
<tr>
<td><strong>Markers using array technology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microarrays (arrangements of small spots of DNA fixed to glass slides)</td>
<td>Whole-genome scanning; high-throughput technology; genotype–phenotype relationship; expression analysis of large numbers of genes.</td>
<td>Expensive; needs gene sequence data; technically demanding.</td>
</tr>
<tr>
<td>Diversity array technology (DArT)</td>
<td>No sequence data required; high Dominant markers; technically</td>
<td></td>
</tr>
</tbody>
</table>
throughput; detects single base changes and indels; rapid germplasm characterization. 

<table>
<thead>
<tr>
<th>Other marker systems</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-strand conformational polymorphism (SSCP)</td>
<td>Detects DNA polymorphisms and mutations at multiple sites in DNA fragments.</td>
<td>Temperature-dependent; sensitivity affected by pH.</td>
</tr>
<tr>
<td>Denaturing gradient gel electrophoresis (DGGE)</td>
<td>Separates individual sequences from a complex mixture of microbes based on sequence differences.</td>
<td>PCR fragment size limited to about 500 bp; difficult to resolve fragments that differ by only one or two bases.</td>
</tr>
<tr>
<td>Temperature gradient gel electrophoresis (TGGE) Methylation-sensitive PCR</td>
<td>Almost identical to DGGE; more reliable; uses temperature gradient. Detects sites of methylated DNA.</td>
<td>Technically demanding; little used in plants.</td>
</tr>
</tbody>
</table>

*The multiplex ratio is the number of independent loci detected in the assay.*
Insights into bamboo genome began with the use of RFLP marker technique by Friar and Kochert (1991) who investigated genetic variability and phylogenetic relationships among different *Phyllostachys* species (Friar and Kochert 1991; Friar and Kochert 1994). While India has largest bamboo forest cover in the world, except few preliminary studies, reports on the species wise genetic diversity and phylogenetic studies are largely non-existing in Indian bamboo.

### 2.6.1 Genetic Diversity and Phylogenetic studies

Assessment of genetic diversity of the germplasm of any crop is a prerequisite for the effective and successful crop improvement programmes, conservation and management strategies (Sui *et al.* 2008). In addition, genetic diversity within different populations of a particular species is the main building block for understanding evolutionary and speciation aspects of that species. However, both the genetic and species divergence have yet to be well understood among different species of bamboo. Although, germplasm collections of few bamboo species have been explored for genetic diversity evaluation and phylogenetic studies, but these are handful considering more than 1200 different bamboo species across the globe. Among different workers, Friar and Kochart (1991, 1994) were pioneers and utilized RFLP markers for studying genetic variation and evolutionary aspects in *Phyllostachys*. In the same year, chloroplast DNA based phylogenetic study of nineteen Asian bamboo species was explored by Kurita *et al.* (1994) and confirmed Potztal's (1964) classification and revealed monophyly and paraphyly in Arundinarieae and bambuseae, respectively. Monophyletic evolutionary pattern among new and old world woody bamboos was also supported in chloroplast *rpl16* intron studied by Kelchner and Clark (1997). Simultaneously, Geillis *et al.* (1997) has explored the utility of RAPD markers for establishing genetic relationships in *Phyllostachys*. In a combined approach using nuclear ITS sequences and AFLP markers in *Phyllostachys*, Hodkinson *et al.* (2000) revealed discrepancies in the taxonomic classification earlier proposed by Wang *et al.* (1980). Considering significance of ITS sequences, these markers were also utilized efficiently in genetic diversity and phylogenetic studies in bamboo by Guo *et al.* (2001, 2002). A wide range of genetic variation among fifteen bamboo species was also observed in an AFLP study by Loh *et al.* (2000). Wide range of genetic diversity within *Dendrocalamus* and polyphyletic origin of genus *Bambusa* was among the major findings of their study. Since then, there are number of reports on phylogenetic and genetic diversity studies in bamboo with various markers namely RAPD (Nayak *et al.* 2003; Biradar *et al.* 2005; Das *et al.* 2005), AFLP (Marulanda 2000; Suyama *et al.* 2000; Huh and Huh
2002; Isagi et al. 2004), transferred SSR or microsatellite markers (Barkley et al. 2005; Sharma et al. 2008; Chen et al. 2010) and Inter Simple Sequence Repeats (ISSR) markers (Lin et al. 2010). There are few more studies in which molecular markers were used in bamboos for the estimation of genetic diversity. Rammayake (2007) has explored nine different bamboo species for genetic diversity and interrelations between them through RAPD in SriLankan Bamboo germplasm. Moreover, in some recent studies ISSR markers were employed to investigate population genetic structure of bamboo species (Tian et al. 2011; Yang et al. 2012). Considering, limited bamboo genomic resources, successful attempts were made for isolation of genic and genomic SSR markers in commercially important bamboo species *Dendrocalamus hamiltonii*, in this study. These markers recorded a high level of cross-transferability in different bamboo species. An account of various molecular markers used in genetic diversity and phylogenetic studies of bamboo species is given in Table 2.6.

**Table 2.6: Status of genetic diversity and phylogenetic studies in bamboo**

<table>
<thead>
<tr>
<th>Species studied</th>
<th>Markers Used</th>
<th>Inferences</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twenty four bamboo species</td>
<td>RFLP</td>
<td>High degree of RFLP variability was detected in bamboo nuclear DNA.</td>
<td>Friar and Kochert (1991)</td>
</tr>
<tr>
<td>Twenty one bamboo species</td>
<td>RFLP</td>
<td>Within species and between species RFLP variability was detected which was useful for establishing relationship in between these species.</td>
<td>Friar and Kochert (1994)</td>
</tr>
<tr>
<td><em>Yushania niitakayamensis</em></td>
<td>RAPD</td>
<td><em>Yushania</em> population were found highly diverse genetically.</td>
<td>Hsiao and Rieseberg (1994)</td>
</tr>
<tr>
<td>Nineteen Bamboo species</td>
<td>RFLP</td>
<td>Study supports the monophyly of Arundinariaeinae in potztals (1964) classification and bambuseae sensu Potztal is suggested as paraphyletic.</td>
<td>Kurita et al. (1994)</td>
</tr>
<tr>
<td>Twenty-three species of <em>Chusquea aurea</em></td>
<td>chloroplast rpl16 intron sequencing</td>
<td>The <em>rpl16</em> intron is most applicable as a phylogenetic tool at the intergeneric level in bamboos.</td>
<td>Kelehner and Clark (1997)</td>
</tr>
<tr>
<td>Fifteen species of bamboo <em>Sasa senanensis</em></td>
<td>AFLP</td>
<td>The wide range of variation within <em>Dendrocalamus</em> the genus <em>Bambusa</em> is polyphyletic.</td>
<td>Loh et al. (2000)</td>
</tr>
<tr>
<td><em>Phyllostachys</em></td>
<td>AFLP</td>
<td>The genotypic diversity and evenness values in <em>S. senanensis</em> were higher than the average values of clonal plants and the <em>S. senanensis</em> population consists of at least 22 clones.</td>
<td>Suyama et al. (2000)</td>
</tr>
<tr>
<td>Twenty-three species of alpine bamboos</td>
<td>AFLP and Ribosomal ITS region ITS5 and ITS4 primers sequencing</td>
<td>This molecular analysis indicated that the taxonomic treatment of Wang <em>et al.</em> (1980) needs revision.</td>
<td>Hodkinson <em>et al.</em> (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The divergence in the ITS region within alpine bamboos ranged from 0 to 4.2%. Except few, all studied alpine bamboos were resolved as a monophyletic clade in the 50% majority rule tree.</td>
<td>Guo <em>et al.</em> (2001)</td>
</tr>
<tr>
<td>Bamboo Species</td>
<td>Methodology</td>
<td>Results</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Thiry three bamboo species</td>
<td>ITS5 and ITS4 region sequencing</td>
<td>Results indicate that re-evaluation of relationships within <em>Thamnocalamus</em> group is necessary.</td>
<td>Guo et al. (2002)</td>
</tr>
<tr>
<td><em>Guadua angustifolia</em></td>
<td>AFLP</td>
<td>A clear genetic differentiation was seen between different species of the <em>Guadua</em> genus.</td>
<td>Marulanda et al. (2002)</td>
</tr>
<tr>
<td><em>Pseudosasa japonica</em></td>
<td>Enzyme electrophoresis</td>
<td>Given limited gene flow, reduced populations are expected to diverge genetically due to drift and the random loss of alleles due to sporadic cutting.</td>
<td>Huh and Huh (2002)</td>
</tr>
<tr>
<td>Twelve bamboo species</td>
<td>RAPD</td>
<td>Relatively large number of polymorphisms obtained seems due to large phylogenetic distance among taxa.</td>
<td>Nayak et al. (2003)</td>
</tr>
<tr>
<td><em>Phyllostachys pubescens</em></td>
<td>AFLP</td>
<td>AFLP analysis of DNA samples showed distinct genets that originated from the previous flowering event and that each genet had its own flowering time.</td>
<td>Isagi et al. (2004)</td>
</tr>
<tr>
<td><em>Bambusa group</em></td>
<td>ITS Sequences</td>
<td>Species of <em>Dendrocalamus</em> were closely related to and nested in a polyphyletic <em>Bambusa</em>.</td>
<td>Sun et al. (2005)</td>
</tr>
<tr>
<td>Forty four bamboo species</td>
<td>Transferred EST-SSRs</td>
<td>These transferred EST-SSR markers were successful in differentiating the various bamboo accessions and determining the level of genetic variation within and between species and genera.</td>
<td>Barkley et al. (2005)</td>
</tr>
<tr>
<td><em>Bambusa arundinacea</em></td>
<td>Genomic SSRs</td>
<td>Three polymorphic microsatellite loci in <em>B. arundinacea</em>, have been identified and characterized for the first time (to our knowledge) in bamboo.</td>
<td>Nayak and Rout (2005)</td>
</tr>
<tr>
<td>Eleven bamboo clones</td>
<td>RAPD markers</td>
<td>Cluster analysis grouped eleven clones of each species into three major groups.</td>
<td>Biradar et al. (2005)</td>
</tr>
<tr>
<td>Fifteen bamboo species</td>
<td>RAPD</td>
<td>Findings suggest that traditional classifications of woody bamboos, often based largely on floral characteristics that may be homoplasious, require in-depth assessment.</td>
<td>Sun et al. (2006)</td>
</tr>
<tr>
<td>Fifteen bamboo species</td>
<td>RAPD markers and phenotypic descriptors</td>
<td>Phylogenetic relationships amongst the 15 bamboo species revealed by the allelic polymorphism data is reasonably in concurrence with the taxonomic classification of Gamble (1896), while the cluster pattern obtained from the key morphological descriptors is not fully in agreement.</td>
<td>Das et al. (2007)</td>
</tr>
<tr>
<td>Nine bamboo species</td>
<td>RAPD</td>
<td>The nine bamboo species were polymorphic and could be distinguished from each other by their RAPD band patterns.</td>
<td>Ramanayake et al. (2007)</td>
</tr>
<tr>
<td>Twenty six taxa from Poaceae family</td>
<td>SSRs</td>
<td>The phylogeny of FT homologs does not resolve monophyly in <em>Bambusoidae</em> because of intercalary positioning by <em>Streptogyneae</em> clade.</td>
<td>Himamoto et al. (2008)</td>
</tr>
<tr>
<td>Twenty three bamboo species</td>
<td>SSRs</td>
<td>Phylogenetic relationship shown between bamboo species was in concordance of existing classification.</td>
<td>Sharma et al. (2008)</td>
</tr>
<tr>
<td><em>Thamnocalamus spathiflorus</em></td>
<td>RAPD markers</td>
<td>Low genetic variability detected between populations and within populations.</td>
<td>Bhattacharya et al. (2009)</td>
</tr>
<tr>
<td>Sixty-four species</td>
<td>multi gene region amplifications</td>
<td>Tribe <em>Bambuseae</em>, the woody bamboos, as currently recognized were not monophyletic because <em>Olyreae</em>, the herbaceous bamboos, were sister to tropical <em>Bambuseae</em>.</td>
<td>Sungkaew et al. (2009)</td>
</tr>
<tr>
<td><em>Phyllostachys pubescens</em></td>
<td>AFLP and ISSR markers</td>
<td>AFLP and ISSR markers could clearly genetically identified ten cultivars of <em>P. pubescens</em>.</td>
<td>Lin et al. (2009)</td>
</tr>
<tr>
<td>Twenty species of bamboo</td>
<td>ISSR and Cross-transferred</td>
<td>Low level of Genetic diversity was detected in all species of <em>Dendocalamus</em> except <em>D. strictus</em>. Genus <em>Bambusa</em> is polyphyletic.</td>
<td>Mukherjee et al. (2010)</td>
</tr>
</tbody>
</table>
Fifteen species from subtribe Bambusinae

Nine bamboo genera

AFLP

The study did not find support for the various earlier infrageneric classifications within *Dendrocalamus*.

Pattanaik and Hall (2010)

Starch synthase I (GBSSI) genes

The climbing Southeast Asian genera, all of which include species previously placed in *Bambusa*, are distinct from the “core Bambusa group.” Results do not support the present subgeneric classification.

Goh *et al.* (2010)

Twenty one bamboo species

SSRs

The dendrogram divided Bamboo species into two major groups on the basis of rhizome types.

Chen *et al.* (2010)

*Phyllostachys pubescens*

EST-SSRs

Characterized were showing limited polymorphism in *Phyllostachys pubescens* cultivars and one marker recommended as species specific.

Tang *et al.* (2010)

*Phyllostachys violascens*

ISSR, SRAP and AFLP Markers

The mean genetic similarity of *Phyllostachys violascens* was 0.872, 0.867 or 0.871 for the ISSR, SRAP and AFLP analyses, respectively.

Lin *et al.* (2011)

Six bamboo species

Chloroplast Genome Sequencing

Phylogenomics based on whole cpgenome could be used to resolve major relationships within the subfamily. Study suggests that three clades of temperate woody bamboos may have diverged very rapidly.

Zhang *et al.* (2011)

*Dendrocalamus giganteus*

ISSR

Genetic diversity within populations was relatively low while diversity was considerably higher among populations.

Tian *et al.* (2011)

3406 ESTs

EST-SSRs

The transferred markers showed 51.4% polymorphism. From the characterized markers, two markers were able to identify inter-species hybrids of bamboo species.

Dong *et al.* (2011)

*Bambusa edulis* and *B. oldhamii*

ISSR

Large proportion of the genetic variation (78.95%) resides among the individuals within populations. Only 21.05% are found among populations.

Yang *et al.* (2012)

2.7 Transposons in Bamboo

The fact that transposons comprise a significant fraction of animals and plant genomes was established during the mid of 20th century. Somatic mutations in bamboo are highly valued in horticulture and are an interesting research aspect for basic studies. The huge variation in stem color and stem morphology are among the common phenomenon in bamboo. There is some reason to believe that these somatic mutations are caused by the transposition events (Okamura 1986). It has been assumed that there are a large number of different transposons present in the bamboo genome. Gielis *et al.* (1997) utilized primers based on sequence information from the 4.5 kb Ac9 transposons from maize and showed that several copies with considerable homology to the original Ac9 transposons were present in bamboo and also successfully used transposon sequences to distinguish bamboo species at the species level (Gielis and Sormann 1997). Nucleotide sequencing of hATbv1 transposon fragments from *Bambusa vulgaris* has shown homology to members of the hAT transposons superfamily which also includes Ac (corn), Tam3 (*Antirrhinum majus*) and hobo
(Drosophila) transposons. The homology was as high as 60% in some regions, which also comply with earlier findings of Ac-like sequences in Bambusa multiplex (Huttley et al. 1995). Further investigations made by Gui et al. (2007) in Phyllostachys pubecens using the 1000 Genomic Survey Sequences (GSS) showed that the bamboo repeat elements were mainly Gypsy/DIRS1 and Ty1/Copia LTR retrotransposons (14.7%), with a few DNA transposons. The cDNA sequence annotation by Peng et al. (2010) also showed the presence of both transposes class-I DNA elements and class-II RNA elements. The widespread distribution and polymorphism of Mariner-like elements (class II transposons) across Bambusoideae sub family was explored by Zhou et al. (2010). They characterized 82 Mariner-like elements in 44 bamboo species and also isolated 79 transposase genes from 63 bamboo species (Zhou et al. 2011a; Zhou et al. 2011b). They further concluded that Ty3-gypsy and Ty1-copia are the two most abundant families of transposons in Phyllostachys pubescens. Recently, while mining of expressed sequence data of D. latiflorus for marker discovery, our group revealed a significant association of both type of transposons with SSR sequences (under communication). Of the 23 transposons detected in 18 unigenes, 13 were class I and 10 were of class II. Further, Gypsy and EnSpm types were predominant among Class I and Class II, respectively. It is evident that many of such types of transposons are expected to occur in bamboo genome which needs to be explored. Since transposons are known to act on the genome in quite a number of different ways (Wessler 1998), the extensive survey and characterization of these elements may unravel the interesting facts about bamboo genome.

2.8 Chloroplast and Mitochondrial DNA sequences

Both chloroplast (cp) and mitochondria (mt) having their own genome and protein-synthesizing machinery, together form the power house of the cell and their genomes are inherited independently of the nuclear genome (Olmstead et al. 1994; Martin et al. 1998; Qiu et al. 2010). Comparative analysis indicates that their gene content and order of genes are highly conserved therefore the cp and mt DNA sequences are often used as ideal experimental tools for investigating phylogenetic and evolutionary relationships in plants (Palmer 1985; Jansen et al. 2005; Qiu et al. 2010). In the beginning, chloroplast DNA restriction site polymorphism were successfully utilized to establish phylogenetic relationships among 31 grass taxa from selected six subfamilies of Poaceae and reported two main clades namely Pooidse and PACC (Panicoideae, Arundinoideae, Chloridoideae and Centothecoideae) that also include woody bamboo Bambusoideae (Davis and Soreng 1993).
DNA sequencing of rDNA subunits (18S & 28S) and selected cp and mt gene(s) have become a routine practice for establishing phylogenetic relationships among different bamboo species (Doebly et al. 1990; Hamby and Zimmer 1998; Backer et al. 1995; Duvall and Morton 1996; Liang and Hilu 1996; Gaut et al. 1997). The Grass Phylogeny Working Group (2001) utilized three chloroplast and three nuclear sequences to establish the comprehensive phylogeny between various species of grass family. Further, to understand relationships among the members of Poaceae, for the first time, complete chloroplast DNA sequences of two of bamboo species namely Dendrocalamus latiflorus and Bambusa oldhamii was generated by Wu et al. (2009). There was no significant difference in the nucleotide length and both chloroplast genomes were of 139365 bp and 139350 bp respectively. Further, to establish phylogeny, six bamboo species were sequenced on Illumina platform (Zhang et al. 2011). The nucleotide sequences of these six species ranged from 139493 bp in Bambusa emeiensis to 139839 bp in Phyllostachys nigra var. henonis. The other four species included in this study were Acidosasa purpurea, Ferrocalamus rimosivaginus, Indocalamus longiauritus and Phyllostachys edulis. The chloroplast sequence data on different platform revealed that organization and gene order of these bamboo species were similar to other grass species. Chloroplast genome sequence data generated through the different sequencing techniques has provided useful insight about bamboo phylogeny and evolution and can be utilized in comparative genomic studies. More recently the mitochondrial genome sequencing of Ferrocalamus rimosivaginus further added additional 432839 bp sequence data to existing genomic resources of bamboo (Ma et al. 2012).

2.9 DNA sequencing and Gene discovery

Advent of next generation sequencing (NGS) technologies has revolutionized the genome sequencing, marker development and plant genotyping efforts (Elshire et al. 2011). NGS technologies have significantly enhanced the pace of DNA sequencing and a large numbers of plant genomes are now available with novel answers to the biological questions. Considering global food safety priorities, such efforts are biased to crop plants. Interestingly, larger section of grass family possesses crop plants. Owing to rapid increase in genome sequence data along with expression data in the crop plants, the grass family, Poaceae becomes an ideal system for comparative studies of genes and genomes (Gaut 2001; Paterson et al. 2009). Although, the bamboo subfamily of Bambusoideae having more than 1500 species, efforts towards the development of genomic resources for all major lineages in the
grasses of the grass family are still in progress and only limited information is available on DNA and protein sequence data (Das et al. 2008; Buell 2009).

Gene discovery efforts in bamboo have started with an aim to dissect its complex flowering mechanism. Bo et al. (2005) performed cloning and expression studies and isolated a cDNA named *DiMADS8*. It was isolated from the young spikelets of the sweet bamboo, *Dendrocalamus latiflorus*. Phylogenetic analysis of plant MADS box genes based on amino acid sequences revealed that *DiMADS8* was grouped into the AGAMOUS-LIKE 6 (AGL6)-like subfamily. It was most likely homologous to the OsMADS6 of rice (*Oryza sativa*), with 88% sequence identity for the entire amino acid sequences and also revealed high amino acid sequence identity (59%) to AGL6 of *Arabidopsis thaliana*. Based on preliminary studies, possible involvement of *DiMADS8* in controlling the flowering time of *D. latiflorus* was indicated. Lin et al. (2006) examined chloroplast genome of an albino mutant isolated from tissue culture of a bamboo species named *Bambusa edulis* Munro to identify aberrations. Comparative 2D electrophoresis study concluded that the repression of protein-expressing *BePsbO* and *BePsbP* is because of a defect in post-transcriptional regulation in the albino mutant. Further, to reveal the complexity in flowering behaviour, a flowering gene namely *FLOWERING LOCUS T* (*FT*) was investigated (Hisamoto 2008). Around 1000 (0.92 Mb) genomic survey sequences (GSS) generated in *Phyllostachys pubescens* by Gui et al. (2007) and their blast hits showed presence of 427 different reported genes. Lin et al. (2009) cloned two novel genes (*PpMADS1* and *PpMADS2*) from *Phyllostachys praecox* and defined that these domain belong to FUL3 and FUL1 clade of Poaceae AP1/SQUA-like genes, respectively. Functional role of these gene domains in flowering have been earlier confirmed in Arabidopsis. RT-qPCR and in situ hybridization analysis revealed distinct expression patterns of these two genes in vegetative and reproductive tissues of bamboo and confirmed their possible role in floral development of *Phyllostachys praecox*. In a breakthrough study, Peng et al. (2010) cloned and sequenced more than ten thousand putative unique FL-cDNA derived primarily from vegetative tissues of Moso bamboo, *Phyllostachys heterocyclos* cv. *Pubescens* and concluded that bamboo diverged from its close relatives through an adaptive radiation. They also found that comparative analysis of the lignin biosynthesis pathway between bamboo and rice suggested that genes encoding caffeoyl-CoA O-methyltransferase may serve as targets for genetic manipulation of lignin content to reduce pollutants generated from bamboo pulping. Wang et al. (2010) cloned six genes related to the development of the bamboo rhizome and concluded up-regulation of fifty two genes which contributed to bamboo rhizome bud development. Xu et al. (2010) isolated another flowering related gene
from bamboo species _Dendrocalamus latiflorus_. Lin et al. (2010) through cDNA sequencing generated 3878 and 4470 EST data from vegetative shoot and flower bud, respectively. Further, all random ESTs were assembled into 6315 unigenes with various annotated functions. Peng et al. (2011) through nucleotide sequencing of young leaves cDNA libraries of _Dendrocalamus latiflorus_ produced 9,574 high-quality ESTs, from which 5,317 unigenes (1,502 contigs and 3,815 singletons) were assembled to further enrich the EST resources of bamboo and functional annotation suggests their involvement in biosynthetic pathways of secondary metabolites and disease resistance. A batch of 1.2 Mb of genomic sequence data was produced from nucleotide sequencing of 13 bacterial artificial chromosome clones (Gui et al. 2010). Functional annotations predicted 112 non-transposable elements related protein-coding genes. Of these, four resistance gene homologs (RGH or RGA), one putative alcohol dehydrogenase (Adh) gene which revealed significant homology with rice Adh3 and two pollen-specific kinase genes, which provide clues of syntenic relationships of bamboo and rice. Zhou et al. (2011) isolated and characterized 79 full length transposase genes from sixty three bamboo species. Considering the fact that formation of the woody stems of bamboo requires the coordinated regulation of cellulose, xylan and lignin biosynthesis, cloning and partial characterization of R2R3MYB transcription factor gene (FfMYB1) from the bamboo species _Fargesia fungosa_ was conducted by Wang et al. (2012). FfMYB1 consists of a coding region of 813 bp and has highest percent amino acid identity to _NtMYBGR1_ of tobacco and both _AtMYB20_ and _AtMYB43_ of Arabidopsis, a putative activator of the phenylpropanoid pathway for lignin production. A phylogenetic study of _R2R3MYB_ sequences available for the bamboo subfamily identified other potential lignin-related _R2R3MYBs_, in particular _bphylf044c24_ of the bamboo genera Phyllostachys. More recently, two independent efforts were made for enriching genomic resources of Ma bamboo (_D. latiflorus_) through _de novo_ transcriptome sequencing to unravel the growth and development phenomenon (Liu et al. 2012) and complex floral mechanism (Zheng et al. 2012). Transcriptome sequencing of tissues from different growth and developmental stages was done by dissecting seeds, flowers and tissues including leaves, stem, shoots and roots (Liu et al. 2012) produced 15,138,726 reads which assembled into 103,354 scaffolds (Liu et al. 2012). A total of 68,229 unigenes were identified, among which 46,087 were annotated in the NCBI non-redundant protein database and 28,165 were annotated in the Swiss-Prot database. Of these annotated unigenes, 11,921 and 10,147 unigenes were assigned to gene ontology categories and clusters of orthologous groups, respectively. Forty five thousand six hundred forty nine unigenes were successfully mapped onto 292 pathways using the Kyoto Encyclopaedia of Genes and
Genomes (KEGG) Pathway database. They also detected 105 unigenes encoding eight key enzymes involved in lignin biosynthesis. However, in comparative study of annotated unigenes some unigenes did not matched to available genomic resources in Moso bamboo, rice and millet and hence considered that these unigenes are unique to Ma Bamboo transcriptome. Further, 621 simple sequence repeats (SSRs) mined from this dataset can serve as a sequence resources for marker development. Concurrently, Zhang et al. (2012) used different floral tissues of D. latiflorus flowers collected from each of the 14 ramets of one flowering genet and grouped into two sized buds sample set for studying differential gene expression. Transcriptome sequencing generated additional 96 million sequencing reads and assembled de novo into 146,395 high quality unigenes. Of these, 80,418 were identified as putative homologs of annotated sequences in the public protein databases, of which 290 were associated with the floral transition and 47 were related to flower development. These sequences and putative function data comprise a resource for future investigation of the floral transition and flower development in bamboo species.

2.10 Genetic improvement and breeding programmes

Diversity of germplasm is an important aspect in breeding programmes as it provides insights about the variations among the different traits which can be explored to create a new dream genotype with combinations of all the possible desirable traits. Being second largest in bamboo genetic resources, India has been considered as hotspot of diversity in terms of genera, species with variations in morphological, physiological, anatomical characteristics (Tewari 1992; Sharma et al. 2008), thus presents ample opportunities of transferring the various desirable traits from one species to another for producing more beneficial genotypes. However, there is a wide gap about the knowledge on these basic aspects of bamboo biology and information on combining ability, crossability pattern, genetics of flowering and hybridization procedures which are prerequisites for successful implementation of genetic improvement programmes in any plant species, is also lacking. The complexities associated with flowering behavior of different bamboo species itself is a great hindrance towards the breeding and genetic improvement studies in this crop. As a result, except few attempts for the production of interspecific bamboo hybrids generated by crossbreeding Bambusa pervariabilis with Dendrocalamus latiflorus, D. hamiltonii with D. latiflorus, B. textilis with D. latiflorus, B. pervariabilis with D. latiflorus or B. textilis (Zhang and Cheng 1980; Muramatsu 1981; Zhang et al. 1985; 1986; Zhang 2000; Wang et al. 2005), Pleioblastus simonii with Phyllostachys violascens, Sasa tokugawana with S. borealis, and Sinobambusa
tootsik with Pleioblastus distichus (Lu et al. 2009), systematic breeding efforts for combining the desirable traits are non-existing in bamboo, worldwide. In India, there is only one report on in-vitro flowering and breeding behaviour of Dendrocalamus strictus (Nadgauda et al. 1990, 1993). More recently, Lin et al. (2010) reported the successful production of hybrids between Phyllostachys kwangsiensis and Phyllostachys bambusoides. More such studies with successful hybrids are required to understand the crossability behavior of different bamboo species and raising mapping populations in bamboo in near future for successful implementation of genetic mapping, dissection of complex traits and successful breeding in this economically important bioresource.

2.11 Future prospects and challenges

India, having a largest bamboo forest cover represents huge employment opportunities to millions of people of the country in different areas namely weaving, food, fuel, pulp and paper and cottage industry. In the recent past, owing to technological innovations, bamboo has emerged as a great source of charcoal, flooring and construction of earthquake resistant housing. This advancement in bamboo technology is having great capacity of generating more employment opportunities in bamboo based industries in future. The fast growing bamboo industry of India will reach the target of worth rupees 26,000 crore by 2015 which indicates that increase in the number of people involved in bamboo related trades is obvious and bamboo industry will continue to grow in the coming years.

Further, fast growth, culm flexibility and lightness are some useful properties of bamboo which present bamboo as multipurpose bioresource used in wide range of applications. Due to wider adaptability in adverse environments and climatic conditions, bamboo is considered as one of the best among different bioresources, which makes it a common forest plant throughout the country except Jammu & Kashmir. This quality can be harnessed for producing sufficient bamboo shoots and culms required for various purposes in those states of country where bamboo do not grow naturally. Some other properties such as soil reclamation and growth in poor quality soils are additional beneficial aspects of bamboo that can be further explored for restoration of bare lands and to prevent soil erosion throughout the country.

Bamboo, although has been proved as one of the very important bioresources across the globe, still it is very challenging to the entire scientific community engaged in bamboo research to crack complex biological question associated with its growth, development and flowering behaviour. Due to unusual flowering behaviour, taxonomic classification is another
limitation in bamboo. Although, efforts have been made in past two decades to employ molecular tools to strengthen, understand and correcting the existing classification systems, which otherwise are based on limited vegetative characteristics. Therefore a common standardized bamboo classification system, which is easy to use, is very much needed consolidating all the criteria so far used by different researchers in various parts of the world (McClure 1966; Ohrenberger and Goerrings 1989; Tewari 1992; Dransfield and Widjaja 1995; GPWG 2001).

In the present era of genomics, cost effective, rapid NGS technologies based genotyping methods can be efficiently employed for solving the problems of systematics. NGS technologies are having advantage of more numbers of nucleotide coverage from all the three types of genomes namely plastid genome, mitochondrial genome and nuclear genome, as compared to Sanger’s sequencing based molecular tools. Thus, adoption of NGS technologies will facilitate progress and accuracy in bamboo systematics, as whole plastome and rDNA cistrons, partial mitochondrial genomes, and low-copy nuclear markers can now be efficiently executed for precise molecular phylogenetic studies (Straub et al. 20012). Among the other challenges, lack in understanding of basic biology related to growth, flowering and disease resistance due to insufficient research work done till date. All these undermined areas of bamboo research should be the focus of future research works in bamboo which can be successfully addressed with the help of NGS and other emerging technologies in plant genomics.