Chapter 1:

Introduction

# **Chapter 1: Introduction**

## **1.1 Bamboo:**

Bamboo is considered as one of the most important group of plants in this world. The diversity, distribution and its uses are mind boggling in its scope and variety. The growth rate of this plant group is truly phenomenal and is one of the fastest growing living being in the world. Apart from the cereal plants which provide staple food and nutrition and the medicinal plant, bamboo comes next in importance to mankind and its ability to influence peoples' lives.

#### **1.1.1 Taxonomy of Bamboo:**

Bamboos are members of the sub-family Bambusoideae within the grass family Poaceae. Grass family is monophyletic and the early diverging lineages recognized within the family are Anomochlooideae, Pharoideae and Puelioideae according to Grass Phylogeny Working Group (Barker et al. 2001). Anomochlooideae lacks a true spikelet and is sister to the rest of the family members. Pharoideae is the earliest lineage from the true Spikelet bearing group and was followed by Puelioideae. The earliest fossil evidence for grasses was reported sometimes between Paleocene and Eocene ages (Crepet et al. 1991).

According to the fossil species of Pharus, the early diversification of the family started between late Eocene and early Oligocene and extensive diversification occurred by Miocene. Most possibly, the major radiations of the grasses including Bambusoideae happened 40–50 million years ago (Piperno et al. 1998). Very recently the first petrified bamboo fossil *Guadua zuloagae*, was reported from the Pliocene age (Brea et al. 2007).

Traditionally, the members of the group share some common features that include rhizomatous habit, hollow segmented culms, petiolate blade with tessellate venation, flowers with three or more lodicules, usually with six stamens, and fruit possess small embryo and linear hilum (Soderstrom 1981). Few synapomorphic features

which are unique for Bambusoideae were reported by Barker et al. (2001). Leaf blade is mainly composed of mesophyll tissue with asymmetrically invaginated arm cells, while pseudo petiole structures are secondary gain for the sub-family. It is broadly divided into two tribes, that is Bambuseae/woody bamboos and Olyreae/herbaceous bamboos depending on the presence (Bambuseae) or absence (Olyreae) of the abaxial ligule (Barker et al. 2001) and (Zhang et al. 2000).

The bamboos represent one out of 6 or 7 main natural groups of the grass family Poaceae or Gramineae. Although this natural group of grasses is sometimes recognised as a tribe viz., Bambuseae yet most taxonomists of today recognised the group as a subfamily viz., Bambusoideae. Species of Bambusoideae are differentiated from other members of the family by the presence of petiolate blades with tessellate venation, three, four, six or more stamens, gynoecium with a single style, one to three stigmas and fruit or caryopsis.

The bambusoid grasses do not comprise only grasses whose culms are lignified, called "woody bamboos" or simply "bamboos", but also of several of grasses with herbaceous culms, called "herbaceous bamboos" or "bamboo allies". In the past only bamboos have, however, been treated in the subfamily Bambusoideae and some botanists even grouped them as a separate family under the name Bambusaceae. The concept of the subfamily Bambusoideae comprising both woody and herbaceous members was put forwarded for the first time by C. G. D. Nees v. Esenbeck in the first half of the 19th century and was based not only on morphological characters but much more on features of the embryo, leaf anatomy, epidermis, and the number of chromosomes. The concept has, however, been redefined recently on the basis of numerous anatomical, cytological and physiological findings (Ohrnberger et al. 1988). Soderstrom et al. (1979) not only published a comprehensive characterisation of Bambusoideae comprising both woody and herbaceous bamboos but also remarked "We became increasingly convinced that the bamboos and herbaceous bambusoid grasses evolved from a common stalk and should therefore be regarded as comprising a single natural subfamily of grasses, technically known as the Bambusoideae".

#### **1.1.2** Global perspective

Bamboo is one of the most widely distributed plant groups in the world. Apart from the natural migration of the plant, it has been spread far and wide by human due to its usefulness and beauty. Being one of the fastest growing plants makes it a gardener's delight and so they are widely planted.

Bamboo is found in all continents except for Antarctica. Without any exception to the congeneric elements, Ohrnberger et al. (1988) have estimated the number of genera and species of Bambusoid grasses to be 110 and 1010-1140 respectively. The herbaceous bamboos comprise of 25 genera with 130-160 species and the remaining 880-980 species belonging to 85 genera are of woody bamboos. According to Clayton et al. (1986), the subfamily Bambusoideae comprises of 91 genera and  $\pm$ 1110 species; the tribe Bambuseae (bamboos having woody culms including 4 genera and  $\pm$  20 species with herbaceous culms) comprises of 49 genera and  $\pm$  840 species, and the remaining 12 tribes comprise of 42 genera and  $\pm$  270 species of herbaceous bambusoid grasses. Soderstrom et al. (1988) on the other hand, estimated the number of genera and species of bamboos as 75 and 1250 respectively.

Bamboos are distributed all over the world, but major species richness is found in Asia Pacific (China: 626, India: 102, Japan: 84, Myanmar: 75, Malaysia: 50 and few others) and South America (Brazil: 134, Venezuela: 68, Colombia: 56 and few others) while least (5) in Africa (Bystriakova et al. 2003). The herbaceous bamboos with about 110 species are mostly concentrated in the Neotropics of Brazil, Paraguay, Mexico, Argentina and West Indies (Judziewicz et al. 2000). Brazil is the most prominent place representing 89% of the genera and 65% of the species that are reported from the New World (Filgueiras et al. 2004). The largest natural bamboo forests, known as 'tabocais' in Brazil and 'pacales' in Peru, cover 600,000 ha across Brazil, Peru and Bolivia (Filgueiras et al. 2004). The woody bamboos are unique with complex branching patterns, woody culm and gregarious, monocarpic flowering. There are about 1290 species and they are universally distributed except in Europe, which has no native species.

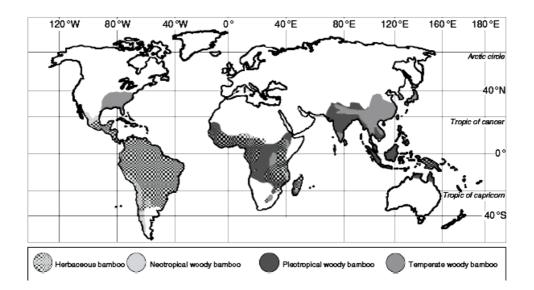


Figure 1.1: World distribution of woody (paleotropical, neotropical, temperate) and herbaceous bamboos (adapted from (Saitou et al. 1987) http://www.eeob.iastate.edu/research/bamboo/maps.html and (Judziewicz et al. 2000))

Woody bamboo is classified into three major groups: the paleotropical woody bamboos (distributed in tropical and sub-tropical regions of Africa, Madagascar, India, Sri Lanka, Southern China, Southern Japan and Oceania, Fig. 1.1), the neotropical woody bamboos (Southern Mexico, Argentina, Chile, West Indies) and the north temperate woody bamboos (mostly in North temperate zone and few at high elevation habitats in Africa, Madagascar, India and Sri Lanka).

#### **1.1.3** National perspective

India is very rich in bamboo diversity. China occupies the top spot in Bamboo diversity followed by India. Together, China and India have 50% of the total bamboo biodiversity in the world. 136 species of bamboos has been reported to be in India. In North Eastern India 58 species of bamboo belonging to 10 genera are found (Moulik 1997).

In India, out of the total forest area, about 13% are made up of Bamboo; this roughly comes out to 9.57 million hectares. The Bamboos of India are predominantly

indigenous, as because 19 genera out of 22 are found to be indigenous with only 3 genera found in India are exotic. The average annual yield per hectare in India is about 0.33 tonnes. (Katwal et al. 2004)

The Himalayan foothills and the Terai region are also very rich both in terms of species diversity and extensive area of growth. The bamboo forest in the Terai region is a big source of livelihood for the local populace. Apart from the above mentioned areas, it is also seen that all the National Parks, Sanctuaries of India have widespread areas under bamboo forest. Such bamboo forests and clumps are the typical characteristic feature of National Parks and Sanctuaries.

#### **1.1.4** North East Perspective

In Northeast India, the number of genera and species of bamboo available are 16 and 58 respectively, although some workers estimated that the number of species in the region is 63. Bamboos are integral part of the culture of NE India, which accounts for nearly 50% of the total bamboo resources of the country possessing largest species diversity. The entire north eastern region is characterized by extensive bamboo forests having considerable socio economic value. The total bamboo cover in the Northeast has been reported to be 38,197 sq km, of which Mizoram occupies the largest forest area under bamboo (30.8%) followed by Tripura (27.1%) and Meghalaya (26.0%).

Bamboo plays an important role in both oral and written traditions as documented in poetry and folk literature throughout South and Southeast Asia. These traditions are a vital part of cultural identity. The north eastern region of India has great diversity of bamboo resource. This resource is owned, managed and grown by the rural masses of the region. Not owning a bamboo orchard is unthinkable in rural Northeast. The mention of bamboo in folk literature, proverbs, adages, tales, folk songs, etc of the Northeast is enormous. More recently, it has exploded into world consciousness as the 'wonder plant of the 21st century' (Nath et al. 2008).

The most commonly available bamboo species in the Northeast are: Arundinaria hirsuta (short, stiff), A. manni (wild climbing), Bambusa tulda (jati – sturdy,

masculine), B. pallida (bijuli - slender, feminine), B. nutans (mokal - slender and sturdy), B. polymorpha (tall growing), B. arundinacea (cultivable non-native species), B. nana (medium short, highly prolific), B. multiplex (hedge bamboo, medium short, highly prolific), B. balcooa (bholuka - strongest tall bamboo), B. variegata (medium tall with variegated leaves), B. vulgaris (tall and thick), B. khasiana (wild representative of bamboo), Cephalostachyum fuschianum (wild climbing), C. pergracile (tall slender), Chimonobambusa callosa (wild drooping), Chimonobambusa armata (a graceful species), Chimono bambusa griffithiana (medium tall), Dendrocalamus longispathus (tall, sturdy), D. hamiltonii (kako abundant species endemic to Northeast), D. hookerii (tall, very dark green), D. giganteus (giant bamboo), D. sikkimensis (tall, sturdy), Phyllostachys mannii (short stiff bamboo), P. assamica (highly cultivated species), Pseudostachyum polymorphum (wild) and Teinostachyum helferii (rare and wild). Out of these species, B. tulda, B. pallida, B. nutans, B. balcoa, D. hamiltonii, D. hookerii are endemic to the Northeast. D. giganteus and D. sikkimensis are semi-wild, C. fuschianum, C. griffithina, C callosa, P. polymorphum and Melocanna baccifera are wild bamboo species. D. sahnii is a rare species, which is found in Lower Subansiri region of Arunachal Pradesh.

The jati (*Bambusa tulda*) bamboo is preferred for cultivation in low altitudes to midhills throughout the region. It is used for pulp, timber, handicraft, furniture, food and miscellaneous uses. The bijuli (*Bambusa pallida*) bamboo is cultivated all over the region. It is medium tall, shows prolific growth and is used for timber, handicrafts, furniture and various domestic purposes (Shukla 1996).

The mokal (*Bambusa nutans*) bamboo is a slender but sturdy and is used for pulping, timber, handicrafts, furniture, food, etc. The bholuka (*Bambusa balcooa*) is the strongest tall bamboo widely cultivated in the plain and valley areas of the Northeast. It is mainly used as timber and furniture. The ornamental species *Bambusa variegata* is characterized by variegated leaves and used as ornamental plants in gardens and avenues. The kako (*Dendrocalamus hamiltonii*) bamboo is one of the abundant species endemic to this region and is used for furniture, handicrafts and miscellaneous purposes (Singha et al. 2008).

## **1.2** Classical identification of Bamboo

The identification of Bamboo on the basis of morphological studies is a very tenuous and rigorous process requiring high level of skill in determining all the characters. There are numerous morphological characters to be studied and determined. Some of the characters are seasonal which requires field visits at different times, for proper study and identification of Bamboo. Some of the important morphological characters are described in the following paragraph. Another important point to be considered while identifying Bamboo is that an error in 1 or 2 characters may lead to wrong identification. Stapleton in his book has included a clarification of Morphological Detail (Stapleton 1997). According to him some important limitations associated with the traditional morphological classifications are:

- a) Morphology-based classifications are often superficial as similarities have frequently gained priorities over dissimilarities.
- b) Reproductive characters have often earned priority with an assumption of having higher evolutionary significance than the vegetative characters. The importance of many vegetative features such as rhizome or branch patterns was understood later and thus many of the early herbarium specimens were incomplete.
- c) In many cases artificiality was enhanced as characters were frequently considered in isolation rather than considered in groups.

### 1.2.1 Rhizomes

Rhizomes are horizontal stems extending from the domain plant that travel underground with the objective of colonizing new territory. As rhizomes spread through the soil they collect and store the primary nutrients for growth. The storage of energy is the primary reason that bamboo exhibit rapid and massive growth. This also gives bamboo plants the ability to utilize energy created from both photosynthesis and that which is stored in the rhizomes. Over time, the rhizomes create an interconnected system of plants, all of which draw on the rhizomes for nutrients. For example, when a single bamboo plant has been introduced into an area, all other bamboo plants that emerge will belong to the same organism. In appearance, rhizomes are segmented and covered by a protective sheath. The leaves are reduced along the sheath, as they provide no photosynthetic benefit underground. The sheath provides the plant with the protection needed to breach the surface to form a culm. A healthy rhizome is usually slightly yellow or ivory in color, although possible colors may include red, brown, green, and purple. The appearance and behavior of rhizomes differs among species, and is divided into two main categories which include the pachymorph system and the leptomorph system.

The pachymorph rhizome system, which is found in clumping bamboos, expands horizontally only by short distances each year. The rhizomes are generally short and thick in appearance. They curve upwards in close proximity to the domain plant. At the nodes, new rhizomes or roots can be produced. New culms can only form at the very tip of the rhizome. It is this feature that causes them to curve upwards and exhibit the clumping behavior. An advanced pachymorph system is very compact near the base of the plant, making removal or transplant of the bamboo exceptionally difficult.

The leptomorph rhizome system is found in running bamboos. In contrast to the pachymorph system, the rhizomes have a tendency to branch away from the domain plant. The rhizomes are generally long and thin in appearance and some species can send the rhizomes up to 20 feet away in a single growing season. At the nodes, they have the ability to produce buds that will form either new culms or rhizomes. Bamboos with a pachymorph rhizome system will be spaced over a wide area. They are invasive by design and it can be extremely difficult to remove a well established plant (Barooah et al. 2003).

#### 1.2.2 Culms

Culms are the most visibly distinguishable feature of a bamboo plant. Culms can vary in size, shape, color, and even smell. The appearance can range from thick or thin, tall or short, erect or bent, and can exhibit irregular patterns such as those found in Tortoise Shell Bamboo (*P. heterocycla* f . *heterocycla* 'Kiko'). Most culms are round in shape, but some species can take on a square like appearance. The color of the culms also has a wide range of characteristics. Although the majority of bamboos are green, they can also be brown, black, yellow, or striped. One of the most popular garden bamboos, Black Bamboo (*Phyllostachys nigra*), is unique in the fact that the culms exhibit a nearly jet black color. The culms can also vary in smell. One of the most interesting examples is Incense Bamboo (*Phyllostachys atrovaginata*), which has a waxy coat on the culms that emits a pleasant fragrance similar to incense.

New culms will generally emerge in the springtime; however timing will vary among species. As the culm shoots from the soil it will have already reached its maximum diameter, or girth. The newly emerging culm will grow rapidly and reach its final height by the end of the first growing season. The final size is determined by the local growing conditions, as well as the age and size of the bamboo grove (Barooah et al. 2003).

#### **1.2.3** Culm sheaths

Culm sheaths are modified leaves, arranged alternately on opposite sides of the growing culms, providing protective cover for the young shoots. The sheath is attached to the node.

It clasps the culm and falls off leaving a scar after the culm becomes mature. Width of the sheath is greater than the circumference of the node so that the two sides of sheath overlap. The back of the sheath is usually covered with hairs that are irritant and easily detached when sheaths become old. The inner side of the sheath is smooth and shining. Early removal of culm sheaths will result in stunted growth of the culm.

Apart from its main protective body, the culm sheath consists of a blade, auricles and ligule. The distinct portion at the top of the sheath is the blade. The blade is smaller at the base of the culm and larger and leaf-like towards top. The blades may be erect or deflexed and are deciduous when the sheaths become old. In some bamboo species, prominent ear-shaped structures are present at the point of attachment of the blade on the sheath. These laterally extended structures are called auricles; which play very important role in identification of bamboo species. The auricles may have hair-like bristles on their edges. The auricles are not deciduous but are fragile and easily broken.

The thin, short upward-growth on the inside top of the sheath, which clasps the culm is known as ligule. The shape, height, margin of the ligule and presence of hair etc. are some of the characteristics facilitating identification of bamboos. These characteristics are more prominent in young culm-sheath. The general appearance, size and shape of culm sheaths, the structure and character of blade, auricle and ligule are considered as the best guide for identification of bamboo species (Barooah et al. 2003).

#### 1.2.4 Leaves

Leaves are present at every main portion of the bamboo plant, which includes the rhizomes, culm, and branches. The anatomy of the leaf itself includes a blade, sheath, and ligule. Leaves are first present in the rhizome where they are almost completely comprised of the sheath. At this stage, leaves serve as a protective cover to encase the rhizome as it travels underground. After the rhizome shoots through the soil and becomes a culm, the blade will become the predominant feature. The blade provides the photosynthetic function of the plant by converting sunlight into energy. The appearance of the blade varies among species. In some species the leaves are very large and less numerous, while other species have a large amount of very small leaves. The appearance of leaves plays a large role in the identification of bamboo (Barooah et al. 2003).

#### 1.2.5 Branching

Branches emerge from the nodes and are arranged on alternate side of the culm. The number of branches at each node varies according to the species. Normally one main branch and two or more secondary branches emerge from each node. The main branch is thick and long as compared to other auxiliary branches. In some species, branching is absent in the lower nodes up to a considerable length. However, in some other species branching starts from fourth inter node above the base. In case of few thorny species the lateral branches are hardened to spines providing natural armour to clumps. The branches and branch lets bear profuse leaves. In many cases the branching pattern is considered as a characteristic for the genus (Barooah et al. 2003).

#### 1.2.6 Flower

Like other plants, bamboo does not flower annually. In general, flowering and fruiting occurs only once during the lifetime at certain age of the clump after which it dies. The flowering cycle of bamboo varies from 7 years to 60 years depending upon the species. There are 3 different types of flowering.

- Annual: The culms remain healthy after flowering.
- Sporadic: Only some clumps in an area flower, bear seeds and die thereafter.
- Gregarious: The whole population of bamboo, over an extensive area flower over a period of time and die out.

Generally most bamboo species flower gregariously at fixed intervals and all culms including those of current year die after flowering .The Bamboo clumps belonging to a particular stock flower simultaneously. It has been reported that the entire population of a given species raised from the same seed source, no matter where they are situated, would flower at the same time. As a precaution, it is required to raise bamboo crop of different species to ensure constant supply of bamboos. In Salia bamboo (*Dendrocalamus strictus*) that is predominant in Odisha forests, both sporadic and gregarious flowering occur at long intervals of 20-65 years. Flowering is very rare in the two important bamboo species cultivated by villagers i.e. *Bambusa nutans* and *Bambusa vulgaris* (Barooah et al. 2003).

## **1.3 Bamboo Application**

Bamboo is one of the most important plants in the lives of people in South East Asia. Particularly for people living in North Eastern part of India, it plays an integral and vital role in their day to day activities and it has been aptly called poor man's timber. A lot of people earn their livelihood based on this plant. Industries at the big, medium and small scale are directly associated with bamboo or its various products. Large scale industries in Assam like the paper mills based at Panchgram (Cachar Paper Mill) and at Jagiroad (Nagaon Paper Mill) is entirely dependent on Bamboo as their raw material.

It is made into houses which is best suited for the hot, humid and rainy weather condition prevalent in this part of the world. Right from big houses constructed of bamboo to small fishing and agricultural implements, bamboo is used. Also things for storage, utensils, baskets, hats and sundry items associated with the daily needs of life are fashioned out of bamboo. It role in the cultural and traditional aspects in the lives of people in North East India and particularly in Assam has to be truly appreciated.

One of the major festivals of Assam, Bihu, has many musical instruments made of bamboo, like the *dhol* and the *pepa*. Cradles, small toys, like rattle are also fashioned out of bamboo. There is also report of a man from Nagaon making a working bicycle completely out of bamboo. Also items like walking sticks, crutches, artefacts and other artistic items are also fashioned out of bamboo. Its plays an integral role in people's lives right from the cradle to the grave (Singha et al. 2008).

One resource book lists over 5,000 uses of Bamboo including paper, scaffolding, diesel fuel, airplane "skins", desalination filters, aphrodisiacs, musical instruments, medicine, and food and it was Alexander Graham Bell's first phonograph needle.

A whole range of economic activity is associated with bamboo. It is seen by many as a tool for social upliftment, as it allows small scale industry to grow up and without the need for major investment. In North East India it is seen that a sizeable portion of the rural population depends on Bamboo to make a living (Shukla 1996).

Bamboo has the fastest growing canopy for the regreening of degraded lands and its culm release 35% more oxygen than equivalent branch of trees. Some bamboo even sequester up to 12 tons of carbon dioxide from the air per hectare per year. Bamboo can also lower light intensity and protects against ultraviolet rays.

Bamboo offers a unique opportunity in Carbon sequestration as it is one of the most productive and fastest growing plants on the planet. The fastest-growing species may grow up to 1.2m or 4feet per day. The unique growing characteristic makes bamboo a valuable and potential sink for carbon storage. Below ground bamboo biomass makes up 25-50% of the total stock. Carbon content comprises usually about 50% of the total biomass. It has been seen that bamboo can be an effective carbon sink, and performs better than Chinese Fir, Eucalyptus and Poplar etc. This fact itself makes Bamboo a worthwhile and important plant to study as it may prove in the future to hold a key in managing climate changes.

Apart from the above mentioned uses, Bamboo is also effective in soil erosion control as its net like root system create an effective mechanism for watershed protection, stitching the soil together along fragile riverbanks, deforested areas, and places prone to earthquakes and mud slides. It has the potential to be effective in saving forest as it is a viable alternative to most of the uses of timber. Bamboo is one of the strongest building materials. Bamboo's tensile strength is 28,000 pounds per square inch versus 23,000 pounds per square inch for steel. In the tropics it is possible to plant and grow ones own home; in Costa Rica, 1000 houses of bamboo are built annually with material coming only from a 60 hectare bamboo plantation. If an equivalent project used timber, it would require 500 hectares of our diminishing tropical rainforests. Using bamboo to replace timber saves the rainforests. With a 10-30% annual increase in biomass versus 2 to 5% for trees, bamboo creates greater yield of raw material for use. One clump can produce 200 poles in the three to five years. Bamboo generates a crop every year.

Bamboo shoots provide nutrition for millions of people worldwide. Young bamboo shoot is considered a delicacy and a part of the diet of a large chunk of the population living in North-East India. In Japan, the antioxidant properties of pulverized bamboo skin can prevent bacterial growth, and it is used as a natural food preservative. Bamboo litter makes fodder for animals and food for fish.

This in short indicates the usage and importance of Bamboo in every sphere of life in North east India. The different type of bamboos has unique characteristics which make it suited for specific usage, e.g. a variety called *Mritinga* with a very thick internode wall is especially suited for making Fishing rod.

## **1.4 Genomic variations**

Molecular data sets can provide useful information for addressing various aspects of plant taxonomy. Considerable progress has already been achieved in bamboo and various molecular tools have been used till date and also the potential pitfalls that need to be critically considered. The major challenge associated with any molecular method is to determine the appropriate taxonomic level at which it is most informative and to correlate it with morphologically definable taxonomic groupings.

### 1.4.1 **RFLP**

In restriction fragment length polymorphism (RFLP), differences in the restriction enzyme recognition site sequences between genomes are the basis of polymorphism. These markers are co-dominant in nature and are useful for marker assisted selection. The technique was introduced to bamboo by Friar et al. (1994) for phylogeny assessment 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 *P. 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.

## 1.4.2 RAPD

In randomly amplified polymorphic DNA technology, a single and short arbitrary primer is used. RAPD was utilized to assess phylogenetic relationships among 73 genotypes of *Phyllostachys* by Gielis J (1997). The resultant phylogeny neither supported the existence of two distinct sections in the *Phyllostachys* species complex nor the placement of *P. nigra* under *Phyllostachys*, thus deviating from the previous proposal by Friar et al. (1994).

However, based on a combined application of RAPD and morphometry, it was confirmed that *P. nigra* belongs to the section *Phyllostachys* (Ding 1998) and it was also confirmed by AFLP and *ITS* sequence data that two distinct sections, *Phyllostachys* and *Heteroclada*, do exist in the *Phyllostachys* species pool. The utility of RAPD was extended to the tropical group as well. *B. ventricosa* was found close to *B. vulgaris var. striata* (Nayak et al. 2003) and was supported by a previous finding that *B. ventricosa* is a cultivated variety of *B. vulgaris*.

#### 1.4.3 AFLP

Amplified fragment length polymorphism (AFLP) is a method described as a combination of RFLP and PCR based techniques (Vos et al. 1995). It generates dominant markers like RAPD and is highly capable of detecting polymorphism among closely related genomes.

It has been demonstrated that this technique is efficient in measuring genetic relationships among 15 bamboo species representing 4 different genera (Loh et al. 2000). Unique banding patterns were obtained in 13 out of 15 species and the cluster pattern helped reveal the polyphyletic nature of the genus *Bambusa*.

#### 1.4.4 SCARs

Sequence characterized amplified regions (SCARs) is an extension of the RAPD procedure (Paran et al. 1993), but is more efficient due to the use of higher annealing temperature. SCARs are co-dominant and have been proved useful for

genotype/varietal identification. Particularly, they are useful at the seedling stage when key morphological features are indistinguishable. Das et al. (2005) used this technique in an attempt to make species specific markers in bamboos.

#### 1.4.5 Microsatellites (SSRs)

Microsatellites or simple sequence repeats are short tandem repeated sequences of 1–6 nucleotides in length. It is highly polymorphic, co-dominant, multi-allelic, presumed selectively neutral and hence widely used in plant genetic diversity studies. Primers are designed from the conserved genomic regions flanking the repeat sequences and the detected polymorphism reflects variation in the number of repeats among genomes. Sharma et al. (2008) have used this technique for phylogenetic and genetic diversity analyses in bamboo using markers used for Rice and Sugarcane studies.

However, the entire procedures that include construction and screening of genomic library prior to primer designing are cumbersome and cost intensive. This severely limits the wide application of the technique in non-crop plants like bamboo, as adequate genomic information is not yet available in the database.

## 1.5 DNA barcoding

DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species. It differs from molecular phylogeny in that the main goal is not to determine classification but to identify an unknown sample in terms of a known classification. Although barcodes are sometimes used in an effort to identify unknown species or assess whether species should be combined or separated, the utility of DNA barcoding for these purposes is subject to debate. Application includes the identification of plant leaves, bark or stem etc. even when flowers or fruit are not available.

### **1.6** Statement of the problem

The correct identification of a species has always been problematic due to many reasons. Particularly in plants the identification has been usually associated with the reproductive organs. Bamboos, also known as woody grasses, are perennial grasses and are currently classified as a subfamily Bambusoideae within the extensive grass family Poaceae. The plant group harbours both herbaceous and woody members. The bamboo subfamily includes approximately 70 genera and ~1400 species worldwide. Classification of bamboo varieties is known to be difficult owing to its long and unusual life cycle. Vegetative characters are used for classification, because in many bamboo species flowering is extremely rare with intervals between inflorescence lasting as long as 120 years or more. Some species have never been known to flower. Taxonomists often must rely solely on vegetative characters such as culm sheath and ligule for classification. The main difficulty in relying on vegetative characters for classification is that these characters are often environmentally influenced. The taxonomy has traditionally been dependent on morphological characters. Classification systems proposed till date need further support as taxonomic delineation at lower levels often lack sufficient resolution. Infrequent flowering events and extensive genome polyploidization are an additional challenge for the woody group. Thus, many accessions of bamboo have been classified and subsequently reclassified in a separate genera or species.

There is limited information available on genetic diversity or population structure of bamboo germplasm. Additionally, the taxonomy and systematics of woody bamboos are incomplete when compared with other grasses. Clearly, more research is required to understand the taxonomy, phylogenetic relationships and genetic diversity of bamboo. The different molecular techniques that have been utilised till now have not been able to reach consensus regarding the phylogenetic relationship as different genes and parts of genome has been targeted. The different genes and parts of genome have different rates of evolution and so diverse results are seen. The website of KEW Botanical Garden has a list of about 1,100 different characters that needs to be looked into as far as applicable in the process of correctly identifying a particular species of Bamboo.

## 1.7 Objectives

- 1. Collection through random sampling of different Bamboo species from different part of Southern Assam, with an aim to identify the species and their uses.
- 2. Study genomic diversity of Bamboo species with special reference to DNA sequences.
- 3. Genomic analysis of the Bamboo DNA sequences.
- Study the phylogenetic relationship among the bamboo species using Bioinformatics tools.