CHAPTER 2. REVIEW OF LITERATURE

Bamboo has played a major role in human civilization since ancient times and is still contributing to the survival of over two billion people living in the tropical and subtropical belts in Asia, Latin America and Africa. It helps people to meet their basic needs and as a widespread, renewable, productive, versatile, easily accessed and environment-friendly resource. It has great potential to improve life, especially in the rural areas of the developing world (Sastry, 2008). Bamboo stands occupy an area of 36 million hectares worldwide which is equivalent to 3.2 per cent of the total forest area in the world. It is estimated that bamboo occupies over one per cent of the tropical and subtropical forest area - over 22 million hectare. Over 80 per cent of the total area covered by bamboo is located in Asia, 10 per cent in Africa and 10 per cent in America (Lobovikov et al., 2007). Bamboo occurs in almost all the states of India, from the tropical to the temperate regions and the alluvial plains to the high mountains. For attaining maximum growth bamboo requires a temperature of about 8-36ºC with a minimum annual rainfall of 1000 mm and high atmospheric humidity (Pandalai et al., 2002).

2.1 Flowering

Bamboo is different from other perennial plants in its typical flowering behaviour where it flowers only once in its lifetime and most of the species die after the gregarious and synchronous blooming. The well defined flowering cycles which vary from 3 to 120 years is supposed to be a genetic trait (Janzen, 1976). The long flowering intervals and the factors that trigger flowering in bamboo are still largely a mystery to scientists. Many causes have been recognized to trigger gregarious flowering in bamboo but it has not been possible to establish their consistency. According to Janzen (1976) who studied natural stands of bamboo and their records of past flowering, there are at least 137 Indian-Asian species that have populations that flower and set seed synchronously at regular and supra-annual intervals. He suggested the presence of an internal mechanism of a biological clock that controls flowering in bamboo. Droughts and depletion of nutrients are some of the causes attributed to trigger bamboo flowering, but again there is no evidence to support this. It has been observed that production of new vegetative culms stops in a few years before and during flowering. During flowering, there is a switching of resources from vegetative to reproductive parts when stored
nutrients are used up in the production of vast quantities of flowers and seeds (Ramanayake, 2006). Analysis of biochemical components of Dendrocalamus stocksii and Ochlandra travancorica indicated the utilization of starch, reducing sugars, crude fat, amino acids, fatty acids and vitamins during flowering and post-flowering stages (Beena, 2011).

Flowering in bamboo is believed to be an ill omen, leading to famine, death and natural disasters in cultures associated with bamboo (Mohan Ram and Hari Gopal, 1981; John and Nadgauda, 2002). Rodents and many other animals get attracted by the large quantities of seeds formed during gregarious flowering and reproduce fast to increase in number. People in these areas also collect the seeds for consumption (Janzen, 1976).

The flowering cycle of many bamboo species have been compiled by Gaur (1987) from the published records in the paper ‘Bamboo research in India’. Flowering of the following species was reported: Bambusa bambos (B. arundinacea) (Rao, 1988); B. nutans (Bahadur, 1980); B. atra (Kurz, 1876 and McClure, 1966); B. balcooa (Rawat, 1987); B. bambos var. gigantea (Bennet and Gaur, 1990); B. burmanica and B. khasiana (Gamble, 1896); B. multiplex (Gamble, 1896 and Holttum, 1956); B. polymorpha (Gaur, 1987); B. vulgaris (Blatter, 1930; Banik, 1987); Chimonobambusa jaunsarensis (Pant, 1987); Dendrocalamus asper (Satsangi et al., 2001); D. sikkimensis (Mohan Ram and Hari Gopal, 1981; Lahiri, 1982); D. strictus (Tatawandi and Kali, 1983; Negi, 1986; Pandey and Chowdhery, 2001); D. hamiltonii (Cavendish, 1905); D. giganteus (Jain and Mohinder, 2002); Oxytenanthera spp. (Gupta, 1987); Thrysostachys regia (Naithani, 1990); Gigantochloa albociliata (Gupta, 1987); G. pseudoarundinacea (Widjaja, 1987); G. rostrata (Banik, 1987); Melocanna baccifera (Kurz, 1876; Gamble, 1896; Troup, 1921; Chatterjee, 1960; Nath, 1968; Vaid, 1972; Sharma, 1992; Ramanayake and Weerawardene, 2003; Jeeva et al., 2009); Ochlandra setigera (Chand Basha and Kumar, 1994), O. sivagiriana (Kumar, 1995); O. talboti (Kumar, 1995), O. travancorica (Asari, 1976; Venkatesh, 1984); Phyllostachys aurea (Chengde and Widjaja, 1995); P. bambusoides (Majumder et al., 1985); Pseudoxytenanthera bourdillonii (Blatter, 1929); Schizostachyum dullooa (Gupta, 1972); Schizostachyum polymorphum (Mohan Ram and Hari Gopal, 1981). Seethalakshmi et al., 2007 described the flowering of eleven bamboo species Bambusa bambos, B. striata, B. tulda, Dendrocalamus giganteus, D. strictus, D. stocksii, Ochlandra ebracteata, O. scriptoria, O. travancorica, Pseudoxytenanthera ritcheyi & P. monodelpha found in Kerala.
Kapoor and Sharma, 1992 reported the early flowering mutants in *Dendrocalamus strictus* after gamma irradiation. The mutants flowered at the age of 15 months, though the normal flowering interval is about 20-40 years. However no seed setting is reported in the premature flowering. *In vitro* flowering has been reported from India (Rao and Rao, 1990; Nadgauda *et al*., 1990). A comparison was made between *in vitro* and *in vivo* flowering in *Bambusa arundinacea* (Nadgauda *et al*., 1997). *In vitro* flowering and seed set has been reported in *Dendrocalamus brandisii* (Nadgauda *et al*., 1993). Subsequently many others reported *in vitro* flowering in other species such as *D. hamiltonii* (Chambers *et al*., 1991), *D. giganteus* (Rajapakse, 1992) in juvenile axillary shoot cultures. Somatic embryos have also given rise to *in vitro* flowering in *B. vulgaris*, *D. giganteus* and *D. strictus* (Rout and Das, 1994). *In vitro* flowering in axillary shoot cultures initiated from field culms of *D. giganteus* (Ramanayake *et al*., 2001) and *B. edulis* (Lin *et al*., 2004) was also reported. Presence of cytokinins and stress has been attributed for flowering *in vitro* (Gielis *et al*., 1997; Ramanayake *et al*., 2001). Reversion of flowering has also been observed under *in vitro* conditions. Contribution of stress and stress release in induction of *in vitro* flowering and its reversion respectively, are discussed in (Ramanayake *et al*., 2001). The main problems for *in vitro* hybridisation seem to be pollen viability and synchronisation of flowering. Pollen viability is always much lower *in vitro*, possibly due to defects in wall formation (Nadgauda *et al*., 1997). Seed setting has been observed (Nadgauda *et al*., 1990; Rout and Das, 1994) but at low percentages, and apparently only when many flowers were open at a particular moment (Nadgauda *et al*., 1997). Studies on the flowering and post flowering behaviour of *Bambusa vulgaris*, *Pseudoxytenanthera monadelpha*, *P. Ritcheyi* and *Dendrocalamus stocksii* revealed that the lack of viable pollen was one of the reason for the poor seed set (Koshy and Pushpangathan, 1997; Beena *et al*., 2007; Jijeesh *et al*., 2009).

Different types of flowering reported in many species are 1. Clump flowering in *Gigantochloa albociliata* (Anantachote, 1987) 2. Vivipary in *Ochlandra ebracteata* (Seethalakshmi, 1993) and 3. *In vitro* flowering in nodal vegetative buds of *Ochlandra travancorica* (Philip and Chacko, 1996). Rai (2009) made a comparative assessment of soil properties after the flowering and death of *Bambusa tulda*, *B. khasiana*, *Dendrocalamus hamiltonii* and *Melocanna baccifera* in a tropical forest of Indo – Burma hot spot. The study revealed that the surface soil in the dead bamboo sites was comparatively more infertile than that in the living bamboo sites. Observations on floral biology in *Dendrocalamus stocksii*, *D. strictus* and *Ochlandra travancorica* revealed dichogamy with protogyny (Venkatesh, 1984;
Nadgauda et al., 1993; Koshy and Harikumar, 2001 and Beena, 2011). Although insects are found visiting the flowers, their role in pollination could not be ascertained. Cross pollination was also observed in *Ochlandra travancorica* (Beena, 2011). There are also reports of albinism and natural selfing in *Bambusa bambos* (Indira, 1988; Adarsh Kumar et al., 1995) and *Ochlandra travancorica* (Abdul Kader et al., 2001). Gielis et al., 1997 studied on the morphological and biochemical aspects of bamboo flowering.

Based on the nature of seed formation, sympodial bamboo species are divided into three distinct groups a). Abundant or profuse seed forming group: Species like *Bambusa bambos*, *Dendrocalamus strictus*, *D. brandisii*, *D. sikkimensis*, *Melocanna baccifera*, *Ochlandra travancorica* in which abundant seed formation is observed after gregarious flowering (Prasad and Gadgil, 1981; Venkatesh, 1984; Nadgauda et al., 1997; Banik, 1998). b) Sparse or diffuse seed forming group: Species like *Dendrocalamus giganteus*, *B. nutans*, *B. tulda* produce limited quantity of seed. c) Sterile group: Both sparse flowering species such as that of *B. vulgaris*, *B. balcooa* or profuse flowering species such as *Pseudoxytenanthera monadelpha* and *D. stocksii* (*P. stocksii*) belong to this group. Seed formation is not reported in any of these species (Banik and Alam, 1987; Banik, 1979; Koshy and Pushpangathan, 1997).

### 2.2 Absence of seed set

Absence of seed set after gregarious flowering is a serious issue with regard to bamboo propagation. Seed set after sporadic flowering was absent in *Bambusa vulgaris*. McClure (1966) reported that *B. vulgaris* is one of the most vigorous bamboos, even though the species has never been “rejuvenated” by sexual reproduction. The vegetative phase is persistent in this species, where only a few plants flower sporadically but set no seed (Ramanayake and Yankandawala, 1995; Koshy and Pushpangathan, 1997). According to Koshy and Harikumar, 2001, high rate of pollen sterility, absence of natural pollination and inhibition of pollen tubes in the stigmatic papillae function as cumulative factors for lack of seed set in *Bambusa vulgaris*. Seed set is not yet reported in *Bambusa balcooa* Roxb. with a flowering cycle of 40±5 years or a multiple of it (Banik and Alam, 1987). No seed set is reported in *Bambusa atra* and *Schizostachyum brachycladum*. The two species flowers regularly but with no seed set and they continue to grow vigorously year after year (Ramanayake, 2006). Seed formation was absent in *Dendrocalamus stocksii* and a few clumps of this species were found reverting to vegetative phase after flowering (Beena et al.,
2007). Similar observation was reported in *Dendrocalamus giganteus*, where a few clumps showed the capacity to regenerate after flowering without the death of the entire population (Seethalakshmi *et al.*, 2010).

### 2.3 Seed morphology

The fruit morphology is one of the important characters for the identification of bamboos. McClure (1966) has given an account of bamboo fruits belonging to 17 genera and 22 species. Detailed fruit and seed morphology of *Melocalamus compactiflorus* has been investigated by Alam (1982). Morphologically, bamboo seeds are classified into Caryopsis, Glans and Bacca (Wen and He, 1991). In caryopsis, the pericarp is membraneous, thin, soft and adhered to the seed coat and with an apparent ventral suture e.g. *Bambusa, Chimonobambusa*, *Gigantochloa, Phyllostachys* and *Thyrsostachys*. Glans has hard, smooth, crustaceous pericarp separated from seed coat and no ventral suture, e.g. *Dendrocalamus* and *Schizostachyum*. Banik (1994) conducted studies on the seed morphology and germination of *Melocanna baccifera*. In bacca the pericarp is fleshy and thick and separated from seed coat, e.g. *Melocanna bambusoides* and *Ochlandra* sp. (Seethalakshmi and Kumar, 1998).

### 2.4 Seed development

Fruit development in *Bambusa tulda, Dendrocalamus giganteus*, *D. hamiltonii*, *D. longispathus*, *Ochlandra travancorica* has been reported by Hari Gopal and Mohan Ram (1987). The ovule is bitegmic, the outer surface of the cells of nucellar epidermis becomes cutinized and forms the seed coat. In a mature fruit, the endosperm is either completely absorbed by the embryo or is present only in small quantity. The developing embryo comes in direct contact with the fruit wall due to the disintegration of the nucellus and integument. The embryo is covered by a thick brown mat from the disorganized cells of the inner layers of the fruit wall.

### 2.5 Seed attributes

The seed characteristics like seed weight and germination of eleven bamboo species viz. *Dendrocalamus strictus, D. giganteus, D. hamiltonii, Bambusa nutans, B. arundinacea, Gigantochloa compressa, G. hasskarliana, G. albociliata, Melocalamus compactiforus, Thyrsostachys siamensis* and *Schizostachyum blumii* were studied in Thailand. Results of the study revealed that there are significant differences in the weight within and between the
species. Higher germination percentage was observed in big seeds than smaller ones and there existed significant correlation between germination percentage and moisture content of seeds (Anantachote, 1987). There are reports from Bangladesh regarding the seed size, weight, germination and longevity of both glumed and deglumed seeds of Bambusa tulda, B. glaucescens, B. arundinacea, Oxytenanthera nigrociiliata and Dendrocalamus longispathus (Banik, 1991).

### 2.5.1 Physical purity

According to Copeland and McDonald, 2001, physical purity is based on physical determination of the components which include percentage by weight of pure seeds, other crop seeds, weed seed and inert matter. Separation of pure seed component is essential before germination tests because the factors that can affect the performance of seed in germination tests include diseased seed, old seed and mechanically damaged seed (Basra, 2006).

### 2.6 Seed testing- Germination and Quick viability tests

Seed testing is considered as the crucial element of intensive seed management for afforestation programmes. In Dendrocalamus strictus, examination of longitudinal section stained with 0.1 per cent tetrazolium chloride for six hours showed several abnormalities of the embryo (Karivaratharaju et al., 1987). Yadav et al., 1987 has described tetrazolium colouring in numerous forest tree species such as Tectona grandis, Acacia catechu, Albizia procera, Butea monosperma and Dendrocalamus strictus. Assessment of potential viability using rapid viability tests such as Tetrazolium and Hydrogen peroxide have gained importance due to the advantage of their simple preparation procedures and quickly obtainable reliable results (ISTA, 1995). Toru Inada and John (2006) found that Oxytenanthera abyssinica seeds treated with one percent TZ solution for two hours were fully viable. Brar et al., 2013, assessed the the loss of viability and vigour of Dendrocalamus membranaceus by treating the seeds using 0.1% of tetrazolium solution for 24 hours at 30°C in dark.

Seasonal differences play an important role in controlling germination capacity of bamboo seeds. Seeds of Oxytenanthera abyssinica sown in the warm season (November in Malawi)
germinated within 11 days, whereas seeds sown in the cold season took 2-4 months to germinate (Anon., 1954).

According to Banik (1987), bamboo seeds start to germinate within 3-7 days and germination will be completed in 15-25 days. Bamboo seeds are considered as negatively photoblastic since they germinate better under shade than in direct sunlight (Banik, 1991).

There are reports about the presence of an endogenous rhythm in the seed germination of *Dendrocalamus strictus* (Rawat and Thapliyal, 2003b). There was no viability loss for seeds of *D. strictus* stored with four moisture contents (2.8, 4.7, 6.3 and 8.9%) and two temperatures (5 and -5°C), but there were seasonal variations in the germination percentage throughout the study period. The germination percentage was higher during hot rainy season (July-August) and decreased during winter season (November-February). The endogenous rhythm was more distinct and prominent in terms of mean germination time as compared to germination per cent. Studies on three bamboo species, viz. *Dendrocalamus membranaceus*, *D. strictus* and *Bambusa nutans* were conducted to determine the optimum conditions for testing the seed germination under laboratory conditions (Rawat, 2005). The seeds were germinated using different combinations of incubation temperatures (20, 25, 30, 35, 40°C and 20–30°C) and sowing media (top of paper, between paper and sand) and also in the presence and absence of light. The incubation temperature of 30°C and top of paper in the presence of light was found to be the ideal conditions for seed germination in the laboratory.

Investigation of thermal effects on germination was carried out in the seeds of *Oxytenanthera abyssinica*. It was found that the temperature 32/22°C regime (light - 8 hours/ dark - 16 hours) was more appropriate for the germination of *O. abyssinica* seeds than the 36/26°C regime. On the other hand, no significant difference was found between the number of germinated seeds in 32/22°C regime and 36/26°C regime. Energy period of the temperature regime of 36/26°C (13 days) was more than that of the regime of 32/22°C (9 days) (Toru Inada and John, 2006).

### 2.7 Classification of seed storage behaviour

According to their physiological storage potential, seeds have traditionally been mainly grouped into two viz. orthodox and recalcitrant (Roberts, 1973). Orthodox seed encompasses seeds that can be dried to low moisture content of around five per cent and can be successfully stored at low or subfreezing temperature for longer intervals. Recalcitrant seeds maintain high
moisture content at maturity (often > 30-50%) and are sensitive to desiccation below 12-30%, hence cannot be successfully stored for longer periods. An intermediate category of seed storage behaviour was mentioned by Ellis et al., 1990. Intermediate seeds can be dried to a moisture content low enough to qualify as orthodox, but is sensitive to low temperatures typical for orthodox seeds.

Further transition groups within the orthodox category were described by Bonner (1990). They are termed as true orthodox and sub-orthodox seeds. True orthodox seeds can be stored for longer periods by reducing the moisture content below 10 per cent. Temperatures of 0 to 5°C are suitable for short term storage of five years or less. A temperature of less than 15°C is used for longer storage. Sub-orthodox seeds can be stored under the same conditions of true orthodox seeds. But due to their fragile structure, thin seed coat and high lipid content, they can be stored only for shorter periods. In bamboos, both orthodox and recalcitrant types have been reported from different species. Caryopsis and glans type of seeds are orthodox and bacca type of seeds are recalcitrant.

2.8 Factors affecting seed viability

Delouche (1973) defined seed deterioration as summation of all physical, physiological and biochemical changes occurring in a seed which ultimately lead to its death. According to Roberts and Ellis (1982) seeds begin to deteriorate at varying rates depending on the conditions of storage environment after physiological maturity. During seed storage, a number of physiological and biochemical changes occur, which is termed as ageing (Silva et al., 2005; Sisman, 2005). The rate at which the seed ageing process takes place depends on the ability of seed to resist degradation changes and protection mechanisms, which are specific for each plant species (Gupta and Aneja, 2004; Sisman and Delibas, 2004; Mohammadi et al., 2011).

2.8.1 Morphological changes associated with ageing

In legumes, changes in the colour of seed coat have been reported as an indication of deterioration (Vaughan and Delouche, 1968; Marzke et al., 1976; Saio et al., 1980). According to Hughes and Sandsted (1975), darkening of the seed coat can be due to oxidative reactions which are accelerated under conditions of high temperature and relative humidity.
2.8.2 Ultrastructural changes

Membrane deterioration and loss of permeability arise at an early stage of seed deterioration. Seed deterioration was found to be associated with coalescence of lipid bodies and withdrawal of plasmalemma in the embryonic axis in a broad group of species including wheat, peas and lettuce (Anderson et al., 1970; Harman and Granett, 1972; Smith, 1983). According to McDonald (1999), disruption of cellular membranes and damage to genetic (nucleic acids) integrity were the major causes of seed ageing. According to Mahajabin (2015), loss in cellular membrane integrity is one of the primary causes for loss of viability. Alterations of membrane systems, such as the tonoplast, plasmalemma and endoplasmic reticulum, result in the decline of normal cell function and energy production. Ultrastructural changes significantly influence cell membrane integrity and speed up the process of seed deterioration and loss of viability.

2.8.3 Cell membranes

The decline of phospholipids occurs only under conditions of high relative humidity but not under long term dry storage (Koostra and Harrington, 1969; Petruzzelli and Taranto, 1984). The loss of phospholipids in deteriorating seeds is generally considered to be due to either phospholipase enzyme activity or lipid peroxidation (Copeland and McDonald, 2005). Increased leakage occurs due to cell membrane disruption associated with loss of membrane phospholipids. According to Copeland and McDonald (2005), one common feature of deteriorating seeds is their inability to retain cellular constituents which leak out during imbibition. Many of these cellular constituents are essential for normal, vigorous germination, some of the exuded compounds are necessary for maintenance of internal osmotic potential which is responsible for normal water uptake and provides the turgor pressure required for radicle protrusion and also the external leakage of these substances encourages the growth of pathogenic microflora.

2.8.4 Loss of enzyme activity

Previous studies in Bambusa bambos and Dendrocalamus strictus seeds revealed that, with accelerated ageing the activity of peroxidase, acid phosphatase and alkaline phosphatase were reduced. Increase in the activity of amylases confirmed the degradation of seed reserves (Ravikumar et al., 1998 and 2002). The enzymes associated with seed deterioration are alcohol dehydrogenase, amylase, catalase, cellulase, diastase, glutamic acid dehydrogenase,
malic dehydrogenase, peroxidase, phenolase, proteinase and phosphatase. These enzymes are associated with breakdown of food reserves and biosynthesis of new tissues during germination (Copeland and McDonald, 2005).

2.8.5 Reduced respiration

Respiration becomes progressively weaker with seed deterioration and ultimately leads to loss of germination. Higher respiratory quotient (RQ) values are often observed in deteriorated seeds, which may be due to increased CO₂ evolution and reduced O₂ uptake. Reduction in respiration is related to a break down in membrane structure, particularly in the mitochondrial cristae (Copeland and McDonald, 2005).

2.8.6 Increases in seed leachates

An increase in electrical conductivity of seed during measurement of leachate concentration was related to low metabolic activity of seed, which is an indication of seed deterioration (Abdul-Baki and Anderson, 1972; Agarwal, 1980). According to Vashisth and Nagarajan (2009), electrical conductance of seed leachates were found to be low in fresh seeds which increases with the ageing process, due to loss of membrane integrity and leading to more loss of electrolytes into the imbibing medium.

2.8.7 Increase in free fatty acid content

According to Christensen et al., 1949, one of the major causes of breakdown of lipids to fatty acids was fungal invasion. Hummel et al., 1954 concluded that the increase in free fatty acids in wheat was due to the production of lipases by microflora. The continual accumulation of free fatty acids results in a reduction in cellular pH which is unfavourable to normal cellular metabolism (Earnshaw et al., 1970). According to Harrington (1973), the hydrolysis of phospholipids leads to the release of glycerol and fatty acids, and this reaction accelerates with increasing seed moisture content. Autooxidation of lipids and increase in the content of free fatty acids throughout storage period are the main reasons for rapid deterioration of seed (Balesevic-Tubic et al, 2005). The deterioration process in seeds occurs, especially under high moisture levels, high temperatures and microorganism infestation, which results in the formation of free fatty acids. Under such conditions, the level of free fatty acids can be suggested as an index of viability (Copeland and McDonald, 2012).
2.8.8 Changes in food reserves

Biochemical analysis of the *Bambusa bambos* seeds stored at different storage conditions showed qualitative and quantitative changes in food reserves specially sugars and proteins (Appasamy, 1993). Similarly, the studies on *Bambusa bambos* and *Dendrocalamus strictus* seeds indicated that with accelerated ageing the total content of food reserves viz., sugars, proteins and lipids were reduced. Increase in total free amino acids confirmed the degradation of seed reserves (Ravikumar *et al.*, 1998 and 2002). Depletion of food reserves is one of the oldest theories of seed deterioration (Copeland and McDonald, 2005).

2.8.9 Temperature and moisture

As a rule of thumb for orthodox seed, it has been suggested that one per cent reduction in moisture content or a 5°C reduction in temperature tends to double the seed longevity in storage (Harrington, 1972). Most of the biochemical and cytological deterioration takes place at high moisture content and temperature. Low temperature (<8-10°C) inactivates most seed insects and storage fungi. Seeds stored at moisture contents above 14 per cent, exhibit increased respiration, heating and fungal invasion that destroy seed viability more rapidly (Schmidt, 2000).

2.8.10 Presence of mycoflora

Most storage fungi attacking orthodox seeds belong to the genera *Aspergillus* and *Penicillium*. Insufficiently cleaned seeds are more susceptible to fungal infection than cleaned seeds (Christensen, 1973). Insects or mites infesting seeds generate heat and moisture by respiration which in turn promotes fungal activity. A natural ‘weak’ site is the chalazal region consisting of easily penetrable parenchyma tissue, through which fungi may invade (Christensen, 1973). Germination of fungal spores requires high humidity. Since spores germinate on the surface of the seed, humidity rather than seed moisture is the critical factor. Some fungal exudates cause damage to the cell membranes, others inhibit vital life processes of the germinating seeds. A moderate infection may reduce germination energy and affect embryo development during germination, eg. causing malformation or discolouration of the seedling (Christensen, 1973, Halloin, 1986). Fruit and seed may be subjected to fungal infection at any time of development but is often more abundant during the later stage of maturation, especially in areas where that stage of development coincides with the season of
high atmospheric humidity (rainy season). The vast majority of seed borne fungi are harmless saprophytes; only a small number are seed transmitted or seed pathogens (Schmidt, 2000).

2.9 Seed longevity and storage

Storage physiology of seeds seems to cover a more or less continuous spectrum, ranging from extremely recalcitrant, which loss viability in few days, to extremely orthodox the viability of which under optimal conditions counts in decades or centuries (Farrant et al., 1988). Seed longevity varies with species from one to eight months. Caryopsis and glans types of bamboo seeds can be stored by controlling moisture content and temperature (White, 1947; Gupta and Sood, 1978; Somen and Seethalakshmi, 1989; Sur et al., 1989; Thapliyal et al., 1991) but for bacca type no storage methods were found to be successful. *Bambusa bambos* seeds are generally viable for a period of 6-8 months.

Viability can be prolonged by adopting suitable storage conditions by controlling moisture content and temperature (White, 1947). Studies were carried out to understand the interrelation effects of temperature and moisture on seed germination of *Dendrocalamus strictus*. A temperature of 30°C and 50 to 75 % moisture level was recorded to be the optimum conditions for germination (Gupta and Kumar, 1977). According to Ramyarangsi (1990), seed viability can be extended by reducing the initial moisture content before storing. *Thrysostachys siamensis* seeds stored at low temperatures (2-4°C and -5°C) were able to maintain the viability for up to 27 months. In an experiment with *Bambusa tulda*, seeds stored with moisture content <10 % at ambient temperature maintained 50% viability after 12 months whereas all seeds stored at higher moisture content lost viability completely in less than four months (Thapliyal et al., 1991).

Varmah and Bahadur (1980) reported that viability of the seeds of *D. strictus* was extended up to 34 months by reducing moisture content to 8% by storing over silica gel or anhydrous calcium chloride in a desiccator, or at 3-5°C ambient temperature after reduction of its moisture content to 8 %. Studies of Banik, 1987 proved that in room temperature longevity period of *Bambusa tulda* was about 30-35 days, 55 days for *D. longispatus* and 65 days for *B. arundinacea var.spinosa*. Banik (1987), was able to increase the seed longevity of *Bambusa tulda* up to 18 months by storing over silica gel in a desiccator. Reports show that storing bamboo seeds over calcium chloride with a moisture content of 10-11 per cent were ideal for maintaining viability. The viability of seeds of *Bambusa bambos* and *B. tulda* was
extended by storing the seeds over calcium chloride at room temperature. Viability and germination of *Dendrocalamus strictus* seeds were found to improve by soaking and drying with low concentration of disodium hydrogen phosphate (Sur et al., 1988).

Storage of seeds of *Dendrocalamus strictus* over silica gel or anhydrous calcium chloride at low temperatures (3 – 5°C) after reducing the moisture content of the seeds to 8% increased the period of viability and recorded germination percentage of 51, 54 and 59 respectively after 34 months (Gupta and Sood, 1978). Somen and Seethalakshmi, 1989 studied the effect of different storage conditions on the viability of seeds of *Bambusa arundinacea*. Germination test using polyurethane sheet proved that seeds stored in room temperature over the laboratory shelf in a plastic container lost viability within 2 months. But the seeds stored with an initial moisture content of 11% over anhydrous calcium chloride in partially evacuated/non evacuated desiccators at room temperature were able to retain viability for more than a year. Boonarutee and Somboon (1990) reported that the *Dendrocalamus brandisii* seeds were able to retain viability for about 18 months by storing under low temperature (2-4°C) whereas seeds stored at 23-40°C storage temperature were not able to retain viability. A study conducted on impact of storage of seeds on the viability of *D. brandisii* revealed that seeds stored for a period of 30 days recorded maximum germination of 77.44%, while the minimum (20.25%) was recorded in seeds stored for 240 days. The occurrence of albinism was observed in the germinated seedlings (Thirtha et al., 2013).

Advanced storage conditions have increased the storage potential of the bamboo seeds. The seeds of *Bambusa arundinacea* were subjected to cryogenic preservation for one year. The germination of fresh seeds was 87.50% and was 65% after one year at -70°C (Midya, 1994).

Experiments conducted to prolong the viability of fleshy recalcitrant bamboo seeds by conventional storage methods were not successful. However in the fleshy seeds of *Melocanna baccifera*, viability was prolonged to 45 days when stored in an air-conditioned room, instead of 35 days in normal room conditions. The viability was prolonged up to 60 days when *M. baccifera seeds* mixed with dry sand, stored in jute bags. The seeds of *M. baccifera* can be carried with dry sand in jute bags during long distance transportation to minimise damage and to retain viability (Banik, 1991).
Storage studies in *Bambusa arundinacea* revealed that seed collection time greatly influences viability. Seeds collected after rains rapidly lose viability due to exposure to excess moisture. Desiccator drying of seeds was found to be more effective to retain viability than sun drying. At ambient storage, seeds were able to maintain viability only for six months. Moisture content of 1.90% and temperature of −5°C and 5°C, was found effective for storage (Warrier et al., 2004).

Seeds when sown soon after collections show more than 90 per cent germination and hence very limited work is available on pre-sowing treatments. Degluming of seeds was found to enhance germination (Karivaratharaju et al., 1987). In stored seeds of *Thrysostachys siamensis* and *Dendrocalamus strictus* low level of GA₃ (5 ppm) was found to enhance germination and IAA was found to enhance seedling vigour (Richa and Sharma, 1994).

Kiruba et al., 2007 had reported the traditional methods of *Bambusa arundinacea* seed storage followed by the tribals of Kanyakumari district. For storing large quantities of seeds huge earthen bins commonly known as Kulukkai were used. The mouth of Kulukkai were covered using mud and cowdung to protect against rodent attack. The leaves of *Azadirachta indica* and *Pongamia pinnata* were used as insect repellant. Similarly, small sized earthen bins called Manpanai hanged in the kitchen premises were used for storing small quantities of seeds.

2.10 Predicting seed deterioration

The ability to predict the seed longevity in long term storage would be extremely valuable for the effective management of gene bank accessions by setting appropriate regeneration periods. Seed viability equations which incorporate the effects of storage temperatures and seed moisture have been developed for many species (Roberts, 1960 and 1973). The general principle of longevity of orthodox seeds is that high quality seeds with low moisture stored under cool dry conditions maintained seed quality better than low quality seeds with high moisture stored under hot humid conditions. To utilize this principle, Ellis and Roberts (1980) developed the following seed viability equation:

\[ V = K i - p/\sigma \]

where \( \log \sigma = K_E - C_W \log_{10} m - C_H t - C_Q t^2 \)
where $V$ is probit percentage viability after $p$ days in storage at $m$ moisture content and temperature, $t$ ($^\circ$C), $K_i$ is the initial probit percentage viability of each seed lot before storage. $K_E$ is a species constant, which is equivalent to $\log \sigma$ at 1% moisture content and $0^\circ$C. $C_W$ indicates the logarithmic response of seed longevity to moisture content. $C_H$ and $C_Q$ are linear and quadratic terms, respectively, that describe the effect of storage temperature on longevity (Ellis et al., 1986). Use of the equation depends on the determination of the viability constants ($K_E, C_W, C_H$ and $C_Q$) which are species specific. Ellis and Roberts (1981) and Dickie et al., 1990, found that the relative effect of temperature on air-dry seed longevity in hermetic storage was similar for many orthodox seeds. The universal values of 0.0329 and 0.000478 for $C_H$ and $C_Q$, respectively, were suggested (Dickie et al., 1990).

Seed survival curves (percentage viability plotted against time) are cumulative normal distributions of negative slope, which become straight lines if the percentage values are transformed to probit. In such plots the slope of the curves is given by $1/\sigma$ (Finney, 1971). Based on this Andreoli and Ramiro, 2004 simplified the Ellis and Roberts equation and proposed a new model as follows:

$$Vt = Vi - (tg\beta)p$$

where the slope $tg\beta$ from equation is a direct measure of the slope $(1/\sigma)$ of the seed survival curves, therefore $tg\beta$ is the seed deterioration rate under any specific storage environment expressed by the angular coefficient of the survival curve, which corresponds to Ellis and Roberts (1980) equation ($V = K_i - p / \sigma$, where $\log \sigma = K_E - C_W \log_{10} m - C_H t - C_Q t^2$). Neither genotype nor seed quality affect the slope ($tg\beta$) of the simplified viability equation, it is only the intercept. $V_p$ is the probit viability at time $p$. $Vi$ is specific for each seed lot and is the mean of the initial quality.

The use of probit analysis for modelling the data from seed storage experiments was first described by Roberts (1972). Probit analysis is basically a regression analysis with probit viability (seed germination) as the dependent variable and time in storage as the independent variable. Roberts, 1972 also suggested different time periods such as $P_{50}, P_{75}, P_{60}$ and $P_{25}$ for determining the seed deterioration. $P_{50}$ is the commonly used time period and it is the time at which viability has fallen to 50 per cent.

Seed viability equation developed by Ellis and Roberts (1980) was applied to *Dendrocalamus membranaceus* seeds (Rawat and Thapliyal, 2003a). The storage behaviour of *Dendrocalamus*
membranaceus seeds were studied by using the seeds stored at three temperatures (5, 15 and 35°C) and three moisture levels (10.6, 7.1 and 5.3%). There was an increase in germination percentage with decrease in moisture and temperature. The association between storage temperature, seed moisture content and mean viability period was quantified and provisional constants were estimated for the improved viability equation. It was evident from the viability equation that the D. membranaceus seeds can be safely stored for around 45 years at ideal conditions.

2.11 Seed pests

Seeds are packages of high nutritive material like starch, protein and fat and thus attract large number of insects and pests. Insect pests reduce seed production causing major loss of seed crops and thus hinder the establishment of new plantations. For instance, the seed bug Udonga montana Distant was reported to cause damage in seeds of Bambusa polymorpha, B. bambos, Dendrocalamus strictus and Melocanna baccifera. Udonga montana is distributed in India, Bangladesh and Myanmar and is considered as a serious pest of bamboo seeds because they affects the seed production in natural stands by eating both developing and mature seeds. The sporadic outbreak of this bug has been reported by many authors (Beeson, 1941; Mathew and Sudheendrakumar, 1992). According to Wang et al., 1996 the bug attack usually occurs during flowering and at the end of flowering insect population declines due to lack of food and other adverse environmental factors. The bug population can be controlled by foliar spray of 0.25% fenitrothion. The natural way of control is the predation by birds (Wang et al., 1998). Mathew and Seethalakshmi (1998) reported Achroia grisella Fb. (Lepidoptera: Galleriidae) as a seed pest of the bamboo reed Ochlandra ebracteata, in natural forest stands in Kerala.

The pentatomid bug Ochrophara montana was reported in the flowering areas of Dendrocalamus strictus from central India, Chandrapur and adjoining areas during 1982-83. Foliar spray of 0.25 per cent fenitrothion or endosulfan was found to be effective in controlling Ochrophara attack (Singh and Bhandhari, 1988). The grain moth Sitotroga cereallela Oliver is a serious pest of stored bamboo seed. According to Beeson, 1941, when the infestation of Sitotroga occurs there is one larva per seed, and the life-cycle is completed within the seed. Fumigating seeds with carbon disulphide or Methyl bromide is effective in controlling the grain moth (Wang et al., 1998).
2.12 Seed mycoflora

Large quantities of bamboo seeds are lost during their development stages and after attaining maturity due to the infection by different seed-borne fungi. The deterioration of seeds during storage is also due to microbial infection. There are many reports from India, Japan and Thailand about the fungal infections such as smut and ergot on the inflorescences of bamboos. Smut infection on the seeds of *Bambusa bambos* and *Bambusa* sp. has been reported from Uttar Pradesh, India. The causal organisms are *Ustilago shiraiana* P. Henn. and *Tilletia Bambusae* Thirum. & Pavgi (Mundkur and Thirumalachar, 1952; Thirumalachar and Pavgi, 1952). Severe infection results in the complete destruction of seeds leaving a fungal spore mass (Thirumalachar and Pavgi, 1952). Slightly infested seeds are able to reach maturity, but they do not produce healthy seedlings. There are reports from Japan on the smut affecting *Phyllostachys heterocla* var. *pubescens*, *Sasa nana* Mak. and *S. ramosa* Mak. et Shib. (Hori, 1905 and Zhu, 1989). Control of smut fungi is possible by the application of systemic fungicides such as carboxin, thiabendazole, etaconazole and also by planting rust resistant species (Mohanan, 1997).

The ergot disease attacking the developing spikelets of *Phyllostachys* spp. and *Dendrocalamus hamiltonii* has been reported from India, Japan and Thailand (Spaulding, 1961; Zhu, 1989; Mohanan, 1995). The causal organisms are *Claviceps purpurea* (Fr) Tul. (Zhu, 1989); *Claviceps* sp. and *Hypocreopsis phyllostachydis* and *Hypocrella semiamplexa* (Berk.) Sacc. (Berkeley, 1856; Spaulding, 1961). The fungi attack the developing spikelets and replace the seeds with a hard mass of fungal mycelium, which ultimately forms the distinctive ergot sclerotium. This disease can be controlled by proper cultural and sanitary measures (Mohanan, 1997). Several fungal species have been recorded on seeds of *Bambusa bambos*, *B. nutans*, *Dendrocalamus strictus*, *Gigantochloa hasskarliana* (Kurz) Backer ex Heyne, *Thrysostachys siamensis* in Thailand (Chalermpongse et al., 1984; Pongpanich and Chalermpongse, 1986; Anantachote, 1987; Pongpanich, 1990) and in *B. bambos* and *D. strictus* in India (Namdeo et al., 1989; Mohanan, 1990). The majority of fungi recorded were storage fungi, which influence the viability of seeds, while others were seed-borne. *Bipolaris* sp., *Exserohilum* sp., *Fusarium pallidoroseum*, *Drechslela* sp. and *Phomopsis* sp. are the common seed-borne fungi that cause seedling infection in bamboos (Mohanan, 1990). Severe *Bipolaris* and *Exserohilum* infection cause seedling deformity at the hilum region of the seed (Mohanan, 1990).
In India and Thailand a total of 65 fungi belonging to 37 genera and two bacteria have been reported on stored seeds of bamboos (Mohanan, 1997). Studies on the seed characteristics of *Bambusa arundinacea* and *Dendrocalamus strictus* carried out revealed the presence of a large number of potential pathogens on the seeds (Mohanan, 1997). Mycofloral studies were conducted in the seeds of *B. arundinacea* stored for four months in closed containers at 15°C and 28± 2°C and seeds of *D. strictus* stored for the same period at 28± 2°C. It was found that fungal species and their frequency of occurrence were more in the seeds of *D. strictus* than those of *B. arundinacea*. The incidence of seed microflora was low in seeds stored at low temperature (15°C) as compared to those stored at 28± 2°C whereas germination percentage was higher in low temperature (82%) when compared to the other (63%). The fungal species and their percentage of occurrence in *Bambusa arundinacea* are *Alternaria* spp.(10.5), *Aspergillus* spp.(4), *Beltraniopsis* spp.(2.5), *Cercospora* sp.(2), *Chaetomium* sp.(3), *Cladosporium* spp.(6), *Curvularia* spp.(11), *Drechslera* spp.(23), *Dactylaria* spp.(6), *Fusarium* spp.(24), *Mennoniella* sp.(2), *Mucor* sp.(0.5), *Nigrospora* sp.(1.5), *Penicillium* sp.(4.5) and *Periconia* sp.(0.5). The fungal species and their percentage of occurrence in *Dendrocalamus strictus* are *Alternaria* spp.(9.5), *Aspergillus* spp.(5.5), *Cercospora* sp.(3.5), *Chaetomium* sp.(5.5), *Cladosporium* spp.(7), *Curvularia* spp.(16.5), *Drechslera* spp.(24.5), *Dactylaria* spp.(8.5), *Epicoccum* spp.(4.5), *Fusarium* spp.(29.5), *Mennoniella* sp.(0.5), *Mucor* sp.(2.5), *Nigrospora* sp.(3), *Penicillium* sp.(6.5), *Periconia* sp.(1), *Phoma* sp.(2), *Phomopsis* sp.(1) and *Pithomyces* sp.(1.5) (Mohanan, 1997).

Studies conducted in the seeds of *B. arundinacea*, *B. nutans*, *Dendrocalamus strictus*, *G. hasskarliana* and *Thyrsostachys siamensis* recorded the occurrence of about 41 fungal species including several seed-borne pathogens such as *Alternaria alternata*, *Ascochyta* sp., *Curvularia lunata*, *Fusarium semitectum* Berk. & Rav., *Myrothecium* sp., *Nigrospora oryza* (Berk. & Br.) Petch, *Phoma* sp., *Phomopsis* sp., *Stemphylium* sp. and *Trichoconis padwickii* Ganguly (Anantachote, 1987). Infection of *Cladosporium graminium* on *Dendrocalamus strictus* seeds was reported. The fungus attack seed between collection and sowing time affecting the germination capacity. Sun drying and storing the seeds in a dry place can effectively control *Cladosporium graminium* attack (Mohanan, 1990). Fungal attack can be controlled naturally by collecting seeds immediately after the seed fall. Seed treatment using fungicides such as Mancozeb, Ceresan D, Hexathir WP, Vitavax 70 WP @4g/kg of seeds is suggested for short term storage of seeds (Mohanan, 1997). About 48 species of seed-borne fungi were reported from Thailand in the seeds of *Dendrocalamus strictus*, *D. giganteus*, *D.*

According to Anantachote (1987), there were more fungal species on bamboo seeds when compared with the other forest tree seeds. Standardizing the time of seed collection, cleaning processes, storage conditions, and duration of bamboo seed storage would help in preventing fungal attack and developing more bamboo plantations in the future.

2.13 Nursery studies

According to Banik (1991) the germination media consisting of soil and cow dung in 3:1 ratio is ideal for the germination of bamboo seeds. Experiments conducted in Terai Zone of West Bengal for the standardization of growing media and seed size for germination and seedling growth of Melocanna baccifera concluded that an equal proportion of soil, sand and FYM (Farmyard manure) gave significantly better germination and seedling growth (Shukla et al., 2010).

Chacko and Jayaraman, 1990 studied the effect of container size (13 × 18 and 18 × 40 cm) on the growth of Bambusa arundinacea seedlings. An increase of 581, 212, 177, 170, 111 and 60 per cent in root biomass, total biomass, shoot biomass, root length, length of longest root and the number of roots was observed in seedlings of larger container. In smaller container the shoot-root-rhizome biomass ratio was 75:10:15, whereas in larger containers it was 65:22:13. There was no significant effect of container size on the number of shoots and the height of the plants.
Seethalakshmi et al., 2008 studied the seed and seedling attributes of two commercially important bamboo species viz. *Ochlandra travancorica* and *Melocanna baccifera*. Results indicated that the growth and biomass accumulation was more for *M. baccifera* than *O. travancorica*. But there was no significant difference in the relative growth rate (RGR) of the two species, while net assimilation rate (NAR) was higher for *M. baccifera*.

In general, even though considerable research has been done for different species of bamboos on development of both macro and micro-propagation protocols like rooting of cuttings, tissue culture methods etc, the studies on seed handling techniques are scanty due to the long flowering intervals and short viability of seeds. Seed handling technologies for many priority species of bamboos are yet to be standardized. Seed size and type vary with species. During last couple of years many bamboo species are flowering in different parts of the country. Several bamboo species are viable replacement for wood, as industrial raw material for traditional and modern sectors. Premature death of the plantations adversely affects the industries which depend on bamboo raw material. This can be avoided if plantations are raised through seedlings. Exchange of seeds is necessary between different states. Many samples sent using the conventional methods are not viable when it reached the users due to several problems like chemical degradation, fungal and insect infestations. Proper planning of the time of seed collection, processing and effective storage methods can reduce the seed deterioration and healthy seedlings can be raised for establishing vigorous plantations.