



## AN APPRAISAL OF PRODIGAL LOSS OF VIGOUR AND VIABILITY OF BAMBOO SEEDS

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Article Received on 14/06/2017

Article Revised on 04/07/2017

Article Accepted on 24/07/2017

### ABSTRACT

Seeds in general play a vital role in man's life since they serve as a source of food, fibre, spices, beverages, oils and drugs. Seeds of cereals contribute about 90% of all the cultivated plants, as the source of up to half of global per capita energy. Bamboo seeds, have very short viability of 1-3 months and are therefore useful as propagules for only a short period of time. Seed deterioration is inevitable and the best that can be done is to control its rate. Seed ageing is affected by a number of factors during seed storage. However, there is very little information on the physiology and biochemistry of seed viability with ageing of bamboo seeds. The speed at which the seed ageing process undergoes depends on the seeds' ability to counterattack the break down changes as well as on species-specific protection mechanisms. In seed ageing, damage to cellular membranes, decrease in sugar, lipid, amino acid, proteins and hormonal contents' activities. Aged seeds show decreased vigour and produce weak seedlings that are unable to survive. Sometimes, non-availability of quality seed is also one of the major snag in enhancing the productivity.

**KEYWORDS:** seed, ageing, storage, vigour, biochemical changes.

### INTRODUCTION

The present review was undertaken with an aim to appraise the physiological and biochemical factors (i.e. metabolites, enzymes, membrane integrity and levels of growth hormones) that lead to loss of seed viability during storage. The knowledge so generated would be helpful in devising techniques for enhancement of vigour and viability of ageing bamboo seeds. Because, most of the bamboos flower at long intervals of 7-20 years. Bamboos comprise the most diverse group of plants in the grass family. They are distinguished from other members of the family by having woody culms, complex branching, a complex and generally robust rhizome system and infrequent flowering. Bamboos are plants of global interest because of their distinctive life form, ecological importance and the wide range of uses and values they have for humans (Bystriakova et al., 2004). They have been variously called as 'The Cradle to Coffin Plant', 'The Poor man's Timber', 'Friend of the People', 'Green Gasoline', 'The Plant with Thousand Faces' and 'The Green Gold'. There are more than 1500 different documented traditional uses of bamboo (INBAR, 1997). The seeds in bamboos are therefore scarcely available. This cyclic flowering in bamboos is gregarious and produces huge quantities of viable seeds. Bamboo seeds have very short viability of 1-3 months and are therefore useful as propagules for only a short period of time.

Several studies on tropical bamboo seeds have reported prompt and high germination rates upon hydration (often around 80%) and/or marked declines in seed viability within a matter of 1-2 months (Banik, 1994; Ravikumar et al., 1998a, b; Koshy and Harikumar, 2001; Rawat and Thapliyal, 2003).

### SEED VIABILITY AND LONGEVITY

Seeds, like any other plant organ, age with time and consequently die. However, the rate at which seeds age depends upon their physiological status, their genetic constitution, and the storage conditions. The availability of an adequate supply of seeds of a uniform high quality is essential for a successful seed industry and the maintenance of a viable and productive agriculture (Barens, 1986). Roberts (1973) recognized two types of seeds on the basis of their storage behaviour viz., orthodox and recalcitrant. More recently, a third category (still undefined), intermediate between the orthodox and recalcitrant categories, has also been identified (Ellis *et al.*, 1990). Orthodox seeds are characterized by their ability to tolerate desiccation and to retain their viability for a long time in the dry state. However, these seeds age during storage and eventually lose their ability to germinate. Lipid peroxidation and the loss of membrane phospholipids are major causes of seed ageing under natural ageing conditions (Wilson and McDonald, 1986;

McDonald, 1999), several studies of long-term storage detected little or no lipid peroxidation and loss of phospholipids from seeds of rice (Matsuda and Hirayama, 1973), soybean (Priestley and Leopold, 1983), and wheat (Petruzzelli and Taranto, 1984).

Under the long-term storage conditions, seeds are likely to be in the glassy state (A glass is an amorphous, non-equilibrium solid and is characterized by its extremely high viscosity. High levels of sugars and other biopolymers in seeds result in a rapid increase in cytoplasmic viscosity during drying, which prevents the cellular biological system from reaching physical and chemical equilibrium in a measurable time frame (Sun and Leopold, 1993)) because of the cool storage environment and low seed water content. The extremely high viscosity and low molecular mobility of the seed cytoplasm could prevent or inhibit many deleterious processes (Williams and Leopold, 1989; Sun and Leopold, 1997; Leopold *et al.*, 1994; Sun *et al.*, 1998).

### PHYSIOLOGY OF SEED GERMINATION

The seed is a propagule by which a seed-bearing plant is dispersed and propagated (Bewley and Black, 1994). The seed also provides protection and nutrition for the quiescent embryo (Taiz and Zeiger, 2002). Bewley (1997) described seed as a critical phase in the life of plants, as the time, place, and nutrient reserves available upon germination mainly determine the successful establishment of plant. According to Bewley and Black (1983), germination is traditionally divided into three basic phases: a) imbibition, the absorption of water needed for hydration of proteins and cell organelles, as well as a substrate for hydrolytic reactions; b) activation of metabolism, involving synthesis of nucleic acids and proteins, increase in enzyme and respiratory activities and initial reserve breakdown (this step was called “*germination sensu stricto*” by Come and Corbineau, 1989); and c) visible growth, usually in the form of root protrusion (Bewley and Black, 1994). Mobilization of reserves during germination is a catabolic process in which the reserve food material is broken down with the help of enzymes and utilized for repair and growth mechanisms by the seed (Bewley and Black, 1994; Job *et al.*, 1997; Gallardo *et al.*, 2001).

Following imbibition and under the control of signals, particularly GA (Gibberellic acid) from the embryo and scutellum, the cells of aleurone layer synthesize an array of hydrolytic enzymes that are transported into the endosperm. These hydrolytic enzymes include  $\alpha$ -amylase,  $\beta$ -amylase and de-branching enzymes.  $\alpha$ -amylase is synthesized *de novo* (Filner and Varner, 1967), probably from amino acids released by proteolysis of storage proteins in the aleurone grains. This enzyme cleaves the internal  $\alpha$ -1, 4-linked bonds of the glycan chains, releasing shorter amylose chains that are then further hydrolyzed to maltose (a disaccharide) by  $\alpha$ -amylase. On the other hand, in dicots, the degradation of starch yields glucose and maltotriose

(Bewley and Black, 1978). The majority of  $\beta$ -amylase is already present in the endosperm in an inactive form in the quiescent grains, but later during germination it is converted into active state by GA induced proteinases from aleurone layer. It cannot hydrolyze native starch grains until they are broken down into large dextrans by  $\alpha$ -amylolytic attack. Nandi *et al.* (1995) reported that long-lived  $\beta$ -amylase plays an important role in starch degradation and helps in initiating early embryo growth. Both  $\alpha$  and  $\beta$ -amylase are unable to hydrolyse  $\alpha$ -1,6 bonds in the branch points in amylopectin. Specific, de-branching enzymes are required to hydrolyze these bonds and release additional amylose chains for further degradation. The  $\alpha$ -glucosidase, limit dextrinase and cell wall hydrolases are synthesized by the aleurone layer and transported along with  $\alpha$ -amylase into the starchy endosperm. In some seeds, the major carbohydrate reserves are in the form of cell wall galacto-mannans and the corresponding hydrolytic enzymes are endo- $\beta$ -mannanase,  $\beta$ -mannosidase and  $\alpha$ -galactosidase (de Miguel *et al.*, 2000; Feurtado *et al.*, 2001; Mo and Bewley, 2002; Adebisi *et al.*, 2008).

In cereals, the protein reserves are stored in two separate sites viz. in the aleurone grains of aleurone layer (about 20%) and in the protein bodies of the endosperm (about 70%). Hydrolysis of storage proteins into amino acids or smaller peptides is carried out by proteinases/proteases (Muntz *et al.*, 2001). The free amino acids released are utilized for protein synthesis or transported to the growing axis. Protein degradation in seed storage tissues during germination does not occur at once in the entire organ. The region where degradation starts varies from species to species (Asghar and DeMason, 1990; Dias *et al.*, 1993).

### EFFECT OF STORAGE ON SEED VIABILITY

The fact that seeds of most species can be dried and stored from year-to-year has been exploited by man since the beginning of agriculture. Indeed, the ability of many orthodox seeds to remain viable for tens or hundreds of years in dry storage (Walters *et al.*, 2005; Daws *et al.*, 2007), indicates that they can be used for the long-term *ex situ* conservation of plant germplasm. Though the causes of deterioration of seed viability during storage has not been fully understood, scientists relate it to bioenergetics disturbance (Ching, 1982), damage to nucleic acids (Cheah and Osborne, 1978), loss of vitamins and hormones (Bewley and Black, 1982; Richa *et al.*, 2000; 2006), and membrane deterioration (Wilson and McDonald, 1986; Richa *et al.*, 2006; 2010). Several comprehensive reports have shown that loss of seed vigour and viability is associated with free radical-mediated lipid peroxidation, enzyme inactivation or protein degradation, disruption of cellular membranes, Maillard reactions and oxidative damage to genetic (nucleic acids) integrity (VanBilsen *et al.*, 1994; Smith and Berjak, 1995; Walters, 1998; McDonald, 1999).

A number of studies have reported potential correlation of seed longevity in dry storage with seed mass, oil content, carbohydrate composition and climate (Horbowicz and Obendorf, 1994; Walters *et al.*, 2005; Richa *et al.*, 2010). However, a purported link between high oil content and short storage life-span has not been supported by recent analyses (Walters *et al.*, 2005). Ravikumar *et al.* (1998a) showed that reducing the moisture content of *Dendrocalamus strictus* seeds to 8.4% and storing them in wax paper bags helped maintain seed viability. Rapid loss in viability of seeds occurred within 5-months under ambient conditions (25-34°C) whereas under vacuum (CaCl<sub>2</sub> in a desiccator at 25-34°C) or cold storage (10°C) conditions the deterioration was gradual. Seeds stored at low temperature i.e. 0 to 5°C showed highest viability percentage after 9-months. Seethalakshmi (1991) suggested two methods for the storage of bamboo seeds viz. cold storage and storing seeds over desiccants like calcium chloride at room temperature and the later was reported to be most effective. Warriar *et al.* (2004) studied that storage of wet seeds of *Bambusa arundinacea* also poses problems. Desiccator drying of seeds was found to retain viability while sun drying proved detrimental. Moisture content of seeds could be reduced to as low as 1.90% for effective storage.

#### MEMBRANE INTEGRITY AND SEED AGEING

Biological membranes with a normal composition and organization regulate the transport of materials into and out of the cell. Therefore, they play a key role in maintaining seed viability and vigour. Solute leakage accompanies seed imbibition during the process of membrane reorganization following rehydration. The rate of leakage depends on the degree of cell membrane damage and repair in response to ageing may constitute an important factor in explaining seed deterioration (Priestley and Leopold, 1979; Senaratna *et al.*, 1988; Ferguson *et al.*, 1990). In seed ageing, damage to cellular membranes, decrease in mitochondrial dehydrogenases activities, chromosomal aberration and DNA degradation increases (Parrish and Leopold, 1978). Electrical conductivity measurements of seed leachates are routinely used to determine seed vigour in a number of species (Pandey, 1992; Hampton and TeKrony, 1995). Ion leakage (e.g., K<sup>+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>, and Mn<sup>2+</sup>) has been shown to relate to seed viability and vigour (Dias *et al.*, 1996; Rehman *et al.*, 1999). Leakage of sugars is considered a less reliable index of membrane integrity than the leakage of electrolytes (Simon, 1974). It is also not clear if sugar leakage from the embryo and endosperm are equally important for seed vigour.

#### METABOLIC CHANGES DURING SEED AGEING

Seed ageing is a natural phenomenon which occurs in all seeds, even if they are stored in dry and low temperature rooms (Machado Neto *et al.*, 2001). The main factors affecting seed ageing are the temperature and relative humidity at which the seeds are stored, the moisture content of the seeds and the seed quality. Generally, high

moisture levels and temperature reduce seed longevity and cause profound deteriorative biochemical changes in seed membrane, DNA and food reserves (Smith and Berjak, 1995; McDonald, 1999; Walters, 1998). Time and again researchers have correlated seed ageing with biochemical changes such as alteration in protein synthesis (Dell'Aquila and Margiotta, 1986; Dell'Aquila and Bewley, 1989); degradation of DNA and RNA (Wilson and McDonald, 1986); degradation of hydrogen peroxide detoxification pathway (Reuzeau and Cavalie, 1995); deterioration in membrane properties (Parrish and Leopold, 1978; Pukacka, 1991).

#### EFFECT OF AGEING ON ENZYME ACTIVITY

Seed deterioration has been associated with chromosome aberrations and changes in RNA synthesis, in proteins and then enzymes. Incomplete protein synthesis occurs due to DNA degradation that impairs the transcription and translation process (McDonald, 1999). There have been reports of differential respiratory and enzymatic activity with ATP production and membrane alterations. A scrutiny of literature suggests that cellular and physiological aberrations are a main cause of loss of viability during seed ageing. Smith and Berjak (1995) showed that with ageing the membrane of the seed become leaky, enzymes lose catalytic activity and chromosomes accumulate mutations. VanBilsen *et al.* (1994) also reported that membranes become more susceptible to imbibition damage with ageing.

Commonly, seed deterioration is reported to accompany changes in enzyme activity during ageing (Richa *et al.*, 2006; Lehner *et al.*, 2008; Afzal *et al.*, 2009; Singh *et al.*, 2010). Although seed deterioration is generally accompanied by loss of enzyme activity (Roberts, 1973, 1979), a few hydrolytic enzymes like  $\alpha$ -amylase and proteases show an increase in their activity (Basavarajappa *et al.*, 1991). Several workers have shown a decrease in amylase activity with ageing (Das and Sen-Mandi, 1992; Richa *et al.*, 2006; 2010; Afzal *et al.*, 2009; Singh *et al.*, 2010). Saxena *et al.* (1985) reported that enzymes catalase, peroxidase and total dehydrogenase showed a decline in activity in aged seeds of sesame (*Sesamum indicum*) subjected to accelerated ageing at 45°C and 10% RH, while invertase, RNA-ase and acid phosphatase showed an increase in their activity with ageing. However, with further increase in age, these enzymes showed a decrease in their activities. Kannababu and Karivaratharaju (2000) reported that accelerated ageing of sunflower seeds showed a decrease in activity of malate dehydrogenase and succinate dehydrogenase in both cotyledons and embryonic axis of germinating seedlings of sunflower (*Helianthus annuus*).

#### EFFECT OF AGEING ON ANTIOXIDANT SYSTEM OF SEEDS

The ascorbic acid (ASC) system functions dynamically in seeds, although the strategies for ASC production and utilization may vary according to seed developmental and functional stages. ASC has been considered almost

uniquely for its antioxidant properties (De Tullio, and Arrigoni, 2003; Pukacka and Ratajczak, 2007), since ASC can react with reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radical and hydroxyl radicals in non-enzymatic reactions. It is now clear that ASC also has a paramount role in both animal and plant cells as a co-substrate necessary for the activity of many 2-oxoacid-dependent dioxygenases (Prescott and John, 1996; Arrigoni and De Tullio, 2000, 2002; Pastori *et al.*, 2003). Plant systems resist toxic oxygen species based on the presence of reduced molecules such as glutathione, ascorbate, enzymes such as superoxide dismutase (SOD), catalase, glutathione reductase (GR) and ascorbate peroxidase (APX). Thiols are first affected by oxidation due to the presence of sulfhydryl groups. Reduced glutathione (GSH) is a major non-protein thiol that plays an important role in storage, transport and maintenance of the redox status in cells (De Tullio, and Arrigoni, 2003; Pukacka and Ratajczak, 2007). Klapheck *et al.* (1990) reported the role of GSH in germinating castor beans during oxidative stress as it degraded H<sub>2</sub>O<sub>2</sub>. Early products of radical-mediated reactions *in vitro* can be detected by electron paramagnetic resonance (EPR) studies. Less direct evidence comes from measurement of specific activities of enzymes such as SOD, GR, APX, etc. EPR studies of high and low vigour dry embryos of rice seeds stored in a warm and humid environment were tested for the presence of free radicals by Nandi *et al.* (1997). The results indicated that high vigour unaged embryos possessed high activity for the antioxidant enzymes, SOD and POX.

It was observed that the balance between free radical/oxidative chain products and the integrity of active oxygen-scavenging enzymes present in dry embryos determined the fate of membranes and macromolecules during imbibition and early germination. Since ASC is known as an antioxidant and APX is known to catalyse the removal of hydrogen peroxide, much attention has been given to their possible involvement in the mechanism of seed defence against oxidative stress occurring during desiccation (De Gara *et al.*, 2003). However, this is not consistent with the fact that both ASC and APX activity decrease during the desiccation stage (Arrigoni *et al.*, 1992; De Gara *et al.*, 2003). APX protein content in barley is associated with early grain filling and then typically decreases during desiccation (Finnie *et al.*, 2002).

#### **EFFECT OF AGEING ON SEED MEMBRANE PHOSPHOLIPIDS**

A perusal of the literature suggests that there may be several mechanisms of seed ageing (Walters, 1998; Walters *et al.*, 2004; Pukacka and Ratajczak, 2007; Rajjou and Debeaujon, 2008). Although lipid peroxidation and the loss of membrane phospholipids are regarded as major causes of seed ageing (Wilson and McDonald, 1986; McDonald, 1999), yet several studies of long-term storage detected little or no lipid peroxidation and loss of phospholipids from seeds of

soybean (Priestley and Leopold, 1983), and wheat (Petruzzelli and Taranto, 1984). Although the longevity of seeds is enhanced by storage under dry conditions, eventually the seeds deteriorate and lose the ability to germinate. One of the most commonly cited hypotheses explaining seed deterioration points to lipid peroxidation as the mechanism by which cellular membranes are disrupted (Priestley and Leopold, 1983; Wilson and McDonald, 1986; McKersie *et al.*, 1988; Ferguson *et al.*, 1990). There are many types of peroxidative reactions in which lipids serve as substrates, but the most commonly accepted view involve breakage of the ester linkage between the acyl chain and the glycerol backbone (McKersie *et al.*, 1988) or attack of unsaturated bonds of the fatty acid chain (Chan, 1987). Both triglycerides and polar lipids are subject to these reactions, and if these reactions occurred, the chemistry of the lipid components of the seeds would change. Unfortunately, studies on the changes in lipid chemistry with seed deterioration have produced mixed results (McKersie *et al.*, 1988; Ferguson *et al.*, 1990), and a consensus regarding the importance of lipid peroxidation in seed deterioration has not been reached. Cellular membranes have been proposed as some of the primary sites of injury during desiccation and storage of seeds (Pukacka, 1991; McDonald, 1999; Bailly, 2004). This is mediated by an oxidative attack which promotes phospholipid degradation and loss of membrane organization (Ratajczak and Pukacka, 2005).

It is unlikely that changes observed in the triglycerides are responsible for changes in seed viability; yet, it is possible that membrane lipids are susceptible to the same reactions. It has been suggested that changes in membrane lipids are involved in the loss of viability of stored seeds (Ferguson *et al.*, 1990; Liu *et al.*, 2006; Sital *et al.*, 2008; Singh *et al.*, 2010) but measurements of the changes in the properties of membranes *in vivo* have not been possible using this technique. The mechanism by which the physical properties of storage lipids change is unknown.

#### **EFFECT OF AGEING ON SEED MEMBRANE PROTEINS**

Ultrastructural studies on seeds have distinguished between dry and imbibed seed tissues of soybean (Chabot and Leopold, 1982). Chabot and Leopold (1982) studied characteristics of the organelles and cells under stress of chilling injury in soybean used electron microscopic (EM) studies (Chabot and Leopold, 1985). Most studies have focused on storage tissues such as cotyledons in soybean (Chabot and Leopold, 1985). Studies by Yaklich *et al.* (2001) on soybean seed anatomy using transmission electron microscopy (TEM) featured testa and the phloem and xylem of the vascular sutures in the soybean pod. It has been observed that protein oxidation can cause modification of amino acid side chains, backbone fragmentation, protein dimerization or aggregation, and the unfolding or altered conformation of proteins (Hawkins and Davies, 2001). These structural changes alter the functional activities of

the modified proteins such as their ability to modulate gene expression, cell signalling, apoptosis, and necrosis. Reactive intermediates from protein peroxides can induce chain reactions that cause damage to other intracellular targets such as DNA, lipids, and other proteins (McDonald, 1999). Protein modifications are often associated with ageing and diseases (Stadtman, 1992). However, protein oxidation may provide a means by which reactive oxygen species are utilized or counteracted e.g. the restoration of metabolic activities following imbibition of mature dry seeds (Job *et al.*, 2005; Khan *et al.*, 2005; Bedi *et al.*, 2006; Kaewnaree *et al.*, 2008). A major cause of deterioration in these seeds is the process that could contribute to the 'cascade' of changes associated with the execution phase of cell death (Kaewnaree *et al.*, 2008; Afzal *et al.*, 2009; Singh *et al.*, 2010).

### EFFECT OF AGEING ON CHANGES IN ENDOGENOUS GROWTH HORMONES

Plant hormones and growth regulators are the chemicals that affect flowering; ageing; root growth; distortion and killing of leaves, stems, and other parts; prevention or promotion of stem elongation; colour enhancement of fruit; prevention of leafing and/or leaf fall; and many other conditions. Even small quantities of these substances produce major growth changes. Growth regulators are known to modify the growth and development pattern of plants by exerting profound effect on various physiological processes and hence regulating the productivity (Clifford *et al.*, 1986; Brenner, 1987; Patrick, 1988; Kucera *et al.*, 2005).

Briggs (1973) suggested that GA<sub>1</sub> and GA<sub>3</sub> are the major gibberellins produced by the germinating embryo though GA<sub>4</sub> and GA<sub>7</sub> were also detected. Further it was suggested that GA<sub>3</sub> and GA<sub>7</sub> activate the aleurone cells where as GA<sub>1</sub> and GA<sub>4</sub> control the embryo growth. Other effective gibberellins are GA<sub>2</sub> and GA<sub>22</sub> while some such as GA<sub>12</sub>, GA<sub>17</sub>, and GA<sub>26</sub> did not have any promotive action. A major role of endogenous GAs in the control of seed germination has also been emphasized by Karssen *et al.* (1989).

Although it is well established that gibberellins (GAs) and abscisic acid (ABA) regulate amylase synthesis in the cereal aleurone layer, very little is known as to how GA and ABA affect amylase synthesis and secretion by the scutellum (Mrva and Mares, 1999). Studies on the expression of  $\alpha$ -amylase genes in barley and rice scutella (Kucera *et al.*, 2005; Huarte and Benech-Arnold, 2010) however, indicate that the mechanism of their regulation is similar to that for the corresponding genes in aleurone layers. Along with regulating the synthesis of secreted hydrolases, ABA and GA influence other functions of aleurone layers and scutella that relate to germination. The aleurone layer is the principal store of mineral elements in the grain of small cereals where K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup> are stored in the vacuole as chelates of phytic acid (Bethke *et al.*, 1998). GA stimulates the synthesis of

phytase, and phytate break down makes cations and phosphate available to the embryo. The viability of the cereal aleurone layer is also tightly regulated by ABA and GA. Haberlandt (1884) was among the first to report that cells of the aleurone layer die after reserves in the endosperm have been mobilized (Fath *et al.*, 2001). ABA promotes aleurone cell viability and GA promotes aleurone cell death (Wang *et al.*, 1998).

Kong *et al.* (1997) identified and quantified endogenous free ABA, IAA and GAs in the whole white spruce (*Picea glauca*) seeds. It was reported that ABA content was high at the early stage of embryo development. Levels of IAA declined in the mega-gametophytes after pollination and through the seed development. Levels of GA<sub>4</sub> slightly decreased while GA<sub>9</sub> increased during this period. Kojima (2005) determined the endogenous level of IAA and ABA in tomato (*Lycopersicon esculentum*). It was found that the concentration of IAA was higher in the symplast (SP) solution than in the apoplast (AP) solution in both upper and lower parts of stems, suggesting that polar IAA transport might be only 19 % of the amount of IAA in stems. Concentration of ABA was high in the pericarp, axis and the locule tissue in the fruits. Warda (2010) reported large amount of GA<sub>3</sub>-like substances and ABA in the developing seeds of cucumber (*Cucumis sativa*). Munoz *et al.* (1990) studied the role of endogenous CKs on reserve mobilization in cotyledons of *Cicer arietinum*. He suggested that CKs are concerned with the metabolism of carbohydrates and proteins. Dewar *et al.* (1998) used HPLC to assay the amount of CKs-Zeatin (Z), Z. reboiside (ZR) and isopentenyladenine (IPA) and combined amounts of GA<sub>1</sub>, GA<sub>3</sub>, IAA and ABA during germination in sorghum. He reported higher concentration endogenous ABA in the embryo prior to germination.

No study has been conducted on the endogenous hormones in bamboo seeds except for the only work by Richa *et al.* (2006), who studied the endogenous levels of IAA and ABA in five bamboo species viz. *Bambusa bambos*, *Dendrocalamus membranaceus*, *Gigantochloa albociliata*, *Thyrsotachys siamensis* and *Dendrocalamus strictus*. These authors reported an increase in the levels of free ABA in all the five bamboo species, with maximum amount in *Dendrocalamus strictus* and minimum in *Bambusa bambos*, after 12 months of storage. A significant decline in the endogenous levels of IAA was also observed in *D. strictus*, which also showed maximum decline in viability.

### CONCLUSION

From the above discussion, it can be concluded that bamboo seeds undergo age-induced biochemical and physiological changes, similar to that of cereals. Age-induced deterioration brings about membrane damage, causes leakage of reserve food material and enzyme degradation. Change in the optimum levels of plant hormones was also discussed. Seed viability could be retained for a greater period by storing the seeds under

controlled conditions. The decline in vigour and viability of seeds is recoverable to some extent by the appropriate application of various seed invigoration treatments.

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