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2. REVIEW OF LITERATURE

Most bamboos flower at long intervals of 7-20 years. The seeds in bamboos are therefore scarcely available. This cyclic flowering in bamboos is gregarious and produces huge quantities of viable seeds. 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 (Venkatesh, 1984; Azmy, 1994; Banik, 1994; Ravikumar et al., 1998a, b; Koshy and Harikumar, 2001; Rawat and Thapliyal, 2003). There is very little information on the physiology and biochemistry of seed viability and vigour enhancement of ageing bamboo seeds. In what follows, a brief review of the available literature relevant to this study is presented under the following heads:

2.1. Seed viability and longevity

2.2. Physiology of seed germination.

2.3. Effect of storage on seed viability and longevity.

2.4. Effect of storage temperature and seed moisture on viability of stored seeds.

2.5. Membrane integrity and seed ageing.

2.6. Metabolic changes during seed ageing.

2.7. Effect of ageing on enzyme activity

2.8. Effect of ageing on ascorbate system of seeds

2.9. Effect of ageing on seed membrane phospholipids

2.10. Effect of ageing on seed membrane proteins

2.11. Effect of ageing on changes in endogenous growth hormones.

2.12. Seed vigour.

2.13. Seed vigour enhancement techniques.
2.1. 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). Seed longevity (i.e. the period of seed survival) varies greatly among different species as also among various accessions within a species because of differences in genotype and provenance. Influence of provenance on potential longevity results from the cumulative effect of environment during seed maturation, harvesting, drying and the pre-storage environment, the time of seed harvest, duration of drying, and the subsequent period before seed is placed in store. How much of the potential longevity established through the effects of genotype and provenance etc. is realized depends on subsequent storage conditions. Seeds of all the species do not respond to the environment in the same way during storage.

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). Seeds of species with orthodox seed storage behaviour can be maintained satisfactorily ex situ over a long term in appropriate environments. The maintenance of the viability of seeds of species with intermediate or recalcitrant seed storage behaviour is however problematic. In general, medium-term storage is feasible for seeds of species with intermediate seed storage behaviour, provided the storage environment is well-defined. Short-term storage is usually the best that can be achieved with seeds which show recalcitrant seed storage behaviour. In order to determine the most suitable storage environment(s), and also the likely duration of successful storage, it is essential to know the seed storage behaviour of the species i.e. whether orthodox, intermediate or recalcitrant.
The loss of viability of seeds in dry storage has traditionally been treated as if all the phases are controlled by same rate-limiting factors. If this was correct, seed survival curves should all be sigmoid. 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. Several comprehensive reviews have identified free radical-mediated lipid peroxidation, enzyme inactivation or protein degradation, disruption of cellular membranes, and damage to genetic (nucleic acids) integrity as major causes of seed ageing (Priestley, 1986; Smith and Berjak, 1995; Walters, 1998; McDonald, 1999). During the last 20 years, considerable research has been conducted to gain better understanding of the physiology of seed ageing. Still however the primary processes and their interactions involved in seed ageing are not yet fully understood (McDonald, 1999).

During storage, seeds undergo ageing processes that lead to a deterioration in seed quality. Aged seeds show decreased vigour and may produce weak seedlings that are unable to survive in the field. Genetic modifications also occur as seeds age (Rao et al., 1987). Such factors may decrease the biodiversity of reintroduced vegetation at rehabilitated sites and thus reduce the effectiveness of an ex situ conservation. Therefore, it is important to determine the storage conditions that maintain initial seed quality for as long as possible. Orthodox seeds are able to withstand drying to low water contents and exposure to low temperatures, both of which increase longevity (Roberts and Ellis, 1989; Walters, 1998). The water content that maximizes seed longevity at a given temperature is species-specific (Ellis, 1998; Walters and Engels, 1998). However, it appears that this critical/optimum water content occurs at a constant relative humidity for all species (Ellis, 1998; Walters and Engels, 1998). Numerous studies of orthodox species suggest that there is a critical water content that (in equilibrium, with approximately 10% RH at 20°C) maximizes longevity at all storage temperatures and that drying seeds below this water
content has no effect on longevity (Ellis et al., 1989, 1995, 1996). Other studies suggest that there is optimum water content for storage in equilibrium with approximately 20% RH at the temperature at which the seeds are to be stored, and that drying seeds below this water content increases the rate of deterioration (Vertucci and Roos, 1990, 1993; Walters, 1998).

Biochemical deterioration during seed ageing has been studied mostly under accelerated ageing conditions using high temperature and high seed water content (McDonald, 1999). Under such storage conditions, seeds typically lose their viability within a few days or weeks. While these studies allowed important progress towards the understanding of seed ageing mechanisms, a major question has been raised whether the mechanisms of seed ageing are the same under natural ageing conditions and under the cool dry conditions where seeds age over many years. For example, while lipid peroxidation and the loss of membrane phospholipids are major causes of seed ageing under natural ageing conditions (Priestley, 1986; 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), peanuts (Pearce and Abdel-Samad, 1980), 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 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, 1993, 1994, 1997; Leopold et al., 1994; Sun et al., 1998). With increasing temperature or seed water content, the solid-like glassy state may soften into the rubbery state or even ‘melt’ into the liquid state, since the glass transition temperature ($T_g$) will fall below the storage temperature. The low viscosity and enhanced molecular mobility in the rubbery or liquid state would permit certain deteriorative reactions to proceed.
rapidly, which are otherwise retarded in the glassy state. Thus, the major primary process that initiates seed ageing could be different under different storage conditions, depending on the $T_g$ of seed cytoplasm (Buitink et al., 1998, 2000).

2.2. 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.

A viable seed upon hydration under suitable conditions reactivates its metabolism and commences germination, giving rise to a new plant (Fig.1). By definition, germination incorporates those events that start with the uptake of water by the quiescent dry seed and terminate with the protrusion of the radicle and the elongation of the embryonic axis (Bewley, 1997). 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).

2.2.1. Imbibition or uptake of water

Dry seeds must absorb water in order to restore their metabolic activities. The rate of absorption of water or imbibition varies from seed to seed. Some seeds hydrate very quickly when in contact with water, while the seeds of some other species go slow with the process of water absorption. Seeds of legumes are surrounded by a permeable
Fig. 1: Diagrammatic representation of various events of physiology of monocot seed germination.

seed coat which allows the water to move in but prevent the damage that can occur due to rapid absorption of water. In some seeds, the seed coats are impermeable which extends the quiescent period until weathering or biological action renders them permeable to water. Thus, the initial rate of imbibition can vary widely depending upon the characteristics of the testa and/or pericarp that surrounds the embryo.

Phase I of imbibition is the initial phase of water uptake which depends upon the water potential (ψ) gradient between the seed and its environment. This occurs equally well in dead and living tissues and is independent of the metabolic activity of seed. Following the initial phase, seed water content usually reaches a plateau level and either remains constant or increases very slowly. This period is known as “lag phase” or phase II of imbibition and is dependent upon the temperature (T) and water potential (ψ) of the seed. Lower T and ψ extend the duration of phase II. Phase III of imbibition is marked by an increase in water absorption due to initiation of embryo growth. The initiation of
radicle emergence marks a ‘point of no return’ for a seed, where it is committed to germination and development. At this stage the young seedlings become highly sensitive to environmental stresses.

Some seeds, such as those of beans and maize, are damaged by rapid influx of water at cool temperatures. This is known as imbibitional injury (Pollock and Toole, 1966) and it leads to rapid leakage of large volume of gases and solutes (sugars, organic acids and amino acids) and low molecular weight metabolites into the surrounding imbibition solution. Under field conditions, this injury might lead to fungal growth around the seed. This is due to the fact that, the membrane in dry seeds is in less fluid state and more prone to leakage when rapid influx of water takes place. When water uptake is slow or the temperature is little warmer, the membranes revert back to more fluid or liquid crystalline state before the damage occurs.

2.2.2. Respiration and metabolic activity

A quiescent dry seed rapidly resumes metabolic activity upon imbibition and follows a pattern similar to that of imbibition. In most cases, initial imbibition is followed by a steep initial increase in oxygen consumption. The mitochondria already present in the cells are capable of oxidative phosphorylation and release ATP (adenosine triphosphate) by aerobic respiration. However, in some cases thick seed coat restricts oxygen penetration and the initial ATP’s are released by glycolysis or anaerobic respiration. This initial rise in oxygen uptake is followed by a decline until the radicle penetrates the surrounding structure. At this time, another burst of respiratory activity occurs (Botha et al., 1992; Bewley and Black, 1994).

The initial substrates for respiration are soluble sugars (sucrose and oligosaccharides), followed by starch and lipids. The ATP and NADPH (Nicotinamide Adenine Dinucleotide Phosphate) generated via respiration, are used to initiate nucleic acid and protein synthesis, which are essential for the support of normal cellular metabolism i.e. ‘growth maintenance’ reactions (Bewley and Marcus, 1990). The synthetic
activities in seeds are associated with repair of damage (such as repair of DNA) that may have accumulated during storage. A marked decrease in number of single ribosomes is observed immediately after rehydration, as they become recruited into polysomal protein-synthesizing complexes (Bewley and Black, 1994). Protein synthesis is initiated using first mRNAs (messenger ribonucleic acid) stored during development, but quickly switching to newly synthesized mRNAs. Many of the enzymes required for reserve mobilization are synthesized de novo, and these are some of the early products of protein synthesis (Job et al., 1997).

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; Eastmond and Graham, 2001; Gallardo et al., 2001). As sucrose is the major form in which the growing embryo assimilates food, the stored reserves like starch, other carbohydrates and lipids have to be converted first into sucrose, before transporting them to the growing regions. In cereals, seeds of a specialized outer layer of thick-walled living cells, called aleurone layer, surrounds the starchy endosperm. 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 α-amylase, β-amylase and de-branching enzymes. α-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 α-1, 4-linked bonds of the glycan chains, releasing shorter amylose chains that are then further hydrolyzed to maltose (a disaccharide) by β-amylase. On the other hand, in dicots, the degradation of starch yields glucose and maltotriose (Bewley and Black, 1978). The majority of β-amylase is already present in the endosperm in an inactive form in the quiescent grains (Rowsell and Goad, 1964), 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 \textit{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 hydrolyze \( \alpha \)-1,6 bonds in the branch points in amyllopectin. 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. Their synthesis and secretion is controlled by gibberellins. The maltose thus released is broken down to glucose by \( \alpha \)-glucosidase and the glucose is converted to sucrose via UDP-Glc Pyrophosphorylase and sucrose-6-P synthetase. In some seeds, the major carbohydrate reserves are in the form of cell wall galactomannnanans and the corresponding hydrolytic enzymes are endo- \( \beta \)-mannanase, \( \beta \)-mannosidase and \( \alpha \)-galactosidase (de Miguel \textit{et al.}, 2000; Nonogaki \textit{et al.}, 2000; Feurtado \textit{et al.}, 2001; Mo and Bewley, 2002; Adebisi \textit{et al.}, 2008).

Major storage lipids in seeds comprise triglycerides. The degradation of lipid reserves is carried out in a specialized organelle called the glyoxisome. Lipases catalyze three-stage hydrolytic cleavage of fatty acid ester bonds in triglycerides and the fatty acids thus released are transported to glyoxisome. Within glyoxisome, the \( \beta \)-oxidation cycle releases acetyl CoA, which is converted into malate and succinate by glyoxylate cycle. Malate and succinate are then transported into the mitochondria where these are converted into glucose and sucrose via gluconeogenesis pathway (Bewley and Black, 1978).

In cereals, the protein reserves are stored in two separate sites \textit{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
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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).

During germination, phosphate is required for energy metabolism and for the synthesis of nucleic acids and phospholipids. Phytic acid (myo-inositol hexaphosphate) is a major phosphate reserve in many seeds (Bewley and Black, 1978). Since phytic acid in seed is present in the form of mixed potassium, magnesium and calcium salt (as phytin or phytate), its hydrolysis by phytase enzyme releases the phosphate group, associated cations and myoinositol. Therefore, it is not only a major source of phosphate for ATP production in the growing embryo but also a major source of macronutrient mineral elements in the seed. In cereals, the major storehouse of phytin is aleurone layer, with little being present in the protein bodies of endosperm.

2.2.3. Initiation of embryo growth

A viable seed, after having repaired the damage sustained during storage and activation of energy production systems in phase II, enters into the IIIrd phase of embryo growth. The initial growth may involve cell elongation or cell division or both, depending upon the species. In some cases the initial protrusion of radicle is the result of cell expansion only, while in others substantial cell division and morphogenesis may occur prior to emergence. De Castro et al. (2000) observed in tomato seeds that soon after imbibition, DNA synthesis was initiated and microtubules were reformed but cell division did not occur until after radicle emergence. Prior to cell expansion, special cell wall proteins called expansins, cause the cell walls to expand by loosening the bonds that hold different cell wall components together (Cosgrove, 1999). Chen et al. (2001) reported the presence of specific expansin genes in the emerging radicle. In many seeds, the embryo is covered by a thick
tissue, which prevents radicle emergence. In such cases, radicle emergence occurs only when the growth potential or turgor of the embryo is sufficient enough to overcome the restrain of covering tissue. In tomato seeds, certain hydrolases were produced which degraded the hard seed coat and helped in radicle protrusion (Bewley, 1997; Bradford et al., 2000). Cell extension is also regulated by certain growth hormones such as auxins and gibberellins. Many workers have reported the release of hydrolytic enzymes on application of GAs (Chen and Bradford, 2000; Toorop et al., 2000; Wu et al., 2001).

The first visible signs of germination are, increase in length and fresh weight of the radicle. The root develops vertically and enters into the soil for water and nutrient absorption. Depending upon the position of cotyledons vis-a-vis the soil, seedlings are defined as epigeal and hypogeal. The cotyledons serve as the source of food material till the first pair of leaves emerge from apical meristem and start synthesizing food. The development of leaves continues between germination and onset of development of reproductive parts.

2.3. EFFECT OF STORAGE ON SEED VIABILITY AND LONGEVITY

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.

The longevity of seeds during dry storage is mainly determined by seed moisture content and storage temperature, with longevity increasing with decreasing temperature and moisture content (Ellis and Roberts, 1980). However, there are also wide inherent differences in seed longevity between different species (Harrington, 1972; Priestley et al., 1985). There is evidence that shows that some species produce seeds with much shorter longevity in dry storage. For example, seeds
(with high initial viability) of *Anemone nemorosa* are predicted to survive for less than one year under seed bank storage conditions (Ali *et al.*, 2007). As there is an increasing aspiration to conserve seeds from wild plant species (Target VIII of the Global Strategy for Plant Conservation; SCBD, 2006), it is likely that more species will be discovered whose seeds have a similarly short life-span.

Seeds have to be stored from year-to-year for crop production and most long-term preservation of plant germplasm is via dry seeds stored at low temperature. During the last 20 years, considerable research has been conducted to understand the physical and chemical changes involved in loss of seed viability during storage. As the seeds are stored, they surely undergo ageing during which symptoms such as reduced rate of germination (Priestley, 1986), reduced seed vigour, and greater susceptibility to attacks by microorganisms are observed (Harrington, 1972). The end result of these deleterious changes of stored seeds is the loss of germinability (Bucharov and Gantcheff, 1984). Heydecker (1972) showed a decrease in the rate of germination and seedling growth while Mackey (1972) showed an increase in the number of morphologically abnormal seedlings as a result of ageing. Sreeramulu (1983) reported that the seeds of bambara groundnut (*Vigna subterranea*) showed a marked decrease in viability after 12 months of storage which was reduced to zero after 24 months of ageing. He showed that decrease in germinability was accompanied by increase in total phenols and inhibitory phenolic acids (p-hydroxybenzoic acid, p-coumaric, ferulic acid and vanillic acid) and a decrease in synergistic phenolic acids (chlorogenic sinapic and protocatechuic acids). He further observed that growth inhibitors appeared after 12 months of storage and increased thereafter and a marked decrease in auxin content was also observed in seeds stored for 18 months or more. Similar results were obtained by Narayanaswamy *et al.* (2000) in seeds of soyabean (*Glycine max*) where a decrease in germinability and vigour index occurred with increase in storage period. Shekhargouda *et al.* (1997) showed that
safflower (*Carthamus tinctorius*) seeds when stored in cloth bags under ambient conditions, showed a decline in germinability by 12.57-17.28% after 8 months and 35.17% after 20 months. Along with decrease in germination percentage, these seeds showed a decrease in shoot length, root length, seedling dry weight and vigour index also.

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 (Copeland, 1976; Bewley and Black, 1982; Richa et al., 2000; 2006), and membrane deterioration (Wilson and McDonald, 1986; Richa et al., 2006; 2010). Several comprehensive reviews 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 (Priestley, 1986; 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, taxonomy and climate (Horbowicz and Obendorf, 1994; Priestley, 1986; 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).

**2.4. EFFECT OF STORAGE TEMPERATURE AND SEED MOISTURE ON VIABILITY OF STORED SEEDS**

The factors which most influence the longevity of seeds during storage are temperature, moisture and oxygen pressure. Many orthodox seeds can be stored for 50-100 years, under conditions of low temperature and low water content (Roos and Davidson, 1992). In general, higher the temperature and higher the humidity of the storage atmosphere and consequently the seed moisture content, the more rapidly is viability lost (Roberts, 1972). With the exception of few
unorthodox or recalcitrant seeds which cannot withstand drying, generally the orthodox type seeds confirm to certain rules and for which the loss of viability in relation to storage environment can be predicted. According to Harrington (1972), as a thumb rule, for orthodox seeds, it has been suggested that a 1% reduction in moisture content or a 5.6°C reduction in temperature tends to double the seed longevity in storage.

Many scientists have studied correlation of seed longevity with seed moisture and storage temperature. Hu et al. (1998) showed that seeds of four crop species viz. wheat (Triticum aestivum), rice (Oryza sativa), millet (Setaria italica) and peanut (Arachis hypogea), when dried to about 2% water content and stored at room temperature, can survive up to 2.5-4 years. Ravikumar et al. (1998) reported that in seeds of the bamboo (Dendrocalamus strictus), high moisture content of 50-65% has a detrimental effect on seed longevity. Sun and Leopold (1995) have shown that soybean (Glycine max) seeds when stored at 11.6-16.4% moisture content and 5°C temperature, maintained 98-100% viability, even after 13-15 years. Studies by Chai et al. (1998) have shown that high water content in seed cultivars of flax (Linum usitatissimum), sesame (Sesamum indicum), soyabean (Glycine max) and durum wheat (Triticum durum), led to complete loss of viability loss within 4 years of storage, but when maintained at optimum moisture content the same seeds showed an increase in their viability over the same storage period. While for wheat (Triticum aestivum) and sesame (Sesamum indicum) seeds, optimum moisture content was 7.6-9.7% and 11.8-12.5% respectively, for the other species the optimum moisture content was below 5%.

Further evidence for a temperature-dependent optimum water content is provided by the studies of Buitink et al. (1998, 2000) on investigating molecular mobility in the cytoplasm of seeds and pollen, demonstrating that the water content at which molecular mobility is minimized (and storage life is suggested to be greatest) increases with decreasing temperature. Many of the processes implicated in seed ageing during storage appear to be free-radical mediated and the lipid
peroxidation is suggested to be a primary cause of deterioration in dry stored seeds (Wilson and McDonald, 1986; McDonald, 1999). Seeds are known to contain numerous antioxidant compounds, both enzymatic and non-enzymatic, that act to prevent oxidative damage by scavenging free radicals before they attack membranes or other seed components (Leprince et al., 1993; McDonald, 1999). In dry seeds, lipid-soluble, non-enzymatic antioxidants (such as tocopherol) are a potential mechanism of defence when enzyme systems may be impaired at low seed water contents (Senaratna and McKersie, 1986). Some studies (Senaratna et al., 1988; Pukacka, 1991) have measured a decrease in the activity of lipid-soluble antioxidants in aged seeds, while others (Priestley et al., 1980) have found no correlation between lipid-soluble antioxidant concentration and seed viability. Thus, while the degree and rate of free-radical-induced injury in seeds may be influenced by antioxidant status, the importance of endogenous antioxidants in limiting seed deterioration during storage remains uncertain.

It has been shown for many diverse species that there is a negative logarithmic relationship between seed longevity and moisture content (Ellis and Roberts, 1980, 1981; Ellis et al., 1989, 1990; Tompsett, 1986). Storage temperature also plays an important role in maintaining seed viability for long period of time and it varies from species to species. While the seeds of jackfruit (Artocarpus heteropyllus) retain viability only if stored at 0°C (Shylla Merlin and Palanisamy, 2000), the seeds of neem (Azadirachta indica) remained viable between 23-25°C (Nayal et al., 2000).

In 1994, FAO (Food and Agriculture Organization of the United Nations)/IPGRI (International Plant Genetic Resources Institute) recommended internationally expectable standards for seed storage. According to this, the seeds should be dried to 5% water content and stored at −18°C to maintain seed viability for longer period of time in storage. Bass (1984) proposed that storage of seeds in sealed containers helped in maintaining the seed moisture content and retain
their viability for a longer period of time than the seeds stored in open. Amuti and Pollard (1977) reported that as the angiospermic seeds approach maturity they accumulate soluble sugars which help the seeds to develop tolerance against desiccation and heat denaturation (Back et al., 1979) and enhance seed longevity (Bernal-Lugo and Leopold, 1992, 1995; Sun and Leopold, 1993).

Studies on the effect of temperature and moisture on the bamboo seeds are very few. Gupta and Sood (1978) reported that the seeds of the bamboo *Dendrocalamus strictus* are viable only for about a year, when kept in gunny bags. They reported that seeds when stored over silica gel and anhydrous calcium chloride in desiccator showed a germination percentage of 51% and 54% respectively, and seeds stored at 3°C to 5°C ambient temperature at 8% moisture content showed a germination percentage of 59%, even after expiry of 34 months.

Ravikumar *et al.* (1998) 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 (CaCl₂ 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. Somen and Seethalakshmi (1989) stored the seeds of *Bambusa arundinacea* under four conditions including low temperature with initial moisture content of 11%, over anhydrous CaCl₂ in a partially evacuated/ non-evacuated desiccator at room temperature (24°C-34°C) and over the laboratory shelf in a plastic container at room temperature as control. A rapid loss of viability of control seeds occurred within 2 months, while in other storage conditions deterioration was gradual reaching 10% or less after 413 days.
Thapliyal et al. (1991) showed that *Bambusa tulda* seeds when stored at their original moisture content (26%) lost viability within 2 months, while seeds with moisture contents of 10.2% and 6.6%, showed a germination percentage of 57% and 70% respectively after 12 months of ageing. Storage of seeds in sealed polybags at 5°C, 15°C and 30°C gave germination percentages of 80%, 82% and 24% respectively. Midya (1994) reported that freshly collected seeds of the bamboo (*Bambusa arundinacea*) when sealed in polythene bags and cryopreserved at -70°C showed 65% germinability even after 1 year. Shanmughavel and Francis (2002) reported that seeds of *Bambusa bambos* (= *B. arundinacea*) maintained a high percentage of viability up to 24 months, when their initial moisture content was reduced and were kept at low temperature. Seeds of the bamboo *Thyrsostachys siamensis* maintained high germinability for up to 27 months when stored at low temperature (Ramyarangsi, 1990).

Rawat and Thapliyal (2003) studied the effect of various storage conditions for increasing viability period of seeds of bamboo *Dendrocalamus membranaceus* which loose their viability within few months under uncontrolled storage conditions. They found that the seeds of this species could be stored for about 45 years at 5% moisture content and -18°C temp., the conditions recommended for germplasm conservation by IBPGR (1976). Warrier 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.

As the decline in viability of a seed lot during dry storage is dependent upon water content and storage temperature, Ellis and Roberts (1980; 1981) have proposed an improved viability equation as given below:

\[ u = K_i e^{-p/\sigma} \]  

where \( u \) is seed viability after \( p \) days of storage, \( K_i \) is the initial viability.
and \(1/\sigma\) is the rate of viability loss. Values of \(\nu\) and \(K_i\) are expressed in probit (probability units) percentage. The \(\sigma\) is an expression of seed longevity and is described as

\[
\log_{10} \sigma = K_E - C_w \log_{10} M - C_H T - C_Q T^2 
\]

(2)

where \(M\) is water content (% wet weight), \(T\) is storage temperature (°C), and \(K_E, C_H, C_Q\) and \(C_w\) are seed viability constants. This equation can be used to determine the seed moisture content and temperature that will be required to retain a certain germination percentage for a set period of time. Further modifications were done to extend the results to seed germination rates (Bradford et al., 1993) and to account for variation in initial viability among seed lots (Mead and Gray, 1999). Tang et al. (2000) proposed an alternative method for predicting seed longevity, which doesn’t require estimation of \(K_E\). This model predicts seed longevity for a given seed-lot as a function of the change in storage conditions (moisture content and temperature) compared with a reference set of conditions under which a storage experiment has been carried out. Thus the ‘intercept’ parameter \(K_E\) is eliminated from this model, whilst the other moisture and temperature parameters are assumed to be constant within species.

### 2.5. 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 (Simon, 1978). Damage to the organization of cell membranes during seed 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⁺, Mg²⁺, Cl⁻, Ca²⁺, and Mn²⁺) 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). Abdul-Baki and Anderson (1970) concluded that sugar measurement was not a reliable index for seed viability barley (*Hordeum vulgare*). However, there are few experiments where sugar composition has been analyzed in detail, so it is still not clear whether all the sugars or only some special sugars correlate to seed vigour. It is also not clear if sugar leakage from the embryo and endosperm are equally important for seed vigour.

The damage caused to membrane through deterioration that provides lower selectivity and hence increase in the leakage of solutes to the environment has been one of the main causes of the decline in the physiological quality of seeds. As a result, the electrical conductivity test is considered as an important tool to evaluate the seed vigour, since it indirectly assesses the cell membrane degradation degree by determining the amount of electrolytes released in the seed soaking solution. One of the major changes during seed storage is membrane deterioration, which leads to the loss of seed viability (Berjak and Villiers, 1972). Simon (1974) suggested that the damage to cellular membranes during ageing could be manifested as an increase of solute and electrolyte leakage from seeds during imbibition. Many authors (Stewart and Bewley, 1980) have suggested lipid peroxidation, the chief cause of membrane deterioration, which in turn is responsible for alteration of unsaturated fatty acids. Niehaus (1978) and Senaratna et al. (1987) reported that the free radicals are produced by de-esterification of phospholipids thereby resulting in accumulation of free fatty acids. Sung (1996) investigated the role of lipid peroxidation during natural and accelerated ageing in seeds of soyabean (*Glycine max*).
Membrane damage that occurs during seed storage contributes to a loss of viability and vigour. An oxidative change in the membrane polyunsaturated fatty acids is one of the reasons for deterioration of the stored seeds (Wilson and McDonald, 1986). However, Shewfelt and Purvis (1995) suggested that membrane lipids are susceptible to both enzymatic and non-enzymatic peroxidation. Seed ageing and loss of viability is closely related to cellular damage and decrease in nucleic acids metabolism. Berjak and Villiers (1972) found that loss of viability was due to degeneration of macromolecules, including nucleic acids and membrane components like appearance of mitochondria, plastids, golgi sticks. The damage to nuclear envelope (Villiers, 1980) and changes in the ultrastructure and membrane properties of seeds of *Brassica napus* (L.) was studied by Dawidowicz-Grzegozewska and Podstolski (1992).

Abdur (1996) studied the effect of seed ageing on the time of emergence, percent emergence, radicle growth, and ion leakage in *Cucumis sativus* seeds ageing at 1, 5, 10 and 15 years. Seeds ageing up to 5 years took increased time to radicle emergence and lower percent emergence but did not affect radicle growth significantly. However, seeds above 5 years of ageing showed a decrease in radicle growth also besides late radicle emergence and lesser %age. These changes were accompanied by increase in ion leakage, indicating that ageing is accompanied by damage to cellular membrane system.

### 2.6. METABOLIC CHANGES DURING SEED AGEING

Seed ageing is a time-dependent process in which the seeds deteriorate during periods of prolonged storage and eventually lose their ability to germinate. 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 Mariotta, 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). The various biochemical products are known to affect seed viability during ageing. These are briefly discussed as follows:

2.6.1. Free Radicals

Free radicals attack membrane lipids and cause major disruption of their viscosity (Dobretsov et al., 1977) and permeability (Van Zutphen and Cornwall, 1973). They can also oxidize proteins (Stadtman, 1992), and even nucleic acids (Reiss and Tappel, 1973). Many workers have reported that accumulation of toxic by-products is a significant cause of loss of viability of seeds during ageing (Bernal-Lugo and Leopold, 1992, 1995; Baker and Bradford, 1994; Madhava Rao and Kalpana, 1994; Sun and Leopold, 1995).

Calucci et al. (2004) reported that seeds of *Triticum aestivum* when stored at 40°C and 100% RH showed complete loss of viability within 10 days. This was accompanied by degradation of soluble and storage proteins associated with a marked decrease in carotenoid content, an antioxidant which exerts a protective effect against free radical content increased in both flour and gluten. An increase in proteinases activity during accelerated ageing of seeds was also reported by in *Zea mays* by Basavarajappa et al. (1991) and in *Triticum durum* by Galleschi et al. (2002).

2.6.2. Glassy state

The onset of dynamic deterioration could result from a gradual
hydrolysis of the soluble sugars. The consequent lowering of the sugar molecular weights would be expected to cause a lowering of the glass transition temperature and thus the melt may gradually occur without obvious changes in temperature or moisture content. In any event, the hydrolysis of the seed’s sugars would lead to an accumulation of reducing sugars which ultimately would threaten the integrity of proteins as a result of the formation of Maillard products (sugar-protein adducts) (Sun and Leopold, 1995; Oge et al., 2008; Almoguera et al., 2009).

The change of glass transition behaviour during accelerated ageing was examined by Lu et al. (2009) in soyabean (Glycine max) seeds. The glass transition temperature (Tg) of soybean axes decreased during ageing and eventually the seed tissue lost the ability to enter into the glassy state, which was followed by a subsequent decrease in seed viability (Sun and Leopold, 1993). The Tg of seeds was correlated with the maximum temperature for long-term storage (Sun and Leopold, 1994). The effect of the glassy state on deteriorative reactions was also studied for soybean seeds (Sun and Leopold, 1995). Deteriorative processes, such as the release of free radicals and sugar hydrolysis, were effectively inhibited during storage in the glassy state (Sun and Leopold, 1997; Buitink and Leprince, 2008).

Vertucci and Farrant (1995) reviewed that glassy state in seeds has provided insight into the understanding of storage stability and Buitink and Leprince (2004) carried out studies to investigate its role. Since the glassy state has been detected in dry biological tissues, it has been put forward as a prominent factor in the control of deterioration rates during storage (Burke, 1986; Williams and Leopold, 1989; Buitink et al., 1998). A glass is a thermodynamically unstable solid state with an extremely high viscosity and its formation is promoted by a low tissue water content and low temperatures (Leprince and Walters-Vertucci, 1995). The presence of glasses has been associated with improved storage stability (Sun and Leopold, 1993; Buitink et al., 1998). It is assumed that the high viscosity of intracellular glasses decreases molecular mobility and impedes diffusion, thus slowing down
degradative processes during ageing (Sun and Leopold, 1993; Sun, 1997). A relationship between longevity and the mobility of molecules in the glassy cytoplasm has been found in pea seeds (Buitink et al., 1998). The presence and amount of the oligosaccharides have been found to correlate with longevity (Bernal-Lugo and Leopold, 1995; Steadman et al., 1996). Oligosaccharides are thought to contribute to the stabilization of intracellular glasses by increasing viscosity and the glass-to-liquid transition temperature \( T_g \) (Leopold et al., 1994; Bernal-Lugo and Leopold, 1995; Sun, 1997). The addition of oligosaccharides to sucrose glasses in a model system will increase the \( T_g \) considerably (Wolkers et al., 1998).

Bernal-Lugo and Leopold (1998) reported that the transition from a period of relative membrane stability to quick seed ageing could occur through the loss of the glassy state. This loss could be affected by an increase in the temperature and in the seed water content or by a separation of phases of the involved sugars. Although it was proposed a decade ago that intracellular glasses might be associated with seed storage stability (Burke, 1986), there is still a lack of physiological evidence to show that the glassy state is crucial to seed storage stability (Williams et al., 1993; Sun and Leopold, 1994). Seed storage stability and the kinetics of seed viability loss are largely dependent upon seed water content and storage temperature. Water content and storage temperature are two major factors affecting the stability of intracellular glasses in seeds.

A study aimed to examine possible relationships between seed storage stability and the glassy state in seeds conducted by Buitink et al. (2000) concluded that long-term storage stability of the orthodox seeds tested was correlated with the presence of a glassy state. Seed deterioration during storage was found to be associated with the glass to liquid transition in seeds, and the rate of deterioration was under the kinetic control of the glass transition in seeds. The effect of water content on storage stability appeared to be largely due to the plasticization effect of water on intracellular glasses in seeds.
Majority of the authors in literature supports the assumption that oxidative reactions are responsible for the deteriorative changes observed in aged seeds. Four types of oxidations are known which might reasonably contribute to the progress of seed ageing. These include free radical oxidations, enzymatic dehydrogenation, aldehyde oxidation of proteins, and Maillard reaction.

2.6.3. Deterioration of repair enzymes

Another factor which can contribute to seed mortality may be the deterioration of repair enzymes with ageing (Osborne, 1980, 1983). One such enzyme which can be relevant to successful germination is DNA ligase (Elder et al., 1987). Another enzyme of potential relevance would be aspartyl methyl transferase, which is involved in repair of proteins damaged by ageing in some organisms (Mudgett and Clark, 1993). Deterioration of either of these enzymes during storage could amplify the damage resulting from the various oxidations mentioned above or the ones arising during germination.

During hydration of the aged seed early in germination, hydroperoxide lyase becomes active and breaks down oxygenated fatty acids accumulated during storage (Wilson and McDonald, 1986). This reaction may increase the level of free radicals to a point in which the detoxifying capacity of the aged seed has been oversaturated (Gidrol et al., 1994). Therefore, it could well be that seed ageing resulted in an impairment of the enzymatic systems involved in the elimination of the toxic O$_2$ intermediate species generated either during dry storage or during germination (Sung and Chiu, 1995; Bailly et al., 1996). This mechanism could explain why intact non-germinable seeds are often metabolically alive, yet the seed dies a day or two after hydration.

Some hydrolysing enzymes and dehydrogenases are active even in good storage conditions. For example, lipases and esterases can show some activity at relative humidity below 10%, and glucosidases, amylase, galactosidase, and proteases show some activity at RH values as low as 20% (Drapron, 1985). Glucosidases (Bernal-Lugo and
Leopold, 1992), ADH, and probably carboxylic-ester hydrolases are active under conditions ranging from 12% to 75% RH at a wide range of temperatures (Zhang et al., 1995). Acetaldehyde, the product of ADH and ethyl acetate hydrolase, is a universal volatile component produced by dry seeds, and may be a factor driving seed ageing, because it can react non-enzymatically with amino groups in protein or DNA and causes the accumulation of acetaldehyde-protein and acetaldehyde-DNA adducts (Zhang et al., 1994).

2.6.4. Protein modification

It is a process that can occur slowly in dry systems and might be expected to contribute to dynamic ageing especially in accelerated ageing conditions (Narayana Murthy et al., 2000). In dry seeds, the protein modification can take place through non-enzymatic glycation with reducing sugars (Sun and Leopold, 1995) and/or by reacting with α, β-unsaturated aldehydes which are products of the free radical-mediated oxidation of polyunsaturated fatty acids (Priestley and Leopold, 1983; Priestley et al., 1985). Also, α, β-unsaturated aldehydes, especially 4-hydroxynonenal may react with the sulphhydryl groups of proteins to form stable thioether derivatives that possess a carbonyl function (Stadtman, 1992; Mudgett and Clark, 1993). Oxidations by reducing sugars, by aldehyde oxidations, and free radical oxidations would all be expected to be facilitated as the glassy state is lost (Sun and Leopold, 1995).

Sheng-Zuo et al. (1998) suggested a co-relation between nutrient content in seeds and seed vigour index and reported that protein content has greatest effect on seed vigour, followed by starch and fats. Loss of seed vigour in terms of delayed germination and increasing proportion of abnormal seedlings with ageing was reported by several workers (DeN’Aquila and Tritto, 1990; Livesley and Bray, 1991).

Dell’Aquila (1994) reported that wheat seeds when stored at 35°C and 12.5-14.5 % RH showed a progressive delay in germination and a decrease of methionine uptake and incorporation into soluble proteins.
of the imbibing embryos. Nautiyal et al. (1985) reported that a lower number of soluble proteins were present in the non-viable seeds than in viable ones. This showed that the proteins with higher mobility were denatured during the ageing process. Chiu et al. (1995) reported that watermelon seeds when incubated at 45°C and 79% RH for 6-days, showed lower germination percentage and slower germination speed. Ageing also increased lipid peroxidation and reduced the activity of peroxide-scavenging enzymes. Abdul-Baki (1980) reported that a decrease in seed germinability and vigour induced by ageing were associated with marked decrease in synthesis of protein and polysaccharides. Bucharov and Gantcheff (1984) reported that lipid peroxidation in embryonic axes may play an important role in the seed deterioration during ageing. Chhetri et al. (1993) reported a decrease in total protein content with concomitant increase in amino acid in accelerated aged seeds of *Phaseolus vulgaris, Pisum sativum, Lens culinaris* and *Panicum miliaceum*.

### 2.6.5. Changes in genetic material

Reuzeau and Cavalie (1995) observed qualitative as well as quantitative alteration of RNA content in sunflower (*Helianthus annuus*) seed lots which could be due to the ageing process during post harvest treatment such as drying and storage. Thompson et al. (1987) showed similar degradation of ribosomal RNA in rice and carrot (*Daucus carota*) seeds during natural ageing. Bray and Chow (1976) observed that in non-viable seeds of pea, the ribosomes failed to dissociate and lesions were formed on the RNA of the seeds. There is also evidence that long lived RNA is lost during the extended seed storage (Osborne, 1982) and the ageing process depresses the synthesis of newly formed m-RNA (Osborne et al., 1980).

Cheah and Osborne (1978) found an increase in chromosomal break in aged seeds of rye (*Secale cereale*) which were incapable of repair during hydration. Similarly, Yamaguchi and Nakatini (1983), observed in barley seeds that DNA polymerase loses activity with
ageing. Murata et al. (1982) have suggested that loss of repair mechanism may be the main reason for increase in chromosomal damage. DNA metabolism in maize (Gutierrez et al., 1993) and pigeon pea seeds (Kalpana and Rao, 1997) was observed to be closely related with seed ageing. In artificially aged seeds of tomato, the loss of viability and vigour was found to be due to break in DNA molecule (Fontes et al., 2001). Boubriak et al. (2000) have suggested that loss of viability may be related to nucleosome cleavage or death with random DNA fragmentation in rye embryo with the ageing.

2.6.6. Role of sugars

Haider and Gupta (1981) reported a decline in the amount of non-reducing sugars in aged seeds. There are reports that during ageing of seeds there is a decrease in the amounts of several oligo and polymeric constituents like proteins, nucleic acids, fats and phospholipids (Maguire, 1977) while an increase in the amounts of amino acids and fatty acids has been reported (Aggarwal, 1981). Lynch et al. (1962) reported a decrease in the amount of total soluble sugars in aged wheat seeds, while a decrease in reducing sugars with storage in Glycine max seeds was observed by Locker and Bucheli (1994). Kumar and Knowles (1993) reported that during long term ageing of potato seed-tubers (up to 30 months at 4°C) the ‘senescent sweetening’ resulted due to the loss of membrane integrity. They showed that tuber ageing was accompanied by the decrease in starch levels, increase in sugar content and an increase in the levels and activity of L-type isoform of α-1,4-glucan phosphorylase.

Basavarajappa et al. (1991) reported a decline in metabolites like carbohydrates, proteins and reducing sugars and an increase in amino acid content in ageing Zea mays seeds. Similarly an increase in amino acid content decrease in protein content, decrease in protein content, with little or no variation in starch content was observed in aged seeds of Trifolium incarnatum, Lolium perenne and Vicia faba (Ching and Schoolcraft, 1968).
Yuri et al. (1996) reported that in lotus (Nelumbo nucifera) seeds, reducing sugars and protein fractions were relatively lower in stored seeds but starch and total sugars did not vary much. Richa and Malik (1985) studied the physiological and biochemical changes in okra (Abelmoschus esculentus) seeds of different ages and concluded that germination decreases with seed ageing. A reduction in the total content of food reserves such as sugars, proteins and lipids were recorded in aged seeds of the spiny bamboo (Bambusa bambos) (Richa et al., 2006; 2010; Ravi Kumar et al., 1998). A decrease in total carbohydrate content with progressive ageing was reported in soybean and barley by Short and Lacy (1976) which was due to an increase in the activity of hydrolytic enzymes and respiration leading to degradation of complex carbohydrates into simpler sugars. Decrease in starch was observed by Rao and Kalpana (1994) in pigeon pea seeds during ageing. Begnami and Cortelazzo (1996) reported increased starch degradation in French bean seeds during ageing. A reduction in soluble oligosaccharides was shown in soybean (Yaklich, 1985). An increase in soluble carbohydrates in seeds of rice (Ray et al., 1990), non reducing sugars (Walters et al., 2008) and glucose in wheat seeds (Petruzzelli, 1986) was reported during ageing.

So with the perusal of literature, many of the authors are of opinion that total carbohydrates contents increased with the ageing due to activity of hydrolytic enzymes and decrease in membrane integrity.

2.7. 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. Reviews by Priestley
and Smith and Berjak (1995) showed that with ageing the membrane of the seed become leaky, enzymes lose catalytic activity and chromosomes accumulate mutations. Van Bilsen et al. (1994) also reported that membranes become more susceptible to imbibition damage with ageing. Food reserves also deplete as the seeds age.

Commonly, seed deterioration is reported to accompany changes in enzyme activity during ageing (Aung and McDonald, 1995; Richa et al., 2006; 2010; Ganguli and Sen-Mandi, 1993; Bailey et al., 2002; 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 α-amylase and proteases show an increase in their activity (Basavarajappa et al., 1991). Several workers have shown a decrease in amylase activity with ageing (Aggarwal and Kharlukhi, 1987; Petruzelli and Taranto, 1990; Livesley and Bray, 1991; 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). Tamura and Mnamikawa (1982) reported an increase in acid phosphatase activity in dry seeds of Vigna mungo. Similar results were observed by Koshiti (1972) in seeds of Bauhinia and Cassia.

Abdul-Baki and Anderson (1972) demonstrated correlations between loss of viability and decline in enzyme activity of aged wheat seeds. Decreased α-amylase activity in aged wheat seeds occurred as this enzyme was synthesized at a reduced rate by the aleurone. Vazquez et al. (1991) reported that repair enzymes such as DNA
ligases may be affected during seed storage and therefore require some lag time to recover. Elder and Osborne (1993) showed that β-polymerase mediated DNA repair occurred on imbibition in rye. With relation to rRNA components of enzymes, total RNA isolated from dry, non-viable rye embryos and the 18S component were degraded and could be a reason for seed ageing (Roberts et al., 1973). The elongation factor-1 (EF-1) was almost without activity while EF-2 had reduced activity. Increased nuclease activity could also lead to amplified lesions and progressive imbibition.

Thorneberry and Smith (1955) and Grabe (1964) reported that in corn seeds, the loss of seed viability was accompanied by decrease in the activity of enzymes like oxidases (catalase, peroxidase and phenolase), hydrolase (amylase), cytochrome oxidase, glutamic acid decarboxylase and dehydrogenases (malic and alcohol dehydrogenase). A decrease in the activity of several hydrolytic enzymes with ageing was reported by Abdul-Baki and Anderson (1972). Decrease in the activity of amylases, cytochrome oxidase, glutamic acid decarboxylase and dehydrogenases also investigated in deteriorating seeds by Batista et al. (1964). Paul and Mukherji (1972) reported decrease in glutamate dehydrogenases, catalases and peroxidase enzyme activity during seed ageing. Gidrol et al. (1994) reported a significant increase in guaiacol, and a slight increase in peroxidase activity in seeds of soybean during ageing. Wheat (Kashem et al., 1995) and soyabean (Gasper et al., 1985) seeds showed similar results. Rice (Oryza sativa) grains showed reduction in glutamic dehydrogenase catalase and peroxidase (Krasnook et al., 1976; Paul and Mukherji, 1972).

Bernal-Lugo et al. (2000) reported that during seed ageing, catalase is more susceptible to loss of activity although peroxidase activity is also impaired. Loss of activity of superoxide dismutase, catalase, ascorbate peroxidase during seed ageing was reported by Sung (1996) and Sung and Jeng (1994). Sung and Chiu (1995) studied the effect of natural ageing on germination and several physiological
characteristics related to peroxidation in the seeds of two edible soyabean (Glycine max) cultivars. The activities of peroxidase, ascorbate peroxidase, superoxide dismutase and lipoxygenase were inhibited during seed ageing. VanBilsen et al. (1994) reported that the loss of seed viability was associated with reduced activity of detoxifying superoxide enzyme such as dismutase, catalase and glutathione reductase. Bailly et al. (1996) showed that sunflower (Helianthus annuus) seeds when subjected to accelerated ageing at 45°C showed accumulation of malonaldehyde, suggesting that loss of seed viability was associated with lipid oxidation. A decrease in the levels of scavenging enzymes (catalase and peroxidase) was seen in aged onion (Allium cepa) seeds by Basra and Malik (1994). Basavarajappa et al. (1991) reported a decrease in activity of peroxidase, acid phosphatase dehydrogenases and phosphonoesterase with seed ageing. Richa and Malik (1985) reported that the seeds of okra (Abelmoschus esculentus) showed low levels of different macromolecules (i.e. total sugars, amino acids, proteins, nucleic acids); low oil percentage and free fatty acid contents; and decreased activity of hydrolases. Chhetri et al., (1993) reported that in seedlings of Phaseolus vulgaris, Pisum sativum, Lens culinaris and Panicum miliaceum reduced activities of dehydrogenase were observed after accelerated ageing. Decrease in peroxidase activity with ageing was observed by Shatters et al (1994).

Ganguli and Sen-Mandi (1993) reported the effects of ageing on amylase activity in wheat seeds. Nandi et al., (1995) showed that β-amylase activity was good parameter of seed vigour in rice grains, since high β-amylase activity was present in seed stocks of 99% germination while zero activity was detected in low viability, low vigour seed stock. However, α-amylase activity was present in both stocks after 24hrs of imbibition. Livesley and Bray (1991) reported an increase in the abnormal seedlings with ageing of wheat (Triticum aestivum) seeds during storage. An increase in the activity of α-amylase was observed in the aleurone cells between 3rd and 6th day of germination of normal seeds which was absent in abnormal seedlings at any point of time up to 12th day of germination. The loss of α—amylase activity was attributed
Review of Literature
to the lesions produced in the aleurone layer in stored seeds during ageing. Lee et al. (1998) after studying different varieties of rice, suggested that high seedling vigour can be correlated with high $\alpha$-amylase activity as also shown by many workers (Petruzzelli and Toranto, 1990; Das and Sen-Mandi, 1992; Ravikumar et al., 1997; Simić et al., 2005; Afzal et al., 2009). Bernal-Lugo et al. (1994) reported that $\alpha$-amylase production by aleurone cells decreases in aged seeds due to decrease in the expression of the $\alpha$-amylase plant genes and this reduction is associated with a decrease in response to GA$_3$.

Vadiraj and Mulimani (1993) reported an increase in protease and $\beta$-amylase activities during germination in seeds of Sorghum and a decrease in their activities during storage. Vimala (1984) studied the changes in the activities of $\alpha$-amylase, protease, IAA oxidase and peroxidase in naturally aged seeds of *Phaseolus aureus*, *P. mungo* and *Vicia faba* and artificially aged seeds of *P. mungo*, in both the cases seed germinability, seed vigour and activities of $\alpha$-amylase and protease were reported to decline. However, an increase in the activities of peroxidase and IAA oxidase was observed in natural ageing whereas a decline was observed in artificial ageing (Sital et al., 2008; Afzal et al., 2009; Singh et al., 2010). Activity of alcohol dehydrogenase increased in the cotyledons of pea (*Pisum sativum*) seeds (Kolleffel, 1968) and lactic dehydrogenase in bean (Sherwin and Simon, 1969) when germinated under wet conditions. Bailly et al. (1996) reported that sunflower (*Helianthus annuus*) seeds when subjected to accelerated ageing at 45°C showed accumulation of malonaldehyde, suggesting that lipid oxidation was associated with accelerated ageing in seeds. A decrease in the activity of scavenging enzymes i.e. dismutase, catalase and glutathione reductase was observed with ageing.

Kalpana and Madhava Rao (1993) reported a decline in arginine decarboxylase activity during accelerated ageing of pigeon pea (*Cajanus cajan*) seeds. Arginine decarboxylase is an important enzyme involved in the biosynthesis of polyamines in plants (Dai et al., 2000). Bernal-Lugo and Leopold (1992) showed that with ageing there was a
decline in antioxidant system of maize (Zea mays) cultivars. Ghosh et al. (1981) reported that aged rice seeds showed a decrease in percentage of germination and seedling vigour with increasing storage time. Although α-amylase and ATPase activity could be detected in 3 year old or older seeds, peroxidase activity could not be detected in seeds more than one year old. Dehydrogenase activity was found to decline as seeds deteriorated.

Nascimento (2005) subjected the lettuce seeds to 41°C and 100% RH for 0,1,3,5 days and examined germination capacity at 20°C and 35°C. It was found that seed ageing led to reduced ethylene and endo-beta-mannanase activity and thus led to thermo-inhibition in lettuce seed germination. Siriwitayawan et al. (2003) reported that seeds of sweet corn (Zea mays) and tomato (Lycopersicon esculentum) naturally aged for 18-months or artificially aged using saturated salt, showed reduced ethylene production compared to non-aged seeds. It was also found that exogenous application of 1-aminocyclopropane-1-carboxylic acid (ACC) to artificially aged seeds reduced the time to radicle protrusion, but did not completely reverse age-related effects on vigour. Merritt et al. (2003) studied the influence of storage environment on seed viability and antioxidant potential in Acacia bivenosa, Anigozanthos manglesii, Banksia ashbyi, and Mesomelaena tetragona. It was found that seed viability remained high for seeds stored at or below 23°C but gradually declined when stored at -18°C. However, no correlation could be found between antioxidant activity and seed viability. Yanping et al. (2000) reported that Welsh onion (Alium fistulosum) seeds after storage for 2 years, showed a decline in seed vigour indices including germination energy, vigour index and emergence percentage. Dehydrogenase activity was also found to decrease with ageing, which further declined with increase in storage temperature and seed moisture content.

Studies on the effect of ageing on enzyme activity in bamboo seeds are limited. Ravikumar et al. (1998) studied the various changes associated with ageing in seeds of the thorny bamboo Bambusa
They reported that enzymes acid phosphatase, alkaline phosphatase and peroxidase showed decline in their activity after ageing. *Dendrocalamus strictus* seeds when kept at 42±1°C and 100% RH for 1 to 8 days showed a loss in viability of seeds; reduction in sugar, starch, proteins and lipids; decrease in the activity of peroxidase and alkaline phosphatase and an increase in total free amino acids and the activity of amylase, confirming degradation of stored reserves (Richa *et al.*, 2006, 2010; Ravikumar *et al.*, 2002; Sital *et al.*, 2008; Afzal *et al.*, 2009; Singh *et al.*, 2010). Decline in the activities of α- and β-amylases, peroxidase and glutamate dehydrogenase with seed ageing of 6 months of bamboo seeds of *Dendrocalamus membranaceus* and *Cephalostachyum pergracile* was reported by Richa *et al.* (2010). Thus the perusal of the literature presented above indicates that during seed ageing there is a decrease in the activity of various enzymes.

### 2.8. EFFECT OF AGEING ON ASCORBATE 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). In orthodox seeds, ASC content and ASC peroxidase activity increase during the early stages of development, then decrease during the desiccation stage so that at quiescence seeds have neither ASC nor ASC peroxidase but retain a small amount of dehydroascorbic acid (DHA) and significant activities of ASC recycling enzymes. Ascorbic acid and ascorbic peroxidise (APX)
are not essential for desiccation, since both ascorbic acid content and APX activity are high in drying-sensitive seeds and low during the desiccation stage of drying-tolerant ones. Therefore, it is more reasonable to hypothesize that other antioxidant molecules such as glutathione which is present at high concentrations in orthodox seeds and enzymes, such as catalase and glutathione reductase (Bailly et al., 2001), or peroxi-redoxins (Finnie et al., 2002) can be responsible for seed protection from reactive oxygen species.

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 (AP) (Halliwell, 1987). Thiols are first affected by oxidation due to the presence of sulphhydryl 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 (Smith et al., 1990; De Tullio, and Arrigoni, 2003; Pukacka and Ratajczak, 2007). Klapheck et al. (1990) reported the role of GSH in germinating castor bears during oxidative stress as it degraded H₂O₂. 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). Carbon-based free radicals derived from quinones affected the activity of scavenging enzymes, such as SOD and anodic peroxidase (POX) decreased with decline in vigour. These authors suggested that a gradual decline in scavenging activity resulted in degradation of protein or enzyme integrity during ageing. The results indicated that high vigour unaged embryos possessed high activity for the antioxidant enzymes, SOD and POX. They concluded 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.
Paula et al. (1996) reported the function of ascorbate-glutathione (AsA/GSH) cycle in aged sunflower seeds. Their study shows that in the embryo, GSH system is the major detoxifying mechanism in dry and imbibed seeds. The loss of viability, lower GSH levels and GR activity indicate that oxidative damage occurred on accelerated ageing of sunflower seeds. 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). In addition, Bailly et al. (2001) reported that APX activity in embryos, isolated from seeds of Phaseolus vulgaris at different developmental stages, was highest at 40DAA (i.e. before the end of mass accumulation), then progressively decreased during desiccation, concomitant with a significant increase in the activity of other antioxidant enzymes (catalase and glutathione reductase). Notably, seeds dried at the stage when APX activity is high, are not able to germinate (Bailly et al., 2001), suggesting that the function of this enzyme is not directly related to drying tolerance.

ASC is required for the activity of a number of dioxygenases involved in the synthesis of the plant hormones ethylene, gibberellic acid (GA) and abscisic acid (ABA), among others. The coordinated action of these phytohormones is necessary to control different aspects of seed development and germination (Rodriguez-Gacio and Matilla, 2001; Peng and Harberd, 2002; Singh et al., 2002; Nambara and Marion-Poll, 2003). The role of GA and ABA in the mobilization of seed reserves was classically studied by Bethke et al. (2002) in the aleurone layer of cereals. It was observed that GA induces programmed cell death in aleurone cells whereas incubation in ABA maintains cell viability. These authors suggested that this phenomenon seems to be...
regulated by increased sensitivity to reactive oxygen species in GA-
treated aleurone cells occurring in parallel with a down regulation of the
transcripts of enzymatic antioxidant defences (superoxide dismutase,
APX, and mainly catalase). Conversely, ABA maintains the level of
expression of the genes coding for antioxidant defences (Bethke et al.,
2002).

2.9. EFFECT OF AGEING ON SEED MEMBRANE
PHOSPHOLIPIDS

Although the longevity of seeds is enhanced by storage under dry
conditions, eventually the seeds deteriorate and lose the ability to
germinate. The mechanism by which seeds lose their germination
potential is not well understood, but most hypotheses involve the
breakdown of cell macromolecules (Priestley, 1986). Under dry storage
conditions, metabolism is restricted (Vertucci and Roos, 1990) and
therefore, it is likely that ageing reactions are stochastic. Studies of the
effects of oxygen on seed deterioration (Priestley, 1986) and of oxygen
uptake by dry seeds (Vertucci and Leopold, 1987; Vertucci and Roos,
1990) suggest that the reactions may be oxidative. One of the most
commonly cited hypotheses explaining seed deterioration points to lipid
peroxidation as the mechanism by which cellular membranes are
disrupted (Spencer et al., 1973; Stewart and Bewley, 1980; Flood and
Sinclair, 1981; Priestley and Leopold, 1983; Priestley, 1986; 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 (Priestley and Leopold, 1983; Priestley, 1986; McKersie
et al., 1988; Ferguson et al., 1990), and a consensus regarding the
importance of lipid peroxidation in seed deterioration has not been reached. Thus, the involvement of lipids in reactions associated with seed deterioration remains an unanswered question.

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 (Priestley, 1986; 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 (Petruzelli and Taranto, 1984).

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 (Priestley, 1986; Wilson and McDonald, 1986; McKersie et al., 1988; 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.

It is noteworthy that even at the low water content of mature dry seeds, the triglycerides within oil bodies remain liquid. The mechanisms that prevent oil body coalescence during seed drying may involve the integral oil body protein, oleosin. Lipid peroxidation is considered to be a major cause of seed deterioration during prolonged storage.
Review of Literature

(McDonald, 1999; Sital et al., 2008). The mechanism of lipid peroxidation is relatively well characterized (Hendry, 1993; Frankel, 2005). Free radicals attack the unsaturated fatty acids of membrane phospholipids. A decline of seed viability may be related to the peroxidation of phospholipids and consequent membrane damage. Loss of membrane integrity is apparent when excessive electrolyte leakage accompanies seed imbibition (Bewley, 1986). The free radicals generated by membrane damage may subsequently attack other subcellular structures in seeds, including organellar membranes, proteins, and DNA. Polyunsaturated fatty acids are more susceptible to peroxidation than are monounsaturated fatty acids, while saturated fatty acids are the most resistant. Thus preferential loss of polyunsaturated fatty acids in seeds during storage may serve as an indication of lipid peroxidation. Lipid peroxidation is commonly used as an indicator of prevalence of free radicals in tissues (Smirnoff, 1993). Lipid peroxidation not only threatens the integrity and function of membranes and membranous proteins but also produces a variety of toxic aldehydes and ketones (Wilhelmova et al., 2006). Some of such products of lipid peroxidation, malondialdehyde and 4-hydroxynonenal cause protein damage by reacting with lysine amino groups, cysteine sulfhydryl groups, and histidine imidazole groups (Refsgaard et al., 2000). The striking chemical change in the membrane lipids is the dramatic increase in the sterol/phospholipid ratio (McKersie et al., 1988), although, content of sterols declines with physiological ageing (Paliyath and Droillard, 1992).

2.10. 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).

While some seed lots retain their full germination capacity during prolonged storage, others deteriorate very rapidly, often with a characteristic abrupt drop in the germination capacity (Kaewnaree et al., 2008). A major cause of deterioration in these seeds is probably due to the oxidation of intracellular macromolecules (lipids and proteins). However, it is very difficult to prove causes and effects of the mechanistic features underlying seed deterioration. It appears that there is an extremely complex cascade involved in cellular and sub-cellular deterioration changes. It is probable that some early changes may be associated with the generation of reactive oxygen species and a compromised capacity of antioxidant-related protective or restorative processes and later changes to sub-cellular constituents will be more associated with cell death per se, particularly the execution phase of cell death. It is possible that lipid peroxidation in seeds is both an early and potentially signal-triggering event involved in seed deterioration. In addition, this process 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).
2.11. EFFECT OF AGEING ON CHANGES IN ENDOGENOUS GROWTH HORMONES

Starling (1905) used the term 'hormone' for the first time and defined it as a substance produced in one organ and transported to another organ where it acts. These structurally diverse compounds include auxins, cytokinins (CK), abscisic acid (ABA), gibberellins (GA), ethylene, polyamines, jasmonates, salicylic acid and brassinosteroids (Davies, 1995).

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. Hormones are produced naturally by plants, while plant growth regulators are applied to plants by humans. Plant growth regulators may be synthetic compounds (e.g., IBA and Cycocel) that mimic naturally occurring plant hormones, or they may be natural hormones that were extracted from plant tissue (e.g., IAA). 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; Setia et al., 1991; Kucera et al., 2005).

Kobayashi et al. (1989) analyzed the endogenous level of gibberellins in shoots and ear of two dwarf rice (Oryza sativa) cultivars. Their results indicated that GA$_1$ is the active gibberellin that regulates vegetative growth of rice. The endogenous level of GA$_4$ in the ears of the two dwarf cultivars of rice was higher than the normal cultivar, suggesting no direct effect of GA$_4$ in vegetative growth of rice.

Metzger and Zeevaart (1980) studied the changes in the level of five endogenous gibberellins (GAs) in spinach in relation to photoperiodic treatment by combined gas chromatography-selected ion current monitoring. A 5-fold decline in the level of GA$_{19}$ and a considerable increase was observed in the level of GA$_{20}$ and GA$_{29}$. 

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under Long-day treatment. These results were consistent with the hypothesis that GA$_{19}$ is converted to GA$_{20}$ during stem growth and that this conversion is under photoperiodic control.

Blechschmidt et al. (1984) detected over 30 compounds in the extracts of the endosperm and embryos from seeds of *Cucurbita maxima* by gas chromatography (GC-MS). These compounds included GA$_4$, GA$_{12}$, GA$_{13}$, GA$_{25}$, GA$_{39}$, GA$_{43}$, GA$_{49}$, trans-ABA, IAA, dihydrophaseic acid, etc. They also deduced the structures of some new compounds e.g. the 12 $\alpha$-hydroxy-derivatives of GA$_{12}$, GA$_{14}$, GA$_{37}$, GA$_{43}$ and the $\beta$-hydroxy-derivatives of ent-7$\alpha$-hydroxy-and ent-6$\alpha$, 7$\alpha$-dihydroxy kaurenoic acids.

Chiwocha et al. (2003) combined high performance liquid chromatography (HPLC) with positive and negative electro spray ionisation-tendem mass spectromerty (ESI-MS/MS) to quantify a broad range of chemically and structurally diverse compounds simultaneously in a single run. The method was applied to analyze various plant hormones and hormonal metabolites associated with seed dormancy and germination in lettuce (*Lactuca sativa* L.) They reported that the germinating seeds transiently accumulated high levels of Abscisic acid glucose ester (ABA-GE). In contrast, thermo-dormant seeds transiently accumulated high levels of dihydrophaseic acid (DPA) after 7 days at 33°C. GA$_1$ and GA$_3$ were detected during germination and levels of GA$_1$ increased during early post-germinate growth. Thermo-dormant seeds also exhibited a striking transient accumulation of indole-3-acetic acid (IAA) after several days of incubation.

Briggs (1973) suggested that GA$_1$ and GA$_3$ are the major gibberellins produced by the germinating embryo though GA$_4$ and GA$_7$ were also detected. Further it was suggested that GA$_3$ and GA$_7$ activate the aleurone cells where as GA$_1$ and GA$_4$ control the embryo growth. Other effective gibberellins are GA$_2$ and GA$_{22}$ while some such as GA$_{12}$, GA$_{17}$, GA$_{26}$ did not have any promotive action (Crozier et al., 1970). A major role of endogenous GAs in the control of seed germination has also been emphasized by Karssen et al. (1989).
Metzger (1983) studied the role of endogenous plant hormones in the seeds of wild oat (*Avena fatua*). GA$_1$ was identified by combined GC-MS as the major biologically active gibberellin in the seeds regardless of the depth of dormancy or stage of imbibition. He suggested that GA biosynthesis in imbibing non-dormant seeds is one of the many coordinated biochemical events, but not necessary prerequisite, that occur during germination.

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 (Appleford and Lenton, 1997; Mrva and Mares, 1999; Sugimoto *et al*., 1998). Studies on the expression of $\alpha$-amylase genes in barley and rice scutella (Sugimoto *et al*., 1998; Bethke *et al*., 2001; Kucera *et al*., 2005; Li *et al*., 2008; 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 (Fincher, 1989). The aleurone layer is the principal store of mineral elements in the grain of small cereals where K$^+$, Mg$^{2+}$ and Ca$^{2+}$ are stored in the vacuole as chelates of phytic acid (Bethke *et al*., 1998). GA stimulates the synthesis of phytase, and phytate breakdown makes cations and phosphate available to the embryo (Gabard and Jones, 1986). 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 (Bush *et al*., 1986; Bethke *et al*., 1999; Bethke and Jones, 2001; Fath *et al*., 2001). ABA promotes aleurone cell viability and GA promotes aleurone cell death (Kuo *et al*., 1996; Wang *et al*., 1996; Wang *et al*., 1998).

Baydar (1998) studied the changes in the amounts of endogenous GA$_3$, IAA and ABA during different growth periods of the safflower (*Carthamus tinctorius*) using HPLC. He confirmed the
correlation between low levels of GA$_3$ and high levels of ABA during initiation of flowering. Endogenous level of IAA was found to be high during bud formation, indicating its role in the differentiation of bud formation.

Baydar and Harmankaya (2005) analyzed the concentration of GA$_3$, IAA and ABA using gas chromatography (GC) in the berries of 3 grape (*Vitis vinifera*) cultivars to elucidate the possible relationship between endogenous hormones and berry set mechanism. They suggested that the levels of GA$_3$ and IAA were high in the initial stages of berry growth but declined to very low levels at the time of ripening. On the other hand changes in ABA levels were closely associated with ripening. A close relationship between hormone contents in berries and the degree of seed development was thus concluded.

Endogenous levels of IAA and Indole-3-butyric acid (IBA) were quantified in the leaves of tobacco (*Nicotiana tabaccum*) by Sutter and Cohen (1992) who found 9 mg per gram of free IBA as compared to 26 mg per gram of free IAA. Slightly less concentration of total IBA than IAA was reported in *Arabidopsis* by Ludvig-Muller *et al.* (1993).

Dullaart (1970) studied the concentrations of IAA and Indole-3-carboxylic acid (ICA) in the acid ether-soluble fraction of methanol extracts of root nodules and roots of *Alnus glutinosa* using 2-D thin-layer chromatography. Substantially more IAA was detected in the nodule tissues than in roots. ICA was present in measurable amounts only in the root extracts. Biochromatographic investigations of the extracts revealed IAA to be the main auxin in the nodule tissue.

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 megagametophytes after pollination and through the seed development. Levels of GA$_4$ slightly decreased while GA$_9$ increased during this period.
Zaffari et al. (2002) analyzed the levels of IAA and CKs in rhizomes of micro propagated normal and dwarf plants of banana (Musa spp.). The levels of IAA were found to be 1.5 times higher than in dwarf plants. While the endogenous levels of total CKs were the same in both the materials.

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 (2005) reported large amount of GA3-like substances and ABA in the developing seeds of cucumber (Cucumis sativa). While low levels of all endogenous hormones during the germination of mango (Mangifera indica) seeds was reported by Sant Ram et al. (1991).

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. reboside (ZR) and isopentenyladenine (IPA) and combined amounts of GA1, GA3, IAA and ABA during germination in sorghum. He reported higher concentration endogenous ABA in the embryo prior to germination. Shendy and Smith (1975) analyzed various phyto hormones using GCMS-MS from the 8days old cotton ovules. ABA, IAA, GA1, GA3, GA4, GA7, GA9, and GA13 were extracted in the first fraction, ethyl indole-3-acetate and indole-3-aldehyde in the second fraction and CKs in the third fraction.

Topcuoglu et al. (1996) assessed the levels of free, bound and total ABA in testa and embryo of sunflower (Helianthus annuus) by GLC. The contents of free ABA were found to be higher than those of the bound ABA in the testa and embryo. The level of ABA was found to be higher as compared to that of the embryos.
Considerable attention has been given to the endogenous level of CKs during the germination of non-dormant kernels of *Zea mays* (Smith and Van Staden, 1978). Many reports have indicated an increased level of CKs in germinating seeds, particularly the butanol-soluble forms, which are result of inter conversion of CKs from water soluble or storage forms (Brown and Van Staden, 1973).

No study has been conducted on the endogenous hormones in bamboo seeds except for the only work in our lab 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*, *Thrysotachys 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.

### 2.12. SEED VIGOUR

Seed vigour can also be defined as "the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence". In any seed lot, losses of seed vigour are related to a reduction in the ability of seeds to carry out all the physiological functions that allow them to perform. This process, called physiological ageing (or deterioration), starts before harvest and continues during harvest, processing and storage. It progressively reduces performance capabilities due to changes in cell membrane integrity, enzyme activity and protein synthesis. These biochemical changes can occur very quickly (within a few days) or more slowly (in years), depending on genetic makeup and environmental factors which are not yet fully understood. The end point of this deterioration is ultimately the death of the seed (i.e. complete loss of germination). However, seeds lose vigour before they lose the ability to germinate. That is why seed lots that have similar high germination values can differ in their
physiological age (the extent of deterioration) and so differ in seed vigour and therefore in the ability to perform. These seed vigour differences exist in seed lots of agricultural, horticultural and silvicultural species.

2.12.1. Factors affecting seed vigour

Seed quality and seed vigour is influenced by many factors such as:

i. Genetic constitution, which determines the quality of seed such as mechanical integrity, protein content, resistance to diseases and seed size;

ii. Environment during seed development which includes effect of soil moisture, fertility, and post maturation/pre-harvest environment.

iii. Seed storage including time of seed storage, type of seeds stored, and seed storage environment i.e. temperature, relative humidity and oxygen levels.

After the seeds have been harvested, the first two factors (i & ii) are no more under control, so it becomes important to monitor the last factor viz. seed storage.

2.12.2. Effect of storage on seed vigour

All seeds stored under air dry conditions suffer some a degree of deterioration which may have been incurred, during delays between collection and processing (if seeds are held under inappropriate conditions) during processing, or in storage. Even in the natural environment, macromolecules within the seed tissues will incur some damage through normal metabolism. However if the damage incurred is not too severe, repair will be possible. Villiers and Edgcumbe (1975) showed that if oxygen is available, fully hydrated seeds of lettuce (*Lactuca sativa*) are able to repair chromosomal aberrations, provided germination is prevented. Similarly, repair of DNA was detected prior to
germination in imbibed seeds of *Zea mays* (Zlatanova et al., 1987) and in embryos of *Avena fatua* and *Secale cereale* (Elder and Osborne, 1993; Boubriak et al., 1997). Seeds that are not quite fully hydrated are also capable of repairing damage (Ibrahim and Roberts, 1983). At equilibrium relative humidities (eRHs) greater than approx. 85 % (approx. -20 MPa water potential), respiration and metabolism occur at a rate that increases, as more water is taken up by the seeds (Vertucci and Leopold, 1984, 1986). Seed longevity also increases over this range, provided oxygen is available (Roberts and Ellis, 1989). However, repair processes are unlikely to be fully functional until water potential reaches between -3 and -1.5 MPa (approx. 98 % eRH) (Vertucci and Farrant, 1995; Pammenter and Berjak, 1999).

Orthodox seeds are characterized by their ability to tolerate desiccation and to retain their viability for a long time in the dry state. Even under uncontrolled storage conditions, seeds of a majority of species are able to survive for up to 4 years (Priestley, 1986). Some lotus seeds nearly 500 years old were found to be still viable by Priestley (1986). A seed is alive, carries on reproduction, and requires oxygen and food, the food coming from stored carbohydrates in the seed and the oxygen from the atmosphere. Apparently, a seed does not require much food, or it would be dead after 500 years. This stability of orthodox seeds in the dry state has been a crucial factor in the development of agriculture and human civilization. However, all seeds age during storage and eventually lose their ability to germinate.

The seeds have to be stored on year to year basis for crop production and most long-term preservation of plant germplasm is via dry seeds stored at low temperature. During the last 20 years, considerable research has been conducted to understand the physical and chemical changes involved in loss of seed viability during storage. As the seeds are stored, they surely undergo ageing during which the symptoms like reduced rate of germination (Priestley, 1986), reduced vigour and greater susceptibility to attacks by microorganisms (Harrington, 1972) are observed. The end result of delirious changes of
stored seeds is the loss of germinability (Bucharov and Gantcheff, 1984). Heydecker (1972) showed a decrease in the rate of germination and seedling growth and Mackey (1972) showed an increase in the number of morphologically abnormal seedlings as a result of ageing. Richa et al. (2006) reported that the seeds of five species of bamboos showed a marked decrease in viability after 12 months of storage. They suggested that decrease in germinability was accompanied by increase in inhibitory substances (ABA) and a decrease in promoters (IAA). Similar results were shown by Narayanaswamy et al. (2000) in seeds of soyabean (Glycine max) where a decrease in germinability and vigour index occurred with increase in storage period was observed.

Loss of vigour and viability during dry storage includes a wide range of degenerative events that accumulate over time, causing loss of viability (Priestley, 1986; Smith and Berjak, 1995). These changes are both physiological and biochemical. The physiological changes induced by seed ageing include decreased rates of germination and seedling growth (Heydecker, 1972), increased number of morphologically abnormal seedlings and decreased ability to emerge when sown under stressful conditions (Mackay, 1972), increased metabolite and ion leakiness (Roberts and Ellis, 1982), and greater susceptibility of seedlings to pathogens (Christensen, 1973). At the biochemical level, seed ageing is accompanied by: decline in metabolic activity upon germination (Ferguson et al., 1990), changes (in most cases a decrease) in enzymatic activities (Ganguli and Sen-Mandi, 1993; Bernal-Lugo et al., 1994; Aung and McDonald, 1995), and a decrease in protein and nucleic acid biosynthesis (Dell’Aquila, 1994; Cruz-Garcia et al., 1995). Lesions in DNA (Roberts, 1988) and loss of membrane integrity have also been reported to cause seed ageing (Basavarajappa et al., 1991; Dawidowicz-Grzegorzewska and Podstolski, 1992). While this information describes the symptoms of seed ageing, the biochemical and molecular basis for loss of seed vigour and longevity still remain enigmatic.
2.13. SEED VIGOUR ENHANCEMENT TECHNIQUES

The development of seed invigouration treatments really started with seed priming first described by Heydecker et al. (1973). The aim of seed priming was to control seed hydration so that all seeds reached the same stage of germination before sowing and that subsequent germination was rapid and synchronous. This was achieved by allowing seeds to imbibe water from a solution of polyethylene glycol (PEG) such that imbibition ceased when the seed water potential equalled that of the PEG solution, and was achieved at a seed moisture content below that required for germination (radicle protrusion). Thus all seeds reached the same stage of "suspended animation" before sowing. Different priming conditions have been applied to many species varying the concentrations of PEG (and therefore water potentials used), temperature and timing of treatment. Improvements in the rate and uniformity of germination have been achieved in a range of species (Heydecker and Coolbear, 1977).

Early priming treatments involved sowing moist seeds immediately after soaking in water. This led to the improvements as the result of an advancement of germination (Heydecker and Coolbear, 1977). However, in some cases when seeds were dried after soaking in water, some degree of improvement was still remained which lead to the suggestion that metabolic repair by water might also be involved in response to priming. Subsequently, priming treatments have consistently involved drying the seeds back as this makes subsequent handling and sowing of the seeds easier.

The principle of using controlled hydration in seed invigouration has also been used in a number of other treatments that differ in their means of controlling hydration (Khan, 1992). These include drum-priming (Rowse, 1996), solid matrix priming (Taylor et al., 1988), hydropriming (van Pijlin et al., 1996) and aerated hydration (Thornton and Powell, 1992). In drum-priming, seed moisture content is raised by the addition of a precise volume of water to the seeds whereas during solid matrix priming and hydropriming seeds imbibe until their water
potential reaches equilibrium with a moist inert medium or humid atmosphere respectively. These treatments can take up to 14 days. Aerated hydration differs from the earlier methods in that it depends on time alone to control the level of seed hydration during a treatment period that is usually less than 36h.

Deterioration of seeds and other plant propagules is a normal natural catabolic process which terminates their life span resulting in complete loss of viability. The process may be accelerated by some pathogenic attack and/or by adverse environmental conditions. The deteriorative events follow their normal course culminating in the production of nonviable seeds. This inevitable natural detrimental process, particularly pathogen and adverse environment induced accelerated ageing; leading to quicker deterioration of seeds is a matter of serious concern to the seed technologists, crop growers, and seed men associated with seed industry. Nowadays some strategies are being adopted to prolong the storage potential of seed by using some physical and chemical manipulative methods (Chhetri et al., 1993; Basu, 1994; Rai et al., 1995; Maity et al., 2000; Chakrabarti et al., 2005).

Desertification and salinization of soil are rapidly increasing on a global scale and currently affect more than 10% of arable land, which results in more than 50% decline in the average yields of major crops (Wang et al., 2009). Therefore, understanding the mechanisms of plant tolerance to stress and high salinity is a crucial area of environmental research topic (Bartels and Sanker, 2005; Wang et al., 2009).

Germination is a critical phase in the life cycle of a plant and salinity tolerance in germination phase may be important for successful establishment for plants growing in this environment (Mandal et al., 2000; Azarrivand et al., 2006; Afzal et al., 2009; Mor et al., 2009). Application of pre-treatments such as salicylic acid and ascorbic acid in addition to hydropriming of seeds may enhance germination under salt stress by neutralizing the excessive super oxide radical or singlet oxygen. An increase in cellular level of an antioxidant such as salicylic
acid and ascorbic acid may cause enhancing stress tolerance (Khan et al., 2005). Salicylic acid is an endogenous plant growth regulator. It is involved in various physiological processes of plant growth and development such as induction of flowering (Cleland, 1974) and root growth stimulation (Gutierrez-Coronado et al., 1998). It also plays a major role during the early stages of Rhizobium-legume symbiosis (Rasmussen et al., 1991). Salicylic acid and ascorbic acid and their related components have been reported to induce significant adverse effects in environmental stress including drought and salinity (Khan et al., 2005; Wang and Li, 2006; Hamid et al., 2008; Wang et al., 2009).

Several studies have shown that salicylic acid, ascorbic acid and hyropriming pretreatment increase tolerance to salinity in wheat (Hamada and Al-Hakimi, 2001), soybean (Gutierrez-Coronado et al., 1998), Atriplex stocksii and Suaeda fruticosa (Khan et al., 2005), sunflower (Hamad and Monsaly, 1998; Kaya et al., 2006) and pigeon pea (Verma and Srivastava, 1998).

An important component of seed vigour is the rate of germination following imbibition. Various prehydration or priming treatments have been developed to increase the speed and synchrony of germination (Bradford, 1986; Heydecker and Coolbear, 1977). In general, these treatments involve imbibing seeds in osmotic solutions or with restricted amounts of water in order to allow sufficient hydration for metabolic advancement to occur, but prevent germination or loss of desiccation tolerance. Seeds treated in this way can be re-dried and will exhibit a considerable reduction in the time required for germination when subsequently re-imbibed. According to a number of tests related to the speed of germination, priming treatments can increase apparent seed vigour in both the laboratory and the field (Argerich and Bradford, 1989; Bradford, 1986; Bradford et al., 1988, 1990).

It is generally considered that the delay in germination, as seeds age, is related to damage accumulated during storage at moisture contents too low for repair processes to occur (Priestley, 1986). Upon imbibition, such damage must presumably be repaired before
germination can commence and there is considerable evidence that repair processes are occurring soon after imbibition of aged seeds (Rao et al., 1987; Davison et al., 1991). A number of studies have indicated that relatively short pre-hydration treatments (either brief imbibition in water or exposure to high relative humidity) can either improve the tolerance of seeds to subsequent adverse storage conditions or improve the vigour of aged seeds (Burgass and Powell, 1984; Rao et al., 1987). Some studies have found that extended priming treatments in osmotic solutions are also advantageous for extending longevity or improving vigour of aged seeds (Rao et al., 1987; Dell'Aquila and Tritto, 1990). Repair of damage during priming may be one of the processes responsible for more rapid germination of primed seeds (Ellis and Butcher, 1988). However, other studies have found that the storage life of seeds of Allium porrum, Daucus carota, Lycopersicon esculentum, Lactuca saliva, and Triticum aestivum is shortened following priming (Alvarado and Bradford, 1988; Argerich et al., 1989). These observations of a reduction in longevity or in resistance to deterioration after priming are inconsistent with the hypothesis that metabolic repair is the primary basis of the advancement effect of the priming treatments.

As in most species, the major cause of a decline in the vigour of seeds is believed to be seed ageing, a hypothesis that is supported by the ability of vigour tests based on ageing (Matthews, 1980). Reduced seed vigour as a result of ageing can have a considerable effect on the timing of harvest, quality and yield of vegetable crops (Finch-Savage, 1994). Seed invigoration treatments were initially developed to overcome these problems and to improve both the rate and uniformity of germination in vegetable species.

Maintenance of seed viability has been a matter of great concern to mankind as seeds have to be stored for short time before sowing or they have to be stored for long term to be sown in the next season. Modern agriculture demands that each and every seed should readily germinate and produce vigourous seedlings ensuring high yield. This can be achieved by manipulating the following:
2.13.1. Storage conditions

Harrington (1972) proposed the basic rule of seed storage which elaborated that reduction of moisture content and temperature minimizes the deterioration of seed during its storage. Roberts (1973) demonstrated that some seeds showed strange behaviour against this rule. The seeds (mostly of tropical trees) referred to as ‘recalcitrant’ would be readily killed by desiccation when moisture content falls below critical value (12.31%) and cannot tolerate freezing temperature unlike the ‘Orthodox seeds’.

Therefore, optimum storage conditions must be known in order to preserve the seed under the best possible conditions while keeping cost to a minimum (Roos et al., 1979; FAO/IBPGR, 1992). For seeds of majority of crops plants, best conditions for storage are low temperature and low atmospheric and oxygen pressure (Ellis and Robert, 1980; Ellis et al., 1990). It was proposed by several workers (Burke, 1986; Williams and Leopold, 1989; Bruni and Leopold, 1991) that survival of the dry seed is associated with their glassy state. Sun and Leopold (1995) reported that germinability of soyabean seed’s could be maintained at around 80-100% after 13-15 years of storage at 5°C and water content of 11.6 – 16.4% i.e. in glassy state. Roos and Davidson (1992) have indicated mean viability period of more than 50 years for seeds of 15 species and 72 cultivars of vegetables under optimum storage conditions. Sveinson and Bjornsson (1994) observed that in the grass Poa pratensis, stage of seed maturity at harvest, drying temperature and storage temperature affect both germinability and rate of germination of stored seeds.

Cryopreservation in liquid nitrogen (-196°C) is also being explored as a potential method to reduce the rate of seed deterioration and thus increasing the storage life of onion seeds (Stanwood and Sowa, 1995).

Post-harvest treatments usually involve retaining seeds within fruits or holding seeds at higher humidity than would be usual in normal seed processing, to delay or slow drying to rates comparable with those
which would occur in situ. Hay et al. (1997) found that in Digitalis purpurea, intact immature capsules placed under field conditions increased in both seed desiccation tolerance (survival at 15 % RH, 15°C) and longevity.

2.13.2. Seed Invigouration

Seed invigouration is ascribed to beneficial treatments applied to the seeds prior to sowing, that improves germination or seedling growth or facilitates the delivery of seeds and other materials required at the time of sowing. Seed invigouration is an improvement in seed performance by post harvest treatment resulting in improved germinability, greater storability and better yield performance than the corresponding untreated lots (Ali et al., 1990). Many seed invigouration treatments are being employed in a number of field crops to improve seedling establishment under normal and stressful conditions. The treatments used to invigourate seeds include hydropriming; seed hardening; on-farm priming; osmopriming; osmohardening; humidification; matricrimping; priming with plant growth regulators, polyamines, ascorbate, salicylate, ethanol, osmolytes; coating technologies; and more recently pre-sowing dry heat treatments. These treatments help in breaking dormancy and improving seedling density per unit area under optimal and adverse soil conditions. Induction and de novo synthesis of hydrolases such as amylases, lipases, proteases; and antioxidants (such as catalases, superoxide dismutase and peroxidases) are reported to be the basis of improved performance using these techniques. Seed priming can be performed by soaking simply in water, in a solution of salts, hormones, osmoprotectants, matric strain-producing materials, and other nonconventional means. Despite certain limitations, such as water potential, oxygen and temperature, rice seed invigouration has been worthwhile in improving rice yield and quality. Nevertheless, in-depth studies are imperative for understanding the physiological and molecular basis of rice seed priming.
Seed invigoration treatments are physiological in nature and are broadly classified as:

i) Pre-sowing treatments for improved field performance.

ii) Pre-storage treatments for better storability.

iii) Mid-storage for improving vigour, viability and productivity of old seeds.

Seed fortification, infusion and osmopriming are the important pre-sowing treatments to accomplish the seed vigour and thereby improve the plant stand in the field and finally increasing the yield and quality of the resultant seeds (Ells, 1963). There are evidences that post-harvest treatments might be used to reproduce on-plant conditions such that seed quality (germinability, desiccation tolerance and/or longevity) continues to develop if they are harvested before the point of natural dispersal (Hay and Probert, 1995; Hay et al., 1997; Hong and Ellis, 1997; Probert et al., 2007).

2.13.3. Seed fortification

It is process of enriching the seeds with bioactive chemicals for improving the germination and seedling vigour. This method involves an impregnation of required substances for invigourating the seed for improved production. Here the seeds are soaked either in water or dilute solutions of bioactive chemicals such as micronutrients, growth regulators, vitamins and seed protectants. Soaking duration of 6-24hrs is required depending upon the crop. Moisture content of seeds is raised to 20-25% just enough for endogenous impregnation of chemicals by endogenous application. The choice of chemicals, its concentration and duration of soaking vary with the species and decide the success of the treatment.

Various pre-sowing seed treatments have been developed to invigourate the seeds (Basra et al., 2002, 2003, 2004, 2005; Farooq et al., 2004, 2005, 2006). The purpose of these treatments is to shorten the time between planting and emergence (Farooq et al., 2004, 2005,
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2006) and to protect seeds from biotic and abiotic factors during critical phase of seedling establishment (Senaratna et al., 2000; Du and Young, 2002; Shakirova et al., 2003). Such treatments synchronize emergence which leads to uniform stand and improved yield (Harris et al., 2002; Du and Young, 2002). Improved seed performance has been achieved by incorporating plant growth regulators during pre-soaking, priming and other treatments in many crops (Jeong et al., 1994). Bhatt et al. (2000) found significant reduction of MGT and substantial improvement in germination when they used 100 ppm GA$_3$ pre-treatment in Myrica esculenta.

2.13.4. Seed infusion

This is a method of impregnation of seeds with bioactive chemicals through organic solvents instead of water. This infusion helps to avoid the damage caused to the seeds due to soaking in water and seed coat injury. Simultaneously, it also provides protective, regulatory and selective functions of the chemicals to improve the performance of seeds with the help of organic solvent. Duration of treatment is 5-24hrs depending upon the species. After the desired period, the solvent is evaporated by air or vacuum desiccator for 30mins to 1 hr. after complete evaporation, seeds are ready for sowing. The chemicals used for treatment are seed protectants, growth regulators, nutrients, herbicide, antidote etc. The organic solvents used in technique include acetone, petroleum ether, ethanol, dichloromethane and dichloroethane.

2.13.5. Seed priming

It is the process of controlled hydration of seeds to a level that permits pre-germinative metabolic activity to proceed but prevents actual emergence of the radicle. Seeds are soaked in variety of solutions including solutions of various inorganic salts, sugars and polyethylene glycol (PEG); a chemically inert, high molecular weight compound which does not penetrate the cell walls. The temperature maintained during priming is suggested between 10°C-15°C. The duration of priming varies with the crop.
Heydecker et al. (1973) used different terms depending upon the methods adopted for priming e.g.

(i) Osmopriming: Soaking the seeds in osmotic solutions.
(ii) Halopriming: Soaking the seeds in salt solutions.
(iii) Biopriming: Coating the seeds with biological agents like bacteria.
(iv) Solid matric priming: This consists of mixing seeds with an organic or inorganic carrier and water for a period of time. The moisture content of the matric is brought to a level just below what is required for radicle protrusion. Seed water potential is regulated by the matric potential of the seed and during priming the water is largely held by the carrier. Seeds can imbibe water from the carrier till the equilibrium is reached.

Increases in the rate of germination through priming have been attributed to the advancement of germination metabolism (Sarocco et al., 1995; Soeda et al., 2005), enhanced antioxidant activity (Bailly et al., 2000) and, particularly where longevity is improved, repair processes (Burgass and Powell, 1984; Ashraf and Bray, 1993; Sivritepe and Dourado, 1995). Negative effects of priming have been explained as the consequence of germination sensu stricto having advanced to a state where seeds have lost desiccation tolerance (Sliwinska and Jendrzejczak, 2002) or are less able to resist damage during air-dry storage (Powell et al., 2000).

Seed priming techniques have been used to reduce the emergence time, to get synchronized emergence, to improve emergence rate, and to have better seedling stand in many horticultural (Bradford et al., 1990; Khan, 1992; Rudrapal and Nakamura, 1998) and field crops such as wheat, maize (Dell’Aquila and Tratto 1990; Chowdhary and Baset, 1994; Basra et al., 2002) and more recently rice (Lee and Kim, 1999, 2000; Basra et al., 2003, 2005; Farooq et al., 2004, 2006).

Drying and subsequent storage of primed seeds is a crucial component of this technology for its commercial application. For proper
storage seeds should be dried to acceptable moisture content. Rate of dehydration and temperature after priming are critical factors affecting the seed quality.

2.13.6. Seed hardening

Seed hardening is a method to overcome adverse conditions like low rainfall, inadequate soil moisture, soil fertility which prevent the germination and establishment of seedling. Seed hardening given as pre-sowing treatment serves as a boon for dry land agriculture. For seed hardening, various chemicals such as KCl, succinic acid, ascorbic acid; growth regulators like IAA, GA, NAA; plant nutrients; and animal and plant waste are used. Methodology slightly varies depending upon the type of seeds. Hydration can be accompanied by spraying the seeds with fixed quality of water, dipping the seed in water or by using moist gunny bags. After hydration, seeds should be soaked in hardening solution at recommended volume for a particular duration. Seeds of sorghum, pigeonpea, cowpea, groundnut soaked for 12, 4, 4, 6 hrs respectively in CaCl₂ (0.4%) and Cycocel (0.2%) registered maximum germination percentage, vigour index, root and shoot length (Rangaswamy et al., 1993).

Seed hardening is the process of hydrating the seed to initiate the pre-germinative metabolism followed by dehydration which fixes the biochemical events. It is done in order to impart resistance against stress conditions viz., drought and cold, to the emerging seedlings. Seeds or grains are allowed to take up a certain amount of water, and then they are kept moist at 10° - 25°C for several hours before drying in a steam of air. The best results can gain in two - three cycles of wetting and drying, although for someone cycle is sufficient. Different amounts of water are recommended for different species and cultivars of seed or grain.

Seed hardening has been used successfully for vigour enhancement in indica (Basra et al., 2003, 2004) and japonica rices (Lee et al., 1998; Lee and Kim, 2000). In hardening, seeds are exposed
to alternate wetting and drying in distilled or tap water (Pen Aloza and Eira, 1993). This hydration-dehydration cycle may be repeated twice, thrice etc. (Lee et al., 1998; Lee and Kim, 2000). Lee and Kim (2000) investigated the effects of osmoconditioning and hardening on the germination of normal and naturally aged seeds by analyzing the total sugars and α-amylase activity. Total sugar content and the α-amylase activity of normal seeds were higher than those of aged seeds. Aged seeds treated with osmoconditioning and hardening increased their total sugar content and α-amylase activity, but hardening was more effective than osmoconditioning. The α-amylase activity was positively correlated with total sugars and the germination rate. Seed hardening for 24 h (two cycles) invigorated the indica rice seeds compared with seed hardening for 18 h, osmoconditioning (−1.1 MPa KNO₃), and traditional soaking of indica seeds (Basra et al., 2003, 2004).

2.13.7. Seed pelleting

Seed coating methods act as efficient carriers, which can be applied on the seed surface. The chemicals involved are mostly fungicides and insecticides (Huijbregts et al., 1995), hormones (Tonkin, 1984), peroxidase (Dadlani et al., 1992), hydrophilic and hydrophobic compounds (Baxter and Waters, 1986; Henderson and Hensley, 1987). One of the seed coating method is seed pelleting (Taylor et al., 1998). Pelleting is defined as the deposition of a layer of inert materials that obscures the original shape and size of the seed, resulting in a substantial weight increase and improved plant ability (Taylor et al., 1998). This method is used to protect rhizobia, increase in seed weight or size (Halmer, 1988), attract moisture (Scott, 1989), stimulate germination, delay germination, and supply oxygen (Halmer, 1988).

Seeds are introduced into a coating drum or pan that resembles a cement mixer. An amalgam of pelleting materials (clays, limestone, calcium carbonate, talc, vermiculite) and cementing adhesives (gum arabic, gelatin, methylcellulose, polyvinyl alcohol, polyoxyethylene glycol-based waxes) are used to form the pellet and other compounds such as innoculants, fungicides, etc. may be added to enhance seed
performance. As drum rotates, the seeds are first sprayed with water followed by the addition of the pelleting materials with binder. The wet seed attracts and becomes coated with the dry pelleting material and the pellet gradually increases in size with each turn of coating drum. Longer rotation times with greater amounts of pelleting materials lead to greater pellet size and roundness. At the end of the pelleting process, a binder is added to harden the outer layer of the pellet. The ingredients of the seed pellets exerts respective positive influence on seed performance eg., Diammonium phosphate stimulates prolific root growth.

The inert material creates natural water holding mediums, protection from insects and birds and provides small amount of nutrients to young seedlings. The main object of seed pelleting is precision, especially in very small seeds, with added advantage of better establishment and increased productivity. There are three basic steps involved in pelleting i.e. stamping, coating and rolling. Materials needed for pelleting are seed, adhesive and filler material. The seeds are uniformly coated with adhesive in correct quantity initially. Then the filler materials are sprinkled on the coated seed and are rolled on the filler material, for effective and uniform coating. Based on the type of filler material pelleting is of different types i.e. Inoculant pelleting, Protective coating, Herbicide coating, Nutrient coating, Hydrophilic coating etc. Probert et al. (2007) reported that fuzz and delinted seeds of cotton pelleted using 5% maida as an adhesive and finely powdered arappu leaf powder, DAP, micronutrient and Azospirillum provided high germination percentage and better seedling growth. Same was true for wheat seeds coated with 0.3g riadimenol/kg of seed and 0.6g of tebuconazole/kg (Gao et al., 2000).

Seed pelleting was found to increase the germination and better stand establishment and seed yield in green gram (Kavitha, 2002). Seed pelleting will be relevant in direct sown irrigated crops which improves initial vigour for sustained crop growth and development to overcome adverse situations.
The assessment of seed vigour has important implications for the seed industry and seed consumers. Vigour tests are commonly used by seed production companies to establish "in house" seed quality standards, to monitor seed quality during the various phases of seed production and processing. This allows them to identify where losses of seed vigour occur, and to identify practices which could subsequently lead to improved seed vigour. Seed store managers may use vigour test results to make better informed decisions about the suitability of seed lots for storage, the possible length of storage time and the storage conditions required. Seed exporters can use vigour information to decide which seed lots can withstand the rigours of transport and thus be expected to arrive in the importing country with quality unimpaired. For the ultimate consumer, the farmer, it would be advantageous to know the vigour status of each high germinating seed lot before making any decision as to which one to buy. Such information will not usually provide an expected field emergence value, but will indicate whether a seed lot is of high or low vigour, and therefore indicate which seed lots will be more likely to perform under sowing conditions which provide some form of stress.

Keeping in mind all the above mentioned vital factors of seed vigour and viability, the present work was undertaken with a view to study the physiological and biochemical aspects of the causes of ageing of bamboo seeds with the following main objectives:

1. To study germination profile and metabolism of ageing seeds of bamboos at different storage intervals.
2. To study ultra-structural changes in ageing seeds.
3. To enhance seed vigour of ageing seeds by the application of vigour enhancement techniques.