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Effect of Seed Ageing in Biochemical and Molecular Changes in Oilseeds: A Review

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ABSTRACT

Seed deterioration is the loss of quality, viability and vigour either due to ageing or effect of adverse environmental factors. The process of deterioration is an irresistible physiological phenomenon. The seeds which are stored under suitable conditions may sometime undergo a series of changes which can ultimately result in the deterioration of the quality of the seed. Hence a thorough study should be done in these deteriorative changes so that proper measures can be adopted to control the variations. Therefore in this article the changes within cellular, biochemical and metabolic aspects of long term stored oilseeds are discussed.

Key words: Antioxidants, Biochemical, Enzyme degradation, Lipid peroxidation, Metabolic, Seed deterioration.

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Seed ageing is an inexorable and irreversible process (Delouche, 1973). Seed being a living entity is vulnerable to ageing and leads to reduction in seed quality, performance and its field establishment. Seed ageing is a natural process which involves cytological, physiological, biochemical and physical changes in seeds. These changes reduce viability and ultimately cause death of the seed. The rate of deterioration fluctuates critically from one species to another and also among varieties of the same species (Justice and Bass, 1978; Preistly *et al*., 1985; Agarwal *et al*., 1987) Deterioration is evident as a reduction in percentage germination, produce weak seedlings, loss of vigor, become less viable and ultimately seed death (Maity *et al.*, 2000; Tilebeni and Golpayegani, 2011).

The speed of deterioration of oil seeds depends on the condition of the storage environment and the particularities of the species which include the seed chemical composition. Once seed deterioration has happened, this catabolic process cannot be reversed It is a sequence of events beginning with a chain of biochemical events, predominantly membrane damage and impairment of biosynthetic reactions and then the resulting losses of various seed performance attributes, starting with reduced germination rate, reduced field emergence, increased numbers of abnormal seedlings and finally seed death. Viability loss results in irreversible chemical and structural changes to cellular constituents (Walters, 1998).

Annual losses due to deterioration can be as much as 25% of the harvested crop. It is one of the basic reasons for low productivity. The loss of viability can have a positive correlation with the changes in genetic diversity, so to prevent this regeneration of the accessions has to be done as fast as possible especially when the viability percentage fallen to 5 to 10 percent. Genetic drift within the population can be observed in most of the long term stored seeds either because of seed aging or due to the increasing proportion of mutation as the seed ages (Kozlowski, 2012).

Among the factors affecting seed quality during storage, the initial quality of seed lot, the environment for conservation with its variation in temperature, moisture, oxygen availability as well as the characteristics inherent to the species should be taken care of. During storage in gene banks, seed ageing. Seeds rich in lipids have limited longevity due to its specific chemical composition and also a trend of declining oil content and seed germination in long termed seeds. Seed deterioration is associated with various cellular, metabolic and chemical alterations including loss of membrane integrity, reduced energy metabolism impairment of RNA and protein synthesis and DNA degradation may result in loss of genetic diversity.

Seed deterioration

Seed deterioration can be defined as “deteriorative alterations occurring with time that increase the seed’s exposure to external challenges and decrease the ability of the seed to survive. It results in the loss of quality, viability and vigour, along with time either due to ageing or effect of adverse environmental factors. The rate of deterioration rapidly increases with increase in either seed moisture content or temperature of storage (Ellis *et al*., 1991).

Mechanism of seed deterioration

(Roberts, 1972) and depletion of food reserves (Barton, 1961; Ravikumar *et al.*, 1998). The possible sequence of events that occur in seed deterioration was proposed by

many workers (Roberts, 1972; McDonald, 1999). Disintegration of the cell membranes is generally the initial step followed by the degradation of cellular organelles and impairment of biosynthetic processes. Some of the internal factors which contribute to the seed deterioration process are loss of membrane integrity, change in the structure of macromolecules (Barton, 1961; Wilson and McDonald, 1986; Wett laufer and Leopold, 1991) , enzyme degradation (Barton, 1961; Bailly *et al.*, 1998; Ravikumar *et al.*, 1998), genetic degradation

Cellular level deterioration

Damage to cell membrane

Cell membrane lose their selective permeability allowing the cytoplasmic contents to leach into the intercellular spaces. Membrane disintegration occurs from both hydrolysis of phospholipids by phospholipase enzyme and phospholipid auto oxidation. Damage to the cell membrane during storage is the most important factor leading to seed deterioration (Priestly and Leopold, 1979; Ferguson *et al.*, 1990; Aiazzi *et al.*, 1997). Among the seed vigour tests the measurement of electrical conductivity has been frequently used to evaluate the physiological activity. As seeds get aged there is an increased memebrane permeability of the seeds which results in an increased EC (Ferguson *et al.*, 1990). Three ultra-structural symptoms related to age induces membrane deterioration were observed in studies conducted in *B.napus* (Alina Dawidowiez *et al.*, 1992) they were, the lowering of electron contrast in all cellular membranes excluding plasma lemma , coalescence of small storage lipid bodies to larger unit as a result of the degradation of enclosing half unit membrane and the appearance of protoplasmic inclusions inside the storage protein bodies, possibly resulting from the rupture of the enclosing unit membrane.

Mitochondrial degradation

Mitochondrial degradation and functional changes appear like it becomes permanently swollen and loose their natural swelling and contracting ability later become pigmented and fragmented. Two important aspects of mitochondrial deterioration are an increase in ATP ase and decline in oxidative phosphorylation ability (Preistly, 1986; Walter, 1998; Murthy *et al.*, 2002). The peroxidative damage to mitochondrial membrane lipids are responsible for decline in respiration rate and inefficiency of energy production of mitochondria in aged soybean seeds (Abu-Shakra and Ching, 1967).

Disfunction of ribosomes

Evidence indicate that dissociation of polyribosomes must occur before attachment of preformed mRNA that will certainly affect the process of protein synthesis. In non-viable seeds the ribosomes fail to dissociate and protein synthesis is retarded which is a measurable symptom of ageing (Smith and Berjak, 1995; Walters, 1998).

Genetic degradation

Presence of chromosomal or DNA aberrations within cells along with loss of seed viability has been observed in plants like broad bean,pea,barley,datura,tobacco and many others (Abdalla and Roberts, 1968). Progressive fragmentation of embryonic nuclear DNA occur during seed ageing. DNA damage can be due to DNA oxidation or due to DNA laddering as in commonly observed in active and genetically controlled PCD. Potential targets for oxidative damage in the DNA chain include the purine and pyrimidine bases as well as as the deoxyribose sugars (Larson, 1997; Roldan – Arjona and Ariza, 2008). As the seeds become older, the DNA repair processes become slower due to inactivation of some of the key enzymes such as DNA ligase and DNA polymerase (Coello and Vazquez- Ramos, 1996; Schoen *et al*., 1998; Chwedorzwwska *et al*., 2002) have also suggested that mutation accumulations occur in long term storage of germplasm and such type of mutations have the ability to reduce the viability of the seeds. The volatile aldehydes which is formed as a result of lipid peroxidation, cross link the macromolecules like the sugars, amino acid or polypeptide chain, thereby altering their structure leading to an increased possibility of genetic mutations during seed ageing.

Several different molecular methods are available for the identification of genetic diversity. The polymerase chain reaction (PCR) methods using primers have been widely used in the last 10 years. The PCR base marker based techniques are used for measuring genetic diversity in many plant species.

Shatters *et al* (1994) observed polymorphism in the RAPD profiles of DNA from different aged soybean seeds. similar observations were also made from the analysis of RAPD and AFLP markers in the long term stored rye seeds (Bednarek *et al.*, 1998). But Zhang *et al.,* 1996 showed that the natural deterioration did not affect the RAPD markers in long term stored soybean seeds. Later Song *et al.,* 1999 has proposed a set off 13 selected SSR loci foe use in DNA profiling of soybean cultivars. These reports further helps to take a deep look into the effects of seed ageing with the help of DNA fingerprinting. With the increasing development in genome sequencing and accessibility to the gene bank, new molecular markers for specific gene or coding regions of the genome are being designed.

Biochemical changes

Lipid peroxidation

Lipid peroxidation is oxidative damage that affects cellular membranes, lipoproteins and other molecules that contain lipids in conditions with oxidative stress Cellular membrane lipids represent most often substrates of oxidative attack (Nawrot *et al*, 2008). Lipid peroxidation is a chain reaction and is created by free radicals influencing unsaturated fatty acids in cell membranes, leading to their damage. Free radicals are initiators and terminators of lipid peroxidation processes. Once activated, reaction continues auto catalytically; it has a progressive course and its final

result is structural and functional changes of substrate (Ognjanovic *et al.*, 2008). Lipoxygenase an oxidative enzyme present in many unimbibed seeds is also capable of producing activated oxygen and subsequently catalyzing lipid peroxidation by using membrane and phospholipid components as substrates (Preistly, 1986).

Changes in chemical constituents of cell have been related to viability of seeds. Srivastava and Gill (1975) studied on physiology and biochemistry of seed deterioration in soybean seeds during storage and they suggested that the increase in electrical conductivity of leachates indicated an increase in deterioration. Paramasivam *et al.* (1990) reported that the germination of groundnut seeds were negatively correlated with electrical conductivity of seed leachates. Volatile aldehydes are the major product of lipid hydroperoxides resulting in the dehydration of fatty acids to smaller and volatile carbon compounds such as hexanal pentanal amd butanal. The accumulation of these volatile aldehydes indicate the occurrence of lipid peroxidation and thereby seed deterioration. Braccini *et al.* (2000) observed a reduction in the protein, lipid and poly unsaturated fatty acids content and an increased amount of hexanal in the storage of soybean seeds.

Enzymatic Changes

Enzymatic changes may seem to be also useful in studies on seed deterioration. Analysis of enzymatic variations within a long term stored seeds can be used to identify the seed ageing process. Many workers have found that there is an increase in some enzymes while in storage but at the same time some enzymes show a reduction in the level. The decrease in the antioxidant enzymes is linked to an increase in peroxidation of lipids and is associated with a positive correlation between the antioxidant capacity of the enzyme and the vigour of the seeds (Bailly *et al*., 1998). The most important hypothesis regarding seed ageing is the degradation of enzymes due to structural changes at the macromolecular level. Correspondingly it has been concluded that aged seed have reduce activity of enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). Free radicals in ageing seeds in the absence of these enzymes leads to decline in seed viability. Decreased activity of enzymes such as catalase, dehydrogenase and glutamic acid decarboxylase were reported by Bailly 2004. The seed ageing has also resulted in the formation and activation of some of the hydrolytic enzymes. As seed moisture content approaches levels necessary for germination, hydrolytic enzymes are activated, however, if these moisture content exceeds then it will leads to deterioration because of energy expenditure and accumulation of breakdown products (Copeland and McDonald, 1995). Under low moisture storage the reduced respiration and enzyme activity may be responsible for accumulation of toxic compounds that reduce seed viability. Bailly *et al.* (1998) has shown that lipid peroxidation resulted in losses of free radical scavenging which is thought to be involved in deterioration of sunflower seeds during accelerated ageing. It was also characterized by a decrease in the activities of catalase and Glutathione reductase (GR). Balesevic *et al.* (2005) determined the degree of biochemicals changes during accelerated and natural ageing on five cultivars of sunflower seeds lipid peroxidation and decrease in SOD and peroxidase activity were caused by both type of ageing.

Metabolic changes

Ageing is an irreversible process which decrease the seed quality as storage period increases. Decline in germination may be due to depletion of food reserves and decline in synthetic activity due to ageing as reported by Heydecker (1972). Changes in proteins are evidenced with ageing of seed (Roberts, 1972; Preistly, 1986). Free radicals produced during lipid peroxidation not only attack fatty acids but can also change the protein structure and lead to decreased protein content in seeds (Kalpana and Madhavarao , 1997).

The chemical protein reactions that may lead to loss of seed viability were investigated by Maillard products. This reaction is catalysed by glycosilation or glycation associated with the covalent attachment of reducing sugars to amine groups of amino acid and protein to form glycated protein. The importance of Maillard reaction for the loss of seed viability was investigated by Wettlaufer and Leopold (1991) who observed that seed germination decreased as Maillard products accumulated in soybean embryos. There has been recent investigation into novel non-destructive methods of technologies to test seed viability and deterioration. The technique is nothing but measuring the products of respiration by seeds (Kranner *et al*., 1996) and assumes that the metabolic activity of seeed changes with increasing age and decreasing viability and quality. Verma *et al* (1987) reported a decrease in carbohydrates and proteins content in deteriorated seeds. Narayanaswamy (2003) concluded that oil, protein and field emergence of groundnut seeds decreased but free fatty acid and EC increased with advancement of storage period.

Control of seed deterioration

As mentioned seed ageing is an irreversible process and it cannot be stopped but measures can be adopted to control it to a certain extent. Some of the measures to control seed deterioration are as follows:

- Use of anti-oxidants to protect from free radicals.
- Important antioxidants are α tocopherol, ascorbic acid, glutathione carotenoids and phenolic compounds such as flavanoids.
- Use of halogen vapours to stabilize unsaturated fatty acids
- Reducing the amount of oxygen around the seed may decrease initiation of free radicals.
- Provision of controlled conditions in storage such as low temperature and relative humidity.
- Seed priming is also an important method to control deterioration.

- Dey and Mukherjee (1988) found that treatments with aminobenzoic acid and hydrobenzoic acid had improved the germination percentage in mustard seeds.

CONCLUSION

In any crop species of seed production ends with safe storage of seeds. Apart from genetic factors, storage potential also varies among genera, species and varieties and even it extend to initial seed lot. Further among different crop seeds, oilseeds are considered to be very poor storer and are classified as medium storer because of the presence of lipids and fatty acids. Loss of seed viability due to ageing and deteriorative changes are irreversible processes but the rate of deterioration can be slowed down by keeping or storing it in controlled conditions of low temperature and relative humidity. While in storage the oilseeds undergo changes in cellular, biochemical and metabolic aspects. Even though many factors contribute to the seed deterioration process in oilseeds, lipid peroxidation and enzyme degradation are the main causes for reduced viability and quality in aged seeds.

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