



RESEARCH ARTICLE

PHYSIOLOGICAL AND BIOCHEMICAL CHANGES DURING SEED DETERIORATION: A REVIEW

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ABSTRACT

Seed deterioration is an undesirable and detrimental attribute of agriculture. This process is a separate event from seed development and germination. Losses in seed quality occur during field weathering, harvesting and storage. Deterioration caused by field weathering is directly related to seed exposure to adverse conditions. Seeds are highly susceptible to damage and mechanical injury during post-harvest handling. Several environmental factors contribute to seed deterioration and these conditions make very difficult to maintain viability during storage. Seed quality depends upon initial seed quality, temperature, moisture content and mycoflora. Rapid deterioration resulting due to environmental conditions make it very difficult to maintain its viability during storage. However, the seed quality and viability during storage depends upon the initial quality of seed and the manner in which it is stored. Seed deterioration is associated with various cellular, metabolic and chemical alterations including lipid peroxidation, membrane disruption, DNA damage, impairment of RNA and protein synthesis and causes several detrimental effects on seed.

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INTRODUCTION

During storage, seed moisture content and temperature are responsible for seed deterioration, many physiological and biochemical changes occur in seeds during seed ageing. Deteriorative changes enhance when seed exposure to external challenges increases and decrease the ability of the seed to survive. It is an undesirable attribute of agriculture. 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 (Shelar *et al.*, 2008). The process has been described as cumulative, irreversible, degenerative and inexorable process (Kapoor *et al.*, 2011). Many physiological and biochemical changes occur in seeds during seed ageing. Some of the physiological changes have been observed during seed deterioration which are measures of seed vigor in many species during storage (Yaklich and Abdul-Baki, 1975; Agrawal and Kharlukhi, 1985; Fernandez and Johnston, 1995; Santipracha *et al.*, 1997). The biochemical changes associated with seed deterioration is the increase in leakage of biomolecules (Chingand Schoolcraft, 1968; Agrawal, 1977; Parrish and Leopold, 1978; Agrawal and Kharlukhi, 1985), decrease in total soluble sugars (Agrawal and Kharlukhi, 1985)

and protein content (Kalpana and Madhava 1997) where as amino acids (Ghosh *et al.*, 1981) and protease activity increases with seed deterioration (Halder and Gupta, 1980; Agrawal and Kharlukhi, 1987) but decrease in - amylase activity has been reported in some species (Mitra *et al.*, 1974; Agrawal and Kharlukhi, 1987; Kalpana and Madhava 1997). As seed deterioration increases, seed performance progressively decreases. The physiology of seed deterioration is a distinguish consequence from seed development and germination. Losses in seed quality occur during field weathering, harvesting and storage. Several factors contribute to the susceptibility for seed deterioration. The basic causes are temperature, relative humidity, seed moisture content and by invasion of and damage to tissues by microorganisms and insects.

The rate of deterioration fluctuates critically from one species to another and also among varieties of the same species (Jatoi *et al.*, 2001). 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 percentage emergence of deteriorated seeds is less than healthy seeds. Hence, deteriorated seed produces uneven stands, spotty fields,

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and fewer plants per hectare than healthy seed (Biabani *et al*, 2011). Plants that have originated from deteriorated seed can also reduce growth rate (Kapoor *et al*, 2010).

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”. Seed deterioration causes loss of seed quality with time. It 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.

Deterioration Types

Deterioration is evident as a decrease in percentage germination, while those seeds that germinate produce weak seedlings. Losses in seed quality occur during field weathering, harvesting and storage (Farhadi *et al*, 2012). Harvesting time of several crops depends on its maturity time and on physiological maturity. Harvesting stage influences the quality of seed, germination, vigor, viability and also storability (Khatun *et al*, 2009). Physiological maturity attainment is a genotypic character which is influenced by several environmental factors (Mahesha *et al*, 2001). Deterioration of seed in the field before harvest (field weathering) begins when the seed reaches physiological maturity and it extends till the seeds are harvested. The moisture content of physiologically matured seed is approximately 50- 55%, because of the high moisture content; the seed cannot be harvested commercially and must remain in storage on the plant through a desiccation period till moisture levels are adequately low to permit mechanical harvest without causing damage to the seed. This desiccation period vary from a few days to several weeks before the seed attains a harvestable moisture level, about 14%. During this post maturation, pre-harvest period weather conditions have a great influence on the quality of the harvested seed. Field conditions are rarely favorable for such storage. Seed quality is influenced by numerous factors that occur in the field before harvesting and during harvesting, drying, processing and storage. The losses are worsened if seeds are stored at high temperatures and high relative humidity conditions (MosaviNik *et al*, 2011).

Weathering

The deterioration of seed quality, vigor and viability, due to high relative humidity and high temperature during the post-maturation and pre-harvest period is referred to as field weathering (Bhatia *et al*, 2010). Weathering occurs in the period between the attainments of physiological maturity till harvesting in the field. Deterioration caused by weathering is directly related to seed exposure to adverse conditions, so that the physiological quality is depending on the environmental conditions preceding harvesting (Pádua *et al*, 2009). Exposure to hot and humid conditions, rainfall, photoperiod after ripening are pre-harvest factors, cause seed quality loss following physiological maturity. Among all these factors, influence of moisture on seeds during ripening appears to exert the major influence on predisposition to weathering. Adverse

environmental conditions during seed filling and maturation result in forced seed maturation, which is associated with low yields, leading to a significant decrease in quality and an extensive reduction in the crop productivity (França-Neto *et al*, 2005; Pádua *et al*, 2009). After physiological maturity if the seeds are retained on mother plant seeds will deteriorate, physiological changes in seed may lead to formation of rigid seeds or off color seeds in pulse crops (Khatun *et al*, 2009). Harvest delays beyond optimum maturity extend field exposure and intensify seed deterioration. Weathering not only lowers seed germination, but also increases susceptibility to mechanical damage and disease infection. Timely harvesting avoids prolonged exposure to moisture, and is the best means of avoiding weathering. Harvest and Post-harvest Deterioration Seed quality is highly affected by harvesting and handling methods.

Deterioration in post-harvest

Comprises threshing, processing machinery, seed collection, handling, transporting and drying. Mechanical damage is one of the major causes of seed deterioration during storage, very dry seeds are prone to mechanical damage and injuries. Such damage may result in physical damage or fracturing of essential seed parts; broken seed coats permit early entry and easy access for micro flora, make the seed vulnerable to fungal attack and reduce storage potential (Shelar, 2008). In its severest form, physical seed damage is exhibited by splitting of the cotyledon, shattered and broken seeds. Large seeded varieties are more sensitive to mechanical damage than small seeds.

Storage of seed

Storability of seeds is mainly a genetically regulated character and is influenced by quality of the seed at the time of storage, pre-storage history of seed (environmental factors during pre and post-harvest stages), moisture content of seed or ambient relative humidity, temperature of storage environment, duration of storage and biotic agents (Shelar *et al*, 2008; Balešević-Tubic *et al*, 2005; Khatun *et al*, 2009; Biabani *et al*, 2011). Damage of seed during storage is inevitable (Balešević-Tubic *et al*, 2005). These environmental conditions are very difficult to maintain during storage. The seed storage environment highly influences the period of seed survival. After planting of deteriorate seeds, seedling emergence may be poor and transmission of pathogens to the new crop may occur. Lower temperature and humidity result in delayed seed deteriorative process and there by leads to prolonged viability period (Mohammadi *et al*, 2011).

Seed Deterioration Mechanisms

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 *et al*, 2010). Structural

changes associated with oxidation are reduced membrane fluidity, altered folding of DNA, lost elasticity of proteins and increased brittleness of the cellular matrix. Molecules oxidation leads to either smaller molecules with reactive carbonyl or nitrogen groups that easily diffuse through cells, or adducts between carbohydrates, proteins and nucleic acids that cause intermolecular cross-linking and further degrade into advanced glycation end-products (Walters *et al*, 2010).

Biochemical Manifestation of Seed Deterioration

Seed deterioration is associated with various cellular, metabolic and chemical alterations including chromosome aberrations and damage to the DNA, impairment of RNA and protein synthesis, changes in the enzymes and food reserves and loss of membrane integrity (Kibinza *et al*, 2006). Some of the major physiological and biochemical events of deterioration are presented below.

Membrane Degradation

It is extensively consented that loss in cellular membrane integrity is one of the primary causes for loss of viability. Under harsh storage conditions loss in membrane permeability leads to increased leaching of seed constituents and hence loss in viability. During seed deterioration, membrane degradation increase electrolyte leakage. Decline in seed germination, field emergence and seedling vigor is associated with high level of electrolytes leakage. Alterations of membrane systems, such as the tonoplast, plasmalemma and endoplasmic reticulum, result in diminishing of normal cell function and energy production. Membrane deterioration and loss of permeability occur at an early stage during the seed deterioration.

Alteration in Enzymes

Enzyme alterations, such as reduced activity of lipase, ribonuclease, acid phosphatase, protease, diastase, catalase, peroxidase, amylase, DNase and dehydrogenase enzymes. Reactive oxygen species (ROS) and hydrogen peroxides are produced from several metabolic reactions and could be destroyed by the activity of scavenger enzymes like catalase and peroxidase. Peroxides activity decreases substantially with ageing. Due to this seeds become more sensitive to the effects of oxygen and free radicals in membrane unsaturated fatty acids and produce lipid peroxidation products such as malondialdehyde and lipid conjugates.

Changes in Cell Chemical Constituents

In deteriorate seeds significant decrease in protein, oil content and total sugars and increase in free fatty acids and reducing sugars has been studied. (Verma *et al*, 2003) showed that carbohydrates increased with decrease in protein content in deteriorated seeds. Some studies indicated that oligosaccharide which has been associated in stabilizing membranes decreased during storage.

Reduced Metabolic Activity

High relative humidity hastens deterioration and results in reduction of nucleic acids with increased storage period.

Metabolic activities of seeds were low in non-viable seeds than in viable seeds. Long term storage decreases the ability to form nucleic acids and nucleotides.

Free Radical Damage

Deterioration is partially associated to the accumulation of free-radicals produced by the metabolic process. Seed storage subjects lipids to slow consistent attack by oxygen, forming hydrogen peroxides, other oxygenated fatty acids and free radicals. The free radicals are unstable and may react and damage nearby molecules. Oxygenated fatty acids in the absence of enzymes activity in the dry seed would accumulate and damage cellular components and leads to deterioration of seeds. Lipid peroxidation and free radicals formation are the major causes for the deterioration of oil seeds in storage.

Chromosome Aberrations

One of the changes linked with seed ageing is aberration of chromosomes, sometimes pertained to as mutagenic effect. Some of the chromosome alterations in seeds comprise fragmentation, bridges, fusion, ring formation of chromosomes and variations in nuclear size. Some other causes of deterioration are:

Degradation of functional structures

Biochemical changes resulting in lower levels of ATP, Decline in sugar content, Inability of ribosomes to dissociate, Enzyme degradation and inactivation (amylase, dehydrogenase, oxidases, phospholipase, glutamic acid decarboxylase), Formation and activation of hydrolytic enzymes, Starvation of meristematic cells, Increases in seed leachates and free fatty acid content, Reduced respiration and Accumulation of toxic compounds. Moreover the main cause of seed damage, lipid peroxidation causes initial biochemical changes in seed that can be observed during storage. Autooxidation of lipids and increase in the content of free fatty acids throughout storage period are the main reasons for rapid deterioration of seed of oil plants (Balesevic-Tubic *et al*, 2005). In sunflower seeds, loss of viability is associated with an accumulation of malondialdehyde (MDA), suggesting that seed deterioration is accompanying with lipid peroxidation related to a decline in the efficiency of the antioxidant defense system (Kibinza *et al*, 2006). Membrane disruption is one of the primary reasons attributed to seed deterioration. As a result, seed cells are not capable to hold their normal physical condition and function. Causes of membrane disruption are enhancing in free fatty acid level and free radicals productivity by lipid peroxidation (Ghassemi-Golezani *et al*, 2010). Lipid peroxidation can result in not only destruction of the lipid itself, but also damage to cell membranes and other cellular components. Free fatty acid can damage lipid bilayer particularly of mitochondria leading to reduce energy production and free radicals have potential to damage membrane, DNA, enzymes, protein and ultimately cellular repair mechanism (Ghassemi-Golezani *et al*, 2010). The rate of seed deterioration is highly influenced by environmental (temperature, relative humidity and seed moisture content) and biological factors (such as fungi that create their own biological

niche) (Ghassemi-Golezani *et al*, 2010). Seed longevity is determined by seed moisture, temperature and seed attributes that are influenced by genetic and environmental interactions during seed maturation, harvesting and storage (Walters *et al*, 2010). Several other factors such as environmental conditions during seed producing stage, pests, diseases, seed oil content, storage longevity, mechanical damages of seed in processing, fluctuations in moisture (including drought), weathering, nutrient deficiencies, packaging, pesticides, improper handling, drying and biochemical injury of seed tissue can affect vigor of seeds (Krishnan *et al*, 2003; Marshal and Levis, 2004; Astegar *et al*, 2011; Simic *et al*, 2007).

Variety of the Seed

The seed storability is considerably determined by the kind or variety of seeds. Some seeds are naturally short-lived, e.g., onion, soybeans, peanuts, etc., whereas some seeds like, tall fescue and annual rye grass, appear very similar but differ in storability. Genetic make-up of varieties also influences storability.

Genotypic Factors

Some types of seeds are inherently long lived; others are short lived, while others have an intermediate life span owing to their differences on genetic makeup.

Seed Quality

High initial viability of seeds maintains their quality in storage longer than those with less initial viability. Vigorous and deteriorated seeds can store longer than deteriorated seeds. Seeds that have been broken, cracked, or bruised due to handling deteriorate more rapidly in storage than undamaged seeds. Cracks in seeds serve as entrance to pathogens causing consequent deterioration. Seeds that have been developed under environmental stress conditions (such as drought, nutrient deficiency and high temperatures) become more susceptible to rapid deterioration.

Temperature

High temperature hastened the rate of these biochemical processes triggering more rapid deterioration that resulted in rapid losses in seed having high moisture content (Shelar *et al*, 2008). Seeds sensitivity to high temperatures is strongly dependent on their water content, loss of viability being quicker with increasing moisture content (Kibinza *et al*, 2006). Temperature is important because it influences the amount of moisture and also enhances the rate of deteriorative reactions occurring in seeds as temperature increases.

Moisture Content

Deteriorative reactions occur more readily in seeds at higher moisture content and subsequently, this condition constitute hazard to the longevity of seed survival (Vashisth and Nagarajan, 2009). Seeds stored at high moisture content demonstrate increased respiration, heating, and fungal invasion resulting in reduced seed vigor and viability. After physiological maturity the rate of seed quality loss depends on

the degree of unfavorable environmental conditions surrounding the seed. Environmental moisture, predominantly intermittent or prolonged rainfall, during the post maturation and pre-harvest period, is quite detrimental to seed quality and cause rapid deterioration. When exposed to humid conditions (heavy rain), dried seeds can absorb enough moisture to reach 27% and subsequently expand in volume. At this moisture level, seed respiration is hastened. Cotyledon reserves will be consumed, not only by the seed itself, but also by fungi allied with the seed. It has been reported that seed moisture content of about 6-8% is optimum for maximum longevity of most crop species. Below 4-6% seed moisture content lipid autoxidation becomes a damaging factor and seeds become more susceptible to mechanical damage. The moisture content of seed during storage is the most persuasive factor affecting the longevity. Storing seeds at high moisture content enhances the risk of quicker deterioration at shorter time. Seeds are hygroscopic in nature; they can pick up and releases moisture from and to the surrounding air. They absorb or lose moisture till the vapor pressure of seed moisture and atmospheric moisture reach equilibrium (Shelar *et al*, 2008). Control of relative humidity is the most important because it directly influences the moisture content of seeds in storage as they come to equilibrium with the amount of moisture surrounding them; a concept known as equilibrium moisture content. The lower the moisture content, the longer seeds can be stored provided that the moisture level can be controlled all through the storage period.

Organisms Associated with Seeds

Organisms associated with seeds in storage are bacteria, fungi, mites, insects and rodents. The activity of these entire organisms can lead to damage resulting in loss of vigor and viability or, complete loss of seed.

Bacteria, fungi, Insect and Mites

There are several factors which favor infection fungi and promote their infestation such as moisture content of seed and interspace relative humidity, temperature, prestorage infection and storage pest. Most storage fungi belong to *Penicillium* and *Aspergillus* genera. They induce seed deterioration by producing toxic substances that destroy the cells of seeds. Mechanically damaged seed allow quick and easy access for microflora to enter the seed (Shelar *et al*, 2008). To minimize the risk of fungi invasion, seeds have to be stored at low moisture content, low temperature, and RH. Researches show that all storage fungi are completely inactive below 62% relative humidity and show very little activity below 75% relative humidity, the amount of fungi in a seed often shows an exponential relationship with relative humidity. The storage bacteria require at least 90% relative humidity for growth and therefore only become significant under conditions in which fungi are already very active.

There is no insect activity at seed moisture contents below 8%, but if grain is infected, increased activity may generally be expected up to about 15% moisture content. The optimum temperature for insect activity of storage insects ranges from 28 to 38°C. The temperatures below 17 to 22°C are considered safe for insect activity. Although it is usually preferable to control insect and mite activity by the manipulation of the seed

environment, i.e., use of fumigants and insecticides. The main problem of chemical control is the adverse effect of chemicals on seed viability and vigor, and some of them are dangerous to handle. However, fumigants which have been used successfully include methyl bromide, hydrogen cyanide, phosphine, ethylene dichloride and carbon tetrachloride in 3:1 mixture, carbon disulphide and naphthalene. Insecticides – used in seed storage include DDT, lindane and Malathion.

Provenance

Seeds obtained from different sources may show differences in viability and storability. Nevertheless, the seed begins its existence before it harvest and it is expected that seeds harvested in different pre-harvest condition.

Fluctuating Environmental Conditions

Fluctuating environmental conditions are harmful for seed viability. Rapid changes in seed moisture content and temperature cause deleterious effect.

Oxygen Pressure

Recent researches on the role of a gaseous environment on seed viability indicate that increases in pressure of oxygen incline to decrease the viability period.

Other Factors

Factors besides those discussed above that affect seed storage life are the direct sunlight on the seed, number of times and kind of fumigation, effect of seed treatment, etc.

Seed Deterioration Symptoms

Seed deterioration is an inexorable, irreversible degenerative change in the quality of a seed. It is observable in their dropped performance during germination such as delayed seedling emergence, slower rate of seedling growth and development, loss of the capacity to germinate was the final phase of deterioration process and final indication of vigor loss.

Deteriorated seeds are also diminished resistance to environmental stresses during germination and early seedling growth. Some of the symptoms of the deteriorated seed are:

Morphological Changes

Changes in seed coat color are apparently owing to oxidative reactions in the seed coat that are enhanced under conditions of high temperature and relative humidity.

Ultra Structural Changes

By electron microscopy examination, two broad patterns of coalescence of lipid bodies and plasmalemma extraction from the cell wall related with deterioration have been observed, both of these events influence cell membrane integrity.

Cell Membrane Changes

As seeds deteriorate, their capability to retain cellular constituents decreased which was attributed to cell membrane disruptions associated with the loss of membrane phospholipids. Some consequence of membrane damage includes: Breaks in the plasmalemma structure and its contraction from the cell wall, Fragmented endoplasmic reticulum lacking of polyribosomes, Lack of dicytosomes, Monosomes arbitrarily dispersed in the cytoplasm, Disintegration of mitochondria and plastids, Coalescence of lipid droplets, Condensation of chromatin and lobed nucleus And Lyses of membranes of lysomic structures.

Loss of Enzyme Activity and Reduced Respiration

The most sensitive test for measuring early seed deterioration is tetrazolium (TZ) and glutamic acid decarboxylase activity test. Other oxidases enzymes such as catalase, peroxidase, amylase and cytochrome oxidase also correlated with seed deterioration. As seeds deteriorate, respiration becomes gradually pathetic and eventually leads to loss of germination. Reduction in respiration rate is closely associated with seed deterioration.

Increase in Seed Leachates and Increase in Free Fatty Acid Content

Increase in leachate content when soaked in water is often noticed symptom of deteriorated seeds. The leachate concentration can be measured by electrical conductance methods and by determines the soluble sugar content of the leachate. The hydrolysis of phospholipids leads to the release of glycerol and fatty acids, and this reaction hastens with increasing seed moisture content. The frequent accumulation of free fatty acids concludes in a decline in cellular pH and is detrimental to normal cellular metabolism.

Methods for Testing Seed Deterioration Germination Test

It is an analytical procedure to evaluate seed germination under standardized, favorable conditions. Standard germination testing includes media, temperature, moisture, light, dormancy breaking and germination counting standard for various crop seeds.

Tetrazolium (TZ) Test

TZ test is extensively accepted as an accurate mean of estimating seed viability. This method was developed by Professor Georg Lakon in the early 1940s. It is quick method to estimate seed viability (Copeland and McDonald, 2001). This test distinguishes between viable and dead tissues of the embryo on the basis of their relative respiration rate in the hydrated state.

Electrical Conductivity Test

As seed deterioration progresses, the cell membranes become less rigid and become more water permeable. It allows the cell contents to leakage into solution with the water and increasing

electrical conductivity. It provides a rapid indication of seed viability for seed lots.

Vital Coloring Test

The principle of this method is the differential coloration of live against dead tissues when exhibited to certain dyes such as sulfuric acid, indigo carmine and aniline dyes. These dyes stain the dead tissue blue and the live tissue leftovers unstained. This method is particularly useful for determining viability of tree seeds.

Enzyme Activity Test

These methods measure enzyme activity (such as lipase, amylase, diastase, catalase, peroxidase and dehydrogenase) of imbibed seeds as an indication of their viability.

Other Tests

Other testing methods are free fatty acid test, hydrogen peroxide test, indoxyl acetate test, fast green test, ferric chloride test, sodium hypochlorite test, excised embryo test and X-ray test. These methods were discussed by (Copeland and McDonald 2001).

Detrimental Effects of Seed Deterioration

Some probable consequences of deteriorative changes in seeds are: decreased percent germination; reduction in vigor and viability; degradation of cellular membranes and loss of permeability control; increased solute leakage; impairment of energy-yielding and biosynthetic mechanisms; reduced biosynthesis and respiration; reduced germination rate and early seedling growth; reduced rate of plant growth and development; reduced storage potential; decreased growth uniformity; increased susceptibility to environmental stresses, especially during germination, emergence, and early seedling development; reduced tolerance under adverse conditions, Loss in seed weight (Mohammadi *et al*, 2011; Ghassemi-Golezani *et al*, 2010; Astegar *et al*, 2011; Farhadi *et al*, 2012; Biabani *et al*, 2011; Shelar *et al*, 2008). It also results in decreasing the quality of seed produced, such as purple stained seeds (contaminated by fungi), wrinkled seeds, fissures in the seed coat, insect damaged seeds, discoloration of seed, etc.

CONCLUSION

Seed quality, germination, vigor and viability are highly influenced by environmental factors in field and storage. There was a distinct reduction in yield, seedling growth, loss of capacity to germinate and increased susceptibility to environmental stresses which cause numerous harmful effect on seed quality.

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