Physiologic alterations in orthodox seeds due to deterioration processes

Luciano Antônio Ebone, Andréia Caverzan*, Geraldo Chavarria

Laboratory of Plant Physiology, Agronomy Post-Graduate Program, University of Passo Fundo, Passo Fundo, Rio Grande do Sul, Brazil

A R T I C L E   I N F O

Keywords:
Vigor
Cell membrane
Genetic material
Lipid peroxidation
Protective capacity

A B S T R A C T

Seed deterioration is a partially elucidated phenomenon that happen during the life of the seed. This review describes the processes that lead to seed deterioration, including loss of seed protection capacity against reactive oxygen species (ROS), damage to the plasma membrane, consumption of reserves, and damage to genetic material. A hypothesis of how seed deterioration occurs was also addressed; in this hypothesis, seed deterioration was divided into three phases. The first is the beginning of deterioration, with a slight reduction of vigor caused by the reactions of reducing sugars with antioxidant enzymes and genetic material. In the second, the cell shows oxidative damages, causing lipid peroxidation, which leads to the leaching of solutes, the formation of malondialdehyde, and, consequently, an increase in damages to genetic material. In the third phase, there is cell collapse with mitochondrial membrane deconstruction and a high accumulation of reactive oxygen species, malondialdehyde, and reducing sugars.

1. Introduction

Seed vigor depends essentially on the ability to resist the deleterious effects of aging (Ventura et al., 2012). Thus, vigor and deterioration are related phenomena. The seed is one of the primordial inputs in agricultural crops because it will originate the individuals that at the end of the cycle will determine the productivity. Seed quality is important for high productivity since it is necessary to guarantee an adequate plant stand under a wide range of environmental conditions, and other quality indicators were also added, like the rapid and uniform emergence of plants (Waterworth et al., 2015). The differences in the stand and morphology of soybean seeds [Glycine max (L.) Merr.] with distinct levels of vigor, such as 48% and 90%, result in differences in productivity of 1376 kg ha$^{-1}$ (Caverzan et al., 2018).

The first concepts of vigor were established in the 50's, as the expression of the set of attributes of the seed that allow obtaining a stand of plants under unfavorable field conditions (Isely, 1957). This concept of vigor was refined by decades of research, acquiring other connotations, such as yielding an adequate plant stand under a wide range of environmental conditions, and other quality indicators were also added, like the rapid and uniform emergence of plants (Waterworth et al., 2015; Marcos-Filho, 2015). Therefore, vigor can be measured by the high seed germination rate, uniformity, and growth of seedlings under adverse conditions (Zhang et al., 2015).

Due the fact that deterioration is not equal for all kind of seeds is important to classify them in groups with similar properties. One of the most common form to segregate seeds to their properties is based on their ability to withstand desiccation, being they classified in two as tolerant to desiccation or orthodox and intolerant to desiccation or recalcitrant (Waterworth et al., 2015). The mainly difference of these two groups is that orthodox seeds acquire their ability to lose water by the syntheses of late embryogenesis abundant proteins, heat shock proteins and sugars (Waterworth et al., 2015). These properties allowed the orthodox to have a quiescent stage when very little metabolic activity is present due the low moisture content in the seed, being this state call the glassy state (Bailly et al., 2008) while recalcitrant maintain higher metabolic activity. When thinking in economic or security food terms the importance of orthodox becomes evident, since the majority of the crops plants, for example rice (Oryza sativa L.), maize (Zea mays L.), wheat (Triticum aestivum L.), soybean and barley (Hordeum vulgare L.) belong to this group.

The highest physiological seed quality is generally obtained at the time of physiological maturation or soon later depending on the species (Demir and Ellis, 1993). After maturity, the metabolic activity, initiating continuous biochemical changes and the predominance of catabolic processes, which cause deterioration (Shelar et al., 2008). The deterioration or reduction of the physiological quality can be considered as the reduction of the germination and vigor of the seed (Pu et al., 2015).

The causes of deterioration and seed death are not fully understood, as aging is a complex biological trait and involves a network of
molecular, biochemical, physiological, and metabolic processes (Fu et al., 2015). However, some processes have been studied to establish a cause and effect relationship with seed deterioration, such as the reduction of mitochondrial activity (Xin et al., 2014), the Amadori and Maillard reactions (Murthy et al., 2003; Murthy and Sun, 2000; Nisarga et al., 2017) and the inactivation of hydrolytic and antioxidant enzymes (Ali et al., 2017; Sharma et al., 2013).

As a way to rationalize the understanding of the phenomena that lead to the deterioration of the seed during storage, this review proposes to separate the deterioration into four events: depression of the protective capacity against oxidative stress, damage to the plasma membrane, consumption of reserves, and damage to the genetic material.

2. Deterioration

2.1. Depression of protective capacity against reactive oxygen species (ROS)

Plants suffer from various stresses during the life cycle, and their response to stress will vary with the increasing duration and severity of stress (Caverzan et al., 2016; Kranner et al., 2010). Seeds also may be vulnerable to stresses during development on the mother plant or during germination. In orthodox seeds, the desiccation during maturation is the first severe stress experienced (Kranner et al., 2010). Thus, stress factors contribute to seed quality and consequently affect plant development and productivity.

The imbalance in the intracellular ROS status and consequent oxidative damages have been associated with the loss of seed viability (Morsch et al., 2015; Sano et al., 2016). During seed deterioration, there is an accumulation of reactive oxygen species (ROS) such as singlet oxygen (O$_2^{ullet}$), superoxide radicals (O$_2^{ullet-}$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radicals (OH$^-$). ROS are molecules highly reactive and toxic, with the potential to damage cell membranes, nucleic acids, proteins, carbohydrates, and lipids, causing irreversible damages to cell systems (Gill and Tuteja, 2010; Sharma et al., 2012). In this way, lipid peroxidation mediated by free radicals, and disruption of cellular membranes have been reported as the major damage during deterioration (Fu et al., 2015). Also, some claim that lipid peroxidation is the first event in the aging (Delouche and Baskin, 1973).

However, this statement is imprecise since the appearance of indicators of physiological quality reduction only occurs with the activation of the antioxidant enzymes responsible for the degradation of ROS (Ali et al., 2017; Sharma et al., 2013). It can be affirmed that the first event in the deterioration is the depression of the protective capacity of the seed, as a consequence of the enzymatic inactivation. Several antioxidant enzymes act to protect cellular components against excess ROS. Major enzymes of the antioxidant defense system include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), peroxidase (POD) peroxidoredoxin (Prx), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Gill and Tuteja, 2010; Mittler, 2017).

SOD is the first enzyme to exert antioxidant activity, performing the dismutation of the superoxide radical (O$_2^{ullet-}$) to hydrogen peroxide (H$_2$O$_2$), although H$_2$O$_2$ is still toxic to the seed, and is reduced to water by CAT or APX (Das and Roychoudhury, 2014). GR catalyzes the reduction of hydrogen peroxide, organic hydroperoxides, or lipid hydroperoxides to water and correspondingly uses reduced glutathione as a reductant (Jain and Bhatia, 2014). When APX reacts with H$_2$O$_2$, it transforms APX into two molecules of monodehydroascorbate, which takes the action of MDHAR to recover as APX. Moreover, monodehydroascorbate is unstable, and when not quickly regenerated, it produces dehydroascorbate, which needs DHAR to regenerate it to APX (Shin et al., 2013).

It has been reported that the depression of the antioxidant system, especially the reduction of the enzymatic activities, may be responsible for the accumulation of ROS, leading to a loss of seed vigor (Yin et al., 2014). The ROS accumulation was investigated in artificially-aged seeds at 50°C (Yin et al., 2014). In rice embryos that underwent 48 h of imbibition, the levels of H$_2$O$_2$ and O$_2^{ullet-}$ in one-month-aged seeds were increased by 9% and 58%, respectively, compared with the control. After aging for 9 months, the levels increased by 26% and 123%, respectively. Moreover, the activity of CAT, MDHAR, and APX of rice seeds aged for 1 month decreased by 12%, 17%, and 20%, respectively, compared with the control. When rice seeds were aged for 9 months, the activity of CAT, APX, and MDHAR enzymes was reduced by 62%, 50%, and 38%, respectively (Yin et al., 2014). The antioxidant systems of mitochondria also suffer depression during the deterioration, with a marked decrease in Mn-SOD (major antioxidant in mitochondria) and GR (Yin et al., 2016).

In wheat seeds, CAT and L-APX proteins were down-regulated under artificial aging conditions (Lv et al., 2016). Similar results were observed in soybean seeds, whereby the abundance of DHAR, APX1, and MDHAR proteins decreased during aging (Min et al., 2017), and exposure to various controlled deterioration treatment conditions reduced the activity of SOD, CAT, and APX in oat (Avena sativa L.) seeds, and down-regulated the transcript levels of these genes (Kong et al., 2015). Furthermore, during aging of pea (Pisum sativum L.) seeds for 15 days, there was modulation of the genes related to oxidative stress, such as POD, thioredoxin (TRX), SOD, MDHAR, GPX, and glutathione-S-transferase (GST) (Chen et al., 2013). Under these conditions, the majority of oxidative stress genes were down-regulated, demonstrating a decline in antioxidant capacity.

The loss of protective capacity is a consequence of Amadori, Maillard (Murthy and Sun, 2000), and Heyns reactions (Frolov et al., 2018). These non-enzymatic reactions involve the glycation of reducing sugars with amino acids or nucleic acids, triggering a plethora of complex molecular processes and, ultimately, the formation of advanced glycation end products (Streleca et al., 2008). Seeds generally exhibit small amounts of reducing sugars that serve to initiate the Amadori and Maillard reactions, and as the seeds age, their constitution is altered, providing more reducing sugars, which rapidly react with proteins and nucleic acids (Murthy and Sun, 2000).

When considering management, the reduction in moisture is beneficial for the maintenance of the protective capacity of the seed, because under conditions of very low humidity there is enhanced preservation of the enzymatic complexes responsible for preventing the cellular aging (Li et al., 2010). The low humidity also suppresses the activity of the hydrolytic enzymes involved in generating the reducing sugars, which initiate the Maillard reaction (Murthy et al., 2003). The enzymes most sensitive to deterioration are SOD and GR (Kibinza et al., 2006). Although there is no current explanation for this trend, it is possible to infer that it results from a higher affinity of reducing sugars to these enzymes at the expense of others.

In addition to temperature and humidity management, chemical strategies can also be employed to avoid the depression of the protective capacity of the seed against ROS, such as the use of vitamins (El-Metwally et al., 2014) or the use of sodium hydrosulfide (NaHS) (Li et al., 2013) in the treatment of seeds. Vitamins C (ascorbic acid) and E (tocopherols and tocotrienols), for instance, act as antioxidant compounds, preventing the damage caused by the ROS (Xia et al., 2015). Vitamin E is an amphipathic compound with an apolar extremity associated with the cell membrane, and it has the function of stabilizing the membrane and reducing ROS, especially singlet oxygens (Sattler et al., 2004). After the reaction with ROS, tocopherol is regenerated by vitamin C or glutathione (Fryer, 1992).

Antioxidant compounds, such as α-tocopherol, could reduce the respiratory rate by blocking the entry of oxygen into the internal tissues of seeds and reduce seed deterioration (Giurizzatto et al., 2012). Plants are classified in two groups: as stress-tolerant plants, which have an increase in tocopherol during all ages, and stress intolerant plants, which only have an increase of tocopherol at the beginning of the aging
or the stress (Giurizatto et al., 2012). In Arabidopsis plants, the antioxidant role played by vitamin E for seed longevity has been confirmed; VTE1 protein (vitamin E synthesis) abundance was lower in aged seeds (Nguyen et al., 2015). Corroborating this information, Sattler et al. (2004) has previously shown that, with mutations in the genes involved in tocopherol biosynthesis (vte1 and vte2), the mutant seeds were more sensible to artificial aging when compared to the wild type.

The exogenous application of tocopherol in priming treatments of sunflower seeds showed as a possibility to increase the total tocopherol content. The treatments with tocopherol a 0.3% and with ascorbic acid, tocopherol and glutathione mixed showed an increased in the tocopherol level of 0.77% and 2.92%, respectively, although all the others treatments with higher tocopherol rates or with the isolated use of de ascorbic acid and glutathione showed reduction in the tocopherol levels (Draganic et al., 2011).

Vitamin C, even though it has a powerful antioxidant capacity, is not a relevant antioxidant in dry seeds because its content is very low in quiescent seeds, and its importance as an antioxidant is present only after germination (Ye et al., 2012). However, their application in seed priming had shown improvements in seed germination and seed quality (Burguiéres et al., 2007; El-Metwally et al., 2014). These positive results of this priming treatment are not linked with the antioxidant activity of ascorbic acid, but rather with its role as an elicitor of glucose-6-phosphate dehydrogenase. This increased the pentose phosphate pathway, resulting in the accumulation of proline, which by the hypothetical proline-linked pentose phosphate pathway, positively influences phenolic synthesis and antioxidant enzyme response (Burguiéres et al., 2007; Randhir and Shetty, 2007).

When the NaHS was used in seed treatment, in the aqueous medium that was released and formed H2S, the H2S enhances the activity of δ-1-pyrroline-5-carboxylate synthetase (P5CS), which is a key enzyme in the glutamate synthesis pathway for proline. The H2S also reduced proline dehydrogenase, which is responsible for proline degradation; this led to an accumulation of proline, which improves germination by the reduction of ROS damage (lipid peroxidation) (Li et al., 2013). Moreover, NaHS also increases the content of antioxidant enzymes such as GR, APX, SOD, CAT, and the non-enzymatic antioxidants (ascorbic acid and glutathione) by gene regulation (Zhou et al., 2018).

Depression of the protective ability against ROS by the seed will lead to some physiological imbalances in the seed, such as lipid peroxidation and increased respiration, leading to loss of seed vigor and viability.

### 2.2. Membrane damages

The plasma membrane consists of a fluid bilayer of discontinuous phospholipids with globular proteins partially incorporated into this matrix (Sych et al., 2018). These proteins play a vital role in protecting the integrity of the cell interior and mictelously regulate the import and export of various substances (Sych et al., 2018). Membrane damage is considered to be the main event occurring in seed aging (Ratajczak et al., 2019). Lipid peroxidation which cause membrane damage is the formation of hydroperoxides through the reaction between oxygen and unsaturated fatty acids (Gschler and Stockwell, 2017), modifying membrane permeability and leading their to destruction (Ali et al., 2017).

Lipid peroxidation causes many changes in the membranes leading to uncertainty of how this could cause cell death, because of that, Agmon et al. (2018) formulated four hypotheses about how lipid peroxidation led to cell death. The first is that the compositional changes caused by lipid peroxidation cause widespread membrane damage, opening the pores and causing the loss of ionic homeostasis. The second is that the membranes lose their integrity by chemical changes in the lipids, disrupting the membrane. The third is that the major impact is not to the lipid fraction of the membrane, but rather to the membrane-embedded proteins, interfering in their function. The last hypothesis is that the oxidized polysaturated fatty acyl moiety fragments are generated, and these fragments act negatively on other primordial cellular processes, causing lethal events.

The membrane damages can occur since the early stages of deterioration, because the slow reduction in the germination rate during the early stages of storage may be due to the impairment of the metabolic processes associated with membrane aberrations and the need for repair mechanisms to compensate the accumulated damages (Kaewnaree et al., 2011). The major enzyme responsible for repairing the lipid peroxidation damage is GPX, which reduces lipid hydroperoxides (Jain and Bhatla, 2014).

For prevail seed deterioration the moisture content is of paramount importance. Below 6% moisture, auto-oxidation due to ROS attack is intensified while above 14% moisture, oxidative enzyme activation is stimulated (Silva et al., 2018). For seeds in the dry state, although ROS accumulate, the membranes do not show high damage, attributable to the extremely low mobility and relatively greater affinity of the ROS with other fractions of the cell, such as proteins and nucleic acids (Bailly et al., 2008).

Temperature is the other environmental factor that modulates deterioration. Low temperatures (< 20°C) during storage or transport are considered adequate for maintaining viability and vigor for seeds from crops (Hartmann-Pilho et al., 2016). For cultures with very higher economic value or with the objectivity of germplasm conservation the use of cryopreservation could and should be employ. It is generally expected that an increase in the temperature increases deterioration, since heat increases the mobility of molecules, promoting molecular reactions (Walters et al., 2010). So, the cryopreservation try to expose seeds to the low temperature possible to reduce molecular reactions and therefore deterioration, although is necessary caution in the moisture content being recommend less than 13% (more common 8–10%) of moisture for mitigate the risk of ice formation (Pritchard and Nadarajan, 2008). However, the adequate moisture to cryopreservation is made by the estimation of unfrozen water content, being this parameter, most impacted by the percentage of lipids in the seed (Her et al., 2005). The temperature of cryopreservation it’s not unanimous, but is generally recommend temperatures less than ~20°C (Pritchard and Nadarajan, 2008).

Lipid peroxidation is the primary factor contributing to membrane damage, so many studies have aimed to evaluate the main byproduct of this reaction, which is malondialdehyde (MDA) (Kibinza et al., 2006). MDA is one of the main biomarkers of oxidative damage and is one of the last products released during the lipid peroxidation process (Min et al., 2017). In soybean seeds exposed to controlled deterioration treatment (3 and 7 days), 28.9% and 37.8% higher H2O2 content, and 57.5% and 64.23% higher MDA content, respectively, was detected when compared with the control seeds (Min et al., 2017), indicating the accumulation of ROS and membrane damage.

Lipid peroxidation leads to a change in cell membrane structure. The phospholipids have a cylindrical shape, but when they undergo lipid peroxidation, the phospholipid head groups become smaller, and the tails become larger by their reaction of ROS, which leads the phospholipids to have a cone shape (Agmon et al., 2018). These shape changes lead to membrane cannot maintain the uniformity of the bilayer, and thus cause severe membrane disruptions (Agmon et al., 2018). At low concentrations of aldehydes, pores appeared in the cell membrane, but were stable; however, with an increase in concentration of aldehydes, the pores destabilized and micellization occurred (Agmon et al., 2018).

Outstanding, in rice, aldehyde dehydrogenases gene plays an important role in maintaining seed viability by detoxifying the aldehydes generated by lipid peroxidation, when analyzed the mutant seeds these were more sensitive to accelerated aging treatment and accumulated more malondialdehyde (Shin et al., 2009). Furthermore, some compounds that have no direct relationship to the lipid peroxidation reaction can also be used as biomarkers. For example, damage to membrane...
integrity has been indicated by the decrease in sucrose content during the initial storage period (Sharma et al., 2007). The possibility of using the decrease in sucrose as an indicator of membrane integrity comes from it indicating the metabolic rate of seeds. In other words, seeds which have larger decreases of sucrose have higher respiration rates, which is directly proportional to a higher ROS production necessary for the lipid peroxidation reaction.

Another form of membrane integrity analysis is the measurement of the polyphenol oxidase (PPO) enzyme or the accumulation of phenols. PPO is present in the plasma membrane of the seeds, so when the membrane system is ruptured, the enzyme comes into contact with phenolic compounds, catalyzing their oxidation and reducing the phenolic content in seeds (Abbade and Takaki, 2014). The PPO activity decreases with increasing storage time (Saath et al., 2014). However, PPO is necessary for rapid germination, since it promotes the transformation of phenols to quinones, which stimulate the synthesis of ATP in the respiratory chain (Kocaqaliskan et al., 1995). Thus, the loss of PPO to the external medium, as a result of the peroxidation, reduces the germination (Abbade and Takaki, 2014).

The leaching of solutes from the seed reflects the state of the cell membranes. Poor retention of solutes, resulting in leaching of embedded seed compounds, can be considered as a result of damaged or incomplete membranes (Rahoui et al., 2010). The leaching of solutes is also used as a vigor test because, in field conditions, the leakage of exudates after sowing stimulates the growth of pathogenic microorganisms and impairs the emergence of seedlings (Marcos-Filho, 2015).

Seed leaching could be divided in three major groups: cations, anions, and sugars. The cations are the most studied, the K⁺ ion is the main inorganic ion leaching from the cell other important representatives of this group also were Ca²⁺, Mg²⁺, and Mn²⁺ (Woodstock et al., 1985). K⁺ is the main ion present in the leaching due to its presence in the cell in the ionic form, small size, and solubility. These properties allow K⁺ to be a good indicator of seed deterioration even better than the electrical conductivity as a whole (Woodstock et al., 1985).

The presence of Ca²⁺ in the leaching brings the question if the lipid peroxidation can cause a generally disrupted of cell homeostasis by degradation of the tonoplast. This question is bringing due the fact that the majority of Ca²⁺ is storage in vacuole to maintain the homeostasis of the cell, because higher contents of Ca²⁺ in cytosol is cytotoxic (Schönknecht, 2013; White and Bradly, 2003). The increase of Ca²⁺ in the cytosol by seed deterioration cause overload of this compound in mitochondria (Kumar et al., 2015). This overload lead the opening of the permeability transition pore by the adenine nucleotide translocator and the voltage-dependent anion channel, which cause a release of cytochrome c and other apoptogenic proteins from mitochondria (Bernardi and Rasola, 2007; Kumar et al., 2015). The release of cytochrome c activates the caspases and downstream proteolytic cascade, which bring cell to death (Kumar et al., 2015).

The main anions leaching from seeds of maize were Cl⁻, PO₄³⁻, isocitric acid, and SO₄²⁻ (Ouyang et al., 2002). The leakage of PO₄³⁻ reached the level of 2752–6727 μg/g when analyzed in the isolated embryo (Ouyang et al., 2002). This has a negative impact on the seed growth, since the phosphorus seed content could be correlated with a faster root growth (White and Veneklaas, 2012). The higher leakage of SO₄²⁻ from deteriorating seeds is not only correlated with an increase in membrane permeability, but also protein and amino acids degradation, since SO₄²⁻ is most prevalent in sulfur amino acids (Ouyang et al., 2002).

In sugar leaching, the most common compounds found were the monosaccharides, such as glucose and fructose, or other sugar disaccharides (sucrose) and trisaccharides (raffinose) (Ouyang et al., 2002). However, despite these sugars being found to leach, they were not a consistent parameter to evaluate electrical conductivity, leaching, or seed quality, because they had higher lengths than ions, and their presence only increased in the final stage of deterioration when seeds are already dead or nearly so at this stage (Ouyang et al., 2002). Nevertheless, Abdul-Baki and Anderson (1970) had shown that sugar leaching was not correlated to seed viability, and they proposed that sugar leaching is regulated by the utilization of sugar by the cell and not by seed deterioration.

Membrane damage does not happen uniformly throughout the seed. In soybean, the embryonic axis is highly susceptible to deterioration. Specifically, the radicle of the embryonic axis is in close contact with the end of the funiculus and ensures the interaction with the environment, resulting in enhanced lipid peroxidation (Hartmann-Filho et al., 2016; Singh et al., 2016). Therefore, damages to the growth of deteriorated seeds are expressed to a greater extent in the roots than the aerial part.

The damage to the plasma membrane is the most important event in the deterioration of the seed. Not only does the damage provide an inadequate exchange of solutes between the cell and external environment, but it also leads to increased damage to the genetic material by MDA and increases the consumption of reserves. Consequently, seeds with an injury to the plasma membrane have more significant losses in vigor and viability.

2.3. Reserve consumption

The reserves stored in the seed act to provide the metabolic needs of the embryo until the seedling reaches autotrophy. Seeds remain metabolically active during storage, and this incurs an energy expenditure, which is supplied by respiration. Respiration is a complex and highly structured series of processes by which energy is converted into a form that can be used for metabolism, besides the development, growth, and maintenance of cells, tissues, and organisms (Perry and Burggren, 2007). The respiratory rate of the seed is impacted mainly by three factors: temperature, moisture (Chidananda et al., 2014), and the structural quality of the seed (Dode et al., 2013).

Soybean seeds stored hermetically at 15 °C and 13% moisture content still maintained 12.7% O₂ in the environment at 250 days, whereas, at 35 °C and 17% moisture content, all the O₂ was consumed in 70 days (Ochandio et al., 2017). Increasing the moisture content (15% and 17%) and temperature (15 and 35 °C) increased the respiration rates (seven-fold increase in oxygen consumption) (Ochandio et al., 2017) by enhancing the action of hydrolytic enzymes (Shekar et al., 2008). Seed dehydration increases viscosity and drastically reduces the reaction rates in aqueous solutions to insignificant levels because the solvent (water) is physically absorbed (Vertucci and Roos, 1990). A cause and effect relationship can be established between temperature and respiration, in which these two factors are directly proportional (Chidananda et al., 2014). The temperature influences the respiration rate by increasing the metabolic activity. Temperature and humidity are the main manageable storage factors, but the seeds naturally present different respiratory rates. Tests on soybean seed lots displayed different respiratory activities and that this activity was negatively correlated with germination (Dode et al., 2013). The increase in respiratory activity lead a higher ROS production, what can result in disruption of the plasma membrane by lipid peroxidation (Dode et al., 2013), producing a reduction in the efficiency of mitochondria energy production from the same substrate (Xin et al., 2014).

The mitochondria, glyoxysomes, and plasma membrane NADPH oxidases are the main sites of ROS in hydrated seeds, whereas, in dry seeds the ROS are synthesized in a nonenzymatic reaction (Kurek et al., 2019). In addition, in mitochondria the oxidative phosphorylation is an important source of ROS in cells. The excess of ROS in the mitochondria of aging seeds reduces the activity of the antioxidant system (Kurek et al., 2019). During deterioration, the mitochondria suffer a series of structural and chemical changes, which are induced mostly by ROS produced inside these organelles. The structural changes promoted by ROS in mitochondria include the inner membrane starting to disrupt
into vesicles inside the external membrane, then the cristae shorten and collapse, and the complexes of the respiratory chain shift to the inner membrane, causing the previously segregated proteins of the inner membrane to begin to mix (Daum et al., 2013). As the deterioration progresses, and as cristae disappear completely, internal membrane proteins lose their cohesion, consequently occurs membranes rupture, leading the cytochrome c and vesicle membranes to leakage to the cytosol (Daum et al., 2013). In addition, a slight reduction of voltage-dependent anion channel 1 in deteriorated seeds has been reported (Yin et al., 2016).

Beyond the physical changes, biochemical changes also occur within the energy system of seeds; the main biochemical changes were in the enzymes responsible for the electron transport chain. Seeds 3 days of age showed a 24% reduction in cytochrome c oxidase, a 10% reduction in malate dehydrogenase activity, and a 44.5% decrease of NADH and a 25% decrease in succinate dependent O2 consumption (Yin et al., 2016). Other main chemical change in the mitochondria during seed deterioration is the lipid peroxidation. The lipid peroxidation is especially important in mitochondria due the presence of Cardiolipin lipid that compose around 11% of the membrane (Horvath and Daum, 2013). The higher susceptibility of this lipid is due the higher number unsaturated acyl chains and their proximity location near the source of ROS (Vähäheikkilä et al., 2018). The peroxidation induced conformational changes that made Cardiolipin no longer match the structural profile of its binding pocket in the host protein, affecting the protein function and reduction mitochondria efficiency (Vähäheikkilä et al., 2018). The chemical or physical inability of the mitochondria to use efficiently the substrate to provide energy reduces the capability of seeds to germinate, and leads to seed death or vigor reduction.

The use of coenzyme Q10 (CoQ10) is a prominent chemical strategy to improve the mitochondrial function, because CoQ10 is the essential cofactor for all activities of the enzyme systems within the mitochondria affecting complex I, II, III and IV in the electron transport chain (Freye and Strobel, 2018). Thus, the application of this compound in seeds could help to restore or maintain part of the efficiency of energy production by enhancing the mitochondrial enzyme system. Furthermore, CoQ10 is also an antioxidant compound that could help to maintain the cell homeostasis by detoxification of ROS (Agmon et al., 2018). In rice to improve the content of CoQ10 the gene encoding the enzyme responsible for the synthesis of 10 isoprene units for the CoQ (Takahashi et al., 2010). The hydroxylation of the C-8 position of guanine (G) to produce 7,8-dihydro-8-oxoguanine (8-oxoG) is considered the main oxygenation of DNA. The 8-oxoG can pair with adenine (A) as well as cytosine (C), and the mispair with an A residue, thus resulting in GC to TA transversion during DNA replication (Sano et al., 2016). Interestingly, the AtOGG1 of Arabidopsis is a DNA glycosylase/apurinic/apyrinimidic (AP) lyase involved in base excision repair to eliminates 8-oxoG from DNA. The overexpression of this gene enhanced seed resistance to controlled deterioration, the 8-oxoG content was reduced in transgenic seeds, indicating a DNA damage repair function, and the seeds exhibited high germination ability under abiotic stress (Chen et al., 2012), demonstrating an important role plays by AtOGG1 gene. The MDA produced in lipid peroxidation is a compound with high mutagenicity capacity and mobility that cause adducts formation with DNA molecules (Ayala et al., 2014). Similar of oxidative damage the adducts are most from the reaction of MDA with guanine resulting series of insertions, deletions and base pair substitution (Niederhofer et al., 2003). The 5-methylcytosine (m5C), is a conserved epigenetic mark in plants, which to regulate gene expression (Plitta et al., 2014). Moreover, ROS influences DNA methylation by m5C oxidation (Kurek et al., 2019). DNA methylation plays a role in seed development and the maintenance of viability (Plitta et al., 2014), further it has be shown that the DNA methylation level induced by desiccation varies to seed category-and tissue-specific when the m5C level was determined (Plitta-Michalak et al., 2018). Thus, to Acer platanoides L. (orthodox) the impact of desiccation on m5C levels in cotyledons no changes were detected. While, in Acer pseudoplatanus L. (recalcitrant) a decline in m5C levels was determined in embryonic axes and cotyledons (Plitta-Michalak et al., 2018). In Quercus robus seeds was verified a decrease in

2.4. Genetic damages

It is generally recognized that the seed vigor expression and the success of emergence depend on the DNA repair mechanisms activated during imbibition (Ventura et al., 2012). Seed aging is associated with chromosome aberration (Abdalla and Roberts, 1968), changes in telomere length, DNA damages, DNA methylation, and abnormal gene expression (Plitta et al., 2014; Fu et al., 2015). All these events are triggered by the reaction of the genetic material, or enzymes responsible for its stability, with ROS, aldehydes, and reducing sugars.

In stored seeds the main DNA damages are from oxidative stress (Kurek et al., 2019). Excess ROS cause oxidative damages at the cellular and molecular levels (Caverzan et al., 2016). ROS-induced DNA damages ensue when DNA is constantly exposed to oxidative stresses, resulting in damage that can halt the growth and cause mutagenesis or even lead to cell death (Waterworth et al., 2016). In addition, ROS can damage nucleic acids and bring about the oxidation of sugar residues, break strands, remove/delete nucleotides, and modify bases and protein–DNA cross-links (Gill and Tuteja, 2010; Sharma et al., 2012). The deoxyriboses and nucleobases are susceptible to ROS (Mittler, 2017), however the most reactive ROS to DNA molecule reported is the OH- causing DNA fragmentation (Gill and Tuteja, 2010). According to Chen et al. (2013) seeds with low water content are prone suffered the oxidation of nucleic acids.

Different types of DNA nucleotide damage are induced by ROS. Guanine (G) is the most commonly oxidized base (El-Maarouf-Bouteau et al., 2013). The hydroxylation of the C-8 position of guanine (G) to produce 7,8-dihydro-8-oxoguanine (8-oxoG) is considered the main mutagenic modification (Sano et al., 2016), this can be also a biomarker of oxidative stress. The 8-oxoG can pair with adenine (A) as well as cytosine (C), and the mispair with an A residue, thus resulting in GC to TA transversion during DNA replication (Sano et al., 2016). Interestingly, the AtOGG1 of Arabidopsis is a DNA glycosylase/apurinic/apyrinimidic (AP) lyase involved in base excision repair to eliminates 8-oxoG from DNA. The overexpression this gene enhanced seed resistance to controlled deterioration, the 8-oxoG content was reduced in transgenic seeds, indicating a DNA damage repair function, and the seeds exhibited high germination ability under abiotic stress (Chen et al., 2012), demonstrating an important role plays by AtOGG1 gene. The MDA produced in lipid peroxidation is a compound with high mutagenicity capacity and mobility that cause adducts formation with DNA molecules (Ayala et al., 2014). Similar of oxidative damage the adducts are most from the reaction of MDA with guanine resulting series of insertions, deletions and base pair substitution (Niederhofer et al., 2003). The 5-methylcytosine (m5C), is a conserved epigenetic mark in plants, which to regulate gene expression (Plitta et al., 2014). Moreover, ROS influences DNA methylation by m5C oxidation (Kurek et al., 2019). DNA methylation plays a role in seed development and the maintenance of viability (Plitta et al., 2014), further it has be shown that the DNA methylation level induced by desiccation varies to seed category-and tissue-specific when the m5C level was determined (Plitta-Michalak et al., 2018). Thus, to Acer platanoides L. (orthodox) the impact of desiccation on m5C levels in cotyledons no changes were detected. While, in Acer pseudoplatanus L. (recalcitrant) a decline in m5C levels was determined in embryonic axes and cotyledons (Plitta-Michalak et al., 2018). In Quercus robus seeds was verified a decrease in
seed viability during the aging, which was correlated with decline in amount of m5C in genomic DNA (Michalak et al., 2015).

RNA is also damaged by ROS, and due to its single-stranded structure and the absence of a repair system, is more vulnerable to oxidation (El-Maarouf-Bouteau et al., 2013; Sano et al., 2016). In addition, the oxidized mRNAs lead to the suppression of protein synthesis and degradation (El-Maarouf-Bouteau et al., 2013). Thus, the translational activity was prejudiced in imbibed Arabidopsis seeds, this was correlated to loss of seed longevity (Rajjou et al., 2008). Seeds RNA suffer damage over time (Fleming et al., 2017, 2018), with effect in the RNA yield and quality (Fleming et al., 2018). When the RNA degradation in embryonic axes of soybean was determined in seeds of the 23-year-old and 2-year-old, strong symptoms of aging was observed. Some transcripts were degraded in both seeds, but more damage was detected in tissues of seeds more old. In addition, the damages were mostly by RNA fragmentation in the longer transcripts (Fleming et al., 2018). Thus, RNA integrity contributed significantly to seed longevity. In addition, genes encoding heat shock proteins, nuclear and chloroplast genes involved in chloroplast activities, like photosynthesis (Pereira-Lima et al., 2017), and antioxidants genes (Lee et al., 2010) has been suggested involved in seed longevity.

The expression of genes encoding protective chaperones and heat shock proteins has been suggested to seed longevity due these proteins are implicated in protein-protein interactions and protein folding (Pereira-Lima et al., 2017). In addition to this, genes involved in the initiation of the degradation of chlorophyll and light harvesting complexes such as chlorophyll b reductase and STAYGREEN1 can be involved in seed longevity (Pereira-Lima et al., 2017). Furthermore, the repression of the genes associated with photosynthesis and chloroplast activity, according to Pereira-Lima et al. (2017), implicated in metabolic changes that dismantle the chloroplasts, through a close interaction between the nucleus and chloroplast genomes. The overexpression of CuZnSOD and APX antioxidant genes in tobacco plastids decreased the amount of ion leakage and maintained membrane integrity during imbibition by aged transgenic seeds (Lee et al., 2010). Thus, the molecular mechanisms coordinates cell longevity, and overexpression of genes linked to antioxidant systems could be used in breeding programs to create seeds less susceptible to deterioration.

The glycation caused by Amadori and Maillard reactions also contribute to the deterioration of the genetic material, by impacting on the enzymes responsible for repairing DNA damage, such as DNA ligase (Murthy and Sun, 2000). Also, the direct reaction of reducing mono-saccharides and DNA occurs. D-fructose 6-phosphate when subject to glycation and subsequent formation of chromophores or fluorophores (Morita and Kashimura, 1991). The occurrence of DNA damage by any of the above-mentioned means, when it reaches a critical proportion of dividing cells, halt root growth, causing germination inhibition (Abdalla and Roberts, 1968).

Although minor DNA damage does not induce loss of viability, it negatively impacts vigor because of the lack of gene expression in germination. For example, the biosynthesis of gibberellin depends on the expression of chromatin-remodeling factors (Han et al., 2015), so the occurrence of damage to genetic material promotes a reduction in its synthesis. The decline in gibberelin synthesis generates imbalances, such as a decrease in the glyoxylate cycle (Gallardo et al., 2001), thereby decreasing the energy available to the embryo.

However, these results are not unanimous, because vigorous hybrid sugar beet seeds (Beta vulgaris L.) exposed to adverse conditions showed a reduction in the availability of carbohydrates, which led to an over-expression of genes linked to synthesis of the isocitrate lyase, and malate synthase enzymes. This would be a way of high-vigor seeds compensating for the reduction in energy supply to the embryo by increased glyoxylate cycle activity (De los Reyes et al., 2003). Isocitrate lyase is the key enzyme in seed lipid mobilization via the glyoxylate cycle. This protein, in Arabidopsis plants, had its abundance decreased in deteriorated seeds (Rajjou et al., 2008). Furthermore, in aged sugarbeet seeds, the protein and enzyme levels were observed to decrease (Catusse et al., 2011). Thus, the isocitrate lyase can be a very good candidate as a diagnostic marker of seed vigor (Catusse et al., 2011; Rajjou et al., 2008). Unlike other processes that develop in peaks, damages to the genetic material are present throughout the deterioration process. The damages are initiated by the Amadori and Maillard reactions and then accentuated by the impacts of oxidative damage and genotoxic lipid peroxidation products.

Primming involves treatment in an osmotic solution, which allows the seed to absorb water and solute during the early stages of germination, and increases seed vigor (Ventura et al., 2012). The mechanisms of early DNA repair are recognized as the major molecular events underlying the beneficial effects of priming (Ventura et al., 2012). Controlled soaking of the seed provides the optimum conditions for DNA repair. As an example of the benefits of priming, primed mung bean (Vigna radiata L.) seeds showed high DNA integrity of the mitochondria and chloroplasts, and enhanced membrane permeability/integrity (Sharma et al., 2018). In addition, changes in ABA, GA, and ethylene biosynthesis gene and GST genes (glutathione S-transferase – involved in stress tolerance) expression were detected in seeds. Thus, the possibility of using ABA genes as an index of seed deterioration and GST expression as an index of priming is highlighted (Sharma et al., 2018), suggesting the mechanisms underlying longevity/priming. Previously, some proteins in Arabidopsis have been identified to be involved in priming treatment with altered abundances, such as degradation products of 125-cruciferin ß-subunits, tubulin subunits (α and β), catalase, class I and other heat shock proteins, linalool-binding protein, and phosphonoxypruvate carboxykinase (Gallardo et al., 2001).

Despite the beneficial properties of priming, the seeds which pass through this process suffer from a major problem that their loss longevity, needing a special storage condition (low temperature or vacuum or low relative humidity of the air) to preserve their viability (Wang et al., 2018). Thus, Sano et al. (2017) detected three quantitative trait locus (QTLs) associated with the reduction of seed longevity by priming. Furthermore, four cell wall modification-related genes (TRGI/XYL1, EXPA1, EXPA2 and DUF642) and three genes related to brassinosteroid synthesis or signaling (REN1, DWFI and EKO) which were highly expressed in the short life populations when compared to the long life populations exposed to priming (Sano et al., 2017). Thus, because the expression of the genes linked with the synthesis of brassinosteroids are related to the reduction of priming seed longevity, it was used a brassinosteroid synthesis inhibitors (brassinazole) during priming as a strategy to increase seed longevity by preserving the seed coat with prominent results (Sano et al., 2017). So, despite the beneficial of the priming when the seed was not immediately sow is necessary the use of physic or chemical strategies to main this seed viable.

Damages to genetic materials occurs by numerous process, they begin with reactions between reducing sugars and DNA forming glycation products. During storage the deterioration are further accentuated by reactions between DNA and ROS causing oxidative stress, triggering to growth arrest, mutagenesis or even cell death. The mRNA also suffers oxidative damage leading the suppression of protein synthesis and degradation. Moreover, the malondialdehyde generated by lipid peroxidation will also react with DNA creating adducts. Thus, damages to genetic material affect the seeds quality, consequently these cannot express its full genetic potential. Several genes involved in different pathways contribute to seed vigor and longevity, so may be an alternative for the development of cultivars with more tolerant seeds to adversities. Furthermore, the priming of seed constitutes an interesting tool to minimize the genetic damages in deteriorated seeds.

3. Hypothesis of the phases of seed deterioration

The processes mentioned above appear in concomitant or non-concomitant forms. To avoid misinterpretation that the processes are
successive events, it is hypothesized that there is a division of the deterioration into three phases (Fig. 1). Phase I is the initial stage after harvest, during which time, deterioration is largely stable (Marcos-Filho, 2015). This stage is associated with the depression of the protective capacity against oxidative damages by Amadori and Maillard reactions and minor injuries to the genetic material (Murthy and Sun, 2000), which do not significantly impact seed viability. In phase II, the deterioration begins. At this time, with the reduction of the protection capacity against ROS, the lipid peroxidation becomes evident, with damage to the membranes and production of MDA (Kibinza et al., 2006). The ROS and the MDA, in turn, trigger severe damage to the genetic material, generating viable seedlings, but with suppressed growth and an aqueous aspect (Shelar et al., 2008). In phase III, the viability reduction curve becomes more pronounced. At this stage, the disruption of the mitochondrial membranes increases the respiration due to the lowered production of energy per substrate, since there is a reduction in the efficiency of electron transport (Xin et al., 2014). With increased respiration, the ROS production is increased, leading to an autocatalytic cycle with lipid peroxidation, and there is a rise in damage to the genetic material, which ultimately inhibits germination completely.

4. Concluding remarks

The deterioration of seeds during transport and storage is inevitable. However, the rate of deterioration can be reduced, mainly through the appropriate handling of temperature and humidity. The deterioration lowers the seed physiological quality. Factors, such as loss of seed protection capacity, lipid peroxidation, consumption of reserves, and damage to genetic material, potentiate the deterioration process.

Nonetheless, many knowledge gaps still need to be filled. For example, it is not yet known how each deterioration process reflects on the seedling, and whether this reverberates or not in the adult plant since almost all of the current literature studies have dealt with the deterioration and the vigor expression in the field, as separate subjects.

Author's contributions

LAE wrote the first draft of the manuscript; AC and GC wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support, PNPD/CAPES for the scholarship of Andréia Caverzan and Prosup/CAPES scholarship of Luciano Antônio Ebone.

References


