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Review Article

Role of Enzymatic Antioxidants Defense System in Seeds

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A B S T R A C T

Antioxidants are the substances that are present in plants or in seeds at lower concentration compared to that of oxidizable substrates, significantly delays or prevent oxidation of substrates. Antioxidants such as tocopherols, phenols, carotenoids, ascorbic acid and thiols are non-enzymatic in nature whereas, catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POD) glutathione reductase (GR), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDAR) are the major enzymatic antioxidants. Enzymatic antioxidants function by breaking down and removing the free radicals from the cells while, non-enzymatic antioxidants work by interrupting free radical chain reactions thus protecting cells from damage. The presence of an antioxidant defense is universal in nature and plants produce it to protect themselves from the ultraviolet light of sun and the reactive oxygen species (ROS) generated during photosynthesis that would cause irreparable damage to the plant tissues. Seeds are miniature plant which when exposed to varied environmental stresses change their moisture content during different stages of development, maturation, desiccation, germination, storage and ageing. This disturbs the photosynthetic electron transport and mitochondrial respiratory chain within its cells which may result in free radical toxicity by enhanced production of ROS. Enzymatic antioxidants work as detoxifying mechanism which curbs the radicals generated at times of stress thus preventing seed from damage or deterioration. There is profound variation in the kind and amount of antioxidants present in different classes of seeds *viz.*, oil seeds, cereals, pulses etc.

Keywords

Enzymatic antioxidants, defense system

Introduction

Reactive oxygen species (ROS) are usually produced due to air pollutants, soil salinity, drought, heavy metals, high light intensity, high temperature and biotic stress and the imbalance between the ROS and antioxidants defense system in plants creates the oxidative stress in the plants or seeds. The accumulation of ROS leads to disturbances in normal physiological processes and leads damage to biomolecules, cells and tissues.

There are different sources for production of ROS not only due to stress conditions but during the normal metabolic conditions also they are produced in mitochondria, chloroplast and peroxisomes of cells due to incomplete reduction of molecular oxygen. They have several targets in cells to modify amino acids, breakage of polypeptide chains, increased proteolytic degradation, inactivation of enzymes, enhanced fluidity and permeability of membranes, breaking of

lipid chains, breaking of DNA strands, depruniation, depyramidation, mutation of bases and protein crosslinks. There are several different members of ROS family with different attributes based on their migration distances, sources, mode of action reaction with DNA, reaction with proteins and their scavenging systems. Autoxidation (Oxidation of lipid in presence of oxygen around the unsaturated fatty acid resulting in formation of peroxides and hydro peroxides) and lipid peroxidation (The oxidative degradation of lipids**,** the process in which free radicles steal electrons from the lipids in cell membranes, resulting in cell damage.

It is stimulated by Lipoxygenase enzyme, occurs in all seeds, more in oil seeds) are the two important sources for seed deterioration. Disturbance in the ratio the ratio of pro-oxidant and anti-oxidant leading potential damage to the cells and tissues. An imbalance between the systemic manifestation of Reactive Oxygen Species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage cause. Disturbances in the normal redox state of cells can cause toxic effects. ROS may also play a role in desiccation-related damage, particularly in dehydration-intolerant recalcitrant seeds.

However ROS has role in signal transduction pathways by cellular antioxidant machinery, which involves detoxifying enzymes and non-enzymatic antioxidant compounds which can control toxic ROS. In orthodox seeds, respiration is intense embryogenesis and decreases during the desiccation phase during germination increase of respiration leads to enhanced production of AOS. Hence there should be defense mechanism to protect the plants or seeds during the oxidative stress either by enzymatic or non-enzymatic antioxidants.

What are Antioxidants?

Antioxidants are the substances that are present in plants or in seeds at lower concentration compared to that of oxidizable substrates, significantly delays or prevent oxidation of substrates. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions.An antioxidant defence differ from species to species. The presence of antioxidant defence is universal. Enzymatic antioxidants work by breaking down and removing free radicals. Non-enzymatic antioxidants work by interrupting free radical chain reactions. It fill electron needs of free radicals that are ferociously searching for their missing electrons, without becoming free radicals themselves. Antioxidants such as tocopherol, phenols, carotenoids, ascorbic acid and thiols are non-enzymatic in nature whereas, catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POD) glutathione reductase (GR), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDAR) are the major enzymatic antioxidants. Enzymatic antioxidants function by breaking down and removing the free radicals from the cells while, non-enzymatic antioxidants work by interrupting free radical chain reactions thus protecting cells from damage. Seeds when exposed to various environmental stresses change their moisture content during different stages of development, maturation, desiccation, germination, storage and ageing. This disturbs the photosynthetic electron transport and mitochondrial respiratory chain within its cells which may result in free radical toxicity by enhanced production

of ROS. Enzymatic antioxidants work as detoxifying mechanism which stops the degradative radicals generated during stress thus preventing seed from damage or deterioration.

Classification of antioxidants

Antioxidants are classified into 3 categories

Primary antioxidants: It is involved in the prevention of oxidants formation

Secondary antioxidants: exhibits scavenger of ROS.

Tertiary antioxidants: repairs the oxidized molecules through sources like dietary or consecutive antioxidants.

Mode of action of antioxidants

Antioxidants may act on 2 folds:

Primary or chain breaking antioxidants: break chain reaction and resulting radical is less reactive

Secondary or Preventive antioxidants: They may act either by, chelators/Deactivate metals, Scavenge singlet oxygen (highly toxic) and Remove ROS.

First line defence: SOD-quenching superoxide radical, CAT - catalysing decomposition of hydrogen peroxide to water, GPX- Se containing enzyme, catalyse reduction of H_2O_2, GR- efficient scavenging of peroxides from cytosol and cell membrane

Second line defence: Glutathione (GSH) good scavenger of free radicals like O_2^-, hydroxyl, and various lipid hyper peroxides. Vitamin E (α-tocopherol) - interacts directly with free radicals like O_2^-, hydroxyl, scavenge peroxyl radical intermediates in lipid peroxidation, also protects LDL. Carotenoid (β- carotene) excellent scavenger of singlet oxygen.

Flavonoid phenolic compound that inhibit lipid peroxidation and lipoxygenase activity

Third line defence: Complex group of enzymes repair damaged DNA, damaged proteins, oxidized lipids and peroxides and also stop chain propagation of peroxyl lipid radical, Enzymes repair the damage to biomolecules and reconstitute the damaged cell membrane, DNA repair enzymes, transferase, methionine sulphoxide reductase

Enzymatic antioxidants

Superoxide dismutase (SOD)

SOD is present in essentially every cell in the body which actually represented by a group of metalloenzymes with various prosthetic groups.

SOD appears in three forms: based on metal cofactors

Cu-Zn SOD: in the cytoplasm
Mn-SOD: in the mitochondria
Fe -SOD: in chloroplast

The Cu/Zn-SOD has a central role in scavenging toxic oxygen radicals. It is the major form in the leaves and is responsible for 65-80% of the total activity.

SOD is catalyse through the action of attracting negatively charged $O_2^{\cdot -}$ Molecule to a site 0f + vely charged amino acid present at the active site of the enzyme.

The metal present at the active site then donates electron directly to the $O_2^{\cdot -}$ reducing $o_2^{\cdot -}$ Molecule which turns forms H_2O_2

Ascorbate peroxidase (APX)

Ascorbate peroxidase that uses ascorbate as its reducing substrate. Ascorbate peroxidase uses two molecules of AsA to reduce H2O2 to water with a concomitant generation of two molecules of MDHA. Ascorbate peroxidase seems to play a more important role in scavenging ROS than other antioxidative enzymes since ascorbate, in addition to reacting with H2O2 may react with superoxide, singlet oxygen and hydroxyl radical. APX get rapid inactivation under conditions where an electron donor is absent. The APX family consists of different isoforms including thylakoid (tAPX) and glyoxisome membrane forms (gmAPX), as well as chloroplast stromal soluble form (sAPX), cytosolic form (cAPX). Similarity between AP X action (in chloroplast and cytosol) and PO X action (in apoplast and vacuole). It's possible that MDA reductase could act as phenoxyl radical reductase in the apoplast to regenerate redox status of phenols PO X uses phenolics as substrates to detoxify H2O2.

Catalase (CAT)

It's a heme containing protein. Catalase is important in the removal of H2O2 generated in Peroxisomes by oxidases involved in beta-oxidation of fatty acids, Photorespiration. It has been found to remove the bulk of H2O2 whereas POX would be involved in the scavenging of H2O2 that is not removed by CAT. *Catalase has* one of the highest turnover rates for all enzymes: One molecule of CAT can convert 6 million molecules of H2O2 to H2O and O2 per minute. Maize has three forms of cat, CAT-1, CAT-2 and CAT-3. The CAT-3 regulates H2O2 levels during metabolic activity at night (Almedia *et al., 2005).*CAT is highly sensitive to light and has a rapid turnover rate. Stress condition that reduce the rate of protein turn over as a result CAT activity reduces

Glutathione peroxidase, GPx

GPx is a selenium-dependent enzyme. The entire process is driven by energy production at the cellular level,It has role in biosynthesis of lignin and defense against biotic stresses by consuming H_2O_2 in cytosol, vacuole and cellwall present both in extra & intra cellular form are participating in the breakdown of H_2O_2.

Glutathione reductase (GR)

Glutathione act as redox sensor of environmental cues & forms part of multi regulatory co-ordinating defence expression.GR has been suggested as an intermediatory in a redox sensing signalling pathway in plants. As increase in GR activity in plants results in the accumulation of GSH and provide stress tolerance. It is involved in the sulphur metabolism and in defence reactions against oxidative stress (Potters *et al.,* 2002). It can also lead to the synthesis of phytochelatins that are important sequesters for certain heavy metals (Cobbett & Goldsbrough, 2002)

Seeds cannot retain their viability indefinitely and so eventually they deteriorate and die due to lipid peroxidation, degradation of functional structures, enzyme degradation and inactivation formation and activation of hydrolytic enzymes, inability of ribosomes to disassociate, breakdown in mechanisms triggering germination, genetic degradation. The enzymes play an important role in the progress of seed deterioration and changes in their activity can be an indication of quality loss (Copeland and McDonald, 1995). The relationship between seed deterioration and the enzymes involved in lipid peroxidation, free radical removal has

been studied (Shen and Oden, 1999). All seeds undergo ageing process during long-term storage which leads to deterioration in seed quality. Aged seeds show decreased vigour and produce weak seedlings that are unable to survive once reintroduced into a habitat (Atici *et al.*, 2007).

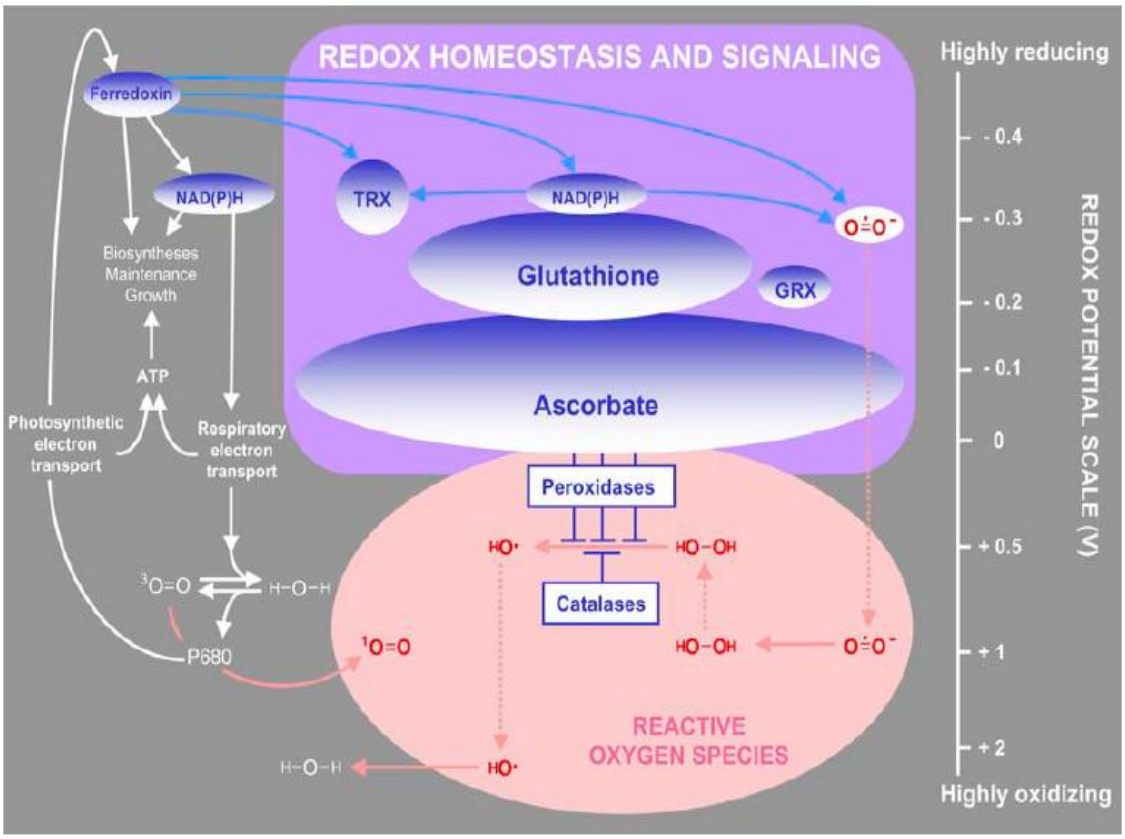
Results and Discussion

The enzymes play an important role in the progress of seed deterioration and changes in their activity can be an indication of quality loss (Copeland and McDonald, 1995). The relationship between seed deterioration and the enzymes involved in lipid peroxidation, free radical removal has been studied (Shen and Odén, 1999). All enzyme activity is positively correlated with germination of seed as ageing progressed germination also decreased and enzyme activity also decreased which showed significant deterioration in both accelerated as well as in natural aged seed lot. All seeds undergo ageing process during long-term storage which leads to deterioration in seed quality, especially in the humid tropical regions. Aged seeds show decreased vigour and produce weak seedlings that are unable to survive once reintroduced into a habitat (Atici *et al.*, 2007).

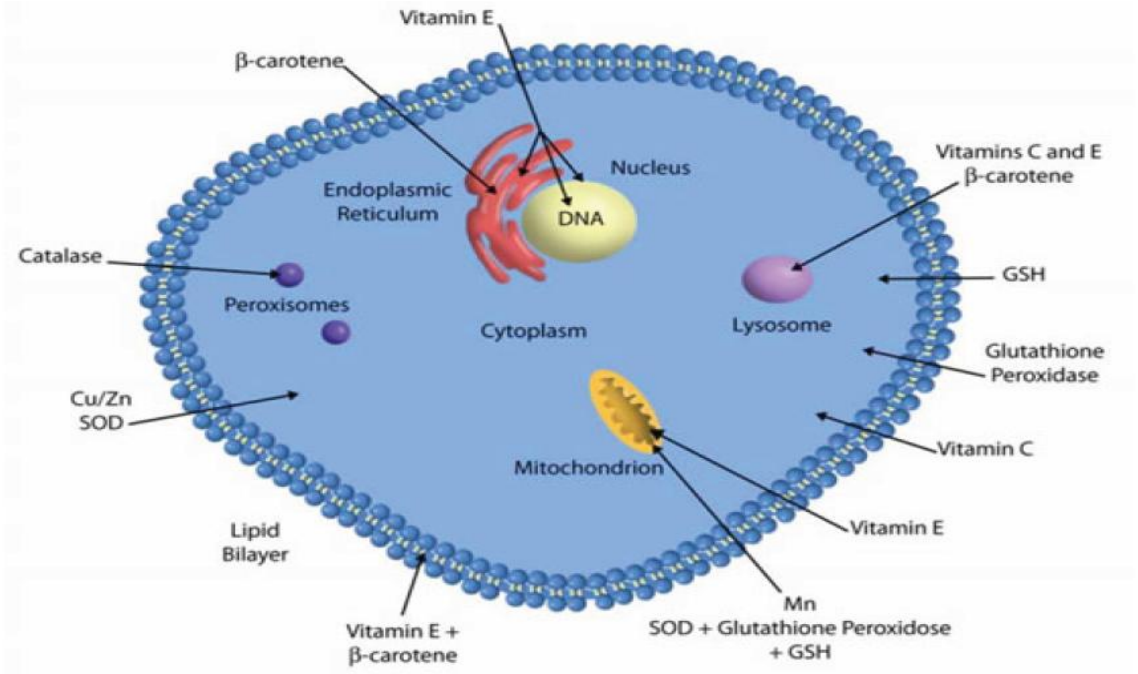
Under normal conditions, cells are protected from free radicals and damages via complex antioxidative system that includes lipid soluble and membrane antioxidants, water soluble reductants, enzymatic antioxidants and enzymes of ascorbant-glutation cycle (Reuzeau and Cavalie, 1995). Special attention is paid to enzyme activities due to their possible usage as significant indicators of vigour or seed longevity. Due to SOD activity low concentration of superoxide in cells is maintained and thus preventing formation of harmful oxidative products in plant cells (Alscher *et al.,* 2002). Level of SOD activity in stored seed can be significant factor in determination of level of seed protection against oxidative stress (Kang and Saltveit, 2002). Both SOD and peroxidase activity decreased in relation to storage condition and duration of soybean seed storage, as well as in sunflower stored seed. Puntralo and Boveris (1990) noticed gradually reduction of peroxidase activity during natural and induced seed aging. Under conventional storage facility, the plastic packaging, sealed with vacuum, has provided better maintenance of physiological quality due to the antioxidant defense enzymes like alcohol dehydrogenase and superoxide dismutase. (Abreu *et al.*, 2013). However at four months, the superoxide dismutase, catalase, alcohol dehydrogenase, and malate dismutase activities decreased regardless of storage conditions in sunflower seed (cv. BRS 122).

This decrease was more obvious in the vacuum-sealed seeds. (Lins *et al.,* 2014). SOD and POD activities increased gradually upto12 weeks after flowering and then declined increase in SOD and POD activities during early stages of seed maturation suggests an increase in oxidative stress for regulating the level of ROS, especially in early stage of seed maturation (He and Gao, 2008). A change in enzyme activity in seeds due to ageing is a topic of scientific importance. Vigour is essentially a physiological phenomenon influenced by the reserved metabolites, enzyme activities and growth regulators. The exact cause of loss of seed vigour and viability is still unknown as deterioration of seed is a complex process. In the presence of oxygen, ageing of seed can lead to peroxidative changes in polyunsaturated fatty acids. The free radical -induced non-enzymatic peroxidation, which has the potential to damage membrane, is likely to be a primary cause of deterioration of stored seeds.

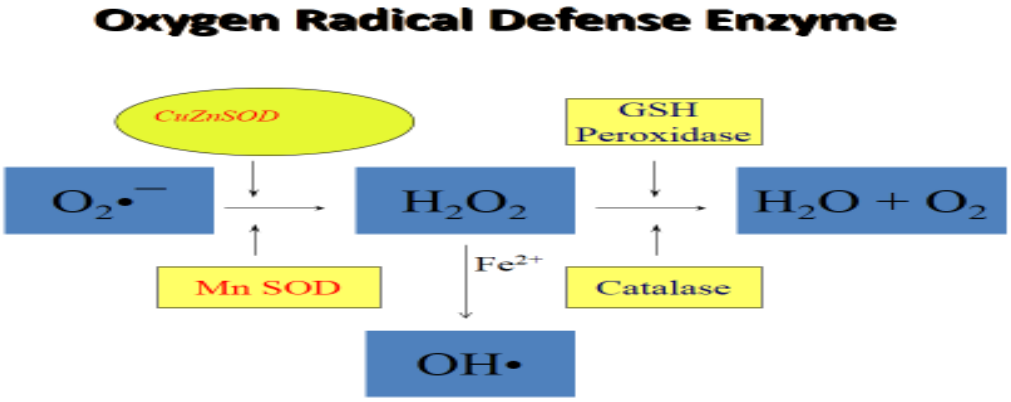
Reductant-antioxidant-oxidant interactions



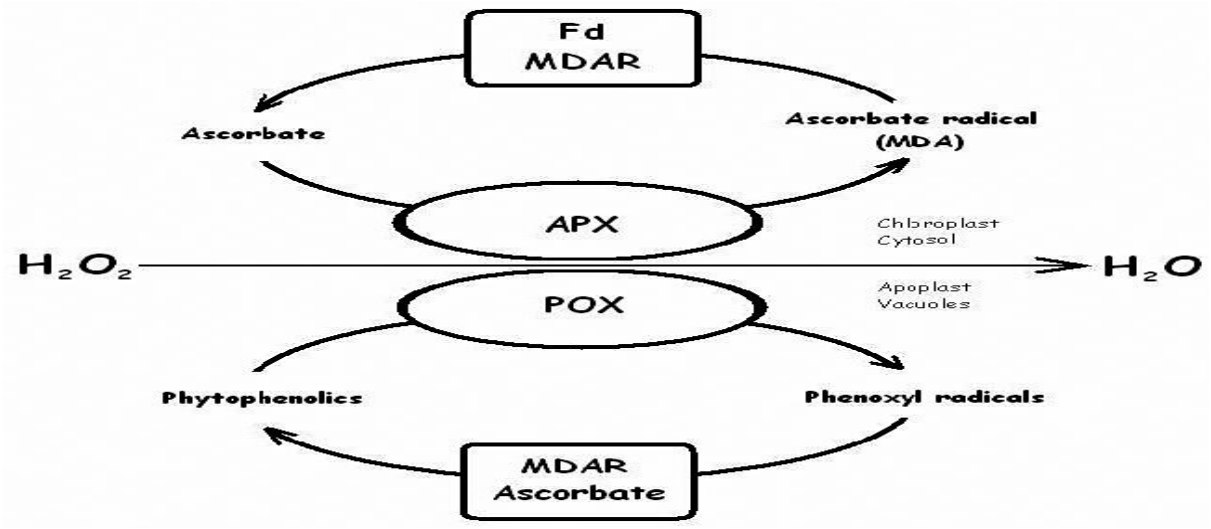
Sources of Enzymatic Antioxidants within a plant cell



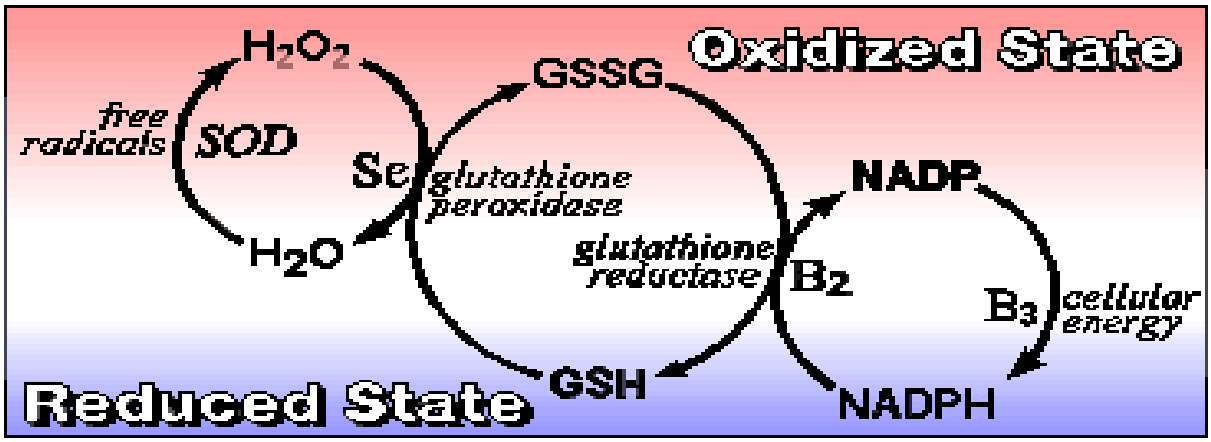
Superoxide dismutase (SOD)



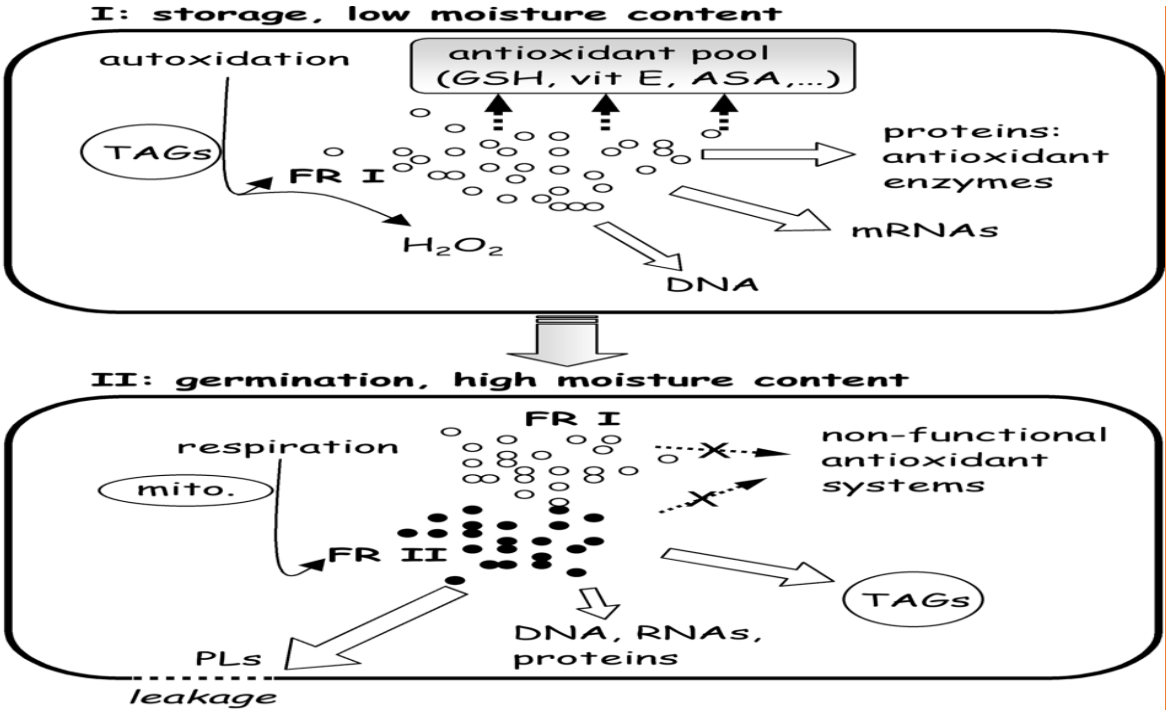
Ascorbate peroxidase (APX)



Glutathione peroxidase, GPx



Proposed involvement of oxidative mechanisms in seed ageing



(Schemes established from the results obtained by Bailly *et al.*, 1996, 1998, 2002).

Certain anabolic enzymes help in maintaining viability while some catabolic enzymes decrease viability. The seed catalase and peroxidase activity seem to be decreased during storage. The results revealed that the peroxidase enzyme activity decreased from 0.236 to 0.444 OD 10 min-1 when storage period increased. A decrease in catalase activity from 0.454 to 0.444 µg H2O2 mg-1 min-1 followed by a small increase from 0.434 to 0.452 µg H2O2 mg-1 min-1 was observed during storage. (Begum *et al.*, 2013)

Peroxidase activity was assayed in crude extracts of integument, cotyledons and embryo axis of radish seeds, deteriorated under accelerated ageing conditions. Over five days of ageing, in which germination decreased from 100 to 52%, the enzyme activity in integument was higher than that in other seed parts, increasing in the first days of ageing and then decreasing sharply in extremely aged seeds. A cytochemical localization of peroxidase activity in the various tissues showed that main differences between unaged and extremely aged seeds occurred in the embryo axis. (Scialabba *et al.*, 2002). After harvesting, the seeds of two rice cultivars (BRS Ourominas and BRSMG Caravera) were dried in the sun, to reach a moisture content around 13%. Then, they were packed in paper and stored in four environments: 5 ± 2 °C / 70 ± 5% RH, 12 ± 2 °C / 70 ± 5% RH, 18 ± 2 °C / 65 ± 5% RH and in a natural condition. Germination and enzymatic activity were assessed at the beginning and at 3, 6, 9 and 12 months of storage. Catalase and ascorbate peroxidase activity increased during the storage period, the most obviously in storage in natural environment to cultivate BRSMG Caravera. (Marques *et al.*, 2014)

Antioxidant enzymes in germinating and non-germinating seeds of *Ceiba pentandra* were evaluated. Enzymatic antioxidants like SOD, CAT, POD, GPx and AO showed

enhanced activities during seed germination. The nutritive utilization of protein and carbohydrates along with efficient participation of antioxidant mechanisms, including the synergistic activities of the different types of SOD, CAT, POD, GPx and AO, might play an important role during seed germination. (Ravi Kiran *et al.*, 2012). Seeds of *A. mongolica* were dried to a 4.67%, 3.73%, and 2.24% moisture content. After storage for 24 months, their level of vigor was measured. To determine whether these low MCs affect the activities of the antioxidant enzymes we evaluated the ability of dried seeds to germinate and to produce normal seedlings. Meanwhile, volatile aldehydes and malondialdehyde (MDA), lipid peroxidation production were also measured. Results indicated that the SOD, CAT, POD, APX and GR activities of ultra-dry seeds were higher than those of control seeds while volatile aldehydes and MDA were lower than in the control. (Li *et al.*, 2010)

Final product of lipid peroxidation is lipid hydroperoxide (ROOH) from which aldehydes are formed, including malonyl-dialdehyde (MDA). Determination of MDA content is the conventional method used for determination of lipid peroxidation (Sung and Jeng, 1994). Many studies confirmed the connection between increased MDA content in seed and prolonged storage period, as well as the application of artificial seed aging (Tian *et al.*, 2008; Li *et al.*, 2008). Obtained results relating to increased MDA content in soybean seed after six and twelve months of storage under both controlled and conventional storage conditions, confirmed the possibility of determining degree of lipid peroxidation in seed via determination of malondialdehyde (MDA) derivatives content. Due to differences existing among varieties, it can be notice that performances of genotypes also influenced the peroxidative changes during seed storage. The above mentioned results are in accordance with the results obtained by other researchers dealing with damages caused during seed storage. Seed susceptibility to peroxidative changes was different, depending on seed fatty acid composition, and lipid peroxidation can be considered as one of the indicators of individual soybean genotype susceptibility to oxidative stress (Malencic *et al.*, 2003). Shorter storage period, as well as the controlled storage conditions slowed down the process of fatty acid peroxidation in soybean seed. However, in the case of prolonged storage period, especially under conditions of variable temperature and air humidity, the rate of decline of seed viability increased with the changes occurring in lipid peroxidation intensity. Based on the obtained relations between increasing of lipid peroxidation and decreasing of seed germination, it can be pointed that storage conditions, at a longer time period, were more pronounced in expressing negative influence on soybean seed viability. Content of MDA in sunflower seed increasing by prolonged storage period indicated that lipid peroxidation was more intensive in aged seed, especially in seed under conventional (uncontrolled) storage.

Under normal conditions, cells are protected from free radicals and damages via complex antioxidative system that includes lipid soluble and membrane antioxidants, water soluble reductants, enzymatic antioxidants and enzymes of ascorbant-glutation cycle (Reuzeau and Cavalie, 1995). Special attention is paid to enzyme activities due to their possible usage as significant indicators of vigour or seed longevity. Superoxide dismutase (SOD) is enzyme belonging to the first group of protective mechanisms of plant cells against oxidative damage. Due to SOD activity low concentration of

superoxide in cells is maintained and thus preventing formation of harmful oxidative products in plant cells (Alscher *et al.*, 2002). SOD serves a protective role in respiring cells through its elimination of the reactive superoxide radical (Blokhina *et al.*, 2003). Level of SOD activity in stored seed can be significant factor in determination of level of seed protection against oxidative stress (Kang and Saltveit, 2002). Both SOD and peroxidase activity decreased in relation to storage condition and duration of soybean seed storage, as well as in sunflower stored seed.

Enzymatic antioxidants are an important class of phyto-chemicals that plays a very crucial role in maintaining seed quality by counteracting the oxidative stress. Activity of enzymatic antioxidants *viz.*, POD, SOD, CAT, and APX is up regulated as an antioxidant defence system against endogenous oxidant radicals that may occur during different stages in seed life *viz.*, seed maturation, desiccation, storage, germination and ageing.

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