Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 3503-3507
Received: 22-05-2019
Accepted: 24-06-2019

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Role of enzymatic antioxidants in seed science and technology: A review

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Abstract
Antioxidants, also known as anti-ageing agents are popular globally for their role in quenching free radicals present inside a cell therby reducing the oxidative stress caused to any living cell. These antioxidants can be present in both enzymatic and non-enzymatic form in nature. The role of antioxidants in biology is focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity. A seed is a living entity and botanically is a mature fertilized embryo embedded in nutritive tissue called as endosperm and enclosed by a protective covering called as the seed coat. Antioxidants present inside the seed affect the growth and development of it by up grading the physiology of seed in terms of seed maturity, vigor and germination. Antioxidants also play a very crucial role in enhancing seed quality by antioxidant priming; it enhances the seed storability and further enzymatic antioxidants may also be used in meeting the new research objectives related to seed science and technology viz., antioxidant gene transfer and development of new varieties of crop species of cultivated plants, predicting the storage life of seeds etc. Therefore, this review presents the role of this very important phyto chemical called as antioxidants in seed science and research.

Keywords: Seed, enzymatic antioxidants, seed storability, oxidative stress

Introduction

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 (Kumar *et al.* 2016) [15]. In 1958 Kaloyereast introduced the (FRTA- Free - Radical Theory of Ageing) into seed science by arguing that lipid oxidation might underlie loss of viability in seed and in late 20th century - role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity. An antioxidant defence differ from species to species and presence of antioxidant defence is universal. Plants produces antioxidants to protect themselves from the ultraviolet light from the sun and the reactive oxygen species generated during photosynthesis that would cause irreparable damage in plants (Gupta and Sharma, 2006) [9]. The various types of antioxidants are present in Fig.1.

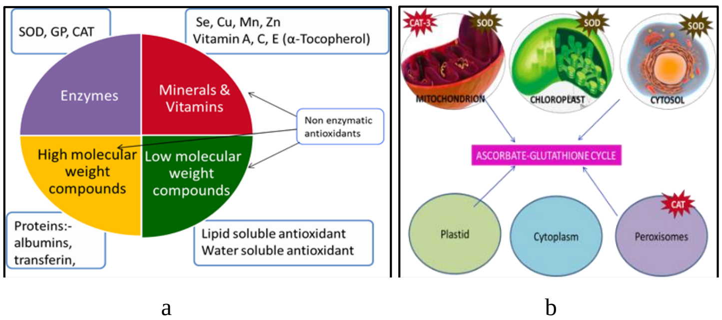

Fig 1 a: Diagram showing a. types of antioxidants: enzymatic and non-enzymatic and **b:** sources of enzymatic antioxidants within a plant cell

Types of enzymatic antioxidants in ascorbate-glutathione cycle includes following:

1. Superoxide dismutase (SOD)
2. Catalase (CAT)
3. Ascorbate peroxidase (APX)
4. Dehydroascorbate reductase (DHAR)
5. Monodehydroascorbate reductase (MDAR)
6. Glutathione reductase (GR)
7. Glutathione peroxidase (GPX)

What is the role of antioxidants in plants/seeds?

The role of these enzymatic antioxidants in the plant can be categorized as first line, second line and third line defence as presented in Table 1.

What is ROS and Lipid peroxidation in plants?

ROS: nature and origin

Reactive Oxygen Species (ROS) are the chemical species formed upon incomplete reduction of molecular oxygen. Accumulation of ROS leads to cell injury and disturbances in seed development or germination processes. These are also called as free radical atoms or molecules with single unpaired electrons as represented in reaction of formation of ROS through energy -transfer and electron-transfer reactions Fig. 2. Another term called as lipid peroxidation refers to the oxidative degradation of lipids, a process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. It is stimulated by autoxidation and lipogenases enzyme and occurs in all seeds, more in oil seeds

The exogenous sources of ROS are UV light, ionizing radiation, environmental toxins, and endogenous sources are mitochondria, peroxisomes, lipogenases, NADPH oxid [1] ase as well as antioxidant defence.

Table 1: Enzymatic antioxidant and their functions in plant cell

Sr. No.	Antioxidant	Function within plant cell
First Line Defence		
1	SOD	Quenching superoxide radical
2	CAT	Catalyzing decomposition of hydrogen peroxide to water
3	GPX	Se containing enzyme, catalyse reduction of H_2O_2 and lipid peroxide to water (produced during lipid peroxidation)
4	Se and Vitamin E	Efficient scavenging of peroxides from cytosol and cell membrane
5	Cu	Act in form of cytosolic Cu SOD
6	Zn	Component of several enzymes viz., cytosolic SOD, alcohol dehydrogenase,
Second Line Defence		
8	Glutathione (GSH)	Good scavenger of free radicals like - O_2^-, hydroxyl and various lipid hydro peroxides
9	Vitamin E (α-tocopherol)	Interacts directly with free radicals like- O_2^-, hydroxyl, scavenge peroxyl radical intermediates in lipid peroxidation, also protects PUFA (poly unsaturated fatty acid) present in cell membranes and low density lipoproteins (LDL) against lipid peroxidation (OIL SEEDS)
10	Carotenoid (β- carotene)	Excellent scavenger of singlet oxygen
11	Flavinoid	Phenolic compound that inhibit lipid peroxidation and lipoxygenases
Third Line Defence		
12	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 e.g. – lipase, proteases, DNA repair enzymes, transferase, methionine sulphoxide reductase

How enzymatic antioxidants acts inside aside?

The enzymatic antioxidants acts within a seed cell through Foyer- Halliwell–Asada cycle, or Ascorbate - Glutathione cycle (Fig. 3.) which refers to a metabolic pathway that detoxifies hydrogen peroxide (H_2O_2- a ROS and a waste product in plant metabolism).

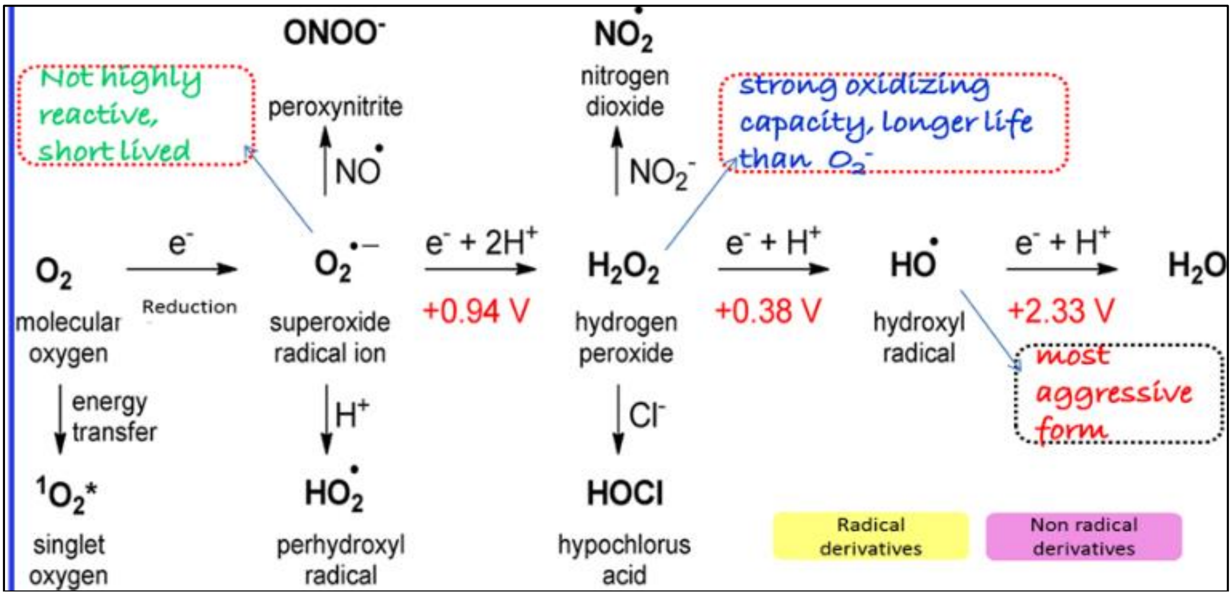

Fig 2: Formation of ROS through energy- and electron-transfer reactions (Krumova and Cosa, 2016).

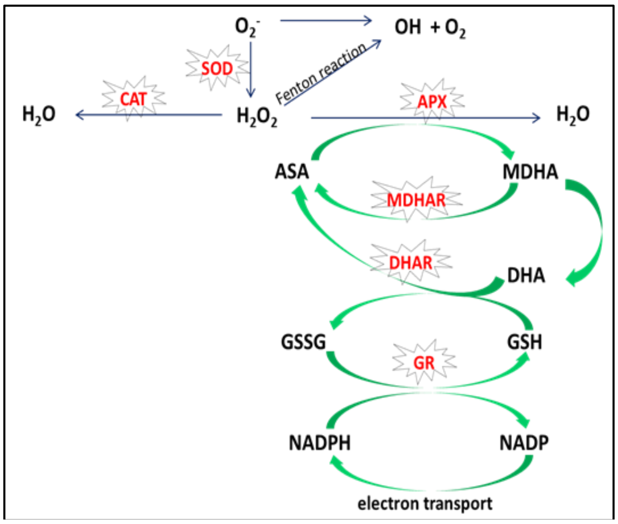

Fig 3: Ascorbate - Glutathione cycle showing the degradation or reduction of hydrogen peroxide into water by the reducing action of various enzymatic antioxidants (full forms given in the foot note[2])

Seed and enzymatic antioxidants: Functions

Seed which is a biological entity is defined in horticulture terms as a plant propagation material and botanically it is fertilized mature ovule consisting of a living tissue known as embryo, embedded in nutritive tissues the endosperm and enclosed a protective covering called as seed coat. The enzymatic antioxidants mentioned earlier plays important role in any living tissue by up grading its growth and physiological activities. Several of these antioxidants have crucial functions without which seed may not develop in a uniform way. The major enzymatic antioxidants within seeds are; Superoxide dismutase (SOD), Catalase (CAT) and POD (Peroxidase). The roles of these antioxidants can be described as follows; SOD has an important protective mechanism against oxidative damage, it works in collaboration with POD and CAT by removing O_2^- and H_2O_2. CAT helps in removal of ROS produced under various stress conditions thus avoiding oxidative damage; also POD and CAT keeps H_2O_2 balance in seeds and in oil seeds it removes H_2O_2 produce during β – oxidation of fatty acid. POD is associated with essential metabolic processes viz., cell elongation, lignification, phenolic oxidation, pathogen defence and defence against stress; it also has important role in seed germination, growth, morphogenesis, senescence and death

There exists variation in these enzymatic antioxidants in seeds of various crop species viz., antioxidant and phenolic contents of nuts and oil seeds, antioxidants in different extracts cereals and pseudocereals as well as extracted meals of cakes (Sreeramulu *et al.*, 2011; Gorinstein *et al.*, 2008 and Schmidt and Pokorny, 2005) [29, 8, 26] as presented in Table 2.

Table 2: Antioxidant activity in various forms in different crop species

Crop name	Form of antioxidant activity
Buckwheat	TRAPW (total radical-trapping antioxidative potential in water extract), CUPRAC (cupric-reducing antioxidant capacity)
Cottonseed	Sinapic, ferulic, p-hydroxybenzoic acid, quercetin
Sunflower seed	Chlorogenic, caffeic, p-hydroxybenzoic acid, p-coumaric, cinnamic, m-hydroxybenzoic, vanillic
Groundnut	phenolic acids, esters e.g. p-hydroxybenzoic acid, p-coumaric, syringic, caffeic acid
Quinoa	TRAPW (total radical-trapping antioxidative potential in water extract)
Green amaranth	FRAP (ferric ion-reducing antioxidant power)
Soybean	syringic, vanillic, ferulic, salicylic, p-coumaric acid, sinapic acids, isoflavones and their glucosides, TRAPAC (total radical-trapping antioxidative potential in acetone extract)
Rapeseed	sinapine, phenolic acid ester and glycosides and benzoic and cinnamic acid derivatives
Linseed	coumaric, ferulic acids, lignans and their glucosides
Pea, chickpea, black gram, green gram, cowpea, horse gram, beans and lentils	DPPH (2, 2-Diphenyl-1-picryl hydrazyl) scavenging activity, FRAP (Ferric reducing antioxidant power)activity

How antioxidants varies among orthodox and recalcitrant class of seeds?

Seeds can be classified on the basis of their desiccation tolerance as well as storability as: long term storer commonly the orthodox seeds, short term storer; the recalcitrant seeds and medium term storer. Further on the basis of antioxidant enzyme activity also seeds can be differentiated as presented in following segment.

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Orthodox seeds- long lived	Recalcitrant seeds- short lived
1. Maximum effective antioxidant system before maturation drying of orthodox seeds, and again when seeds take up water upon imbibition.	1. Maximum effective antioxidant system when shed from the mother plant at a high moisture content and very active metabolism. This declines in case of stress
2. Seeds are devoid of both ascorbate peroxidase (AP) at end of desiccation period	2. Seeds contain high ascorbate peroxidase (AP) activity to eliminate toxic H_2O_2as they never reach the quiescent phase
3. They are desiccation tolerant	3. This leads to desiccation senstivity
4. E.g. – Pinus, Broad bean, Oats	4. E.g. – Gingko, Oak, Aesculus,Cycas

How activity of enzymatic antioxidants affects seed storability?

As per some researchers there is a relation between enzymatic antioxidants and seed storability. There is a sharp decline in the activity of peroxidase as the seeds ages whereas younger seeds have comparatively higher peroxidase activity. Some of research work carried out in this aspect is presented in Table 3.

Table 3 Summary of work done in relation to enzymatic antioxidants and seed storability

Crop	Scientist	Summary of work done
Pinus sylvestris L.	Shen and Oden, 1999 [28].	There exist relationship between seed deterioration and the enzymes involved in lipid peroxidation, free radical removal, and seed respiratory metabolism
Radish	Scialabba *et al.*, 2002 [27].	Peroxidase activity decreased in aged seeds as compare to fresh seeds
Sunflower	Pallavi *et al.* 2003 [21]. Abreu *et al.* 2013 [1].	Sharp decline in peroxidase enzyme during ageing The oil content in seeds of sunflower decreases along time
Chenopodium rubrum	Mitrovic *et al.*, 2005 [19].	Peroxidase and catalase activities were found higher in younger seeds
Arabidopsis	Rajjou *et al.*, 2008 [17].	Ageing induced deterioration increase the extent of protein oxidation thus inducing loss of functional properties of proteins and enzymes
Soybean	Chandel *et al.*, 2016 [4].	Activities of catalase, peroxidase and superoxide dismutase and ascorbate peroxidase enzymes decreased during seed storage thus play an important role in determining seed longevity

Role of enzymatic antioxidants in seed science and research?

Enzymatic antioxidants hitherto have a very important function in seed science and research aspects viz., seed maturation, germination, ageing, seed storage, seed vigour and physiological stress conditions. Thus findings of some of the research workers are enlisted in Table 4.

Table 4: Enzymatic antioxidants and seed functions in various agricultural crops**.**

Crop	Scientist	Summary of work done
Chimonanthus praecox (ornamental plant)	He and Gao, 2008 [10]	Increase in SOD and POD activities during the early stages of seed maturation suggest an increased oxidative stress It also play a major role in regulating the level of ROS
Jatropha curcas (Oil from seeds used to make high quality biodiesel)	Cai *et al.*, 2011 [3]	SOD, POD and CAT, might play an important role during seed germination Their activities are up regulated as an antioxidant defence system against endogenous oxidant radicals generated during seed germination
Sunflower	Draganic and Lekic, 2012 [12].	Lower accumulation of deterioration when the cold test is applied, as opposed to cases when accelerated ageing is performed, which provides the opportunity for the seeds to restore vigor after priming
Tea	Jamalomodo *et al.*, 2013	Desiccation induced rapid accumulation of antioxidant enzymes, APX, SOD, CAT and POD Reducing ROS to appropriate concentration will efficiently reduce desiccation damage
Soybean	Chandel *et al.*, 2016 [4].	Activities of catalase, peroxidase and superoxide dismutase and ascorbate peroxidase enzymes decreased during seed storage thus play an important role in determining seed longivity
Maize	Radha *et al.*, 2014 [23]	Change in storage enzymes activities in natural and accelerated aged seed.
Oat	Kong *et al.*, 2015 [13]	Antioxidant response and related gene expression in aged seed
Sunflower	Morscher *et al.*, 2015 [20]	Glutathione redox state, tocochromanols, fatty acids, antioxidant enzymes and protein carbonylation embryos associated with after-ripening and ageing
Date palm	Eldin and Ibrahim., 2015 [6]	Some biochemical changes and activities of antioxidant enzymes in developing somatic and zygotic embryos in vitro
Chenopodium murale	Bogdanovic *et al.*, 2008	Changes in activities of antioxidant enzymes during Chenopodium murale seed germination
Ceiba pentandra (Kapok)	Kiran *et al.*, 2012 [12]	Impact of Germination on Biochemical and Antioxidant Enzymes of Seeds
Nigella sativa	Kushwah *et al.*, 2014 [16]	Evaluation of antioxidant properties at different germination stages of seeds
Erythrina velutina Willd.	Ribeiro *et al.*, 2014 [25]	Activity of antioxidant enzymes and proline accumulation in seeds subjected to abiotic stresses during germination

Conclusion

Enzymatic antioxidants are important class of phytochemicals 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 are up regulated as an antioxidant defence system against endogenous oxidant radicals generated during seed germination, desiccation, maturation, storage and ageing. Further quantification of antioxidants present in seeds of wild and cultivable species of different plants can be useful in seed research. The enzymatic antioxidants present in wild species can be transferred of the to the cultivable species *viz.*, cereals, oilseeds and pulses for making these species more economically useful. Enzymatic antioxidants can be used to enhance seed quality through treatment with enzymatic antioxidants with standardized priming duration and combinations of antioxidant substances. Prediction of seed longevity based on antioxidant content of seed in storage also proves to be an innovative technique to the seed researchers. Thus, looking into the vast number of applications antioxidants it has in the present day seed science and research and technology, antioxidants can be considered as an important phytochemical that regulates the growth and development in the plant species.

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