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Primary Antioxidant Enzymes and Their Important Role in Oxidative Stress in Plants and Mammalian

Mohammad Behrouzi Varjovi*, Mostafa Valizadeh** and Ali Bandehagh**

**Young Researchers and Elite Club, Maragheh Branch, Islamic Azad University, Maragheh, IRAN.*
*** Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.*

(Corresponding author: Mohammad Behrouzi Varjovi)
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ABSTRACT: Enzymatic and non-enzymatic pathways are existed within cells to eliminate reactive oxygen species. Primarily, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase have important rolls in theses pathways. Plants as well as mammalians respond to oxidative stresses by changing expression levels of these enzymes. Investigations indicate that, in most cases cells which are exposed to oxidative stresses up regulate SOD, CAT and GPx. So that they can resist against stress. Some manipulation have been conducted to artificially over-express theses primary antioxidant enzymes and the results were promising. Transformed plants are relatively resistance against stress and some extracts/agents have been introduced to reduce mammalian oxidative stress. Since high concentrations of ROS could damage cells and cause to diseases such as cancer, ischemia, and failures in immunity and endocrine functions, these results could offer a bright future, finding permanent, cheap and firm cures.

Key-words: Superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, oxidative stress, plants, mammalian

INTRODUCTION

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions can produce free radicals which leads the cell to damage or death. Antioxidants could end these chain reactions. Plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Failing of these systems could cause oxidative stress. Oxidative stress seems to play a significant role in many human diseases, including cancers and many cellular damages in plants which lowers quantity and quality of crops. Although studies suggested that antioxidant supplements improve human health, large treatments of antioxidant supplements such as beta-carotene, vitamin A, and vitamin E singly suggest that supplementation has no effect on mortality or possibly increases it (Williams *et al,* 2004; Frei. 2009; Bjelakovic *et al,* 2013) external application of some antioxidants (e.g. polyphenols), on plants have been done (Abner *et al,* 2011; Bjelakovic *et al,* 2007). Although these trials had significant effects on plant production it seems to be costly using this kind of antioxidants externally in a large scale. Here, investigation of enzymatic systems for detoxification of reactive oxygen species (ROS) and applying the achievements could be an alternative. In this review, an overview of enzymatic systems of elimination of free radicals and possible trials of improving these systems in mammalian and plants is presented.

A. Enzymatic pathways

Reactions to neutralize ROSs are catalyzed by several systems including superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT). These antioxidant enzymes and antioxidant agents are located in various parts of a cell, which is depicted in Fig. 1. The Cu-Zn SOD isoform is located in the cytosol, whereas the Mn SOD isoform is found in the mitochondria (Grisham & McCord, 1986; Ohno *et al,* 1994; Ji, 1995). Although CAT is widely distributed in the cell, high concentrations are found in both peroxisomes and mitochondria (Halliwell & Gutteridge, 1989).

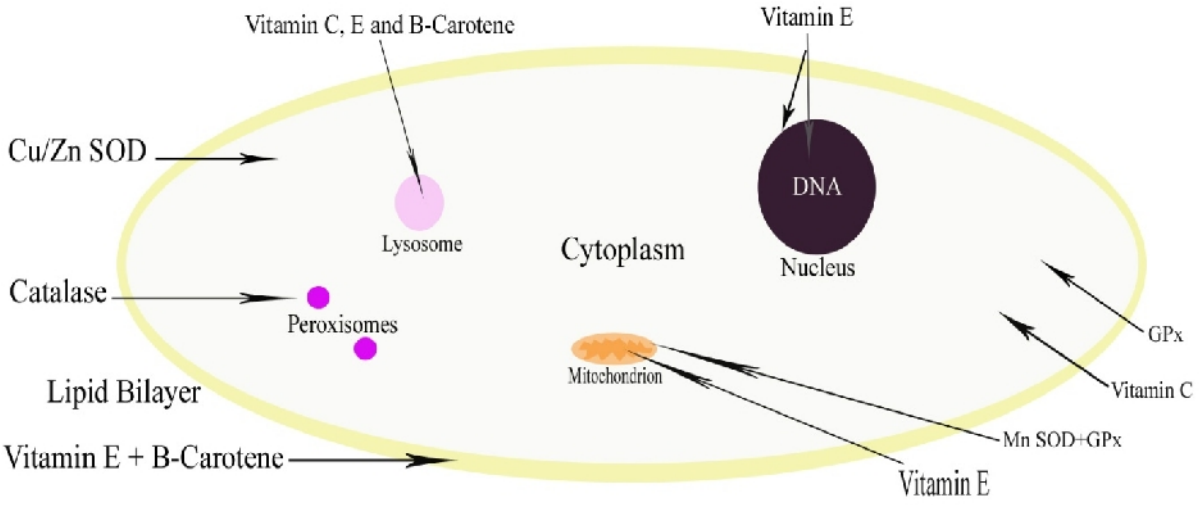

Fig 1. Cellular localization of different antioxidants: Cu/Zn SOD enzyme is found throughout cell cytoplasm but Mn SOD wherewith glutathione peroxidase is located in mitochondrion. Also, glutathione peroxidase is found in cytoplasm. Peroxisomes are organelles in which contain antioxidant enzymes like catalase. Antioxidant agents, Vitamin C, Vitamin E and β-carotene, have their cellular locations, too.

Antioxidant enzymes work as a complex to remove types of ROS. First step is to convert ROS into less-active molecules and block them to be transformed into harmful forms such as hydroxyl radicals. SOD, GPx and CAT are known as primary antioxidant enzymes and each one performs reduction of particular ROS. Superoxide radicals are reduced by SOD so that H_2O_2 and O_2 are formed. GPx is the next player in which has a role to reduce H_2O_2 or organic hydro-peroxides to water and alcohol respectively (Halliwell & Gutteridge, 1989). Glutathione, an important water-soluble antioxidant, is synthesized from the amino acids glycine, glutamate, and cysteine. Glutathione can directly neutralize ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism (Xenobiotics are toxins that the body is exposed to). Exposure of the liver to xenobiotic substances means the body prepares itself by increasing detoxification enzymes, i.e., cytochrome P-450 mixed-function oxidase. In order to function, GPx requires the GSH as electron donor. Since GSH is oxidized by GPx to form glutathione disulfide (GSSG), cells require a method of regenerating GSH. Regeneration of GSH is accomplished by the enzyme GSSG reductase which uses NADPH to provide the reducing power.

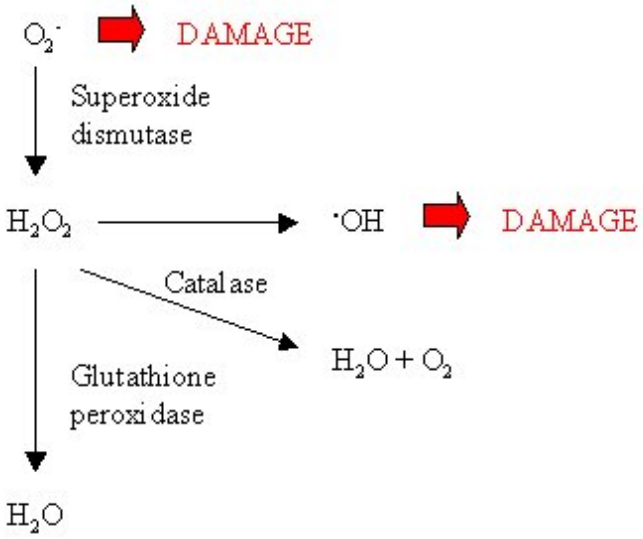

Fig. 2 Primary antioxidant enzymatic reactions to reduce ROS-related cell damage: hydroxyl radicals (˚OH), superoxide anions (O_2˚) and hydrogen peroxide (H_2O_2) could damage cell compartment and destroy vital molecules. Superoxide dismutase, catalase and glutathione peroxidase are the primary antioxidant enzymes which react with these damaging molecules to transform them to other molecules including water and oxygen.

Production of water and molecular oxygen from H_2O_2 is catalyzed by CAT. CAT and GPx have different affinity for H_2O_2 as their substrate. GPx in mammalian cells has a greater affinity for H_2O_2 at low concentrations than CAT. CAT is recruited, when high concentrations of H_2O_2 is found (Fig. 2).

B. ROS production and enzymatic response in plants

Abiotic stresses such as drought, salinity, extreme temperature, heavy metals and toxic chemicals affect plant growth and its production (Kochhar & Kochhar, 2005). The majority of lab experiments related to plant stress response to changes in environmental conditions have focused on a single stress. In contrast, a number of different stresses, e.g. drought, salinity and high temperature stresses, occur simultaneously in the field and often induce similar cellular damage (Zhu, 2002; Mittler *et al*, 2004). For example, oxidative stress, which frequently accompanies high temperature, salinity, or drought stress, activates similar cell signaling pathways (Shinozaki & Yamaguchi-Shinozaki, 2000; Zhu, 2001; Zhu, 2002) and cellular responses, e.g. production of heat-shock proteins (HSPs) (Qu *et al*, 2013), up-regulation of antioxidants to detoxify reactive oxygen species (ROS), and accumulation of compatible solutes (Kochhar, & Kochhar, 2005; Nahakpam, & Shah, 2012). Accumulation of ROS in plants leads to oxidative stress, inducing superoxide and hydrogen peroxide production (Panchuk *et al*, 2002). Plants response to this by enhancing of ROS detoxification systems (Munne´-Bosch *et al*, 2004).

Recently, it was identified that ROS play an important signaling role in plants during growth, development, response to stresses and apoptosis. In recent years, it is recognized that rapid production of ROS is an essential process, and molecular studies indicated that respiratory burst oxidase homolog (Rboh) genes, encoding NADPH oxidases, are the key producers of signal transduction-associated ROS in cells (Miller *et al*, 2009). Although accumulation of H_2O_2 is involved in stress signaling and mediating the cellular redox status (Panchuk et al, 2002). Thus, a powerful strategy is required to control the steady state level of ROS in cells, therefore providing an additional powerful strategy to enhance the tolerance of crops to these environmental stress conditions (Suzuki & Mittler, 2006).

C. Transgenic plants for oxidative stress tolerance

Zhao *et al.* (2009) introduced the transcription factor YAP1, originally from yeast (Saccharomyces cerevisiae), into Arabidopsis thaliana (ecotype Columbia).

When treated with various NaCl concentrations, transgenic plants showed increased activities of antioxidant enzymes catalase, superoxide dismutase, ascorbate peroxidase, peroxidase, glutathione S-transferase and glutathione reductase compared with the wild-type Arabidopsis. This demonstrated that an active oxygen scavenging system was enhanced to protect plants from salt stress by equilibrating ROS metabolism. Transgenic Arabidopsis maintained higher photosynthesis levels and lower amounts of H_2O_2, suggesting that ROS production was reduced (Zhao et al, 2009).

Wang *et al.* (2010) introduced a MnSOD gene (TaMnSOD) from Tamarix androssowii, under the control of the CaMV35S promoter, was introduced into poplar (Populus davidianax *P. bolleana*). They showed that SOD activity was enhanced in transgenic plants and the MDA content were significantly decreased when exposed to NaCl stress (Wang *et al*, 2010).

Nagamiya *et al.* were able to produce salt tolerant japonica rice (at 100 mM salt concentration) by over expressing the catalase gene katE (Nagamiya *et al*, 2007). Prodhan *et al.* introduced katE, a catalase gene of *Escherichia coli*, into the indica rice cultivar Kasalath (Prodhan *et al*, 2008). Transgenic rice plants at a very young stage (three-four days) were able to grow up to 15 days in 100 mM NaCl solution and seven days in 250 mM NaCl solution whereas, control plants died within five days in 100 mM and seven days in 50 mM NaCl. Moriwaki *et al.* integrated the katE gene into BR5 rice plants using an Agrobacterium tumefaciens-mediated method (Moriwaki *et al*, 2008). The introduced katE gene was actively expressed in the transgenic BR5 rice plants and catalase activity in transgenic rice was approximately 150% higher than in non-transgenic plants. Under NaCl stress conditions, the transgenic rice plants exhibited high tolerance compared with non-transgenic rice plants.

The Key Laboratory of Plant Stress, P. R. China, obtained a GST from Suaeda salsa cDNA library (accession number BE859255) and showed that it played an important role in salt stress resistance (Wang *et al*, 2002; Qi *et al*, 2004). Zhao and Zhang proved that the expression of Suaeda salsa GST gene in transgenic rice (*Oryza sativa* L.) could confer resistance to salt stress (Zhao & Zhang, 2006).

Other studies have been conducted in order to achieve enhanced tolerance to oxidative stress in plants. Lee *et al.* had investigated the effect of simultaneous expression of genes encoding three antioxidant enzymes, copper zinc superoxide dismutase, ascorbate peroxidase (APX), and dehydroascorbate reductase (DHAR), in the chloroplasts of tobacco plants under oxidative stress conditions (Lee *et al*, 2007).

Their results indicate that the simultaneous expression of multiple antioxidant enzymes, such as Cu/Zn SOD, APX, and DHAR, in chloroplasts is more effective than single or double expression for developing transgenic plants with enhanced tolerance to multiple environmental stresses.

In another interesting approach a hyper-thermostable SOD isolated from a polyextremophile higher plant *Potentilla atrosanguinea* Lodd. Var. argyrophylla (Wall. ex Lehm.) was engineered by mutation of a single amino acid that enhanced the thermo-stability of the enzyme to twofold. The engineered enzyme was functional from sub-zero temperature to .506C, tolerated autoclaving (heating at 1216C, at a pressure of 1.1 kg per square cm for 20 min) and was resistant to proteolysis. The present work is the first example to enhance the thermo-stability of a hyper-thermostable protein and has potential to application to other proteins for enhancing thermo-stability (Kumar *et al*, 2012).

D. ROS production and enzymatic response in mammalian

Small amounts of ROS, hydroxyl radicals, superoxide anions and hydrogen peroxide, are constantly generated in aerobic organisms in response to both external and internal stimuli (Mills et al, 1998; Hurst et al, 1997; Chopra & Wallace, 1998). Low levels of ROS are essential in many biochemical processes, including intracellular messaging in the cell differentiation and cell progression or the arrest of growth, programmed cell death (Ghosh & Myers, 1198), immunity (Yin *et al*, 1995), and defense against micro-organisms (Bae *et al*, 1197; Lee *et al*, 1998). On the other hand, oxidative stress in humans, likewise plant cells, may lead to various malfunctions or damages to cellular functions (Czene *et al*, 1997; Wojtaszek, 1997).

Presence of high concentrated ROS in different parts of intracellular or extracellular spaces or in organelles could have various effects or lead to diseases such a reaction may lead to cytotoxicity, allergy, mutagenicity, and/or carcinogenicity, depending on the properties of the epoxide in question (Oesch, 1984). In addition, oxidative events may play an important role in the mechanism of action of ether lipids, and ability to oxidize may contribute to cellular drug sensitivity (Wagner *et al*, 1998). Some of ROS-Antioxidant enzymes related diseases are presented in table 1. Having a look at table 1 indicates the importance of ROS-related pathways, in which many disorders or diseases are due to antioxidant enzymes and their pathways.

Table 1: Having a look at Table 1 indicates the importance of ROS-related pathways, in which many disorders or diseases are due to antioxidant enzymes and their pathways. Allergies, cancers, cardiac and vessels injuries, infectious diseases, neurodegenerative diseases and Ophthalmologic problems are just examples of the diseases related to ROS and antioxidant enzymes.

Human Disease
Allergy
Bronchial asthma
Intolerance to aspirin
Intolerance to foods
Response to mercury
Response to other drugs
Response to other oxidants
Cancer
Bladder
Bowel
Breast
Colorectal
Esophageal
Kidney
Leukemia
Liver
Lung
Prostate
Skin
Cardiac and vessels injuries
Atherosclerosis
Ischemia
Genetic and metabolic diseases
Chronic granulomatous disease
Diabetes
Down's syndrome
Infectious diseases
Helicobacter pylori
Hepatitis
HIV
Influenza virus
Pneumonia
Rheumatoid arthritis
Neurodegenerative diseases
Allergic encephalomyelitis
Alzheimer's disease
Amyotrophic lateral sclerosis
Huntington's disease
Parkinson's disease
Prion disease
Ophthalmologic problems
Cataract
Glaucoma

Allergies, cancers, cardiac and vessels injuries, infectious diseases, neurodegenerative diseases and Ophthalmologic problems are just examples of the diseases related to ROS and antioxidant enzymes.

E. Increasing antioxidant enzymes activity

Researchers try to find agents that could enhance activity of antioxidant enzymes to give a higher protection level to cells which are exposed to ROS concentration and some were successful. In a study Lee et al. Realized that Dieckol enhances the activities of antioxidant enzymes, and the expression of detoxifying enzymes including heme oxygenase - 1 (HO-1), NAD(P)H: quinine oxidoreductase 1 (NQO1), and glutathione S-transferase (GST) in HepG2 cells. Enhanced expression of antioxidant and detoxifying enzymes by Dieckol was presumed to be the activation of the nuclear factor erythroid-derived 2-like 2 (Nrf2) demonstrated by its nuclear translocation and transcriptional activity via activation of mitogen-activated protein kinases in HepG2 cells. Furthermore, they demonstrated that dieckol induced the expression of HO-1 in mouse liver. These results demonstrate that the dieckol-mediated cytoprotection in HepG2 cells is mediated through a ROS independent up-regulation of antioxidant and detoxifying enzymes via Nrf2 activation as well as its intrinsic antioxidant activity, suggesting that dieckol may be used as a natural cytoprotective agent (Lee *et al*, 2014).

In another study, in order to determine the gene expression of enzymatic antioxidants by acetone extract from the stem bark of three Acacia species in hydrogen peroxide (H_2O_2)-induced human hepatoma (HepG2) cells, the expression of antioxidant enzymes such as superoxide dismutase containing copper- zinc SOD, manganese SOD, CAT and GPx in HepG2 cells was evaluated by real-time PCR. The results of antioxidant enzyme expression in real-time PCR study revealed that the H_2O_2 challenged HepG2 cells reduced the expression of enzymes such as SOD, GPx and CAT. However, the cells pre-treated with acetone extracts of all the three Acacia species significantly up-regulated the expression of antioxidant enzymes in a concentration dependent manner. In conclusion, the findings of their study demonstrated that the acetone extract of Acacia species effectively inhibited H_2O_2 mediated oxidative stress and may be useful as a therapeutic agent in preventing oxidative stress mediated diseases (Sowndhararajan *et al*, 2015).

The objective of another study was to evaluate the bioactivity of eggshell membrane (ESM) peptides digested with Alcalase and Protease S (AL-PS) at the cellular level. Effects were tested for inhibition of lipid and protein oxidation, synthesis of glutathione (GSH), and cellular antioxidant enzyme activity in H_2O_2-stimulated Caco-2 cells. AL-PS and its ultra-filtered fraction AL-PS-I significantly suppressed the formation of hydrogen peroxide - induced malondialdehyde (MDA) and protein carbonyl derivatives. AL-PS and AL-PS-I also increased glutathione peroxidase, glutathione S transferase, and glutathione reductase activity. The peptides also elevated cellular GSH levels via up-regulation of γ-glutamylcysteine synthetase (γ-GCS) activity and its mRNA expression. This study confirmed that ESM peptides are able to reduce intestinal oxidative stress and thus validates their use as a valuable source material of ESM waste (Shi *et al*, 2014).

CONCLUSION

Environmental stresses in plants lead to higher levels of ROS in cells. Thus cells respond to stress with changing expression levels of antioxidant enzymes. Plants which express higher levels of SOD, CAT and GPx could relatively resist against these stresses. These promising results of transgenic plants indicate that it could be possible to gain tolerant plants which could resist against higher levels of ROS production (oxidative stress). So that, it seems that genomic and proteomic approaches (e.g. genomic mapping and protein engineering) could be more successful in the future for this goal, making another green revolution.

Mammalian cells are exposed to oxidative stress, too. Reactive oxygen species (ROS) are known to be involved in the cell growth, differentiation, progression, and death. Low concentrations of ROS may be beneficial or even indispensable in processes such as intracellular signaling and defense against micro-organisms. Nevertheless, higher amounts of ROS play a role in the aging process as well as in a number of human disease states, including cancer, ischemia, and failures in immunity and endocrine functions. As a safeguard against the accumulation of ROS, several non-enzymatic and enzymatic antioxidant activities exist. Diet or in general life style could affect antioxidant enzymes expression level, so that ROS production is controlled and maintained in a low levels than harmful.

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