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Reactive Oxygen Species and Antioxidant Enzymes Involved in Plant Tolerance to Stress

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Abstract

Plants are continuously exposed to several stress factors in field, which affect their production. These environmental adversities generally induce the accumulation of reactive oxygen species (ROS), which can cause severe oxidative damage to plants. ROS are toxic molecules found in various subcellular compartments. The equilibrium between the production and detoxification of ROS is sustained by enzymatic and nonenzymatic antioxidants. Due to advances in molecular approaches during the last decades, nowadays it is possible to develop economically important transgenic crops that have increased tolerance to stresses. This chapter discusses the oxidative stress and damage to plants. In addition, it reports the involvement of antioxidant enzymes in the tolerance of plants to various stresses.

Keywords: ROS, abiotic and biotic stress, oxidative stress, antioxidative mechanisms, tolerant plants

1. Introduction

Crop yield depends on the plant's ability to adapt to different types of environmental adversities, which generally induce oxidative stress. Environmental stress induces the accumulation of reactive oxygen species (ROS) in the cells of plants, which can cause severe oxidative damage to the plants, thus inhibiting growth and grain yield. ROS are involved in processes such as growth, development, response to biotic and abiotic environmental stimuli, programmed cell death, and may act as signal transducers. Stressors, hormones, development, and other several metabolic routes can stimulate ROS production that in turn may induce other routes or act directly as defense compounds [1].

Knowledge about the oxidative mechanisms in plants may contribute to the development of plants most well adapted to the environment and resistant to pathogens. Plants have defense mechanisms against oxidative damage that are activated during stress to regulate toxic levels of ROS. Antioxidant and nonantioxidant systems are involved in ROS detoxification.

During the last decades, antioxidant enzymes have been used to develop transgenic plants that have increased tolerance to several stresses. Therefore, this chapter will address the relation between abiotic and biotic stresses and ROS generation. The ROS production, major antioxidant enzymes involved in detoxification, and defense under stresses will be described. The involvement of the antioxidant enzymes in the tolerance of plants to various stresses will be also discussed.

2. Crop production and stress

Global agricultural production has tripled in the last 50 years because of increased demand due to population growth. Genetic breeding has improved crop yields per unit area. In 1960, the food requirement per capita was approximately 2,200 kcal/day. In 2009, the global food requirement per capita increased to more than 2,800 kcal/day. The global public spending on agricultural research and development rose markedly from 26.1 billion dollars in 2000 to 31.7 billion dollars in 2008; however, many challenges still remain in the agricultural sector [2].

Despite the efforts and progress achieved in recent decades in agriculture, growth and crop productivity are still negatively affected by several stress factors. Most crop plants grow in suboptimal environmental conditions, which prevent the plants from expressing their full genetic potential for development and reproduction, and consequently, these abnormal conditions lead to decreased plant productivity [3]. These stresses cause considerable production and economic losses worldwide.

Biological stress is an adverse force or condition that inhibits normal functioning of a plant [4]. These stresses may be biotic or abiotic. Biotic stresses include pathogens (viruses, bacteria, and fungi), insects, herbivores, and rodents. Abiotic stresses comprise cold (chilling and frost), heat (high temperature), salinity (salt), drought (water deficit condition), water excess (flooding), radiation (high-intensity ultra-violet and visible light), chemicals and pollutants (heavy metals, pesticides, and aerosols), oxidative stress (reactive oxygen species, ozone), wind (sand and dust particles in the wind) and soil nutrient deprivation [4, 5]. All of these factors may affect plant development and reproduction at different levels of severity.

Tolerance can be achieved by plant breeding or cultural practices that reduce losses, which is in turn accomplished by understanding the plant's response to its stressors and how they affect individual plants and plant processes [6]. Yield losses by oxidative damages occur because of an imbalance in plant synthesis and quenching. However, attributing this loss to the oxidative damage is difficult taking into account the several processes involved in ROS synthesis; however, stresses and oxidative damage are interlinked and are responsible for the yield losses [7] (Figure 1).

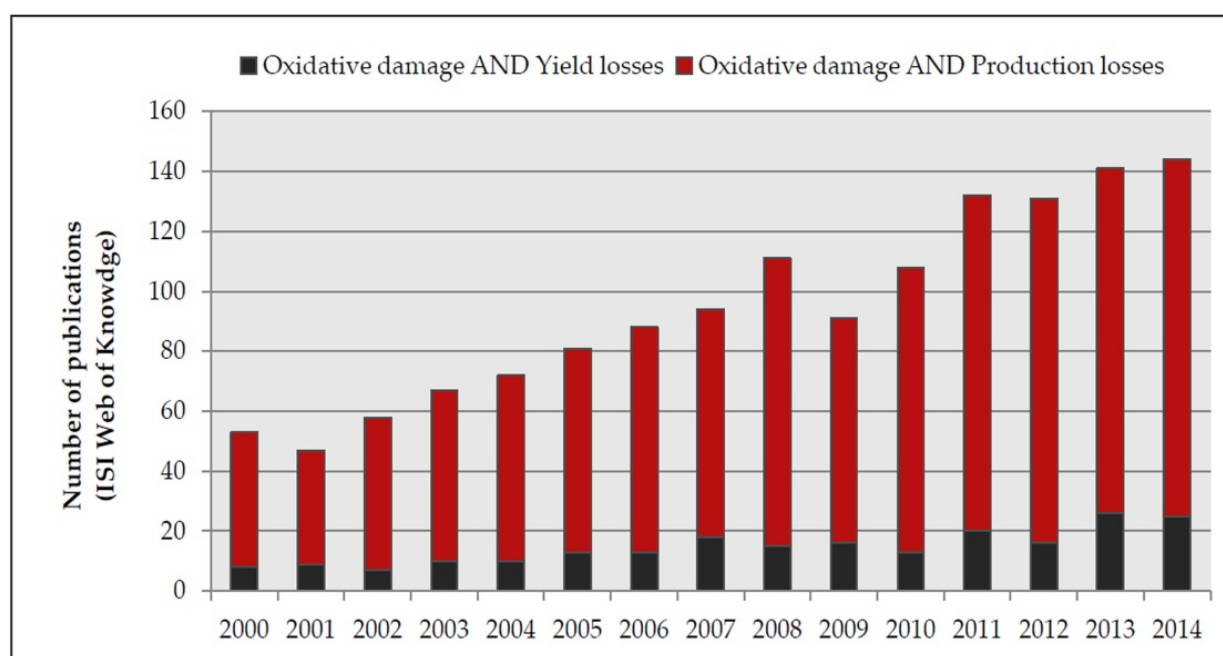


Figure 1. Evolution of the number of publications (2000-2014) addressing oxidative damage and yield/production losses. Total number of publications in 2000-2014 is 1,418. Source: ISI Web of Knowledge.

3. ROS generation

Environmental stress is directly correlated with increased accumulation of ROS. The equilibrium between production and scavenging of ROS may be disturbed by a number of biotic and abiotic factors, which may increase the intracellular levels of ROS [8]. When the level of ROS is increased and exceeds the defense mechanisms, the cell is in a state of oxidative stress [8, 9, 10, 11]. High concentrations of ROS are highly harmful to organisms, and when the symptoms persist, irreversible damage to the cells occurs, resulting in loss of physiological capacity and eventual cell death. Therefore, defense mechanisms against oxidative damage are activated during stress to regulate toxic levels of ROS [12] (Figure 2).

ROS are a group of free radicals, reactive molecules, and ions derived from oxygen. The most common ROS include singlet oxygen ($^1\text{O}_2$), superoxide radical ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^{\cdot}). These substances are highly reactive and toxic and can lead to oxidative destruction of the cell [8, 13]. ROS are found in various subcellular compartments such as chloroplasts, mitochondria, and peroxisomes due to the high metabolic activity that normally occurs in these compartments [13]. ROS are generated in chloroplasts via the Mehler reaction, in mitochondria via electron transport, and in peroxisomes via photorespiration.

The glycolate oxidase reaction, fatty acid β -oxidation, enzymatic reactions of flavin oxidases and disproportionation of $\text{O}_2^{\cdot-}$ radicals are all metabolic processes responsible for the gener-

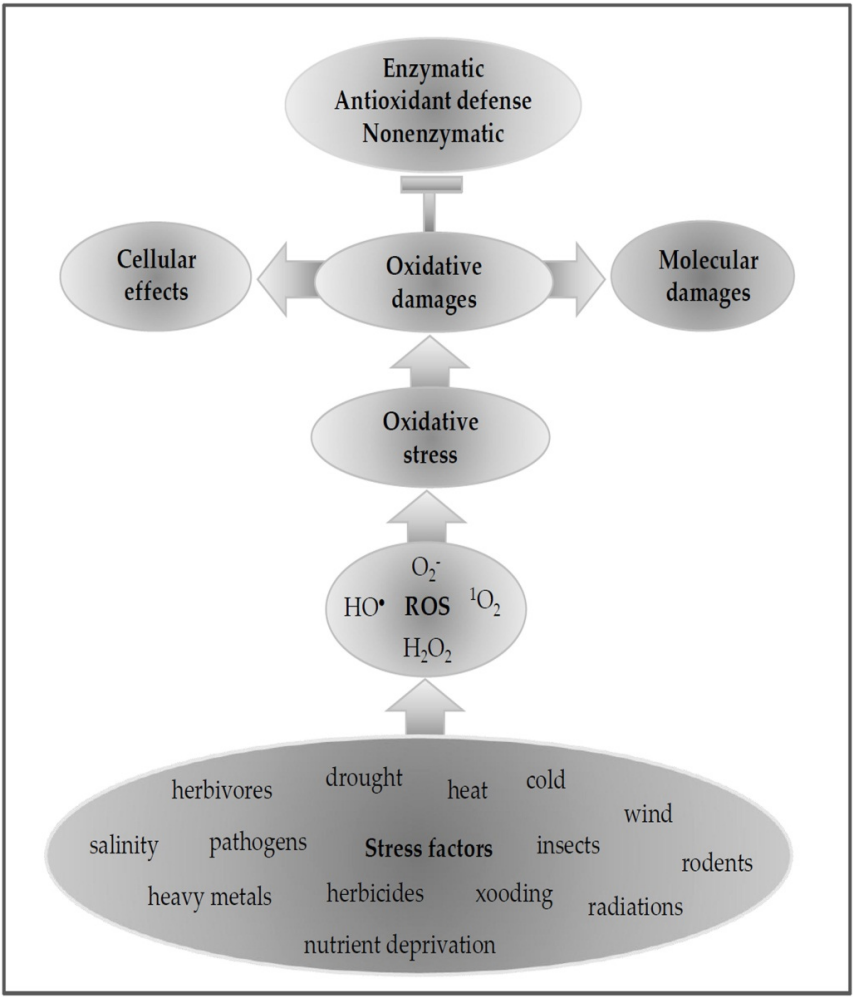


Figure 2. Stress factors, ROS generation, oxidative damage, and antioxidant defense. Several stress factors increased the ROS production, such as HO[•], O₂^{•-}, ¹O₂, and H₂O₂. The increased ROS levels lead to oxidative stress. Consequently, oxidative damage at the molecular and cellular levels occurs. Defense mechanisms against oxidative stress are activated to neutralize toxic levels of ROS. Singlet oxygen (¹O₂), superoxide radical (O₂^{•-}), hydrogen peroxide (H₂O₂), and hydroxyl radical (OH[•]).

ation of H₂O₂ in different types of peroxisomes [14]. Cytoplasm, plasma membrane, apoplasts, endoplasmic reticulum, and extracellular matrix are also sources of H₂O₂. In the cytoplasm, the electron transport chain associated with the endoplasmic reticulum is the main source of H₂O₂/ROS [11]. H₂O₂ generation can also be via enzymatic sources such as plasma-membrane-localized NADPH oxidases, amine oxidases, and cell wall peroxidases [15, 16]. Different organelles and cellular compartments possess potential targets for oxidative damage, as well as mechanisms for eliminating excess ROS. However, the balance between production and elimination of ROS can be severely disturbed by several biotic and abiotic stresses [9, 15]. These disturbances in the ROS equilibrium can lead to a rapid increase in intracellular ROS levels, which can cause significant damage to cell structures [17]. The redox homeostasis is the equilibrium between the production and scavenging of ROS; however, when ROS production overcomes the cellular scavenging capacity, there occurs an unbalancing of the cellular redox

homeostasis resulting in a rapid and transient excess of ROS, known as oxidative stress [11, 12]. Thus, the antioxidant defense imbalance disrupts metabolic activities [18], causing severe oxidative damages to cellular constituents, which can lead to loss of function and even cell death [12].

ROS may affect many cellular functions, for example, they can damage nucleic acids (oxidation of deoxyribose, strand breaks, removal/deletion of nucleotides, modification of bases, and cross-linked protein-DNA), lipids (breaking of the chain and increasing the fluidity and permeability of the membrane), and proteins (site-specific amino acid modification, fragmentation of the peptide chain, aggregation of cross-linked reaction products, alteration of the electric charge, inactivation of enzymes, and increasing the susceptibility of proteins to proteolysis) and can activate programmed cell death [10, 11].

The balance between production and elimination of ROS at the intracellular level must be tightly regulated and/or efficiently metabolized. This is necessary to avoid potential damage caused by ROS to cellular components as well as to maintain growth, metabolism, development, and overall productivity of plants. This equilibrium between the production and detoxification of ROS is sustained by enzymatic and nonenzymatic antioxidants [13, 15].

In plants, the major ROS-scavenging pathway is the ascorbate–glutathione cycle (AsA-GSH) in chloroplasts, cytosol, mitochondria, apoplast, and peroxisomes. This cycle plays a crucial role in controlling the level of ROS in these compartments [15]. The AsA-GSH cycle involves successive oxidation and reduction of ascorbate, glutathione, and NADPH catalyzed by ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) [15, 18]. Thereby, the AsA-GSH cycle plays an important role in combating oxidative stress induced by environmental stress. Many components of the antioxidant system of plants are already well characterized into plant models, and disturbances or alterations in this system are an excellent strategy to investigate the different signaling pathways involving ROS.

3.1. Nonenzymatic antioxidants

Nonenzymatic antioxidants are found in all cellular compartments. These compounds may act directly in the detoxification of ROS and radicals, or they can reduce substrates for antioxidant enzymes [15]. Nonenzymatic components of the antioxidative defense system include the major cellular redox buffers ascorbate (AsA) and glutathione (GSH) as well as tocopherol, carotenoids, and phenolic compounds [10, 13, 18].

Ascorbate is found in organelles of most plant cell types and in the apoplast. AsA is a crucial component of the detoxification of ROS in the aqueous phase due to the ability to donate electrons in enzymatic and nonenzymatic reactions. AsA can directly eliminate $O_2^{\cdot-}$, OH^{\cdot} , and 1O_2 , and thus reduce H_2O_2 to water via the ascorbate peroxidase reaction [19]. AsA is generally maintained in its reduced state by a set of NAD(P)H-dependent enzymes, including monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase [13, 20, 21]. Moreover, AsA is involved in the regulation of cell division, the progression of G1 to S

phase of the cell cycle and cell elongation, and it participates in multiple functions in photosynthesis [22].

Glutathione is oxidized by ROS to form oxidized glutathione (GSSG), which is present in all cellular compartments. Along with its oxidized form, GSSG, GSH maintains the redox balance in cellular compartments. Several studies indicate that GSH is involved in regulating gene expression and the cell cycle due to the properties of the GSH:GSSH pair [15]. The glutathione and AsA antioxidants are abundant and stable and have appropriate redox potential to interact with numerous components and pathways.

Tocopherols (α , β , γ , and δ) is a group of lipophilic antioxidants [11]. The α -tocopherol is the largest scavenger of peroxy radicals in lipid bilayers. The α -tocopherol present in the membrane of chloroplasts protects them against photooxidative damage [19].

Phenolic compounds are abundantly found in plant tissues, such as flavonoids, tannins, hydroxycinnamate esters, and lignin, and possess antioxidant properties [23].

3.2. Enzymatic antioxidants

Enzymatic components of the antioxidative defense system comprise several antioxidant enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPX, EC 1.11.1.9), guaiacol peroxidase (POX, EC 1.11.1.7), and peroxiredoxins (Prxs, EC 1.11.1.15), which catalyze ROS degradation, and enzymes of the ascorbate-glutathione (AsA-GSH) cycle, such as ascorbate peroxidase (APX, EC 1.1.1.1), monodehydroascorbate reductase (MDAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1), and glutathione reductase (GR, EC 1.8.1.7), that regenerate soluble antioxidants [13, 15, 18, 24]. This antioxidant system plays an important role in the maintenance of cell homeostasis and in the antioxidant response in plants.

Superoxide dismutases are enzymes that catalyze the dismutation of $O_2^{\cdot -}$ to H_2O_2 ; therefore, they constitute a frontline in the defense against ROS. These enzymes may be attached to a metal ion (Cu/Zn, Mn, Fe, and Ni); thus, they are classified according to their subcellular location and metal cofactor. SODs are present in many organisms, such as bacteria, yeast, animals, and plants. Plants have multiple genes encoding SODs that can be regulated by development, tissue-specific and environmental signals [10, 25].

Catalases are responsible for the removal of H_2O_2 by reducing H_2O_2 to $2H_2O$. CATs are largely, but not exclusively, localized to peroxisomes. Plants possess multiple CATs encoded by specific genes, which respond differentially to various stresses that are known to generate ROS [9, 10].

Ascorbate peroxidases are enzymes that play a key role in catalyzing the conversion of H_2O_2 into H_2O and use ascorbate as a specific electron donor. Plants have different APX isoforms that are distributed in distinct subcellular compartments, such as chloroplasts, mitochondria, peroxisomes, and the cytosol. The APX genes are differentially modulated by several abiotic stresses in plants [26, 27, 28]. The balance between SODs, CATs, and APXs is crucial for

determining the effective intracellular level of $O_2^{\cdot -}$ and H_2O_2 , and changes in the balance of these appear to induce compensatory mechanisms [8, 9, 10].

Glutathione peroxidases are nonheme thiol peroxidases that catalyze the reduction of H_2O_2 or organic hydroperoxides to water. The GPX proteins have been identified in many life species [29]. In plants, the GPX proteins are localized to mitochondria, chloroplasts, and cytosol.

Peroxiredoxins are a family of thiol-specific antioxidant enzymes that are involved in cell defense and protection from oxidative damage. These enzymes are widely distributed in plant cells and are important proteins in chloroplast ROS detoxification [30]. The peroxiredoxins are a group of peroxidases that have reducing activity in their active sites via cysteine residues. These enzymes do not possess a prosthetic group and catalyze the reduction of H_2O_2 , peroxynitrite, and a wide variety of organic hydroperoxides to their corresponding alcohols [31].

Guaiacol peroxidases are involved in H_2O_2 detoxification. The POX proteins are heme-containing enzymes that belong to class III or the “secreted plant peroxidases.” These enzymes are able to undertake a second cyclic reaction, called the hydroxylic reaction, which is distinct from the peroxidative reaction. Due to the use of both cycles, class III peroxidases are known to participate in many different plant processes, from germination to senescence, auxin metabolism, cell wall elongation, and protection against pathogens [32].

Monodehydroascorbate reductase is a flavin adenine dinucleotide enzyme that catalyzes the regeneration of AsA from the monodehydroascorbate radical using NAD(P)H as an electron donor. Thereby, MDAR plays an important role in the plant antioxidant system by maintaining the AsA pool [24]. Isoforms of MDAR have been reported to be present in chloroplasts, the cytosol, peroxisomes, and mitochondria [33, 34].

Dehydroascorbate reductase is a thiol enzyme that maintains AsA in its reduced form. DHAR catalyzes the reduction of dehydroascorbate to AsA using GSH as a reducing substrate [18, 24]. It is present in various plant tissues, and its modulation activity has been reported in various plant species [35].

Glutathione reductase is an NAD(P)H-dependent enzyme. GR catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH); it is a key enzyme of the AsA-GSH cycle; it protects cells against oxidative damage; and it maintains adequate levels of reduced GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress [20].

The great increasing number of publications addressing APX, SOD, CAT, POX, GPX, Prxs, MDAR, DHAR, and GR enzymes are examples of positive responses to biotic and abiotic stresses by these enzymes. Over the past fourteen years, significant efforts have been made to understand plant antioxidant system mechanisms related to stresses, so the number of publications reporting antioxidant enzymes and biotic and abiotic stresses has increased substantially (Figure 3A and 3B, ISI Web of Knowledge database). These data show the relevance of studying these enzymes assisting in the understanding of its involvement with scavenging of cell toxic products in diverse species and the relation between oxidative stress and biological processes.

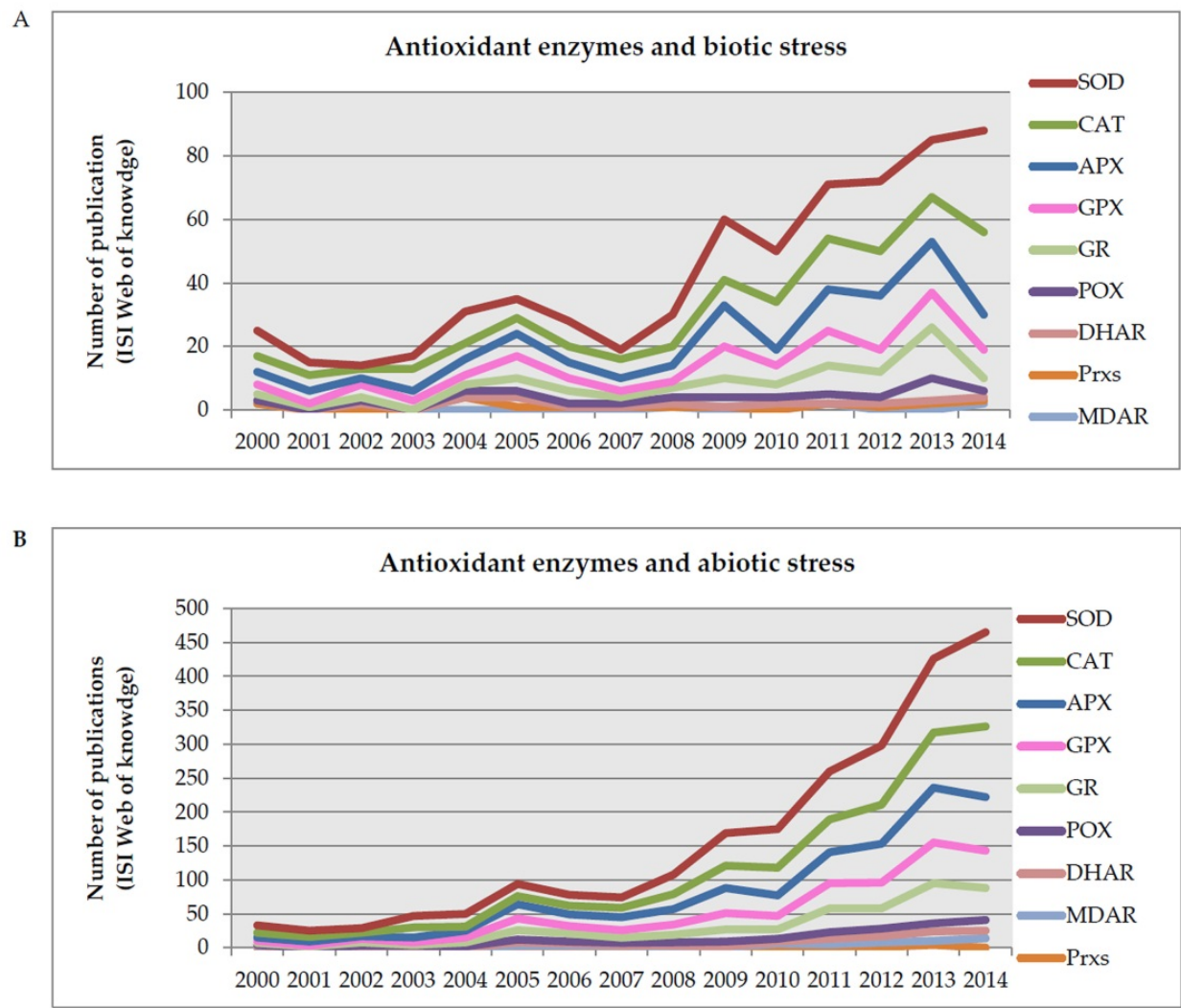


Figure 3. Evolution of the number of publications addressing antioxidant plant enzymes and biotic and abiotic stresses in the last fourteen years. (A) Data of the antioxidant enzymes and biotic stresses; (B) Data of the antioxidant enzymes and abiotic stresses. SOD (superoxide dismutase), APX (ascorbate peroxidase), CAT (catalase), GPX (glutathione peroxidase), POX (guaiacol peroxidase), Prxs (peroxiredoxins), MDAR (monodehydroascorbate reductase), DHAR (dehydroascorbate reductase), and GR (glutathione reductase) enzymes. Source: ISI Web of Knowledge.

4. Stress conditions and plants tolerant to stress

Stressful conditions are the main factor limiting agricultural productivity because plants do not reach their full genetic potential [4, 17]. Environmental conditions affect growth and development and trigger a series of morphological, physiological, biochemical, and molecular changes in plants. The metabolic pathways of plant organelles are sensitive to changes in environmental conditions [36]. Consequently, all environmental adversities have led to the world's agriculture facing serious challenges to meet demand. The increased consumption, allocation of land for other uses, and use of chemical products with implications for health safety are some examples these challenges [37].

The estimated world population for the year 2050 is nine billion people [2], and, consequently, the food demand will rise again. Therefore, it is necessary to increase the production and quality of food. Currently, the goal of many studies is the understanding of defense/tolerance mechanisms to different stresses in plants and to develop technologies and products that enable the generation of resistant/tolerant and more productive plants. Due to advances in molecular approaches, several crops of economic importance are being produced containing genes that encode stress tolerance using transformational technologies. Thus, several stress signaling and regulatory pathways have been elucidated and better understood.

Knowledge about the oxidative mechanisms in plants may contribute to the development of plants most well adapted to the environment. The maintenance of high antioxidant capacity to remove toxic levels of ROS has been related to increased stress tolerance of crop plants. Several studies show that maintaining a high level of antioxidant enzymes will help a plant to protect itself against oxidative damage by rapidly scavenging the toxic levels of ROS in its cells and restoring redox homeostasis.

Considerable progresses have been achieved in the development of plants tolerant to oxidative stress due to transgenic plants with altered levels of antioxidant genes to improve tolerance and productivity. This fact can be observed in Figure 4, which shows the increasing number of publications addressing antioxidant genes and its relation to tolerant plants in the last fourteen years (Figure 4). It highlights that SOD, CAT, and APX genes are the main antioxidant genes involved in the tolerance of plants to stresses, followed by GPX, GR, POX, DHAR, MDAR, and Prxs, respectively. These studies reflect the importance and advances in comprehension of the antioxidant mechanisms and tolerance to stresses.

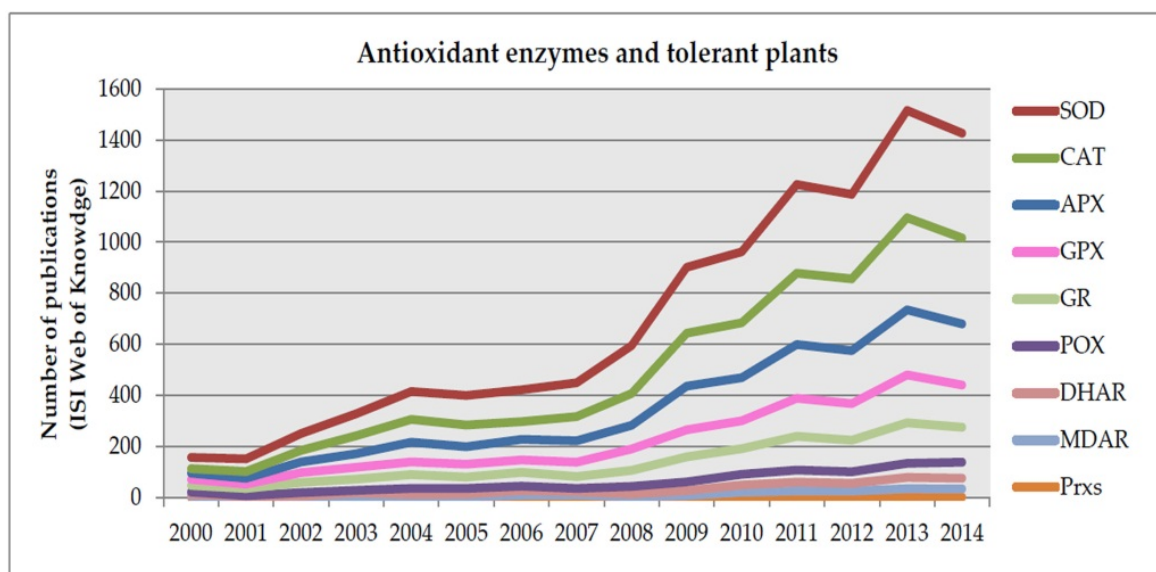


Figure 4. Evolution of the number of publications addressing antioxidant enzymes and plants tolerant to stresses in the last fourteen years. SOD (superoxide dismutase), APX (ascorbate peroxidase), CAT (catalase), GPX (glutathione peroxidase), POX (guaiacol peroxidase), Prxs (peroxiredoxins), MDAR (monodehydroascorbate reductase), DHAR (dehydroascorbate reductase), and GR (glutathione reductase) enzymes. Source: ISI Web of Knowledge.

Furthermore, the increased antioxidant activity has been reported to lead to better performance or tolerance response to several stresses. Using transgenic approaches, several species were studied aiming at the improvement of tolerance to stress enhancing antioxidant capacity of antioxidant genes. Table 1 shows some examples of the successful and positive responses obtained with regard to increased tolerance to cold, drought, heat, salt, hydrogen peroxide, methyl viologen, and metals stresses (Table 1). Improved tolerance using antioxidant genes are attributed by high antioxidant activity and more efficient ROS elimination. Plants expressing or overexpressing one or more antioxidant genes have more antioxidant capacity; consequently, plants can more efficiently eliminate excess ROS and protect their cellular components against toxic effects of ROS produced during the exposure to stress. As a consequence, plants suffer less oxidative injury and can tolerate a stress condition more effectively.

Gene	Native specie	Target specie	Stress tolerance	Reference
<i>Ascorbate peroxidase</i>	<i>Brassica campestris</i>	<i>Arabidopsis thaliana</i>	heat	[38]
	<i>Puccinellia tenuiflora</i>	<i>Arabidopsis thaliana</i>	salinity, hydrogen peroxide	[39]
	<i>Jatropha curcas</i>	<i>Nicotiana tabacum</i>	salinity	[40]
	<i>Hordeum vulgare</i>	<i>Arabidopsis thaliana</i>	zinc, cadmium	[41]
<i>Superoxide dismutase</i>	<i>Arachis hypogaea</i>	<i>Nicotiana tabacum</i>	salinity, drought	[42]
	<i>Tamarix androssowii</i>	<i>Populus davidiana</i> × <i>P. bolleana</i>	salinity	[43]
	<i>Pisum sativum</i>	<i>Oryza sativa</i>	drought	[44]
	<i>Oryza sativa</i>	<i>Nicotiana tabacum</i>	salinity, water, PEG-treatment	[45]
<i>Catalase</i>	<i>Brassica oleracea</i>	<i>Arabidopsis thaliana</i>	heat	[46]
	<i>Brassica juncea</i>	<i>Nicotiana tabacum</i>	cadmium	[47]
	<i>Triticum aestivum</i>	<i>Oryza sativa</i>	cold	[48]
<i>Glutathione peroxidase</i>	<i>Triticum aestivum</i>	<i>Arabidopsis thaliana</i>	salinity, hydrogen peroxide	[49]
<i>Peroxiredoxins</i>	<i>Solanum tuberosum</i>	<i>Solanum tuberosum</i>	heat, methyl viologen	[50]
	<i>Festuca arundinacea</i>	<i>Festuca arundinacea</i>	heat, methyl viologen	[51]
	<i>Suaeda salsa</i>	<i>Arabidopsis thaliana</i>	salinity, cold	[52]
<i>Monodehydroascorbate reductase</i>	<i>Malpighia glabra</i>	<i>Nicotiana tabacum</i>	salinity	[53]
	<i>Acanthus ebracteatus</i>	<i>Oryza sativa</i>	salinity	[54]
	<i>Avicennia marina</i>	<i>Nicotiana tabacum</i>	salinity	[55]

Gene	Native specie	Target specie	Stress tolerance	Reference
<i>Dehydroascorbate reductase</i>	<i>Oryza sativa</i>	<i>Oryza sativa</i>	salinity	[56]
<i>Glutathione reductase</i>	<i>Brassica campestris</i>	<i>Nicotiana tabacum</i>	methyl viologen	[57]
<i>Ascorbate peroxidase/ Superoxide dismutase</i>	<i>Rheum austral/Potentilla astrisanguinea</i>	<i>Arabidopsis thaliana</i>	cold	[58]
	<i>Manihot esculenta</i>	<i>Manihot esculenta</i>	methyl viologen, hydrogen peroxide, cold	[59]
	<i>Solanum tuberosum</i>	<i>Solanum tuberosum</i>	heat, methyl viologen	[60]
<i>Catalase/Superoxide dismutase</i>	<i>Gossypium hirsutum</i>	<i>Gossypium hirsutum</i>	salinity, methyl viologen	[61]

Table 1. Some examples of the transgenic plants with potential stress tolerance expressing antioxidant genes

Some antioxidant enzymes such as SOD, CAT, APX, and GPX are better studied (Figure 3 and 4), but in general all enzymes have potential defense antioxidant activity helping in scavenging ROS in different ways, either by dismutation of O_2^{\bullet} to H_2O_2 , reduction of H_2O_2 , maintenance of the AsA pool, or of the adequate levels of GSH and GSSG, that all together maintain the antioxidant balance. In addition, antioxidant enzymes act in different subcellular compartments, thereby assisting in the ROS detoxification in organelles such as chloroplasts, mitochondria, peroxisomes, and in the cytosol. Besides, ROS-scavenging enzymes in various subcellular compartments might have a synergistic effect to improve stress tolerance in plants [59].

Many attempts aiming to increase the tolerance of plants to environmental stresses using antioxidant genes have been made by researchers. However, due to the great complexity of the antioxidant system and plant stress tolerance, we cannot state that ROS scavenging is the only factor that determines the level of tolerance, because other factors and several genes pathways are involved in the stress tolerance in plants. Furthermore, it must be emphasized that stresses often occur in combination; thus, the relation between ROS signaling mechanisms in different stress responses is very complex [62]. When under the effect of a combination of stresses, the plants respond differently than when experiencing just a unique type of stress [63]. Moreover, this can range depending on the plant species and cultivation area. Complexity of the tolerance mechanisms in plants is also a key factor because sometimes the alteration of one gene in the pathway can influence the expression of others, various genes and pathways being involved [64].

5. Conclusions

Plants activate antioxidant defense mechanisms under stresses, which helps in the maintenance of the structural integrity of the cell components and presumably alleviates oxidative

damage. Several antioxidant enzymes contribute to plant defense. The manipulation of ROS-scavenging enzyme systems is a worthwhile approach to produce transgenic plants with enhanced tolerance to a wide range of stress conditions; however, this needs to be further explored as many enzymes and isoforms can be involved, and ROS is only one of the potential parameters of plant tolerance against environmental variations and biotic stresses.

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References

- [1] Bailey-Serres J, Mittler R. The roles of reactive oxygen species in plants cells. *Plant Physiol.* 2006;141(2):311. DOI: 10.1104/pp.104.900191
- [2] FAO, editor. FAO STATISTICAL YEARBOOK 2013 World Food and Agriculture. Rome: Food and Agriculture Organization of the United Nations; 2013. 289 p. ISBN 978-92-5-107396-4
- [3] Atkinson NJ, Urwin PE. The interaction of plant biotic and abiotic stresses: from genes to the field. *J Exper Botany.* 2012;63(10):3523-3544. DOI: 10.1093/jxb/ers100
- [4] Mahajan S, Tuteja N. Cold, salinity and drought stresses: an overview. *Arch Biochem Biophys.* 2005;444:139-158. DOI: 10.1016/j.abb.2005.10.018

- [5] Cancado GMA. The importance of genetic diversity to manage abiotic stress. In: Shanker A, editor. *Abiotic Stress in Plants – Mechanisms and Adaptations*. InTech; 2011. p. 351-366. DOI: 10.5772/22397
- [6] Peterson RKD, Higley LG, editors. *Biotic Stress and Yield Loss*. 1st edn. Washington: CRC Press; 2000. 261 p. ISBN 0-8493-1145-4
- [7] Wahid A, Farooq M, Siddique KHM. Implications of oxidative stress for plant growth and productivity. In: Pessarakli M, editor. *Handbook of Plant and Crop Physiology*. 3rd edn. LLC 6000 Broken Sound Parkway, Suite 300, Boca Raton, FL 33487 USA: Taylor & Francis Group; 2014. p. 549-556. DOI: ISBN13:978-1-4665-5328-6
- [8] Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol.* 2004;55:373-399. DOI: 10.1146/annurev.arplant.55.031903.141701
- [9] Scandalios JG. The rise of ROS. *TRENDS Biochem Sci.* 2002;27(9):483-486. DOI: 10.1016/S0968-0004(02)02170-9
- [10] Scandalios JG. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. *Brazilian J Medic Biologic Res.* 2005;38(7): 995-1014. DOI: 10.1590/S0100-879X2005000700003
- [11] Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J Botany*. 2012;2012:1-26. DOI: 10.1155/2012/217037
- [12] Mullineaux PM, Baker NR. Oxidative stress: antagonistic signaling for acclimation or cell death. *Plant Physiol.* 2010;154(2):521-525. DOI: 10.1104/pp.110.161406
- [13] Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. Reactive oxygen gene network of plants. *TRENDS Plant Sci.* 2004;9(10):490-498. DOI: 10.1016/j.tplants.2004.08.009
- [14] Baker A, Graham IA, editors. *Plant Peroxisomes: Biochemistry, Cell Biology and Biotechnological Applications*. 1st edn. Dordrecht, The Netherlands: Springer Netherlands; 2002. 505 p. DOI: 10.1007/978-94-015-9858-3
- [15] Mittler R. Oxidative stress, antioxidants and stress tolerance. *TRENDS Plant Sci.* 2002;7(9):405-410. DOI: 10.1016/S1360-1385(02)02312-9
- [16] Neill S, Desikan R, Hancock J. Hydrogen peroxide signalling. *Curr Opin Plant Biol.* 2002;5(5):388-395. DOI: 10.1016/S1369-5266(02)00282-0
- [17] Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem.* 2010;48(12):909-930. DOI: 10.1016/j.plaphy.2010.08.016
- [18] Gratão PL, Polle A, Lea PJ, Azevedo RA. Making the life of heavy metal-stressed plants a little easier. *Function Plant Biol.* 2005;32(6):481-494. DOI: 10.1071/FP05016

- [19] Blokhina O, Virolainen E, Fagerstedt, KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann Botany*. 2003;91(2):179-194. DOI: 10.1093/aob/mcf118
- [20] Foyer CH, Noctor G. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *The Plant Cell*. 2005;17(7):1866-1875. DOI: 10.1105/tpc.105.033589
- [21] Foyer CH, Noctor G. Ascorbate and glutathione: the heart of the redox hub. *Plant Physiol*. 2011;155(1):2-18. DOI: 10.1104/pp.110.167569
- [22] Smirnoff N. Vitamin C: the metabolism and functions of ascorbic acid in plants. Advances in Botanical Research. In: Rebeille F, Douce R, editors. *Biosynthesis of Vitamins in Plants: Vitamins B6, B8, B9, C, E, K*, Part 2. 1st edn. USA: Academic Press; 2011. p. 107-177. ISBN 978-0-12-385853-5
- [23] Grace SC, Logan BA. Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. *Philos Trans Royal Soc Lon. Series B*. 2000;355(1402):1499-1510. DOI: 10.1098/rstb.2000.0710
- [24] Asada K. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annu Rev Plant Physiol Plant Mol Biol*. 1999;50(NaN):601-639. DOI: 10.1146/annurev.arplant.50.1.601
- [25] Menezes-Benavente L, Teixeira FK, Kamei CLA, Margis-Pinheiro M. Salt stress induces altered expression of genes encoding antioxidant enzymes in seedlings of a Brazilian indica rice (*Oryza sativa* L.). *Plant Sci*. 2004;166(2):323-331. DOI: 10.1016/j.plantsci.2003.10.001
- [26] Rosa SB, Caverzan A, Teixeira FK, Lazzarotto F, Silveira JAG, Ferreira-Silva SL, Abreu-Neto J, Margis R, Margis-Pinheiro M. Cytosolic APx knockdown indicates an ambiguous redox responses in rice. *Phytochemistry*. 2010;71(5-6):548-558. DOI: 10.1016/j.phytochem.2010.01.003
- [27] Caverzan A, Passaia G, Rosa SB, Ribeiro CW, Lazzarotto F, Margis-Pinheiro M. Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection. *Genet Mol Biol*. 2012;35(4):1011-1019. DOI: 10.1590/S1415-47572012000600016
- [28] Caverzan A, Bonifacio A, Carvalho FEL, Andrade CMB, Passaia G, Schünemann M, Maraschin FS, Martins MO, Teixeira FK, Rauber R, Margis R, Silveira JAG, Margis-Pinheiro M. The knockdown of chloroplastic ascorbate peroxidases reveals its regulatory role in the photosynthesis and protection under photo-oxidative stress in rice. *Plant Sci*. 2014;214(NaN):74-87. DOI: 10.1016/j.plantsci.2013.10.001
- [29] Margis R, Dunand C, Teixeira FK, Margis-Pinheiro M. Glutathione peroxidase family – an evolutionary overview. *FEBS J*. 2008;275(15):3959-3970. DOI: 10.1111/j.1742-4658.2008.06542.x

- [30] Foyer CH, Shigeoka S. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiol.* 2011;155(1):93-100. DOI: 10.1104/pp.110.166181
- [31] Wood ZA, Schroder E, Harris JR, Poole LB. Structure, mechanism and regulation of peroxiredoxins. *TRENDS in Biochem Sci.* 2003;28(1):23-40. DOI: 10.1016/S0968-0004(02)00003-8
- [32] Passardi F, Longet D, Penel C, Dunand C. The class III peroxidase multigenic family in rice and its evolution in land plants. *Phytochem.* 2004;65(13):1879-1893. DOI: 10.1016/j.phytochem.2004.06.023
- [33] Jiménez A, Hernández JA, del Río LA, Sevilla F. Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. *Plant Physiol.* 1997;114(1):275-284. DOI: 10.1104/pp.114.1.275
- [34] Leterrier M, Corpas FJ, Barroso JB, Sandalio LM, del Río LA. Peroxisomal monodehydroascorbate reductase. Genomic clone characterization and functional analysis under environmental stress conditions. *Plant Physiol.* 2005;138(4):2111-2123. DOI: 10.1104/pp.105.066225
- [35] Anjum NA, Gill SS, Gill R, Hasanuzzaman M, Duarte AC, Pereira E, Ahmad I, Tuteja R, Tuteja N. Metal/metalloid stress tolerance in plants: role of ascorbate, its redox couple, and associated enzymes. *Protoplasma.* 2014;251(5):1265-1283. DOI: 10.1007/s00709-014-0636-x
- [36] Suzuki N, Koussevitzky S, Mittler R, Miller G. ROS and redox signalling in the response of plants to abiotic stress. *Plant Cell Environ.* 2012;35(2):259-270. DOI: 10.1111/j.1365-3040.2011.02336.x
- [37] Curtis T, Halford NG. Food security: the challenge of increasing wheat yield and the importance of not compromising food safety. *Ann Appl Biol.* 2014;164(3):354-372. DOI: 10.1111/aab.12108
- [38] Chiang CM, Chien HL, Chen LFO, Hsiung TC, Chiang MC, Chen SP, Lin KH. Overexpression of the genes coding ascorbate peroxidase from *Brassica campestris* enhances heat tolerance in transgenic *Arabidopsis thaliana*. *Biol Plant.* 2015;59(2):305-315. DOI: 10.1007/s10535-015-0489-y
- [39] Guan Q, Wang Z, Wang X, Takano T, Liu S. A peroxisomal APX from *Puccinellia tenuiflora* improves the abiotic stress tolerance of transgenic *Arabidopsis thaliana* through decreasing of H₂O₂ accumulation. *J Plant Physiol.* 2015;175(1):183-191. DOI: 10.1016/j.jplph.2014.10.020
- [40] Liu Z, Bao H, Cai J, Han J, Zhou L. A novel thylakoid ascorbate peroxidase from *Jatropha curcas* enhances salt tolerance in transgenic tobacco. *Int J Mol Sci.* 2014;15(1):171-185. DOI: 10.3390/ijms15010171
- [41] Xu W, Shi W, Liu F, Ueda A, Takabe T. Enhanced zinc and cadmium tolerance and accumulation in transgenic *Arabidopsis* plants constitutively overexpressing a barley

- gene (HvAPX1) that encodes a peroxisomal ascorbate peroxidase. *Botany*. 2008;86(6): 567-575. DOI: 10.1139/B08-025
- [42] Negi NP, Shrivastava DC, Sharma V, Sarin NB. Overexpression of CuZnSOD from *Arachis hypogaea* alleviates salinity and drought stress in tobacco. *Plant Cell Rep*. 2015;34(7):1109-1126. DOI: 10.1007/s00299-015-1770-4
- [43] Wang YC, Qu GZ, Li HY, Wu YJ, Wang C, Liu GF, Yang CP. Enhanced salt tolerance of transgenic poplar plants expressing a manganese superoxide dismutase from *Tamarix androssowii*. *Mol Biol Rep*. 2010;37(2):1119-1124. DOI: 10.1007/s11033-009-9884-9
- [44] Wang FZ, Wang QB, Kwon SY, Kwak SS, Su WA. Enhanced drought tolerance of transgenic rice plants expressing a pea manganese superoxide dismutase. *J Plant Physiol*. 2005;162(4):465-472. DOI: 10.1016/j.jplph.2004.09.009
- [45] Badawi GH, Yamauchi Y, Shimada E, Sasaki R, Kawano N, Tanaka K, Tanaka K. Enhanced tolerance to salt stress and water deficit by overexpressing superoxide dismutase in tobacco (*Nicotiana tabacum*) chloroplasts. *Plant Sci*. 2004;166(4):919-928. DOI: 10.1016/j.plantsci.2003.12.007
- [46] Chiang CM, Chen SP, Chen LFO, Chiang MC, Chien HL, Lin KH. Expression of the broccoli catalase gene (BoCAT) enhances heat tolerance in transgenic *Arabidopsis*. *J Plant Biochem Biotechnol*. 2014;23(3):266-277. DOI: 10.1007/s13562-013-0210-1
- [47] Guan Z, Chai T, Zhang Y, Xu J, Wei W. Enhancement of Cd tolerance in transgenic tobacco plants overexpressing a Cd-induced catalase cDNA. *Chemosphere*. 2009;76(5): 623- 630. DOI: 10.1016/j.chemosphere.2009.04.047
- [48] Matsumura T, Tabayashi N, Kamagata Y, Souma C, Saruyama H. Wheat catalase expressed in transgenic rice can improve tolerance against low temperature stress. *Physiol Plant*. 2002;116(3):317-327. DOI: 10.1034/j.1399-3054.2002.1160306.x
- [49] Zhai CZ, Zhao L, Yin LJ, Chen M, Wang QY, Li LC, Xu ZS, Ma YZ. Two wheat glutathione peroxidase genes whose products are located in chloroplasts improve salt and H₂O₂ tolerances in *Arabidopsis*. *PLoS One*. 2013;8(10):e73989. DOI: 10.1371/journal.pone.0073989
- [50] Kim MD, Kim YH, Kwon SY, Jang BY, Lee SY, Yun DJ, Cho JH, Kwak SS, Lee HS. Overexpression of 2-cysteine peroxiredoxin enhances tolerance to methyl viologen-mediated oxidative stress and high temperature in potato plants. *Plant Physiol Biochem*. 2011;49(8):891-897. DOI: 10.1016/j.plaphy.2011.04.001
- [51] Kim KH, Alam I, Lee KW, Sharmin SA, Kwak SS, Lee SY, Lee BH. Enhanced tolerance of transgenic tall fescue plants overexpressing 2-Cys peroxiredoxin against methyl viologen and heat stresses. *Biotechnology Letters*. 2010;32(4):571-576. DOI: 10.1007/s10529-009-0185-0
- [52] Jing LW, Chen SH, Guo XL, Zhang H, Zhao YX. Overexpression of a chloroplast-located peroxiredoxin Q gene, *SsPrxQ*, increases the salt and low-temperature toler-

- ance of Arabidopsis. *J Integrat Plant Biol.* 2006;48(10):1244-1249. DOI: 10.1111/j.1744-7909.2006.00357.x
- [53] Eltelib HA, Fujikawa Y, Esaka M. Overexpression of the acerola (*Malpighia glabra*) monodehydroascorbate reductase gene in transgenic tobacco plants results in increased ascorbate levels and enhanced tolerance to salt stress. *S Afr J Botany.* 2012;78(NaN):295-301. DOI: 10.1016/j.sajb.2011.08.005
- [54] Sultana S, Khew CY, Morshed MdM, Namasivayam P, Napis S, Ho CL. Overexpression of monodehydroascorbate reductase from a mangrove plant (*AeMDHAR*) confers salt tolerance on rice. *J Plant Physiol.* 2012;169(3):311-318. DOI: 10.1016/j.jplph.2011.09.004
- [55] Kavitha K, George S, Venkataraman G, Parida A. A salt-inducible chloroplastic monodehydroascorbate reductase from halophyte *Avicennia marina* confers salt stress tolerance on transgenic plants. *Biochimie.* 2010;92(10):1321-1329. DOI: 10.1016/j.biochi.2010.06.009
- [56] Kim YS, Kim IS, Shin SY, Park TH, Park HM, Kim YH, Lee GS, Kang HG, Lee SH, Yoon HS. Overexpression of dehydroascorbate reductase confers enhanced tolerance to salt stress in rice plants (*Oryza sativa* L. japonica). *J Agron Crop Sci.* 2014;200(6):444-456. DOI: 10.1111/jac.12078
- [57] Lee H, Jo J. Increased tolerance to methyl viologen by transgenic tobacco plants that over-express the cytosolic glutathione reductase gene from *Brassica campestris*. *J Plant Biol.* 2004;47(2):111-116. DOI: 10.1007/BF03030640
- [58] Shafi A, Dogra V, Gill T, Ahuja PS, Sreenivasulu Y. Simultaneous over-expression of PaSOD and RaAPX in transgenic *Arabidopsis thaliana* confers cold stress tolerance through increase in vascular lignifications. *PLoS One.* 2014;9(10):e110302. DOI: 10.1371/journal.pone.0110302
- [59] Xu J, Yang J, Duan X, Jiang Y, Zhang P. Increased expression of native cytosolic Cu/Zn superoxide dismutase and ascorbate peroxidase improves tolerance to oxidative and chilling stresses in cassava (*Manihot esculenta* Crantz). *BMC Plant Biol.* 2014;14(208). DOI: 10.1186/s12870-014-0208-4
- [60] Tang L, Kwon SY, Kim SH, Kim JS, Choi JS, Cho KY, Sung CK, Kwak SS, Lee HS. Enhanced tolerance of transgenic potato plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against oxidative stress and high temperature. *Plant Cell Rep.* 2006;25(12):1380-1386. DOI: 10.1007/s00299-006-0199-1
- [61] Luo X, Wu J, Li Y, Nan Z, Guo X, Wang Y, Zhang A, Wang Z, Xia G, Tian Y. Synergistic effects of GhSOD1 and GhCAT1 overexpression in cotton chloroplasts on enhancing tolerance to methyl viologen and salt stresses. *PLoS One.* 2013;8(1):e54002. DOI: 10.1371/journal.pone.0054002

- [62] Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ.* 2010;33(4): 453-467. DOI: 10.1111/j.1365-3040.2009.02041.x
- [63] Petrov V, Hille J, Mueller-Roeber B, Gechev TS. ROS-mediated abiotic stress-induced programmed cell death in plants. *Front Plant Sci.* 2015;6(69):1-16. DOI: doi: 10.3389/fpls.2015.00069
- [64] Bakhsh A, Hussain T. Engineering crop plants against abiotic stress: Current achievements and prospects. *Emirates J Food Agric.* 2015;27(1):24-39. DOI: 10.9755/ejfa.v27i1.17980