Modulation of redox signal transduction in plant system through induction of free radical /ROS scavenging redox-sensitive enzymes and metabolites

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Abstract

Antioxidant phytochemicals in plant cells are mainly glutathione, ascorbate, tocopherols, proline and betaine etc. which are information-rich redox buffers and important redox-signaling components that interact with the cellular compartments. It is known that life evolved in the reducing environment, as an unfortunate consequence of aerobic life for higher plants, reactive oxygen species (ROS) are formed by partial reduction of molecular oxygen. The enzymatic and non-enzymatic antioxidants in higher plants can protect their cells from the oxidative damage by scavenging ROS. In addition to their crucial roles in plant defense system and also as enzyme cofactors, antioxidants influences higher plant growth and development by modifying processes from mitosis and cell elongation to senescence and death. Most importantly, they provide essential information on cellular redox state, and regulate gene expression associated with the biotic and abiotic stress-responses to optimize defense for survival. An overview of the literature is presented here in terms of the primary antioxidant free radical scavenging and modulation of redox signaling in plant cells. Special attention is given to ROS and ROS-antioxidants interaction as a metabolic interface for different types of signals derived from metabolisms as well as from the changing environment. This interaction regulates the appropriate induction of acclimation processes or execution of cell death programs, the two essential directions for higher plant cells.

Keywords: Plant cells; Antioxidant; Reactive Oxygen Species (ROS); ROS-antioxidant interacting interface; Soil-plant interface; Signaling; Carotenoids; Glutathione; α- tocopherol.
Abbreviations: ROS-Reactive oxygen species; MAP-Mitogen-activated protein; SOD-superoxide dismutase; CAT-Catalase; PUFA-Polyunsaturated fatty acid; AsA-Ascorbic acid; GSH-Glutathione.

Introduction

Stress is an altered physiological condition caused by the factors that tend to disrupt the equilibrium. Plants are frequently exposed to many stresses such as drought, high and low temperature, salt, flooding, oxidative stress and heavy metal toxicity - stress is rule, normalcy is exception. Stress being a constraint or highly rather unpredictable fluctuations imposed on regular metabolic patterns cause injury, disease or aberrant physiology. However, flexibility of normal metabolism allows the response initiation to the environmental changes, which fluctuate regularly and are predictable over daily and seasonal cycles. Crop plants survive in a constantly fluctuating environments, which has driven the evolution of a highly flexible metabolism and growth (basic growth curve of slow-fast-slow) and development necessary for their sessile life style (Pitzschke and Hirt, 2006; Chinnusamy et al., 2004; Liu and Bush, 2006; Geigenberger et al., 2005; Buchanan and Luan, 2005; Kwon et al., 2006, Kumar et al., 2012). Plants have developed a series of pathways at different levels that combat with the environmental stresses by producing more ROS (Halliwell, 2006; Coupe et al., 2006). Primary intracellular plant antioxidants expression (or levels or location) are closely related to their metabolic state and responds very well to the fluctuating environment (Wang et al., 2003; Kwak et al., 2006). The pathways to combat the different environmental stresses include - photorespiratory pathway, enzymatic and non - enzymatic pathways, corresponding responsive-gene regulation and anatomical ways (Geigenberger et al., 2005; Grun et al., 2006). Currently, in contrast to the situation in the natural world, the detailed dissection of the regulatory networks that govern crop plants responses to the abiotic and biotic stresses have been studied almost exclusively in the controlled environments where a single challenge has been applied (Zheng and Wang, 2001; Halliwell, 2006; Kwak et al., 2006; Mullineaux et al., 2006; Chaves et al., 2003; Kwon et al., 2006; Grun et al., 2006). In real situation, multiple stresses are more common eg. water - deficit with salt stress or high temperature. Plant cell metabolism must be highly regulated in order to allow effective integration of a diverse spectrum of biosynthetic pathways that are reductive in nature (Wingler et al., 2006; Scheibe et al., 2005; Foyer and Noctor, 2003; Millar et al., 2003), in which the regulation does not completely avoid photodynamic or reductive activation of molecular oxygen to produce ROS, particularly superoxide, H_2O_2 and singlet oxygen (Scheibe et al., 2005; Geigenberger et al., 2005). However, in many cases, the production of ROS is genetically programmed, induced during the course of development and by environmental fluctuations, and has complex down-stream effects on both, primary as well as secondary metabolism (Buchanan and Luan, 2005; Mur et al., 2006; Li and Jin, 2007; Millar et al., 2003). Adams *et al.*, (2013) reported a mechanistic link between plant photo-protection and the synthesis of oxylipin hormones as

regulators of development and defense; characterized plants included a mutant deficient in reactive-oxygen-detoxification *via* the antioxidant vitamin E (tocopherol). Plant cells produce ROS, particularly superoxide and H_2O_2, as secondary messengers in many processes associated with plant growth and development (Grun et al., 2006; Foyer and Noctor, 2005; Schurmann, 2003). Moreover, one of the major ways in which plants transmit information concerning change in the environment is *via* the production of bursts of superoxide at the plasma membrane (Barnes and Mayfield, 2003; Terman et al., 2006, Kumar et al., 2013). Conditions which provoke enhanced ROS production have, in the past, been categorized under the heading of the oxidative-stress, which is a negative connotation implying a harmful process (Pfannschmidt et al., 2007; Foyer and Noctor, 2003; Millar et al., 2003). Rather than involving simple signaling cassettes, emerging concepts suggest that the relationship between metabolism and redox state is complex and subtle (Foyer and Noctor, 2005; Kiffin et al., 2006). Antioxidants and protein molecules are involved in influencing the different biological processes through modulating the redox signal transduction pathways inside plant system and ultimately decide the fate of the cells under normal and stressed conditions (Fig. 1).

Production of ROS and modulation of redox signaling pathways

The steady-state-level of ROS in the different cellular compartments is determined by a complex interplay between multiple ROS-producing pathways, and ROS scavenging mechanism; these are controlled by the ROS-signal transduction pathway and constitute the "basic ROS cycle". During the normal growth and development, this pathway monitors the level of ROS production metabolism and controls the expression and activity of ROS - scavenging pathways (Fig. 2). During the reduction of O_2 to H_2O, reactive oxygen species (ROS), namely superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) are formed (Fath et al., 2002; Cvetkovska et al., 2005). The basic ROS cycle may also perform fine metabolic tuning, e.g. suppression of photosynthesis to reduce the production rate of ROS. There are many potential sources of ROS in plants (Table 1). The cellular state is defined as the total active level that regulates cells to adapt to their surroundings in a narrow sense (Zheng and Wang, 2001; Halliwell, 2006; Wingler et al., 2006; Fey et al., 2005; Scheibe et al., 2005; Coupe et al., 2006). Redox state is an important part of the cellular state and one of the most important indicators for evaluating the above state (Zheng and Wang, 2001; Kwak et al., 2006, Wingler et al., 2006; Fey et al., 2005; Scheibe et al., 2005; Coupe et al., 2006). Plants, as other aerobic organisms, require oxygen for the efficient production of energy (Shcolnick and Keren, 2006). Most cellular compartments in crop plants have the potential to become a source of ROS. Environmental stresses that limit CO_2 fixation, such as drought, salt stress, ozone and high or low temperatures, reduce the $NADP^+$ regeneration by the Calvin cycle, consequently, the photosynthetic electron transport chain is diminished, producing superoxide radicals and singlet oxygen in the chloroplasts (Wu and Tang, 2004; Bechtold et al., 2005; Shao et al., 2005; Li and Jin, 2007; Hare et al., 1998). To prevent diminished electron transport chain under the conditions that limit CO_2 fixation, plants have evolved the photorespiratory pathway to regenerate $NADP^+$ (Ledford and Niyogi, 2005; Shao and Chu, 2005); as part of this pathway, H_2O_2 is produced as a by-product of ß-oxidation of fatty acids during the catabolism of lipids in the peroxisomes

(Foyer and Noctor, 2005; Wu et al., 2007). Because of the highly cytotoxic and reactive nature of ROS, their accumulation must be kept under tight control. Crop plants possess very efficient enzymatic and non-enzymatic antioxidant defense systems that allow scavenging of ROS and protection of plant cells from the oxidative damage (Foyer and Noctor, 2005; Shao et al., 2005; Shao et al., 2006; Shao et al., 2007a; Pourcel et al., 2007, Kumar et al., 2013). The distinct sub-cellular localization and biochemical properties of antioxidant enzymes, their differential inducibility at the gene expression level and enzyme level along with a plethora of non-enzymatic scavengers render the antioxidant system a very versatile and flexible unit that can control ROS accumulation temporally and spatially (Shao, 2007b; Foyer and Noctor, 2003; Millar et al., 2003); controlled modulation of ROS levels is significant specially in the light of the recent evidence for the signaling capacity of ROS (Shao et al., 2006; Pourcel et al., 2007; Millar et al., 2003). Redox signaling is responsive to myriad environmental signals through influences on metabolism and the triggered activation of the suite of oxidative burst-generating enzymes whose function is to enhance the oxidation state of the apoplast/cell wall environment (Foyer et al., 2012). Crop plants can sense, transduce, and translate the ROS signals into appropriate cellular responses, the process which requires the presence of redox-sensitive proteins that can undergo reversible oxidation/reduction and may switch 'on' and 'off 'depending upon the cellular redox state (Foyer and Noctor, 2003; Shao et al., 2005). ROS can oxidize the redox-sensitive proteins directly or indirectly *via* the ubiquitous redox-sensitive molecules, such as glutathione (GSH) or thioredoxins, which control the cellular redox state in higher plants (Shao et al., 2005; Foyer and Noctor, 2005; Nakashima and Yamaguchi, 2006; Uemura et al., 2006). The intracellular concentration of main redox couples in plant cells has been observed to fluctuate with the external environments (Table 2). Redox-sensitive metabolic enzymes may directly modulate the corresponding cellular metabolism, whereas redox-sensitive signaling proteins execute their function *via* downstream signaling components, such as kinases, phosphatases, and transcription factors (Li and Jin, 2007; Foyer and Noctor, 2005; Shao et al., 2005). Currently, two molecular mechanisms of redox-sensitive regulation of protein function have been observed in living organisms (Delledome et al., 2003; Link, 2003; Yabuta et al., 2004; Cvetkovska et al., 2005; Pourcel et al., 2007; Foyer and Noctor, 2005; Millar et al., 2003). Signaling mediated by ROS involves hetero-trimeric G-proteins (Pfannschmidt et al., 2003) and protein phosphorylation regulated by specific MAP kinases and protein tyrosine phosphatases (Foyer and Noctor, 2005; Kiffin et al., 2006). The biochemical and structural basis of kinase pathway activation by ROS remains to be fully established in higher plants, but thiol oxidation possibly plays a key role (Gapper and Dolan, 2006; Igamberdiev and Hill, 2004; Foyer and Noctor, 2005). The best-characterized redox signal transduction system in higher plants is the stromal ferredoxin - thioredoxin system, which functions in the regulation of photosynthetic carbon metabolism. Signal transmission involves disulfide - thiol conversion in target enzymes and is probably achieved by a light-induced decrease in the thioredoxin redox potential from about -0.26 V to about -0.36 V (Barnes and Mayfield, 2003; Foyer and Noctor, 2003; Yabuta et al., 2004). Thiol groups are likely to be important in other types of redox signal transduction including ROS sensing by receptor kinases, such as ETR1 (Noctor, 2006; Pourcel et al., 2007). ROS in crop plants must utilize and/or interfere with other

Table 1. Production and scavenging of Reactive oxygen intermediates (ROI) in plants.

Production of ROI	Localization	Primary ROI
Photosynthesis ET and PSI orII	Chl	O_2^-
Respiration ET	Mit	O_2^-
Glycolate oxidase	Per	H_2O_2
Excited chlorophyll	Chl	O_2^-
NADPH oxidase	PM	O_2^-
Fatty acid β oxidation	Per	H_2O_2
Oxalate oxidase	Apo	H_2O_2
Xanthine oxidase	Per	O_2^-
Peroxidase, Mn^{2+} and NADH	CW	H_2O_2, O_2^-
Amine oxidase	Apo	
Scavenging of Reactive oxygen intermediates (ROI)		
SOD	Chl, Cyt, Mit, Per, Apo	O_2^-
APX	Chl, Cyt, Mit, Per, Apo	H_2O_2
CAT	Per	H_2O_2
GPX	Cyt	H_2O_2, ROOH
Peroxidase	CW, Cyt,Vac	H_2O_2
Thioredoxin peroxidase	Chl, Cyt, Mit	H_2O_2
Ascorbic acid	Chl, Cyt, Mit, Per, Apo	H_2O_2, O_2^-
Gluthione	Chl, Cyt, Mit, Per, Apo	H_2O_2
α-Tocopherol	Membranes	ROOH, O_2^-
Carotenoid	Chl	O_2^-
AOX	Chl, Mit	O_2^-

Abbreviation: Chl-Chloroplast, Mit- Mitochondria, Per- Peroxisome, PM-Plasma membrane, Apo-Apoplast, CW-Cell wall, Cyt- Cytosol, Vac-Vacuole, ET- Electron transport, PS- Photosystem
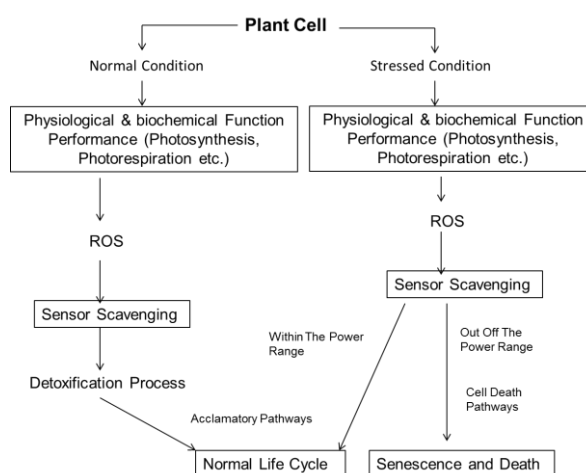

Fig 1. Related biological processes influenced by antioxidants and redox signaling in plant cells under control and stressed conditions; ROS acts as sensor molecules and modulate the signal transduction pathways through induction of enzymes and antioxidant molecules and ultimately decides the fate of the cells.

signaling pathways or molecules, forming a signaling network (Foyer and Noctor, 2003; Millar et al., 2003). Oxidants and antioxidants do not, as normally depicted, operate in isolated linear redox signaling pathways; rather, they are part of a much larger stress signaling network that integrates information from many pathways including hormones and sugars to regulate plant growth and defense responses (Foyer et al., 2012). Of late, increasing evidence shows that plant hormones are positioned downstream of the ROS signal. H_2O_2 induces accumulation of stress hormones such as salicylic acid (SA) and ethylene (Kiffin et al., 2006; Yabuta et al., 2004). Crop plant hormones are not only located downstream of the ROS signal, but ROS themselves are secondary messengers in many hormone signaling pathways (Coupe et al., 2006; Kwon et al., 2006; Millar et al., 2003; Nakashima and Yamaguchi, 2006; Uemura et al., 2006); feedback or feed forward interactions may conceivably occur between different hormones and ROS

(Delledome et al., 2003; Terman and Brunk, 2006; Rio et al., 2006; Shao et al., 2005).

Biological function of antioxidant molecule in plant cells α- tocopherol

α-tocopherols (Vitamin E) found in green parts of plants scavenges lipid peroxy radicals through a concerted action of other antioxidants (Kiffin et al., 2006; Hare et al., 1998). Further, tocopherols are also known to protect lipids and other membrane components by physically quenching and chemically reacting with O_2 in chloroplasts, thus protecting the structure and function of PSII (Ivanov, 2003; Igamberdiev and Hill, 2004). Researchers have reported a two-fold increase in α-tocopherol in turf grass under the water stress (Ledford and Niyogi, 2005; Kiddle et al., 2003). α-tocopherols are lipophilic antioxidants synthesized by all plants and interacts with the polyunsaturated acyl groups of

Table 2. Approximate redox potential and intracellular concentration of main redox couples in plant cells.

Redox Couples [a]	Redox Potentials (V)	Concentration Range (μM) [b]
O_2/H_2O	+0.82	200-300 (O_2)
$O_2/O_2^{\cdot-}$	-0.30	<0.001 ($O_2^{\cdot-}$)
$O_2^{\cdot-}/H_2O_2$	+0.94	1-100 (H_2O_2)
H_2O_2/OH^-	+0.54	Negligible (OH^-)
OH^-/H_2O	+2.20	—
DHA/ASC	-0.10	10, 000-20, 000
GSSG/GSH	-0.24	2, 000-5, 000
TRX_{ox}/TRX_{red}	-0.33	10-100
NAD (P)/NAD (P)H	-0.32	200-500
Fd_{ox}/Fd_{red}	-0.42	10-100
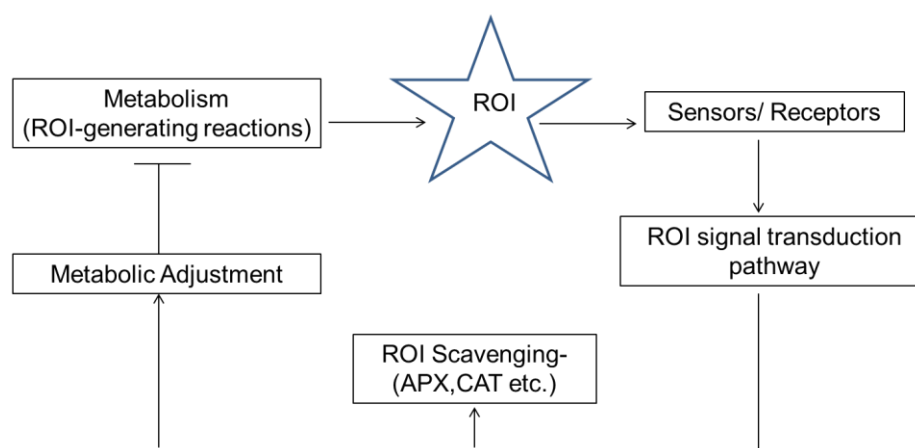
Fig 2. The basic pathways involved in generation of Reactive Oxygen Species (ROS) and its cycling in the system influencing various signal transduction pathways (ROI=ROS); Biological processes generates reactive oxygen intermediates which acts as sensors influencing redox balance of the cells by manipulating the scavenging protein molecules as well as antioxidants. The metabolic adjustment of the cells ultimately represses the generation of ROI from various biological pathways.

lipids, stabilize membranes, and scavenge and quench various reactive oxygen species (ROS) and lipid soluble byproducts of the oxidative stress (Fath et al., 2002; Cvetkovska et al., 2005). Singlet oxygen quenching by tocopherols is highly efficient, and it is estimated that a single α-tocopherol molecule can neutralize up to 120 singlet oxygen molecules *in vitro* before being degraded (Wu and Tang, 2004). Because of their chromanol ring structure, tocopherols are capable of donating a single electron to form the resonance - stabilized tocopheroxyl radical (Bechtold et al., 2005). These also function as recyclable chain reaction terminators of polyunsaturated fatty acid (PUFA) radicals generated by lipid oxidation (Hare et al., 1998) and scavenge lipid peroxy radicals and yield a tocopheroxyl radical that can be recycled back to the corresponding α-tocopherol by reacting with ascorbate or other antioxidants (Igamberdiev and Hill, 2004). α-tocopherols are major lipid soluble antioxidants present in the PUFA-enriched membranes of chloroplasts and are proposed to be an essential component of the plastid antioxidant network. However, most attributed function of tocopherols is their involvement in various mechanisms in protecting PUFAs from oxidation (Ledford and Niyogi, 2005). ROS generated as by-product of photosynthesis and metabolism are potential sources of lipid peroxidation in plant cells. α-tocopherol level has been found to increase in photosynthetic plant tissue in response to various abiotic stresses (Noctor, 2006) and have been reported to scavenge and quench various ROS and lipid oxidation products, stabilize membranes, and modulate signal transduction (Shao and Chu, 2005; Noctor, 2006). Synthesis of low molecular weight antioxidants, such as α-tocopherols has been reported in drought-stressed plants (Shao and Chu,

2005). Oxidative stress activates the expression of genes responsible for synthesis of tocopherols in higher plants (Shao and Chu, 2005; Wu et al., 2007). Antioxidants including α-tocopherol and ascorbic acid have been reported to increase following triazole treatment in tomato and thus may have a role in protecting membranes from oxidative damage contributing to chilling tolerance (Shao et al., 2007). Similarly, increase in the levels of antioxidants and antioxidant enzymes were observed in wheat in response to triazole (Shao et al., 2006). It has also been shown that water deficiency may result in an increase of tocopherol concentration in plant tissues (Wu et al., 2007; Shao et al., 2007). Some evidence implies that tocopherol content of soybean leaves increases with the decrease of rainfall (Shao et al., 2006): which is consistent with the reports (Shao et al., 2007) that subjecting spinach to water deficit increased the content of α-tocopherol in the leaves. Based on the studies of 10 different grass species under the water stress, the researchers found that drought stress led to an increase in α-tocopherol concentration from 1 to 3 fold in 9 out of 10 species (Shao et al., 2007; Pourcel et al., 2007) and it was pointed out that the species with a high stress are protected through tocopherol. Moreover, highly significant correlations have been observed between the stress tolerance and α-tocopherol concentration (the precursor of α-tocopherol; Spearmans rank correlation coefficient $r = 0.731$).

Ascorbic acid (AsA) as ROS scavenger

Ascorbic acid (Vitamin C) is one of the most extensively studied antioxidants and has been detected in the majority of plant cell types, organelles and apoplast (Aro and Ohad,

2003; Borland et al., 2006). Ascorbate occurs in all plant tissues, usually being higher in photosynthetic cells and meristems (and some fruits). About 30 to 40% of the total ascorbate is in the chloroplast and stromal concentrations as high as 50 mM have been reported (Foyer and Noctor, 2005). It is highest in the mature leaf where the chloroplasts are fully developed and the chlorophyll levels are highest. Although D-glucose is the precursor of L-AsA, the synthetic pathway has not been fully understood (Foyer and Noctor, 2003; Foyer and Noctor, 2005). AsA has effects on many physiological processes including the regulation of growth, differentiation and metabolism of plants and has a low cost in terms of synthesis and toxicity, and its benefits include protection of the glutathione pool and proper functioning of a range of enzymes (Stevens et al., 2013). A fundamental role of AsA in the plant defense system is to protect metabolic processes against H_2O_2 and other toxic derivatives of oxygen. Acting essentially as a reductant and reacting with and scavenging many types of free radicals, AsA reacts non-enzymatically with superoxide, hydrogen peroxide, and singlet oxygen. As mentioned earlier, it can react indirectly by regenerating α-tocopherol or in the synthesis of zeaxanthin in the xanthophyll cycle. Ascorbic acid is synthesized in the mitochondria and is transported to other cell components through a proton – electrochemical gradient or through the facilitated diffusion. Further, it has also been implicated in the regulation of cell elongation (Yabuta et al., 2004; Rio et al., 2006). In the ascorbate-glutathione cycle, two molecules of AsA are utilized by APX to reduce H_2O_2 to water with concomitant generation of monodehydroascorbate which is a radical with a short life time and can disproportionate into dehydroascorbate and ascorbic acid, the electron donor is usually NADPH and the reaction is catalyzed by monodehydroascorbate reductase or ferredoxin in water-water cycle in the chloroplasts (Gapper and Dolan, 2006). In plant cells, the most important reducing substrate for H_2O_2 removal is ascorbic acid (Sagi and Fluhr, 2006; Foyer and Noctor, 2005; Rio et al., 2006); its direct protective role has been demonstrated in rice also where partial protection against damage caused by a release from flooding conditions was provided by the pretreatment with ascorbic acid (Shcolnick and Keren, 2006). A continuous oxidative assault on plants during the drought stress leads to the appearance of an arsenal of enzymatic and non-enzymatic plant antioxidant defenses to counter the oxidative stress in plants (Shao et al., 2007). AsA is an important antioxidant which reacts not only with H_2O_2 but also with O_2^-, OH and lipid hydroperoxidases; it is water soluble and also has an additional role in protecting or regenerating oxidized carotenoids or tocopherols (Shao et al., 2006; Shao et al., 2005). Water stress has been shown to result in significant increase in AsA concentration in the turf grass. AsA showed a reduction under drought stress in maize and wheat, suggesting its vital involvement in deciding the oxidative response (Shao et al., 2005). It can also directly scavenge $1O_2$, O_2^- and •OH and regenerate tocopherol from tocopheroxyl radicals, thus providing membrane protection (Li and Jin, 2007; Shao et al., 2005). It also acts as a co-factor of violaxanthin de-epoxidase, thus sustaining dissipation of excess excitation energy (Li and Jin, 2007; Pourcel et al., 2007). Antioxidants such as AsA and glutathione are also involved in the neutralization of secondary products of ROS reactions (Foyer and Noctor, 2003; Hare et al., 1998). Therefore AsA influences many enzyme activities, and minimizes the damage caused by oxidative process in synergistic mode with other antioxidants (Foyer and Noctor, 2003; Foyer and Noctor, 2005).

Glutathione (γ-glutamyl cysteinyl glycine)

Glutathione is a tripeptide which has been detected virtually in all cell compartments such as cytosol, chloroplasts, endoplasmic reticulum, vacuoles and mitochondria (Millar et al., 2003). It is the major source of non-protein thiols in most plant cells. The chemical reactivity of the thiol group of glutathione makes it uniquely suitable for broad range of biochemical functions in all organisms. The nucleophilic nature of the thiol group is important in the formation of mercaptide bonds with metals and for reacting with selected electrophiles. This reactivity along with the relative stability and high water solubility of GSH makes it an ideal biochemical to protect plants against stress including oxidative stress, heavy metals and certain other exogenous and endogenous organic chemicals (Foyer and Noctor, 2005; Millar et al., 2003). Glutathione takes part in the control of H_2O_2 levels (Foyer and Noctor, 2005; Shao et al., 2005) and has been reported to play potentially important roles in setting signaling strength through the jasmonic acid (JA) pathway. Noctor *et al.*, (2013) implicated glutathione as a factor determining basal jasmonic acid gene expression and suggested a novel glutathione-dependent control points that regulate JA signaling in response to intracellular oxidation. The change in the ratio of its reduced (GSH) to oxidized (GSSG) form during the degradation of H_2O_2 is important in certain redox signaling pathways (Millar et al., 2003); the GSH/GSSG ratio, indicative of the cellular redox balance, is involved in ROS perception (Foyer and Noctor, 2005; Millar et al., 2003). GSH acts as an antioxidant and is involved directly in the reduction of most active oxygen radicals generated due to stress; it has been found to help to withstand oxidative stress in transgenic lines of tobacco (Foyer and Noctor, 2005; Rio et al., 2006).

Carotenoids

Carotenoids are a large class of isoprenoid molecule, which are *de novo* synthesized by all photosynthetic and many non-photosynthetic organisms (Andrew et al., 2008). They are divided into hydrocarbon carotenes, such as lycopene and β-carotene or xanthophylls typified by lutein (Jaleel et al., 2007). Oxidative damage generated by drought stress in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant systems. These include β-carotenes, ascorbate (AsA), α-tocopherol (α-toc), reduced glutathione (GSH) and enzymes including superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), polyphenol oxidase (PPO) and glutathione reductase (GR) (Prochazkova et al., 2001). Carotenes form a key part of the plant antioxidant defense system but are very susceptible to oxidative destruction. β-carotene present in the chloroplasts of all green plants is exclusively bound to the core complexes of PSI and PSII (Havaux, 1998) and protection against damaging effects of ROS at this site is essential for chloroplast functioning. Here, β-carotene, in addition to its function as an accessory pigment, acts as an effective antioxidant and plays a unique role in protecting as well as sustaining photochemical processes (Havaux, 1998). A major protective role of β-carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and protects from oxidative damage (Farooq et al., 2009). Water stress, among other changes, has the ability to reduce the tissue concentrations of chlorophylls and carotenoids (Havaux, 1998; Kiani et al., 2008), primarily through the production of ROS in the

thylakoids (Niyogi, 1999; Reddy et al., 2004). However, reports dealing with the strategies to improve the pigment contents under the water-stress are scarce. However, available reports show that exogenous application of brassinolide, uniconazole and methyl jasmonate improve the drought-tolerance with increased activities of SOD, CAT, APX, ABA and enhanced carotenoid contents in maize (Li et al., 1998), while methyl jasmonate brought about a three-fold increase in the β-carotene synthesis as well as degradation of the chlorophyll contents in the epidermal peels (Perez et al., 1993). Likewise, an important role of tocopherols, the lipid-soluble antioxidants in chloroplasts, has been envisioned through the improved pigments contents under stress conditions in the photosynthetic organisms including tobacco (Tanaka et al., 1999) and *Arabidopsis thaliana* and *Synechocystis* sp. PCC6803 (DellaPenna and Pogson, 2006).

Future research

Although steps in the biosynthetic pathways resulting in the antioxidant accumulation in higher plant cells have been primarily characterized at the physiological and molecular levels the full cast of participants involved in the complex regulation remains to be identified. It will require not only greater understanding of the degradation and transport of antioxidants, but also the elucidation of the molecular events responsible for stress perception and stress-related signal transduction *via* wider scope of tested plants. Basic research leading to the characterization of tightly-regulated stress-inducible promoters that are also responsive to appropriate tissue-specific regulation and endogenous developmental programmes, is likely to be critical in improving the overall field performance of transgenic crops. The future will determine more precisely how ascorbate, glutathione, and tocopherol are involved in initiating and controlling redox signal transduction and also how they trigger the gene expression of other related responses to optimize survival strategies. In addition, other problems remaining to be resolved are: how antioxidants coordinate growth and development of higher plants in a constantly changing environment; how redox signaling is linked with hormonal regulation, nutrient status and redox potential of higher plants; and also how their redox signaling is cooperated with inter-and intracellular signaling, transport capacity, developmental and environmental cues to maintain an appropriate dynamic homeostasis for stress-tolerance and efficient survival.

Conclusion

Redox signal transduction is a universal characteristic of aerobic life through the evolution under the natural and selecting pressures to balance information from metabolism and the changing environment. There are sets of discrete locations where signaling is controlled (or buffered) independently in plant cells which permits redox-sensitive signal transduction to occur in locations such as the appoplast, thylakoid, and endoplasmic reticulum, whereas other highly buffered spaces have a much higher threshold for ROS signals. Both oxidants and antioxidants fulfill signaling roles to provide information on improved plant health, particularly in terms of robustness for defense, using kinase-dependent and independent pathways that are initiated by redox-sensitive receptors modulated by thiol status. Differential concentrations of antioxidants between the compartments make antioxidant-driven vectorial signaling through processes such as ascorbate-driven electron transport

or futile cycles. Undoubtedly, transgenic plants will prove very valuable in assessing the precise role that main antioxidants and ROS play in the functional network that controls stress-tolerance.

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