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Protein Oxidation and Redox Regulation of Proteolysis

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Abstract

Reactive oxygen species (ROS), beyond the role of toxic by-products of aerobic metabolism, contribute to cell redox homeostasis and are signalling molecules in pathogen defence and abiotic stress tolerance. The putative mechanism of cell responses to ROS is thiol modifications of cysteine residues, which cause changes in protein conformation and activity. These post-translational modifications include generation of disulphide bridges and formation of sulphenic, sulphinic, and sulphonic acids, as well as S-glutathionylation and S-nitrosylation. S-nitrosylation or reversible modification may change the activity of enzymes related to the metabolism of nitric oxide, ROS, and cellular metabolism, whereas S-glutathionylation regulates the activity of proteins that contain in their structure the active cysteine residue, regulates the oxidoreductive pathway of signal transduction, and participates in the regeneration of antioxidant enzymes. Carbonylation, an irreversible, non-enzymatic modification of proteins is the most commonly occurring oxidative protein modification. The formation of carbonyl groups can be linked to abnormal translation, altered chaperone system and responses to stress factors. Carbonylated proteins are marked for proteolysis mediated by different pathways in different cell compartments to counteract the formation of high molecular weight aggregates and accumulation of inactive proteins. However, products of proteolysis of carbonylated proteins could function as secondary ROS messengers that target the cell nucleus.

Keywords: ROS, carbonylation, S-glutathionylation, protein hydrolysis, plant abiotic stresses

1. Introduction

The current literature reveals that the extreme changes in water content experienced by dehydration tolerant plants are accompanied by equally extreme fluctuation in cellular redox

state. Regulation of redox potential is essential not only for proper plant development but is required for plant survival under extreme environmental conditions. It is widely accepted that drought induces oxidative stress. This implies that water scarcity in environment disturbs a delicate balance between endogenous generation and elimination of reactive oxygen species (ROS). Reactive oxygen species are produced by a large number of physiological processes, mainly by the electron transport chains in chloroplasts and mitochondria. Under stress conditions, proteins may be modified by ROS in large number of reactions. The putative mechanism of cell responses to ROS is the modifications of protein cysteine residues, which can be oxidized to varying degrees. These post-translational modifications of proteins include formation of disulphide bridges, sulphenic, sulphinic and sulphonic acids as well as S-glutathionylation and S-nitrosylation. The above modification cause changes in protein conformation and activity, although these modifications seem to be potentially reversible (with the exception of sulphonic acid). Additionally, oxidation of protein by direct oxidative attack on Lys, Arg, Pro, and Thr or by secondary reactions on Cys, His, and Lys residues leads to the formation of protein carbonyl derivatives. Carbonyl (CO) groups (aldehydes and ketones) are chemically stable and thus, carbonylation is irreversible and unrepairable. Protein carbonyl derivatives can also be generated through oxidative cleavage of proteins by either the a-amidation pathway or by oxidation of glutamyl side chains. Carbonylation of proteins seems to lead to inhibition or alteration of the activities of the proteins and to increase of their susceptibility to proteolytic attack. Protein hydrolysis can be mediated by different pathways in different cell compartments. In nucleus it engages mainly ubiquitination and proteasomes activity, whereas in mitochondrion and chloroplast specific enzymes like ATP-depended protease La, ClpAP and others has been described. In cytosol hydrolysis once again is maintained by ubiquitination and proteasomes, and probably other means. One of the most effective ones is the vacuolar hydrolysis based on specific for these compatment proteases like cysteine, serine, aspartate proteases and metalloproteases. Therefore, in this chapter the changes in cellular redox state and their impact on modifications of protein functions and efficient removal of abnormal and/or non-functional proteins are discussed.

2. Functional activities of ROS in plants under abiotic stresses

Plants, as sessile organisms, are commonly exposed to diverse unfavorable environmental factors including drought, cold, and heat that may induce stress conditions. Throughout the life cycle of plants, reactive oxygen species (ROS) are continuously produced as a consequence of aerobic metabolism and they play a role in cellular redox regulation [1]. However, stress of any kind, biotic or abiotic, leads to an increased level of ROS production and/or to inactivation of antioxidants, particularly enzymes. The oxidative stress that accompanies unfavorable environmental conditions has been suggested to be associated with harmful effects on cellular components including metabolic activities and integrity of organelles. However, recent findings indicate that transient oxidative stress may have beneficial effects in the response of plants to unfavorable environmental conditions. Oxidative stress acting transiently seems to be a signal-activating pathway that enables plants to acclimate to unfavorable environmental

conditions [2–4]. Therefore, ROS seem to play a dual role because they may either be involved in the stress-induced oxidative damage of plants or may also activate genes facilitating the development of plant tolerance to abiotic and biotic stresses. Transient oxidative stress seems to participate in the control of seed dormancy, germination and seedling growth [5–6] and other processes involved in physiological transitions [7]. It should be underlined that whether ROS acts as a damaging, protective, or signaling factor depends on sensible equilibrium between ROS production and scavenging at the proper site and time. This equilibrium depends largely on proper cooperation between photosynthesis, respiration, and photorespiration linking their redox state and energy balance in the cell. Antioxidants found in almost all cellular compartments demonstrating the importance of ROS detoxification for cellular survival [8].

Aerobic organisms are evolved mechanisms that minimize the levels of oxygen or delay the generation of ROS [9]. Mobile organisms are able to avoid oxygen by move from high O_2 regions, but it is not the case of plants. Comparing the redox regulation in plants and animals on the cellular level, it seems that they evolved common mechanisms. The most important source of ROS in vivo is the mitochondrial electron-transport chain in aerobic animal cells and chloroplast electron transport chain in plant cells. Plant and animal cytochrome oxidase does not release ROS, but other components of the mitochondrial electron-transport chain can leak electrons directly to O_2, thus generating ROS [10]. However, plants are able to minimize mitochondrial ROS formation by alternative oxidase [11]. Uncoupling proteins located in the inner mitochondrial membranes of both plants and animals allow protons to leak, preventing the escape of electrons to oxygen. Other mechanisms involved in delayed ROS formation in both animals and plants are suppression of Fenton reaction and lipid peroxidation by metal binding proteins such as transferrin, ferritins, and metallothionins [12].

It is widely accepted that proteins are the most abundant cellular targets of the ROS, constituting about 70% of the oxidized biological molecules in the cell [13]. The extent of protein damage depends on the rate at which any particular reaction occurs and on the intensity of oxidant-scavenging and repair reactions [14]. The rate constants for the reaction of reactive species with proteins vary, but they are useful in computational models to predict sites and selectivity of damage between different oxidants [14]. On the basis of kinetic data, one can predict that the least selective are the most reactive oxidants, whereas the more selective are less reactive oxidants. Thus, sulphur-containing and aromatic side chains will be depleted rapidly [14].

ROS-induced protein modifications can result in the unfolding or alteration of the protein structure, but some of them may be harmless. Proteins can be oxidized in reversible or irreversible ways. Protein oxidation chemistry, based on 20 different types of amino acid side chains, results in many potential reaction sites and products that may be grouped into four categories, i.e., aliphatic, aromatic, cysteine and cystine residues, and methionine residues [14]. Amino acids containing sulphur in the side chain are the most oxidation-susceptible and therefore are the most commonly modified [15]. The oxidation of cysteine residues can result in a number of redox-based post-translational protein modifications such as glutathionylation or S-glutathionylation, S-nitrosylation and sulphenic acid, sulphinic acid and disulfide formation [16]. Side chains of proline, threonine, lysine and arginine can be oxidized to

aldehyde or ketone derivatives in the process known as carbonylation of proteins. Protein carbonylation, irreversible post-translational protein modifications, is commonly regarded as a good measure of intensity of protein oxidation [17].

3. Oxidation of sulphur-containing amino acids

The most frequently modified amino acids are cysteine and methionine containing sulfur in the side chain. The oxidation of protein cysteine thiol groups can generate thiyl radicals (-S.), disulfide bonds (-S-S-), as well as sulfenic (-SOH), sulfinic (-SO_2H), and sulfonic (-O_3H) acid derivatives. Methionine residues can be oxidized to methionine sulfoxides and less frequently to methionine sulphones. An increase in free cysteine levels in response to various abiotic stress factors has been reported [18]. In most studies, this increase was reported together with increased reduced glutathione (GSH) concentrations, leading to the conclusion that cysteine is mainly needed for the biosynthesis of Sulphur-rich compounds with anti-stress activity, such as GSH and stress-related proteins. Cysteine plays an important role as an extracellular reducing agent and is also a critical substrate for protein synthesis being the precursor of taurine. Thiol-containing substances are normally considered as antioxidants. However, metabolites containing -SH group exhibit a double effect of antioxidant and prooxidant properties. Cysteine is a potent chelator of heavy metals ions, but cysteine-metal ion complexes can trigger the Fenton reaction, thereby producing the highly toxic ·OH radical. Furthermore, free cysteine is often irreversibly oxidized to different by-products [19] and reversibly to cystine (cysteine disulphide). However, it remains unclear whether cystine reductase (EC 1.8.1.6), the enzyme that catalyzes the reduction of cystine to cysteine, is active in plants [20].

Methionine, similar to cysteine, can undergo ROS-mediated oxidation to methionine sulfoxide and this can lead to changes in protein conformation and activity [21]. The conversion of methionine sulfoxide to methionine is mediated by methionine sulfoxide reductases (MSRs; EC 1.8.4.11), a class of cytosolic and plastidic enzymes that are involved in ameliorating oxidative damage [22]. Methionine is also a substrate for the synthesis of various polyamines with important roles in stress tolerance, the most prominent being putrescine, spermidine, and spermine [23]. The oxidized methionine residue is readily reduced back to methionine by methionine sulfoxide reductase. Methionine residue is proposed to be a "last chance" antioxidant defense system to protect proteins from oxidation under oxidative stress. Surface exposed methionine residues effectively scavenge oxidizing agents while generally preserving the biological function of the molecule.

4. Protein glutathionylation as a mechanism of antioxidant defense

Glutathionylation of proteins is the modification of a reactive protein cysteine thiol by the reduced glutathione (GSH) and occurs under normal or oxidative stress conditions [24]. It was assumed as a by-product of oxidative or nitrosative stress (S-glutathionylation). Under

oxidative stress conditions, GSH may interact in a reversible manner with protein cysteinyl thiols [25]. Non-enzymatic glutathionylation reactions are non-specific and seem to be associated with oxidation stress, whereas enzymatic glutathionylation reactions catalyzed by glutaredoxin in both directions are highly specific, reversible [26], and also protect sensitive SH groups from oxidation to sulphinic and sulphonic acid [27]. Thus, various mechanisms may be involved in the formation of glutathionylated proteins and it is not clear which one is prevalent in vivo [24]. The thiol-disulfide exchange seems to be a major one, although evidences against a role of GSSG in the formation of protein-SSG have been given [26, 28].

There is still some controversy about the difference between oxidative stress and redox regulation and signalling. It seems that low levels of ROS play a regulatory role, e.g., the reversible oxidation of cysteines to mixed disulfides represents redox regulation, whereas high levels of ROS lead to oxidative stress and toxicity, i.e., irreversible oxidation to sulfonic acids is toxic [29].

Glutathionylation seems to be involved in such processes as glycolysis, signal transduction, protein degradation, intracellular trafficking, and protein folding [30]. It can protect protein thiols from irreversible inactivation but can also alter the activity of many proteins. In *Arabidopsis thaliana* glutathionylation of glycolytic enzyme, cytosolic triose-phosphate isomerase and fructose-1,6-bisphosphate (FBP) aldolase, a Calvin cycle enzyme, inhibited their activity [31]. The inactivation of a soybean protein tyrosine phosphatase and inhibition of transcription factors (such as Jun and NF-kB) by glutathionylation was noticed [32]. However, glutathionylation activated the transcription factor/cyclic AMP-responsive element binding protein [33].

Glutathionylation results in the regulation and redox signaling in plants. It has been shown that glutatione-S-transferase from *Arabidopsis thaliana* undergoing glutathionylation possess a dehydroascorbate reductase activity and/or a glutathione-dependent thiol transferase activity [34]. These enzymes, which contain a catalytically essential cysteine, are glutathionylated in vitro in the presence of GSSG with a concomitant loss of enzymatic activity would constitute an intermediary step of the catalytic mechanism, allowing glutathione-dependent reduction of dehydroascorbic acid [30].

It has also been proposed that glutathionylation of some photosynthetic enzymes regulates photosynthetic metabolism and thus allows for rearrangement of NADPH and ATP within chloroplasts under oxidative stress [35]. Glutathionylation of the f-type thioredoxin (TRX f) prevents its reduction by ferredoxin-thioredoxin reductase and these thioredoxins are involved mainly in the light-dependent regulation of carbon metabolism enzymes, including several Calvin cycle enzymes [36]. Therefore, it seems that the glutathionylation of TRX f, glyceraldehydes-3-phosphate dehydrogenase and fructose-1,6-bisphosphate aldolase suggest that this posttranslational modification could constitute a new mechanism of regulation of Calvin cycle enzymes under oxidative stress [30]. The in vivo proteomic study on photosynthesizing cells performed in *Chlamydomonas* supported such a possibility [35]. This experiment has shown that more than 20 glutathionylated proteins, mostly located in the chloroplast, are involved in diverse processes such as photosynthesis, chloroplast translation, amino acid and ATP metabolism, protein folding, acetate metabolism, and oxidative stress. Two new Calvin

cycle enzymes, phosphoglycerate kinase and ribose-5-phosphate isomerase distinct from those known to undergo glutathionylation, were also identified [30].

Glutathionylation of glycine decarboxylase, a key enzyme in the process of photorespiration [37], resulted in the inactivation of plant mitochondria. This points to the role of glutathionylation in the temporary protection of enzymes against oxidative stress. Glutathionylation can also regulate the activity and function of nuclear proteins, including transcription factors, as well as influence the chromatin structure and dynamics of the process of condensation [20]. Some of the best-characterized plant proteins regulated by glutathionylation are annexins. Annexins are Ca^{2+} and phospholipid binding proteins forming an evolutionary conserved multigene family with a presumed function of the enzyme associated with signal transduction. Different stress conditions cause different changes in gene expression of annexin [38]. Glutathionylation of annexin A1 in *Arabidopsis thaliana* modifies its ability to bind Ca^{2+} and leads to the reduction or inhibition of enzyme activity.

5. Protein carbonylation and its role in plant development and stress response

Carbonylation is an irreversible, non-enzymatic modification of proteins and the most commonly occurring oxidative protein modification. Carbonyl groups are introduced into proteins by a variety of oxidative pathways. Lysine, arginine, proline, and histidine side chains can be oxidized to reactive aldehyde or ketone groups via carbonylation causing inactivation, crosslinking, or breakdown of proteins [39–40]. Carbonyl groups can be generated through oxidative cleavage of proteins by α-amidation pathway or by formation of peptides with α-keto derivatives at the N-terminus [41]. In living organisms, the common mechanism of carbonylation is metal-catalyzed oxidation (MCO) occurring during the interaction of reduced metal ions, such as Fe^{+2} or Cu^{+} with H_2O_2, in the Fenton reaction producing extremely reactive hydroxyl radicals oxidizing amino acid side chains or causing protein backbone cleavage [41]. In many cases, oxidative modification of proteins is a detrimental process in which irreversibly inactivated proteins lead to cellular dysfunction. On the other hand, it is now clear that oxidative modification of proteins can be specific and reversible, playing a key role in metabolic regulation and normal plant physiology. However, little is known about the involvement of protein carbonylation in developmental processes and in the response of plants to adverse environmental conditions. Oxidatively modified proteins were identified at all stages of the plant life cycle and their involvement in the control of common biological functions such as dormancy, germination, and aging has been suggested [42–43]. The intracellular level of protein carbonyl groups increases with age, reaching typical values of 1–4 nmol mg^{-1}protein but under oxidative stress may increase to 8 nmol mg^{-1} protein, i.e., about 40% of all protein molecules have one carbonyl group [41]. The increased level of oxidized proteins in aged organisms seems to be the result of higher susceptibility of proteins to ROS due to alterations in the protein structure as well as the diminished ability for protein removal [44]. Protein carbonylation seems to be the consequence of age-induced transcriptional and translational

errors and increased levels of aberrant misfolded proteins resulting in the level of oxidized proteins [44, 45].

Carbonylated proteins have been found in all plant cell cellular compartments: cytosol, chloroplasts, peroxisomes, nucleus, and mitochondria [42, 43, 46-48]. Interestingly, it has been found that in wheat leaves, the concentration of carbonylated proteins per mg protein was higher in the mitochondria than in chloroplasts and peroxisomes [46]. It may suggest that mitochondrial proteins are more susceptible to oxidative damage or are more tolerant to oxidation or mitochondrial proteases are less efficient in protein removal.

Carbonylated proteins have been identified mainly in *Arabidopsis thaliana* leaves [42], germinating seeds [43], and whole shoots [49]. However, aging and stress-induced carbonylation does not affect proteome in the same way [50]. Specific plant proteins such as ribulose-1, 5-bisphospate carboxylase/oxygenase, enzymes of the cycle of Krebs, and electron transport chains may be classified as sensitive to carbonylation [42, 45]. The findings that such carbonylated proteins as glycolytic enzymes, aldose reductase, methionine synthase, and molecular chaperons characterized in mammals, yeast, and bacteria found in plants point out the participation of carbonylation in the control of common biological functions [51]. Carbonylation of plant proteins seems to also be involved in such physiological transitions such as dormancy, germination, and aging and can act as a signal in physiological transitions [42, 43, 48, 52].

Abiotic stresses such as dehydration, heat, salinity, heavy metals gamma irradiation, and others, as well as biotic stresses, result in protein carbonylation but the extent of carbonylation correlates to the exposure time and the severity of stress [51, 53]. The inactivation of the Calvin cycle, such as ribulose-1, 5-bisphosphate carboxylase oxygenase (RuBisCO), by protein carbonylation would allow plant adaptation to stress conditions. Furthermore, several components of the glycine decarboxylase complex (GDC), a key enzyme involved in photorespiration in photosynthetic tissues, are highly prone to carbonylation, especially under stress conditions [42, 54]. The peroxisome fraction isolated from castor bean endosperm and subjected to harsh metal catalyzed oxidation, a sharp increase in carbonylated malate synthase, and isocitrate lyase were observed. Also, antioxidants proteins such as catalase, manganese superoxide dismutase (MnSOD) and peroxiredoxin (Prx), which are associated with the defense against oxidative stress, are themselves sensitive to oxidative attack [55]. Molecular chaperones such as Hsp60 can be also inactivated by carbonylation. It seems that these proteins are fairly susceptible to carbonylation and under prolonged oxidative stress they could lose structural integrity and become dysfunctional. The loss of function of the chaperones and MnSOD combined with the ongoing oxidative stress would aggravate the damaging effect to the cell, eventually resulting in cell death.

6. Degradation of oxidized proteins

Enhanced modification of proteins has been reported in plants under various stresses [56]. To maintain the cellular metabolism, it is highly required to possess the efficient degradation and

removal of oxidized proteins. The fate of carbonylated proteins in plant cells is of paramount importance. If not degraded these proteins tend to form high molecular weight aggregates due to covalent cross-linking and/or increased surface hydrophobocity. What is more, the accumulation of such inactive proteins could functionally compete with their active counterparts. To circumvent this, several distinct proteolysis pathways balance the extent of protein carbonylation [57].

Different pathways can mediate protein hydrolysis in different cell compartments. In the nucleus, it engages mainly in ubiquitination and proteasomes activity. In mitochondrion- and chloroplast-specific enzymes such as ATP-depended protease La, ClpAP and others have been described [58–60]. In cytosol, hydrolysis once again is maintained by ubiquitination and proteasomes [61]. One of the most effective ones is the vacuolar hydrolysis based on proteases such as cysteine, serine, aspartate proteases, and metalloproteases specific for this compartment. These different proteolytic pathways do not exclusively target carbonylated proteins. However, some of them as 20S proteasome pathway seem to preferentially degrade oxidized rather than non-modified proteins [62]. In this way, protein carbonylation could promote degradation of mistranslated, damaged, and aberrant or even no longer required proteins in plant cells.

7. Removal of oxidized proteins by proteasome

The ubiquitin 26S proteasome system is the major mechanism of intracellular protein degradation in eukaryotes, playing a key role in basic cellular processes. Plant cells contain a mixture of 26S and 20S proteasomes that are responsible for proteolysis [63]. The 26S proteasome is proteolytic component of the ubiquitin (Ub)-dependent proteolytic system, essential in plants and also in animals. It degrades functional proteins, negatively controls the abundance of regulatory proteins involved in signaling and metabolic pathways, and degrades abnormal and denatured proteins produced under biotic and abiotic stress. This illustrates why proteasome is essential for protein quality control [64]. The 26S proteasome is an ATP-dependent, multi-subunit protease complex composed of two subcomplexes, the 20S core protease (or multi-catalytic protease) and the 19S regulatory particles. The 20S core protease is ATP- and ubiquitin-independent protease that consists of a cylindrical stack created by the assembly of four heptameric rings [61]. It has been reported that reduction in 26S proteasome abundance is connected with the increasing cell expansion and with the decreasing cell division rates [65]. It is known that abiotic stresses lead to inhibit 26S activity directly by inhibiting 26S proteasome function or indirectly by increasing the substrate load. Heat shock and other stresses can cause protein misfolding which leads to a substrate overload. Oxidative stress directly leads to 26S proteasome causing a decrease in cell division. These mechanisms seem to be relevant when the intensity of the stressor is high [63].

A strong candidate for the removal of damaged proteins is the 20S proteasome, a 700 kDa proteolytic complex responsible for degradation of short-lived and abnormal intracellular proteins. It is composed of a barrel-shaped protein comprising of four stacked rings with the

catalytic side in the lumen. Two outer and two inner rings are made up of seven subunits ranging from 20 to 35 kDa each. The first and third rings contain alpha subunits; the second and fourth rings contain beta subunits.

The 26S proteasome is responsible for the degradation of misfolded proteins and the free 20S proteasome is needed for the removal of oxidized proteins [66]. Plant cells contain a mixture of the 26S and free 20S proteasome and the adequate proteasomes ratio seems to be important for plant development and stress responses. The comparative analyses of mutants showed that optimal 26S proteasome levels are needed to maintain tolerance to stresses, such as heat shock, that cause protein misfolding, while increased levels of the free 20S proteasome lead to better tolerance to oxidative stress [63].

For the first time, the importance of developmental regulation of the 26S to 20S proteasome was studied for *Drosophila*. It was proved that during aging, the 26S proteasome decreases while 20S proteasome increases [67]. Similarly, a decline in 26S proteasome levels and increase in 20S proteasome levels was presented in a proteomic study of potato tuber aging [68].

An increased production of free radicals and increased oxidative damage to cellular components are obtained during aging. Under these conditions, a shift in the proteasomes ratio in favor of the free 20S proteasome would be beneficial because it increases the cellular capacity to remove oxidized proteins that are potentially cytotoxic when allowed to accumulate. The regulation of 26S proteasome to 20S proteasome levels during aging and senescence is still poorly understood. From what we know, we can conclude that the mild loss of 26S proteasome activity strongly impacts plant growth; that is why plants need to maintain an optimal 26S proteasome activity level to ensure developmentally-programmed cell division and expansion rates. Finally, the loss of the 26S proteasome function does not disturb plant survival because it provides to the 20S proteasome biogenesis and at the same time increases oxidative stress tolerance [63]. Not all proteins are degraded after the carbonylation; some of them may form aggregates that accumulate in the cell. Inhibition of proteolysis may be a result of the creation of groups of modified proteins susceptible to degradation, effectively blocking the proteasome. Accumulation of such protein aggregates may result in cell death.

8. Autophagic degradation of carbonylated proteins

Autophagy, a non-specific protein degradation pathway, could degrade oxidized proteins [69, 70]. However, the potential role of autophagy in plant responses to abiotic stresses is still unknown. Although autophagy-related proteins function in plant responses to salt and osmotic stress, the relationship to autophagy is not clear [71, 72]. This process plays a critical role in the acclimation of plants to changing environmental stresses such as oxidative stress, drought, salt, and pathogen occurrence [73]. Autophagy is a process in which cytoplasmic components are taken up into the vacuole (yeast, plants) or lysosome (mammals) for degradation. There are three major forms of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy [74–76]. In plants, chaperone-mediated autophagy has not yet been established. Microautophagy is in intravacuolar vesicles (autophagic bodies) that came

from the invaginated tonoplast. The isolated body containing the cytoplasmic materials and membranes is degraded by vacuolar hydrolases. Macroautophagy (hereafter called autophagy) is a cellular mechanism of the removal of unnecessary cytosolic components in vesicles formed de novo. These double membrane-bound vesicles, called autophagosome, are fused with the tonoplast to release the internal vesicles into the lumen of the vacuole, where they are degraded by hydrolases residents in the vacuole [77]. Recently, new type of autophagy-dependent bodies specifically involved in the delivery of chloroplast (chlorophagy), mitochondria (mitophagy), and ribosome (ribophagy) components to the vacuole has been described [77].

Nutrient deprivation was one of the first common abiotic stresses shown to induce autophagy. Very common and frequent environmental stresses encountered by plants are high salinity and drought stress [78]. These stresses reveal some common features but are regulated differently. Autophagy-deprived AtAtg18a-RNAi plants show high sensitivity to these two stresses. It is commonly accepted that diverse environmental stresses may induce autophagy, but it may be regulated by different signaling pathways. It has been shown that the salinity and osmotic stress-induced autophagy in *Arabidopsis thaliana* [79] and autophagy induced by starvation and salt stress has been dependent on NADPH oxidase activity, enzyme generating superoxide by transferring electrons from NADPH to molecular oxygen to produce superoxide anion. However, it was not the case in autophagy induced by osmotic stress. To date, the isoforms of NADPH oxidase responsible for the induction of autophagy have not been found. Thus, in nutrient-starved and salinized plants, autophagy seems to be regulated by NADPH oxidase-dependent pathways and involved ROS, whereas in osmotically stressed plants, autophagy is regulated by an NADPH oxidase independent pathway in which ROS may not be involved as a signal [79]. Evidences show that autophagy is induced by high salinity and osmotic stresses and that autophagy-defective plants are more sensitive to these conditions [80].

9. Role of the vacuole

Vacuolar proteolysis also plays a major role in the turnover of proteins oxidized by endogenously generated ROS. These oxidized proteins, probably nonessential to survival, are selectively recognized and degraded by proteolytic enzymes [81]. Thereafter, amino acids may be released for synthesis of new proteins, aberrant proteins formed under water deficit may be degraded, and certain proteins may be activated. Controlled protein hydrolysis has therefore been recognized as essential for the adaptation of plants to environment [82]. The MEROPS database (http://merops.sanger.ac.uk/) reflects the increasing number of proteases classified based on their catalytic type, e.g., aspartic (A), cysteine (C), serine (S), threonine (T) and glutamic (G) peptidases and asparagine peptide lyases (N), all based on the amino acid residue at the active site involved directly in peptide bond hydrolysis, and into metallopeptidases (M) that require a divalent metal ion as part of the active site. Drought has been shown to induce large increases in acid protease activity in leaves of a susceptible wheat cultivar, probably associated mainly with cysteine proteinases involved in playing a house-keeping

function to remove abnormal and misfolded proteins arising from unfavorable conditions [83, 84]. One may suppose that carbonylated proteins could promote degradation of misfolded, damaged, or even no longer required proteins that are nonessential to survival and should be replaced by newly formed proteins [84, 85]. However, detailed studies in this respect are needed.

10. The effects of vitamins present in the plants on ROS

The Food and Nutrition Board of the National Institute of Medicine in the U.S.A. has defined a dietary antioxidant as "a substance in foods that significantly decreases the adverse effects of reactive species (oxygen and nitrogen species) on normal physiological function in humans." Among the most commonly studied dietary antioxidants playing a vital role against ROS are ascorbic acid (vitamin C), α-tocopherol (vitamin E), and β-carotene. Vitamin C is a very important water-soluble antioxidant in extracellular fluids and is able to neutralize ROS in the aqueous phase before they can attack lipids. Vitamin E is a lipid-soluble antioxidant. It is important as the chain-breaking antioxidant within the cell membrane; it plays an important role in protecting membrane fatty acids from lipid peroxidation and has the highest antioxidative activity because of the presence of three methyl groups in its molecular structure [86]. Vitamin C, moreover, is able to regenerate vitamin E. β-carotene and other carotenoids also have antioxidant properties and may work in synergy with vitamin E [87]. Among the most important non-enzymatic antioxidants in plants are the glutathione (GSH), proline, carotenoids, and flavonoids. Glutathione (GSH) is aimed to be the most important intracellular defense against ROS-induced oxidative damage. It is involved in the control of H_2O_2 levels. Flavonoids belong to one of the most reactive secondary metabolites of plants. Flavonoids play an important role as a ROS scavenger by locating and neutralizing radicals before they damage the cell structure. Proline is considered to act as an osmoprotectant, a protein stabilizer, a metal chelator, an inhibitor of lipid peroxidation, and $OH^{\cdot}$ and $1O_2$ scavenger. Moreover, it also plays an important role as a ROS quencher as well as a signaling molecule. Carotenoids are lipid soluble antioxidants that play a multitude functions in plant metabolism including oxidative stress tolerance [88].

Arabidopsis thaliana, contained in leaf flavonoids is a highly diverse polyphenolic secondary metabolite and glucosinolate - another class of secondary compounds [89, 90]. Glucosinolates, due to their flavor and medicinal properties, and coumarins characterized by their antimicrobial and antifungal activities as well as pharmacological effects have attracted much interest [91]. Flavonoids appear to play a fundamental role during environmental interactions and plant coping with abiotic stresses [90].

It is possible to measure antioxidant assay value for food. There are numerous methods of assessment for dietary antioxidants. These assays can be divided into two groups depending on the reactions involved: assays based on hydrogen atom transfer (HAT) reactions and assays based on electron transfer (ET). The first group includes low-density lipoprotein autoxidation, oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter

(TRAP), and crocin bleaching assays. ET-based assays include the total phenols assay by Folin–Ciocalteu reagent (FCR), Trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP), "total antioxidant potential" assay using a Cu(II) complex as an oxidant, and diphenyl-1-picrylhydrazyl radical –DPPH [87]. The antioxidant values of foods listed are expressed in ORAC (Oxygen Radical Absorbance Capacity) units, a unit of measurement for antioxidants developed by the National Institute on Aging in the National Institutes of Health [92, 93]. Numerous health food and marketers have erroneously capitalized on the ORAC rating by promoting products claimed to be "high in ORAC."

11. Conclusion

Redox regulatory mechanisms are a necessary part of the intracellular communication system activating the plant stress responses. Redox-based post-translational protein modification plays a fundamental role in redox regulation of protein function by disulfide bridge formation and in control of protein activity by redox-based Cys-modification such as S-glutathionylation. Another oxidative protein modification marks carbonylated proteins to proteolysis. On the other hand, carbonylation of mistranslated and aberrant proteins seems to be an important mechanism of protein quality control. It seems that plant tolerance to stress factors are mediated by both, redox modifications of proteins and their contribution to the maintenance and control of cellular homeostasis.

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