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REVIEW ARTICLE

Lipids and proteins—major targets of oxidative modifications in abiotic stressed plants

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Abstract Stress factors provoke enhanced production of reactive oxygen species (ROS) in plants. ROS that escape antioxidant-mediated scavenging/detoxification react with biomolecules such as cellular lipids and proteins and cause irreversible damage to the structure of these molecules, initiate their oxidation, and subsequently inactivate key cellular functions. The lipid- and protein-oxidation products are considered as the significant oxidative stress biomarkers in stressed plants. Also, there exists an abundance of information on the abiotic stress-mediated elevations in the generation of ROS, and the modulation of lipid and protein oxidation in abiotic stressed plants. However, the available literature reflects a wide information gap on the mechanisms underlying lipid- and protein-oxidation processes, major techniques for the determination of lipid- and protein-oxidation products, and on critical cross-talks among these aspects. Based on recent reports, this article (a) introduces ROS and highlights their relationship with abiotic stress-caused consequences in crop plants, (b) examines critically the various physiological/biochemical aspects of oxidative damage to lipids (membrane lipids) and proteins in stressed crop plants, (c) summarizes the principles of current technologies used to evaluate the extent of lipid and protein oxidation, (d) synthesizes major outcomes of studies on lipid and protein oxidation in plants under abiotic stress, and finally, (e) considers a brief cross-talk on the ROS-accrued lipid and protein oxidation, pointing to the aspects unexplored so far.

Keywords Abiotic stress · Lipid peroxidation · Oxidative modifications · Protein oxidation · Reactive oxygen species

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Introduction

Abiotic stress, reactive oxygen species, and biomolecules

Abiotic stresses (such as drought, salinity, radiations, extreme temperatures including freezing/low and high temperatures, and chemicals such as metals/metalloids) continue to have a significant impact on plant growth and development, limiting the global crop production significantly (reviewed by Mahajan and Tuteja 2005; Gill and Tuteja 2010; Cramer et al. 2011; Anjum et al. 2012, 2014a; Krasensky and Jonak 2012). Normal aerobic metabolism in plants results in the generation of reactive oxygen species (ROS; such as $O_2^{\bullet-}$, H_2O_2, and $^{\bullet}OH$), where antioxidant defense system (comprising enzymes such as superoxide dismutase, SOD; catalase, CAT; guaiacol peroxidase, GPX; glutathione sulfo-transferase, GST; ascorbate peroxidase, APX; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione reductase, GR; and non-enzymes such as ascorbate, AsA; glutathione, GSH; carotenoids; tocopherols; phenolics) efficiently scavenge ROS and maintain their levels at non-damaging levels (Mittler 2002; Gill and Tuteja 2010; Anjum et al. 2012). However, incapability of plants to escape stress exposures makes them fall prey to previous abiotic stresses. In isolation or combination, these stresses can cause severe damage to plants, either directly or indirectly, by triggering an increased production of ROS and their reaction products (Fig. 1). Although minimal levels of ROS may act as important signal transduction molecules and trigger and/or orchestrate plants' responses to varied (abiotic) stresses, a disturbance in the ROS/antioxidant homeostasis in any cell compartment leads to a situation called oxidative stress (Gill and Tuteja 2010). Thus, oxidative stress (via unmetabolized and/or excess ROS and their reaction products within cells) can cause significant physiological challenges including cell death, and the arrest of plant growth and development, mainly by provoking oxidative modification of vital biomolecules including membrane lipids, cellular amino acids, proteins, and DNA (Mahajan and Tuteja 2005; Gill and Tuteja 2010; Anjum et al. 2012; Krasensky and Jonak 2012). The major metabolic sources (subcellular sites) of ROS in plants have been summarized in Fig. 2, whereas Fig. 3 presents a scheme of the major energy transfer by generation of the ROS and their reaction products, their chief characteristics, and the role they play in the oxidative modification of lipids and proteins in plants.

Being a highly elaborated structure (with a lipid bilayer and the integral and peripheral proteins) and arguably the most diverse membrane of the plant cell, plasma membrane functions as the point of exchange with adjoining cells, cell walls, and the external environment and helps the plant to develop and regulate plant–environment interactions (Barkla and Pantoja 2011; Furt et al. 2011; Murphy et al. 2011). In particular, the lipid bilayer constitutes the hydrophobic barrier that prevents arbitrary exchange of solutes, whereas the regulation of exchange of solutes or the transduction of signals from one side of the membrane to the other is tightly regulated by the transmembrane proteins (Kleinschmidt 2013). Notably, the peroxidation of membrane (phospho) lipids and the degradation/oxidation of proteins are among the most investigated consequences of ROS action on membrane structure and function (Blokhina et al. 2003; Davies 2005; Rinalducci et al. 2008; Foyer and Noctor 2009; Foyer and Shigeoka 2011). Regarding the interaction of ROS with lipids and proteins, superoxides ($O_2^{\bullet-}$) react primarily with protein Fe–S centers, while singlet oxygen ($^1O_2$) is particularly prone to react with conjugated double bonds such as those found in polyunsaturated fatty acids (PUFAs) (Rinalducci et al. 2008; reviewed by Braconi et al. 2011). Enhanced protein oxidation and lipid peroxidation may take place in membranes of the cell and the organelle, which, in turn, can affect the normal cellular functioning and metabolism. On the one hand, oxidative stress may be aggravated by lipid peroxidation via the production of lipid-derived radicals (e.g., alkyl, peroxyl, and alkoxyl radicals) that can react with and damage the proteins and the DNA (reviewed by Gill and Tuteja 2010; Sharma et al. 2012). On the other hand, in the ROS-accrued protein oxidation, there may occur site-specific amino-acid modification, fragmentation of peptide chain, aggregation of cross-linked reaction products, altered electric charge, and increased susceptibility of proteins to proteolysis. Nevertheless, lipid peroxidation and protein oxidation are closely linked, as the proteins could be modified by their direct conjugation with the breakdown products of fatty-acid (such as PUFA) peroxidation in membrane (Yamauchi et al. 2008). Owing to the short-lived nature of toxic intermediates and ROS, and to the inefficiency of methodologies for their direct estimation, the quantification of stable end-products of reactions of the ROS (and their reaction products) with cellular macromolecules has been advocated as an alternative approach (Orhan et al. 2004). In this context, plant-tissue contents of malondialdehyde (MDA, one of the final products of peroxidation of unsaturated fatty acids in phospholipids) and carbonylated proteins [reactive carbonyls (RCs)] are considered as the biochemical markers of lipid peroxidation/cell-membrane damage (Taulavuori et al. 2001; Sochor et al. 2012) and protein oxidation (Møller and Kristensen 2004), respectively. Increased peroxidation (degradation) of lipids and the elevated protein oxidation are common in plants growing under environmental stresses (Romero Puertas et al. 2002; Han et al. 2009; Tanou et al. 2009; Mishra et al. 2011; reviewed by Sharma et al. 2012).

This review (a) critically examines the various aspects of oxidative damage to lipids (membrane lipids) and proteins in abiotic-stressed plants, (b) summarizes the current technologies used to evaluate the extent of lipid and protein oxidation, (c) evaluates the cross-talks of lipid and protein oxidation and their oxidation products, and (d) highlights the aspects that are relevant but so far unexplored. The discussion is focused mainly on the

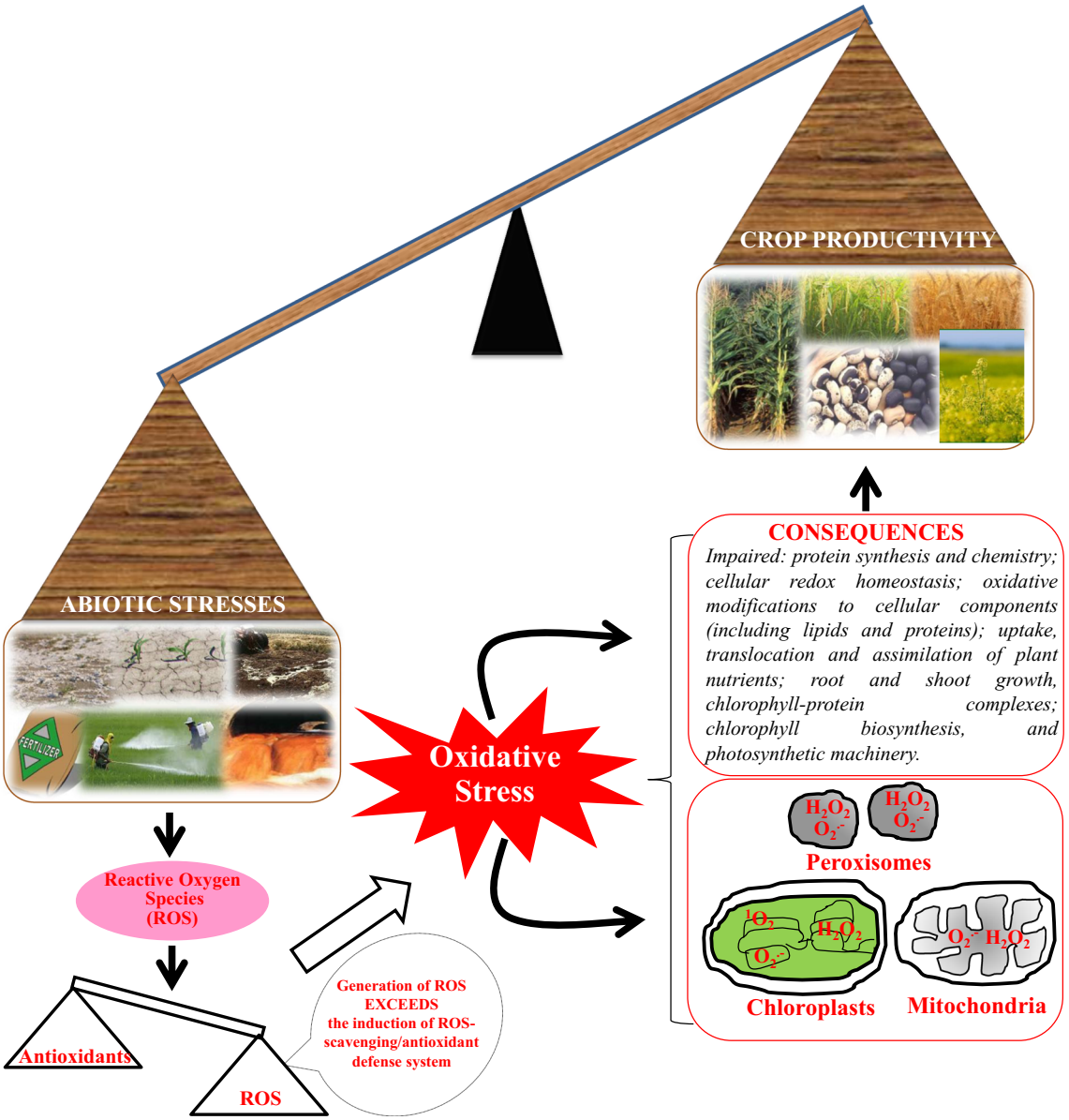

Fig. 1 Schematic representation of relationships between abiotic-stress factors, and oxidative stress, their cumulative consequences in plants, and subsequent impact on crop productivity

oxidation of lipids and proteins due to ROS (and their reaction products) generated by the above-mentioned abiotic stresses.

Lipid peroxidation in plants

Plant lipids—an overview

Plant lipids include fats, waxes, steroids, phospholipids, hydrocarbons, and free higher fatty acids and their salts (soaps). Membrane lipids constitute a major chemical component of all cell membranes and are represented mainly by phospholipids (the more abundant) and sterols (particularly stigmasterol), which are amphipathic and spontaneously form bilayers in an aqueous environment (López et al. 2011). Thylakoid membranes of chloroplasts are an exception and contain primarily galactolipids, which are also amphipathic and most stable in a bilayer configuration (Tetlow et al. 2004; Robinson and Mant 2005). The amphipathic nature of membranes permits the formation of membranous sheets that self-anneal their edges into a sealed compartment. The inner and outer surfaces of both plasma and organelle membranes differ considerably in chemical composition (Evert 2006). Owing to the dynamic nature of plant plasma membrane, and the sensitivity of its lipid composition/structure to the external environment cues (such as abiotic stresses), the changes in the membrane lipids act as stress markers and help the plant in tuning with the potential abiotic stress impacts (Barkla and Pantoja 2011; Furt et al. 2011; Murphy et al. 2011).

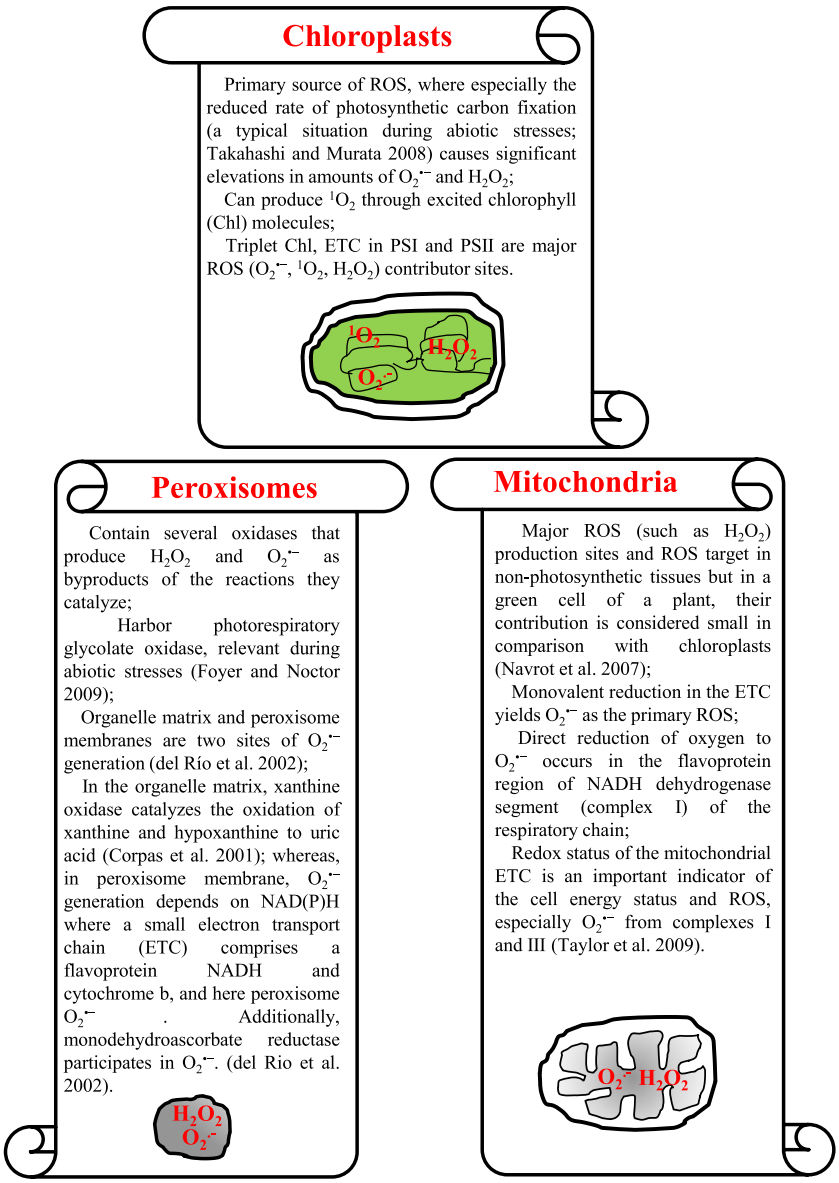

Fig. 2 Major metabolic sources (subcellular sites) of reactive oxygen species in plants (based on del Río et al. 2002; Corpas et al. 2001; Foyer and Noctor 2009; Taylor et al. 2009; Gill and Tuteja 2010; Jaspers and Kangasjärvi 2010)

Basic mechanisms and estimation strategies

Peroxidation of lipids can be brought about by both enzymatic and non-enzymatic (chemical) ways; the ROS-mediated lipid peroxidation falls under the latter category. The ROS (such as free oxygen radicals) have the tendency to cause peroxidation of lipids, where polyunsaturated fatty acids (PUFAs) of lipids are chemically damaged by free radicals and oxygen, giving way to lipoperoxides formation. Depending on the type of lipids and oxidants, and on the severity of oxidation, a variety of lipid-peroxidation products are formed (Sharma et al. 2012; Hameed et al. 2013). The major products of lipid peroxidation are moieties containing hydroxyls, hydroperoxyls, aldehydes, ketones, caroxylic acids, and trans double bonds (Borchman and Sinha 2002). In fact, in comparison to the saturated fatty acids (with no double bonds) and monounsaturated fatty acids (with one double bond), PUFAs are more vulnerable to ROS-mediated peroxidation because of the presence of greater number of double bonds in a fatty-acid chain, and the easy removal of a hydrogen atom (Wagner et al. 1994; Porter et al. 1995). Owing to their unstable nature, lipoperoxides decompose to form a wide range of compounds including the reactive carbonyl compounds, especially certain aldehydes [such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal or hydroxynonenal (4-HNE)], which in turn fetch severe consequences to cells by binding free amino groups of amino acids of proteins (reviewed by Sochor et al. 2012). With particular reference to mitochondria, lipid peroxidation principally refers to peroxidation of PUFA of membrane lipids such as linoleic acid, linolenic acid and arachidonic acid, where various cytotoxic aldehydes, alkenals, and hydroxyalkenals can be yielded (Taylor et al. 2004). Nevertheless, among the ROS, mainly

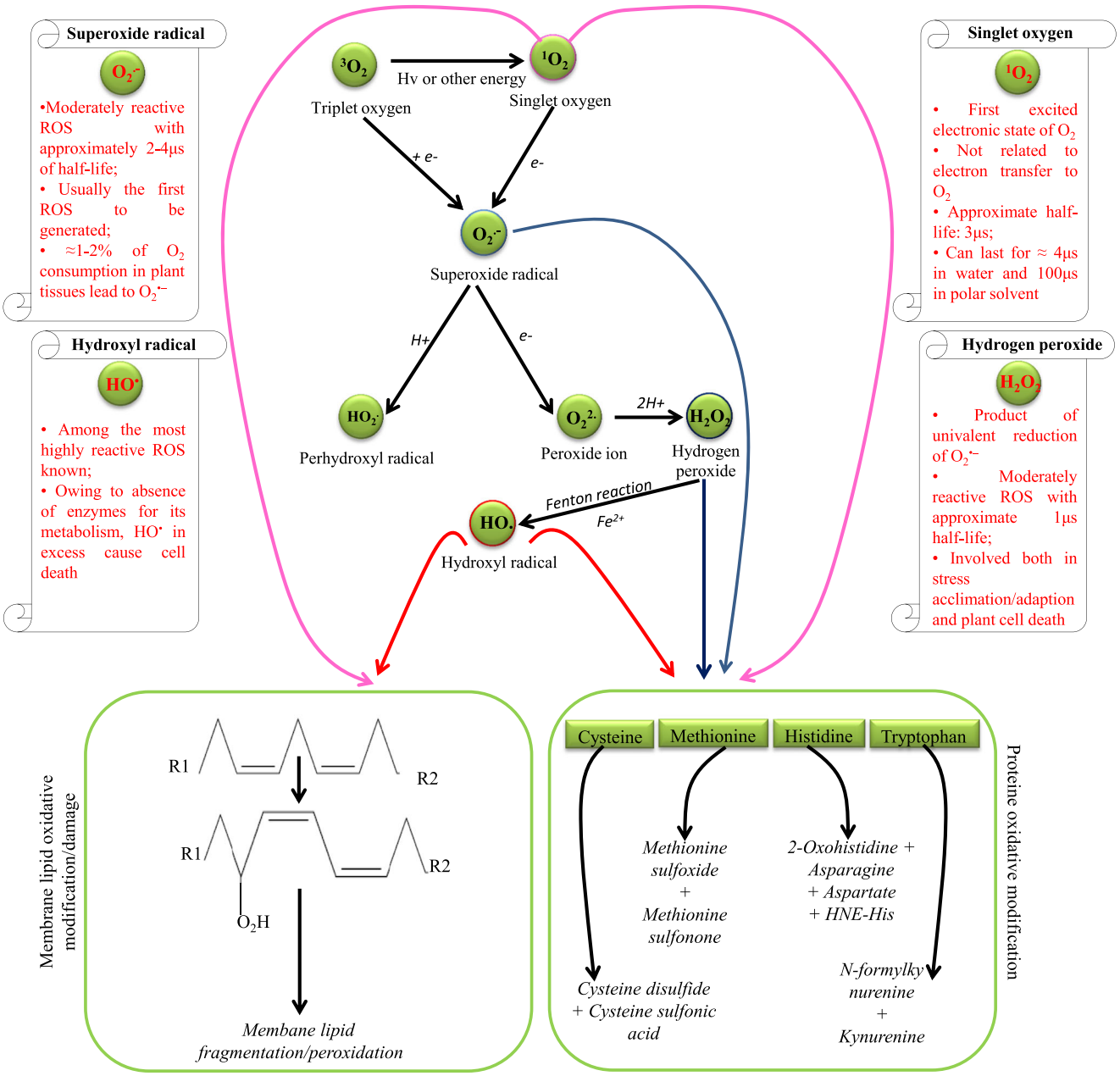

Fig. 3 Energy-transfer concept of reactive oxygen species (ROS) and their reaction products, their chief characteristics and the role in oxidative modification of lipids and proteins in plants (based on Gill and Tuteja 2010; Sharma et al. 2012; Sabater and Martin 2013)

hydroxyl radicals have been reported to initiate the peroxidation of mitochondrial membrane PUFAs by abstracting a hydrogen atom and eventually leading to the formation of cytotoxic lipid aldehydes, alkenals, and hydroxyalkenals (such as HNE and MDA) (Rhoads et al. 2006). In general, plant-lipid peroxidation is mainly due to ROS activity, where the primary target of the ROS attack on lipids is the 1,4-pentadiene structure of PUFAs, which are either free or esterified to cholesterol or glycerol (Browne and Armstrong 2002). The overall mechanism of the ROS (free radicals)-mediated lipid oxidation consists of: (a) initiation (activation), i.e., the formation of free radicals; (b) propagation (distribution), i.e., the free-radical chain reactions; and (c) termination (cleavage), i.e., the formation of nonradical products (Fig. 4).

Enzymatic lipid peroxidation is catalyzed by the enzymes lipoxygenase (LOX, EC 1.13.11.12) and cyclooxygenase (EC 1.14.99.1), which are involved in the formation of eicosanoids, which represent a group of biologically active lipid compounds derived from unsaturated fatty acids containing 20 carbon atoms (reviewed by Sochor et al. 2012). In particular, LOXs (linoleate/oxygen oxidoreductases) are ubiquitously occurring non-heme Fe-containing fatty acid dioxygenases, soluble in water, constituted by a single polypeptide associated with an atom of Fe(III), and catalyze the

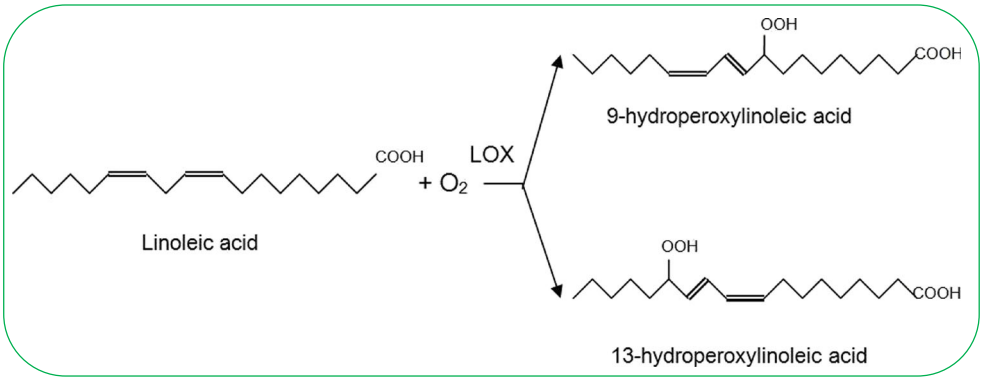

Fig. 4 Schematic representation of the basic mechanism of reactive oxygen species (ROS)-mediated peroxidation (oxidative/non-enzymatic modification) of lipids (based on Gutteridge 1995; Södergren 2000; Mäkinen 2002; Sochor et al. 2012)

addition of molecular oxygen to PUFAs via regio- and stereo-specific oxygenation, and thereby produce hydroperoxy fatty acids and oxy-free radicals (Garder 1991; Sofo et al. 2004a). In fact, owing to the presence of *cis*, *cis*-1,4-pentadiene moiety (–CH=CH–CH_2–CH=CH–), PUFAs become the major target of LOX. LOX reactions exhibit high stereospecificity, with most lipoxygenases catalyzing the formation of (S)-configured fatty acid hydroperoxides (LOOHs) (Bannenberg et al. 2009) (Fig. 5). Extensive reports including that of Feussner and Wasternack (2002), Liavonchanka and Feussner (2006), Skórzyńska-Polit et al. (2006), Vellosillo et al. (2007), and López et al. (2011) have evidenced the enzymatic modification of these intermediates into oxylipins, bioactive compounds involved in growth, development, and responses to (a) biotic stress conditions. Three LOX isoenzymes, namely, LOX-1, LOX-2, and LOX-3, were considered common in plants. However, LOX-3 is the isoform with most abundance and highest activity (Sofo et al. 2004a). *Arabidopsis thaliana* has been reported to exhibit six LOX isoforms (At-LOX-1 to At-LOX-6) (Bannenberg et al. 2009).

Contingent upon the lipid (per) oxidation-product types (such as conjugated dienes and lipid hydroperoxides, LHP; or secondary products, such as MDA; alkanes or isoprostanes), a number of methods/state-of-the-art equipments are employed for estimation of lipid-oxidation level in plant samples. A brief description of the basic methods/principles and their limitations follows.

Spectrophotometry assay

Spectrophotometry (UV–visible) is a simple, rapid, reproducible, and low-cost technique that can be employed for estimating MDA via the thiobarbituric acid reactive substances (TBARS) method. This method can also be employed to estimate the levels of lipid hydroperoxides (LHPs), the primary oxygenated products of PUFAs, and the key intermediates in the octadecanoid signalling pathway in plants. The principle underlying the TBARS method is that thiobarbituric acid (TBA) reacts with MDA in acidic conditions and forms a pink MDA-(TBA) 2 complex at a higher (100 °C) temperature (Fig. 6) that can be quantified spectrophotometrically at 532 nm. In fact, the level of MDA/TBARS is frequently utilized as a suitable biomarker for lipid peroxidation in stressed plants (Sofo et al. 2004a, b; López et al. 2011; Diwan et al. 2012; Hakeem et al. 2012; Sorkheh et al. 2012). However, it has been argued that the estimation of MDA via TBARS method/TBA assay may underestimate the actual extent of peroxidation because (a) MDA can only be formed from fatty acids with three or more double bonds (Halliwell and Gutteridge 1989, cited in Griffiths et al. 2000), and (b) the occurrence of high levels of the predominant acyl constituent such as linoleic acid [18:2 ($\Delta^{cis,9,12}$)] that is the major PUFA and is used as substrate by LOX, and hence can restrict the estimation of actual extent of peroxidation (Leverentz et al. 2002). Additionally, TBA assay yields little information on the levels of LHPs.

Ferrous oxidation in xylenol-orange assay

In general, total LHPs may be determined using the ferrous oxidation in xylenol orange (FOX) assay, which can be used for hydroperoxides present in the aqueous (FOX1) and the lipid (FOX2) phases (Wolff 1994; Nourooz-Zadeh et al. 1994). To overcome the above-mentioned issue and correctly estimate LHPs in plant samples, Griffiths et al. (2000) developed this spectrophotometric method, employing the FOX2-assay technique with some modification. Herein, the LHPs estimation was based on their reaction with the ferric-ion-indicator dye "xylenol orange" [o-cresolsulphonphthalein-3,3′-bis (methyliminodiacetic acid) sodium salt] that binds ferric ion to produce a colored (blue-purple) complex with the maximum absorbance at 560 nm (a: Fe^{2+}+LOOH→Fe^{3+}+LO•+OH^-; b: Fe^{3+}+XO→blue-purple complex). To determine the LHP present in plant tissues, the authors first extracted total lipids in an acidified chloroform–methanol-based solvent system, and subsequently, the isolated lipids were assayed spectrophotometrically with FOX2 reagents. In another instance, modified FOX2 and iodometric assays were applied to detect LHP in methanol extract of a range of plant tissues including the pericarp (avocado; *Persea americana*; European pear, *Pyrus communis*), periderm (potato; *Solanum tuberosum*), leaves (cabbage,

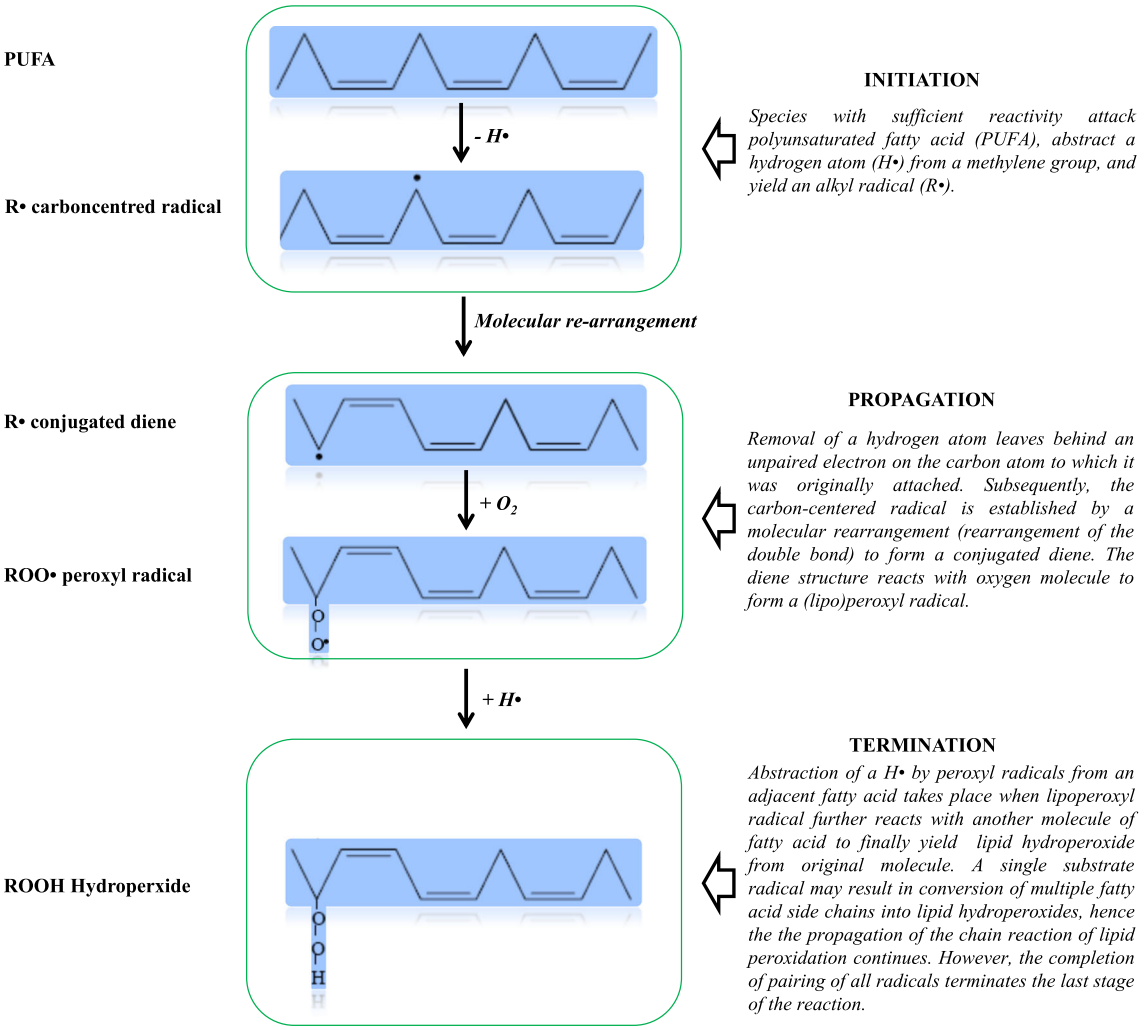

Fig. 5 The primary reaction catalyzed by lipoxygenase, using linoleic acid as substrate, indicates two possible products

Brassica oleracea convar. capitata var. rubra; spinach, *Spinacia oleracea*), and fruits (red pepper; *Capsicum annuum*). The impact of 10–12 days exposure of excised pieces of these plant species to 83 kJ m^{-2} day^{-1} of biologically effective ultraviolet-B irradiance (UV-BBE) on both LHP and TBARS was monitored (DeLong et al. 2002). The FOX assay was advocated to be employed for the detection of early membrane-associated stress events in plant tissues owing to its insensivity to ambient O_2 or light levels, its efficiency to generate LHP measurements rapidly (during the initial rather than more advanced fatty-acid-oxidation phase), and its being relative inexpensive.

Infrared spectroscopy assay

Infrared spectroscopy (IS) technique can detect the major products of lipid peroxidation and is uniquely sensitive in detecting the lipid hydroxyl and hydroperoxyl groups (Borchman and Sinha 2002). The detection of these groups is especially useful for quantifying the oxidation of mono-unsaturated lipids, such as those found in the ocular lens, where secondary products of lipid oxidation, such as MDA, are not readily formed. Indeed, many plant lipids are highly unsaturated, containing as many as six C=C bonds per hydrocarbon chain, and are very sensitive to lipid oxidation, particularly under stress conditions.

Reverse-phase high-performance liquid chromatography assay

This technique can separate regioisomeric species of lipid hydroperoxides (LHP) and lipid hydroxide (LOH) derived from plant PUFA. Details of the reverse-phase high-performance liquid chromatography (RP-HPLC) assay have been described by Browne and Armstrong (2002) and Armstrong (2002).

Lipid oxidation vs. abiotic stresses

Various abiotic stresses lead to the overproduction of ROS in plants, which cause damage to lipids that ultimately results in oxidative stress (Nishida and Murata 1996; Gill and Tuteja

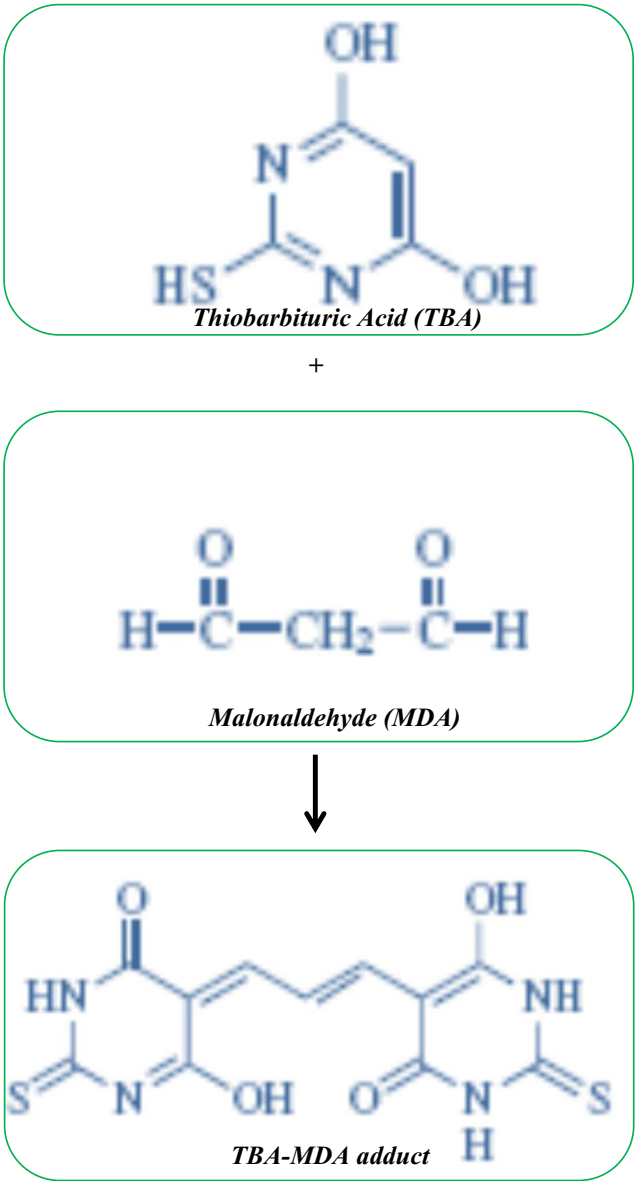

Fig. 6 The basic principle of thiobarbituric acid (TBA) test. *TBA* 2-thiobarbituric acid, *MDA* malonaldehyde)

2010; Miller et al. 2010; Foyer and Shigeoka 2011; Sharma et al. 2012). Instances of abiotic-stress-mediated lipid oxidation via ROS production are being discussed hereunder, critically analyzing the experimental results and synthesizing the basic underlying mechanisms.

Metals/metalloids

Oxidative stress caused in plants by exposure to elevated metal concentrations in the environment coincides with a constraint on plastidial and mitochondrial electron transport, which enhances lipid peroxidation in these two compartments and in the whole cell (Nagajyoti et al. 2010; Yadav 2010; Keunen et al. 2011). Metals/metalloids can be potent inducers of lipid peroxidation in plants since they have been extensively reported to promote the formation of ROS (Gill and Tuteja 2010; Anjum et al. 2012). Notably, redox active metals (such as Cu, Cr, and Fe) can cause lipid peroxidation via generating ROS through redox cycling. However, redox inactive metals (such as As, Cd, Co, Hg, Al, Ni, Pb, Se, Zn, etc.) bring significant impairments in antioxidant defense components such as thiol-containing antioxidants and enzymes, and eventually cause lipid peroxidation. Many studies on lipid peroxidation have shown that Cd produces strong alterations in the functionality of membranes by inducing lipid peroxidation and disturbances in chloroplast metabolism by inhibiting chlorophyll biosynthesis and reducing the activity of enzymes involved in CO_2 fixation (Cuypers et al. 2010, 2011; Ahmad et al. 2011; Gallego et al. 2012; Gill et al. 2013). Enhanced LOX activity has been reported in a number of Cd-exposed plants (Skórzyńska-Polit et al. 2006; Smeets et al. 2008; Tamas et al. 2009; Remans et al. 2010; Keunen et al. 2013; Liptáková et al. 2013). Upregulation of lypoxigenase (LOX) was an important component of stress response of barley (*Hordeum vulgare*) roots to toxic Cd, but was not responsible for the Cd-induced harmful lipid peroxidation (Liptáková et al. 2013). Cd-accrued enhanced LOX activity can mediate Cd-induced root growth inhibition (Tamas et al. 2009). Cd exposure can also cause a strong upregulation in the transcription level of the cytosolic LOX1 gene (Smeets et al. 2008; Remans et al. 2010). The Cd (25, 50, and 100 mg Cd kg^{-1} soil)-induced elevated TBARS production in Cd-tolerant mung bean (*Vigna radiata*) cv. Pusa 9531 was assumed to be the outcome of a strong cellular redox homeostasis, as compared with Cd-susceptible *V. radiata* cv. PS16 (Anjum et al. 2011). In another study, the same Cd treatments elevated the TBARS level in the leaves of mustard (*Brassica campestris* L.) cv. Pusa Gold, which was reduced by ROS scavengers such as ascorbate and glutathione (Anjum et al. 2008a). Organs of the same plant may exhibit different levels of lipid peroxidation under Cd exposure. For example, an increased accumulation of MDA (a lipid-peroxidation product) was more pronounced in shoots than in roots of the Cd-exposed lentil (*Lens culinaris*) seedlings (Talukdar 2012). Plant species significantly differing in their tolerance to Cd may exhibit different extent of membrane lipid peroxidation; Cd-induced increase in MDA level was lower in the less-sensitive pea (*Pisum sativum*) genotypes 3429 and 1658 than in the more-sensitive genotypes 4788 and 188. However, the maximum value of lipid peroxides was associated with genotype 8456, which showed a relatively high tolerance to Cd, as deduced from growth parameters (Metwally et al. 2005). Thus, the Cd-induced oxidative stress varied among *P. sativum* genotypes. In a recent study of *B. campestris* and *V. radiata*, the difference in the Cd-accrued elevation in TBARS level was attributed to the variation in the Cd-accumulation capacity of roots and in the balanced tuning between the enzymatic

and non-enzymatic components of the antioxidant defense system (Anjum et al. 2014b).

Lipid peroxidation appeared as an early symptom triggered by aluminium (Al)-induced oxidative stress (Yamamoto et al. 2001; Boscolo et al. 2003), but the major mechanisms underlining the Al toxicity remain unidentified. In this context, Yin et al. (2010) verified the participation of the lipid-peroxide-derived aldehydes (especially highly electrophilic α,β-unsaturated aldehydes, 2-alkenals), in Al toxicity. In the roots of Al-exposed transgenic tobacco (*Nicotiana tabacum*) (overexpressing 2-alkenal reductase gene obtained from *A. thaliana*), *N. tabacum* cultivar SR1, and an empty vector-transformed control line (SR-Vec), ROS-led aldehydes such as MDA were considered as the major cause of injury to root cells (Yin et al. 2010). In *H. vulgare* seedlings, Al (2.5, 5, and 10 mM; 6 days) exposure resulted in a dose-dependent significant increase in lipid peroxidation (Achary et al. 2012). In a recent study of peanut (*Arachis hypogaea*), the Al-induced lipid-peroxidation damage of mitochondria was more serious, showing a significantly higher mitochondrial MDA content, in a sensitive (Zhonghua 2) cultivar than in the tolerant (99–1507) one (Zhan et al. 2014). Lipid peroxidation (measured as MDA level) due to Al exposure has been reported in a number of plants including barley (*H. vulgare*; Achary et al. 2012), black soybean (*Glycine max*; Wu et al. 2013), cucumber (*Cucumis sativus*; Pereira et al. 2010), maize (*Zea mays*; Giannakoula et al. 2010), pea (*P. sativum*; Yamamoto et al. 2001), and rapeseed (*B. campestris*; Basu et al. 2001), among others. In contrast, no significant change in MDA level was observed in *Plantago algarbiensis* leaves and roots, while it decreased in *Plantago almogravensis* roots, exposed to Al (Martins et al. 2013), suggesting the existence of protective mechanisms in this species. In *N. tabacum* cells, Al-mediated enhanced lipid peroxidaion was considered as a direct cause of cell death (Ikegawa et al. 2000). Contrarily, Boscolo et al. (2003) held that lipid peroxidation is not essential for cell death in Al (6, 12, 36, and 60 μmol l^{-1})-exposed *Z. mays* and that the oxidative stress can induce cell injury by several other pathways, as pointed out by Halliwell and Gutteridge (1999) for mammalian cells.

In the roots of Hg-exposed alfalfa (*Medicago sativa*), Hg ions at low concentration (1.0 μM) did not cause any significant lipid peroxidation; however, higher concentrations (5 and 20 μM) in growth medium did it markedly, as indicated by TBARS accumulation as well as lysil oxidase (LOX) activity, measured in non-denaturing polyacrylamide gel electrophoresis (Zhou et al. 2007, 2008). Alterations in activities of antioxidant enzymes due to high Hg concentrations are ascribed to Hg-accrued differential lipid peroxidation. Additionally, owing to their transition property, mercuric ions induce oxidative stress by triggering generation of ROS in plants, which can be correlated to the Hg-accrued disruption of biomembrane lipids and cellular metabolism (Patra and Sharma 2000; Israr and Sahi 2006), as observed recently in salt marsh macrophytes *Halimione portulacoides* (Anjum et al. 2014c) and *Juncus maritimus* (Anjum et al. 2014d, e). In environmentally exposed two grass species (*Eriophorum angustifolium* and *Lolium perenne*), a differential extent of lipid peroxidation was argued as a result of a differential efficiency of ROS-metabolizing system (Anjum et al. 2013). A dose-dependent increase in TBARS content could be seen in boron (B)-exposed wheat (*Triticum aestivum*; Gunes et al. 2007) and *Artemisia annua* (Aftab et al. 2012) and Se-exposed *H. vulgare* seedlings (Akbulut and Çakır 2010). Se at higher concentrations can elevate lipid peroxidation (Gomes-Junior et al. 2007; Akbulut and Çakır 2010).

Not only the known toxic elements elevate lipid peroxidation but even such elements as Cu, Fe, Ni, Se, and Zn, which are beneficial in low concentration for plant physiological and biochemical processes, may enhance lipid peroxidation (Cuypers et al. 2010, 2011; Sofo et al. 2013). Increasing concentration of Fe (40, 80, and 160 mM) enhanced lipid peroxidation in *Bacopa monnieri* (Sinha and Basant 2009). Similarly, elevation in lipid peroxidation in a number of plants due to their exposure to Cu (Posmyk et al. 2009; Opdenakker et al. 2012; Singh et al. 2012; Thounaojam et al. 2012; Elleuch et al. 2013; Ansari et al. 2013a), Ni (Kazemi et al. 2010; Gajewska et al. 2012), Se (Malik et al. 2012), As (Ansari et al. 2013b), Pb (Qureshi et al. 2007; Maldonado-Magaña et al. 2011), Cr (Diwan et al. 2008; 2010), and some other heavy metals/metalloids was reported to be dependent on their doses and plant types (Sytar et al. 2013). The main site of attack by any redox active metal in a plant cell is usually the cell membrane. Being a redox active metal, Cu can catalyze the formation of ROS through Fenton and Haber–Weiss-type reactions (Van Acker et al. 1995), which in turn can induce lipid peroxidation (Valko et al. 2005). Cell membrane is considered as the primary site of Cu toxicity in plants (Thounaojam et al. 2012; Elleuch et al. 2013). At 2.0 and 5.0 μM, Cu could significantly stimulate MDA formation in the cyanobacteria *Phormidium foveolarum* and *Nostoc muscorum*, after 24 h of experiment (Singh et al. 2012). Cu treatment (200 and 500 μM) for a period of 1 and 7 days initiated the process of lipid peroxidation (measured as MDA level) in the root and shoot of rice (*Oryza sativa*) seedlings in a dose-dependent manner (Thounaojam et al. 2012). Enhanced levels of lipid peroxides (such as TBARS) were reported in *B. oleracea* tissues treated with 2.5 mM Cu^{2+}; excess accumulation of Cu caused the ROS-mediated oxidative damage to plasma membrane (Posmyk et al. 2009). In three wild-type plant species, namely, *Datura stramonium*, *Malva sylvestris*, and *Chenopodium ambrosioides*, grown on Cu mine, a significant increase in the leaf MDA was corresponded to Cu loads as well as to activities of antioxidative enzymes (Boojar and Goodarzi 2007). Lipid peroxidation (measured as TBARS) was argued as a first indication of oxidative damage

observed under Cu (and also Cd) exposure (Cuypers et al. 2011). Compared to Cd, the exposure of plants with Cu led to a higher cytotoxicity in *A. thaliana* roots, where a major increase in lipid peroxidation (and also a striking decrease of the K content) was noted. Interactive effects of elements on MDA may differ from those of isolated elements. Cu and Zn (10, 50, and 100 μM) together increased the MDA content in duckweed (*Spirodela polyrhiza*) in a dose-dependent manner (Upadhyay and Panda 2010). However, Zn supplementation had a declining effect on the MDA levels, reducing the lipid peroxidation, thereby indicating that Zn has a property of existing in a univalent state and being stable in a biological medium, and hence, the membrane-lipid packing was protected from ROS (Teisseire and Guy 2000). Recently, Malecka et al. (2014) reported a differential content of MDA in hydroponically grown *P. sativum* under Cd, Pb, Cu, and Zn alone and joint exposures. The observed difference in MDA level was attributed to the variation in the Cd, Pb, Cu, Zn, as well as Cu+Pb, Cu+Cd, Cu+Zn, Pb+Cd, Zn+Pb, and Zn+Cd (25 μM for each metal ion)-accrued ROS generation and to the ROS-metabolizing components of the antioxidant defense system.

Drought and salinity

Expression of the drought and/or salinity-induced metabolic alteration in plants involves oxidative stress. In the drought-stressed plants, membranes are considered to be a primary target of desiccation injury, and the ability of desiccation-tolerant organisms to avoid membrane damage during a dehydration-rehydration cycle is related to changes in membrane fluidity. Membrane bilayer structure in drought-tolerant organisms is considered as being stabilized due to interactions of the polar head groups with sugars and proteins. Such interactions create space between phospholipids and prevent membrane-phase changes. Membranes thus remain in the liquid-crystalline phase when the hydration shell is lost (Golovina and Hoekstra 2003). Moreover, under conditions of high vapor-pressure deficit, plants can reduce excessive water loss by closing their stomata and thus enhancing the oxidative stress due to excess energy. Drought stress of various degrees can cause damage to lipid structure and functioning in cereals (Fukao et al. 2011; Hameed et al. 2011, 2013; Csiszár et al. 2012), tree species (Štajner et al. 2011), forage plants (Slama et al. 2011), and medicinal plants (Tian et al. 2012).

Plants differing in photosynthetic capacity may exhibit varied extent of lipid peroxidation under drought exposure. In this context, decreasing field capacity was correlated indirectly with increased lipid peroxidation (in terms of TBARS) in *V. radiata* genotypes, where high-photosynthetic-capacity genotype (Pusa 9531) experienced less increase in TBARS and electrolyte leakage than the relatively more sensitive low-photosynthetic-capacity genotype (PS 16) (Anjum et al. 2008b). Interestingly, exogenous cinnamic acid and derivatives of jasmonic acid were effectively used in improving the drought-stress tolerance of plants by modulating the membrane-lipid peroxidation and antioxidant activities (Sun et al. 2012). Arbuscular mychorriza could also alleviate the detrimental effect of drought by reducing the MDA content and membrane permeability and by increasing the proline content and antioxidant enzyme activities (Zhu et al. 2011).

Salt stress, even at mild levels, is able to cause lipid peroxidation in cereals (de Azevedo Neto et al. 2006; Ashraf et al. 2010), vegetables (Sergio et al. 2012; Tayebimeigooni et al. 2012), and tree species (Ahmad et al. 2010; Ayala-Astorga and Alcaraz-Meléndez 2010). Indian mustard (*Brassica juncea*) cultivars differing in ATP-sulfurylase activity exhibited a differential extent of TBARS level under salinity stress. Exhibition of low TBARS in *B. juncea* cv. Pusa Jai Kisan (vs. *B. juncea* cv. SS2) was argued to be due to higher activity of ATP-sulfurylase, which in turn increased the content of glutathione, a reduced form of inorganic sulfur and an essential component of cellular antioxidant defense system (Khan et al. 2009). Plants differing in salinity tolerance could exhibit a varied extent of lipid peroxidation. Increase in lipid peroxidation, expressed by MDA content, is normally significantly higher in salinity-sensitive cultivars than in salinity-tolerant ones, as for instance, in *Brassica napus* (Rasheed et al. 2014). The lower level of TBARS in salinity (50 mM)-exposed *B. juncea* cv. Alankar (salt-tolerant) vs. *B. juncea* cv. PBM16 (salt-sensitive) was assumed to be due to its lower content of leaf Na^+ and Cl^- as well as the plant capacity to maintain tuning among the antioxidant defense-system components (Syeed et al. 2011). Similar conclusion was drawn in the case of *B. campestris*, showing increase in TBARS content with increase in the degree of soil salinity (Umar et al. 2011). Earlier, Sekmen et al. (2007) reported a similar differential response of salt-tolerant *Plantago maritime* and salt-sensitive *Plantago media* exposed to 100 and 200 mM NaCl.

Other abiotic stresses

The membrane lipid perturbation can also be performed by a range of other abiotic stress factors such as UV radiation, temperature extremes, nutrient deficiency, air pollution, and chemical toxicants (Gill and Tuteja 2010; Yan et al. 2010; Tripathi et al. 2011; Li et al. 2012; Szarka et al. 2012; Bashir et al. 2014; Majid et al. 2014). The contribution of membrane lipids, particularly the unsaturated membrane lipids, in protecting the photosynthetic machinery from photoinhibition under cold conditions has been intensively discussed (Nishida and Murata 1996). The membrane-lipid profiles and phospholipases have a role in freezing-induced lipid changes in *Arabidopsis* (Welti et al. (2002), contributing significantly to plant tolerance under cold conditions. Exposure of the excised

pieces of various plant species (*B. oleracea*, *C. annuum*, *P. americana*, *P. communis*, *S. tuberosum*, and *S. oleracea*) to 83 kJ m^{-2} day^{-1} of biologically effective ultraviolet-B irradiance for 10–12 days enhanced TBARS concentration (DeLong et al. 2002). Transgenic *N. tabacum*, overexpressing citrus (*Citrus unshiu*) dehydrin could exhibit a lower lipid peroxidation (measured as MDA content), compared with the control, under freeze (−4 °C, 3–24 h) stress (Hara et al. 2003). Plant exposure to herbicides also showed linkage with increase in lipid peroxidation (McCarthy-Suárez et al. 2011; Pazmiño et al. 2011). The degree of lipid peroxidation was used effectively as a biomarker of environmental pollution due to herbicides (Spoljaric et al. 2011). Lipid peroxidation was elevated significantly in peroxisomes during senescence. It was argued that the peroxisomal NADH-dependent production of $O_2•^-$ radicals is intensified by the reverse transition of leaf peroxisomes to glyoxysomes, which occurs when plants experience adverse environmental conditions, since more NADH would be available as a result of induction of fatty acid β-oxidation and the glyoxylate cycle (Zentgraf 2007; Sharma et al. 2012; Hameed et al. 2013). Elevation in CO_2 concentration can also cause an increase in lipid peroxidation (Farfan-Vignolo and Asard 2012). A significant increase in lipid peroxidation (measured as malanoaldehyde, MDA) was recently reported in potato (*S. tuberosum* cv. Kufri chandramukhi) when grown under conditions with maximum ozone (O_3) flux, ambient CO_2, and elevated O_3 (ACO_2+EO_3) (Kumari et al. 2015).

Protein oxidation in plants

Overview

Proteins are the key players in majority of cellular events; hence, in order to survive under stress conditions, functional conformations of cellular proteins are maintained and the aggregation of non-native proteins is prevented (Timperio et al. 2008; Ghosh and Xu 2014). However, since proteins are continuously oxidized even under normal physiological conditions, their oxidation is not necessarily a deleterious phenomenon in plants (Job et al. 2005; Johansson et al. 2004). In several instances, protein oxidation (measured as increased protein carbonyl levels) has been reported as a result of changes in factors related to plant development (Johansson et al. 2004; Job et al. 2005; Oracz et al. 2007). Protein dysfunction and inhibition of protein synthesis are among the earliest usual metabolic responses to abiotic stresses in plants (Timperio et al. 2008; Cramer et al. 2011). However, this is not the case always. In response to abiotic stress factors, plants may also accumulate and/or synthesize protein types (such as heat shock proteins (HSPs), zinc-finger proteins, mitogen-activated protein kinase (MAPK), late embryogenesis abundant (LEA) proteins; dehydrins, late embryogenesis abundant (LEA) proteins, chaperones, etc.). These proteins have been evidenced to help plants to run cellular metabolism and eventually to tolerate potential abiotic stress impacts (reviewed by Timperio et al. 2008; Fatehi et al. 2012; Hossain et al. 2012; Ghosh and Xu 2014). However, owing to their great abundance and high rate constants of a range of reactive radicals, proteins are one of the major cellular constituents targeted by ROS (Davies 2005). In fact, proteins are among the most abundant cellular components vulnerable to oxidation and constitute up to 68 % of the oxidized molecules in the cell (Rinalducci et al. 2008). Nevertheless, owing to the ubiquitous occurrence and diverse roles (structural, catalytic, and regulatory) of proteins in the cell, oxidation of proteins may affect almost all important cell functions and, in view of its wide occurrence, is often used as a diagnostic marker for oxidative stress. Oxidative modifications in proteins (such as oxidation of thiol residues and formation of carbonyl derivatives) caused by oxidative stress due to environmental factors have a special significance in eco-toxicological assessments (Braconi et al. 2011). The ROS-accrued oxidation of proteins in the abiotic-stressed plants will be discussed in the following sections.

Basic mechanisms and estimation strategies

ROS can cause damage (by modifying covalent bonds) and/or inhibit proteins by directly oxidizing amino acids (such as the oxidation of Cys residues to form disulfide bonds, oxidation of Met residues to form Met sulfoxide, and oxidation of Arg, Lys, Pro, and Thr residues) and creating carbonyl groups in the side chains (Berlett and Stadtman 1997; Dean et al. 1997; reviewed by Rhoads et al. 2006) (Fig. 7). However, other protein oxidation-causal events can also be possible as a result of ROS-mediated impairments in the peptide backbone (Dean et al. 1997), protein reactions with both lipid peroxidation products (such as HNE) and with reactive nitrogen species (Sakamoto et al. 2003), and direct ROS interaction with metal cofactors (Verniquet et al. 1991; Flint et al. 1993) (reviewed by Rhoads et al. 2006). The list of major ROS, leading to protein oxidation, includes radical species such as superoxide ($O_2•^-$), hydroxyl (OH•), peroxyl ($RO_2•$), alkoxyl (RO•), hydroperoxyl ($HO_2•$), and non-radical species such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1O_2$), and peroxynitrite ($ONOO^-$) (Berlett and Stadtman 1997; Dean et al. 1997; Dalle-Donne et al. 2003). Owing to very different properties, the ROS can damage protein differently. HO•, generally produced from hydroperoxides through the Fenton reaction, is known to be the most reactive of these species and cause non-specific protein oxidation in contrast to specific one brought about by the other ROS, causing low-oxidation rate (Requena et al. 2001; Davies 2005; Rinalducci

et al. 2008). ROS modify proteins in a variety of direct and indirect ways. Several processes, including nitrosylation or nitration of tyrosine residues, carbonylation of specific amino-acid residues, formation of disulfide cross-links and glycoxidation adducts, and glutathionylation, have been shown to affect protein's activity and subsequently cause direct modification (Davies 2005). Indirect oxidation of proteins can be caused by protein conjugation with the breakdown products of fatty-acid peroxidation (Davies 2005). The ROS attack on specific proteins may cause chemical modifications that may be reversible or irreversible (Ghezzi and Bonetto 2003). Irreversible modifications are responsible for permanent loss of function of the damaged proteins; whereas reversible modifications may have a role in redox regulation (Ghezzi and Bonetto 2003). Examples of protein oxidation leading to irreversible modifications include lysine and arginine carbonylation, tyrosine and tryptophan nitration, dityrosine formation, and protein–protein cross-linking, whereas some other oxidative modifications such as glutathionylation and S-nitrosylation are reversible. The basic principles and major strategies for estimating the irreversible (carbonylation, nitration of tyrosine and tryptophan, protein–protein cross-linking) and reversible (glutathionylation, S-nitrosylation) oxidative protein modifications are described below.

Protein carbonylation involves oxidation of (a) protein backbone, yielding the formation of protein fragments with an N-terminal α-ketoacyl amino acid residue, and (b) some amino-acid side chains (particularly, histidine, arginine, and lysine), yielding ketone or aldehyde derivatives. Additionally, protein carbonylation may also be caused by protein reaction with lipid-peroxidation products (such as 4-hydroxy-2-nonenal) and by protein conjugation with reducing sugars (glycation) or their oxidation products (glycoxidation) (Berlett and Stadtman 1997; reviewed by Ghezzi and Bonetto 2003; Richter et al. 2005). The protein-carbonyl content has been a general indicator and the most commonly used marker of ROS-mediated protein oxidation in abiotic-stressed plants (Qiu et al. 2008; reviewed by Rinalducci et al. 2008) as well as in diseased/stressed animals and humans (Berlett and Stadtman 1997; Chevion et al. 2000; Beal 2002). In plants, cellular compartments such as cytosol, chloroplasts, peroxisomes, nucleus, and mitochondria may exhibit presence of carbonylated proteins (Bartoli et al. 2004; Job et al. 2005; Rajjou et al. 2008). The highest concentration of oxidatively modified proteins has been reported in mitochondria of *T. aestivum* leaves (Bartoli et al. 2004) and legume nodules (Matamoros et al. 2013).

Spectrophotometric quantification of hydrazones, the reaction product of protein-carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH), has been a classical approach for estimating the carbonylated/oxidized proteins (Levine et al. 1994; Reznick and Packer 1994). Additionally, the protein DNPH moieties can also be detected with high sensitivity and specificity, using the Western or slot blotting techniques (Levine et al. 1994). Information on the extent of oxidative damage to a particular protein in a complex mixture can be obtained by a more sensitive and simple method developed by Gladstone and Levine (1994), wherein separation of proteins by gel filtration HPLC follows the spectrophotometric detection of DNP-carbonyl derivatives. A highly sensitive and reproducible method, enzyme-linked immunosorbent assay (ELISA), was developed by Buss et al. (1997) and Winterbourn and Buss (1999), where an anti-DNP antibody is used to measure the total protein CO groups. One- (1D) or two-dimensional (2D) sodium dodecyl sulfate (SDS) gel electrophoresis, followed by Western blot immunoassay (oxyblot), can also be used to assess the carbonyl content in individual proteins (Nakamura and Goto 1996). The specific labeling of carbonylated proteins after 2-DE via biotinylation is considered to be a good strategy to detect carbonylated proteins (Yoo and Regnier 2004). Nevertheless, affinity selection and chromatography at the protein level before proteolysis and mass spectrometric protein identification can also yield significant information on protein carbonylation (Mirzaei and Regnier 2007). The use of 2D gels coupled with peptide fragment fingerprinting approaches based on the matrix-assisted laser desorption/ionization (MALDI-TOF)-MS/MS, or the nano-electrospray liquid chromatography (LC)-MS/MS approaches can be recommended both for separating proteins through quite small variations in the isoelectric point, and detecting the potential post-translational modifications (Rinalducci et al. 2008). Recently, using the dot blot, Wehr and Levine (2012, 2013) developed a method for immunochemical quantitation of carbonylated protein in homogenates or purified proteins. Employing dimethyl sulfoxide as the solvent [to extract proteins efficiently from tissues and keep them soluble, and to dissolve readily the 2,4-dinitrophenylhydrazine and wet polyvinylidene difluoride (PVDF) membranes], they could detect 0.19±0.04 pmol of carbonyl in even 60 ng of protein.

Tyrosine and tryptophan are among amino acids that are most prone to ROS-mediated irreversible oxidation (Berlett and Stadtman 1997; Davies 2003; Stadtman and Levine 2003; Todorovski et al. 2011). Indole ring is the normal target of the ROS attack on tryptophan, where 5-hydroxy-tryptophan (5-HTP), oxindolylalanine (Oia), dioxindolylalanine, *N*-formyl-kynurenine (NFK), kynurenine (Kyn), hydroxykynurenine, and hydroxy-*N*-formyl-kynurenine are produced as the tryptophan-oxidation products (Simat and Steinhart 1998; Garrison 1987). Figure 7 summarizes the commonly observed ROS-mediated modifications of protein amino acids. Owing to incapability of the cellular machinery for their reduction, the above-mentioned oxidation products modulate the function of the protein by changing the local protein structure, since they are not usually degraded by enzymes or the proteasome (Chang et al. 2000) and can also accumulate and aggregate in the cells (Squier 2001). A number of state-of-the-art techniques, such as the reverse-phase

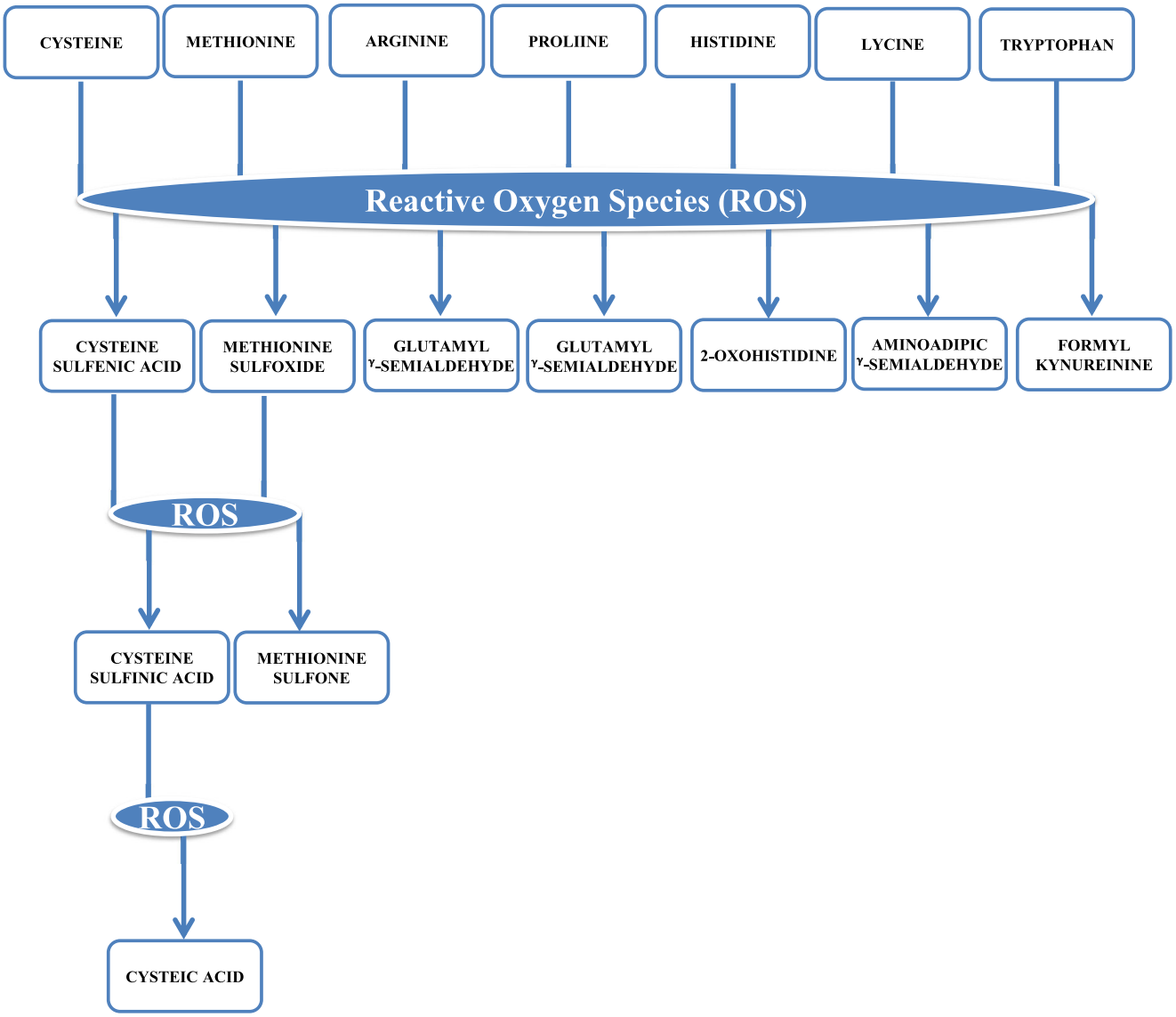

Fig. 7 Commonly observed reactive oxygen species (ROS)-mediated modifications of protein amino acids (based on Møller et al. 2007)

high-performance liquid chromatography (RP-HPLC) combined with UV and fluorescence detection (Simat and Steinhart 1998; Simat et al. 1994) or mass spectrometry (MS) (Van de Weert et al. 1998; Dominques et al. 2003; Szuchman-Sapir et al. 2008; Mouls et al. 2009), are available for efficient analysis of tryptophan oxidation products. In fact, oxidized tryptophan residues, such as 5-HTP, Oia, NFK, and Kyn, have been reported to exhibit different fragmentation characteristics in both MALDI- and ESI-tandem MS that in turn help in their identification by specific marker ions, in addition to their increment masses within a given ion series (Todorovski et al. 2011). Todorovski et al. (2011) were able to distinguish the isomeric 5-HTP- and Oia-containing peptides.

Examples of reversible oxidation of protein include the oxidation of thiol to yield mixed disulfides, such as those with GSH, cysteine, and homocysteine, as well as the formation of intra-/intermolecular protein disulfides (Davies 2005; Eaton 2006). Re-reduction of disulfide bonds can be accomplished by a number of enzymes such as thioredoxin reductase (Konrad et al. 1996; Banze and Follmann 2000) and 2-oxoglutarate dehydrogenase (Møller and Kristensen 2004). Detection of reversible protein oxidation is based on the major principle of relatively reactive nature of thiol group on cysteine residues; hence, many reagents have historically been designed to target it (Fig. 8). Two-dimensional electrophoresis (2DE)-based techniques (with either IEF/SDS or diagonal nonreducing/reducing gel electrophoresis) were extensively applied for reversible protein-oxidation detection (Yano et al. 2001; reviewed by Bykova and Rampitsch 2013). Successive labeling with two different alkylating reagents of distinct

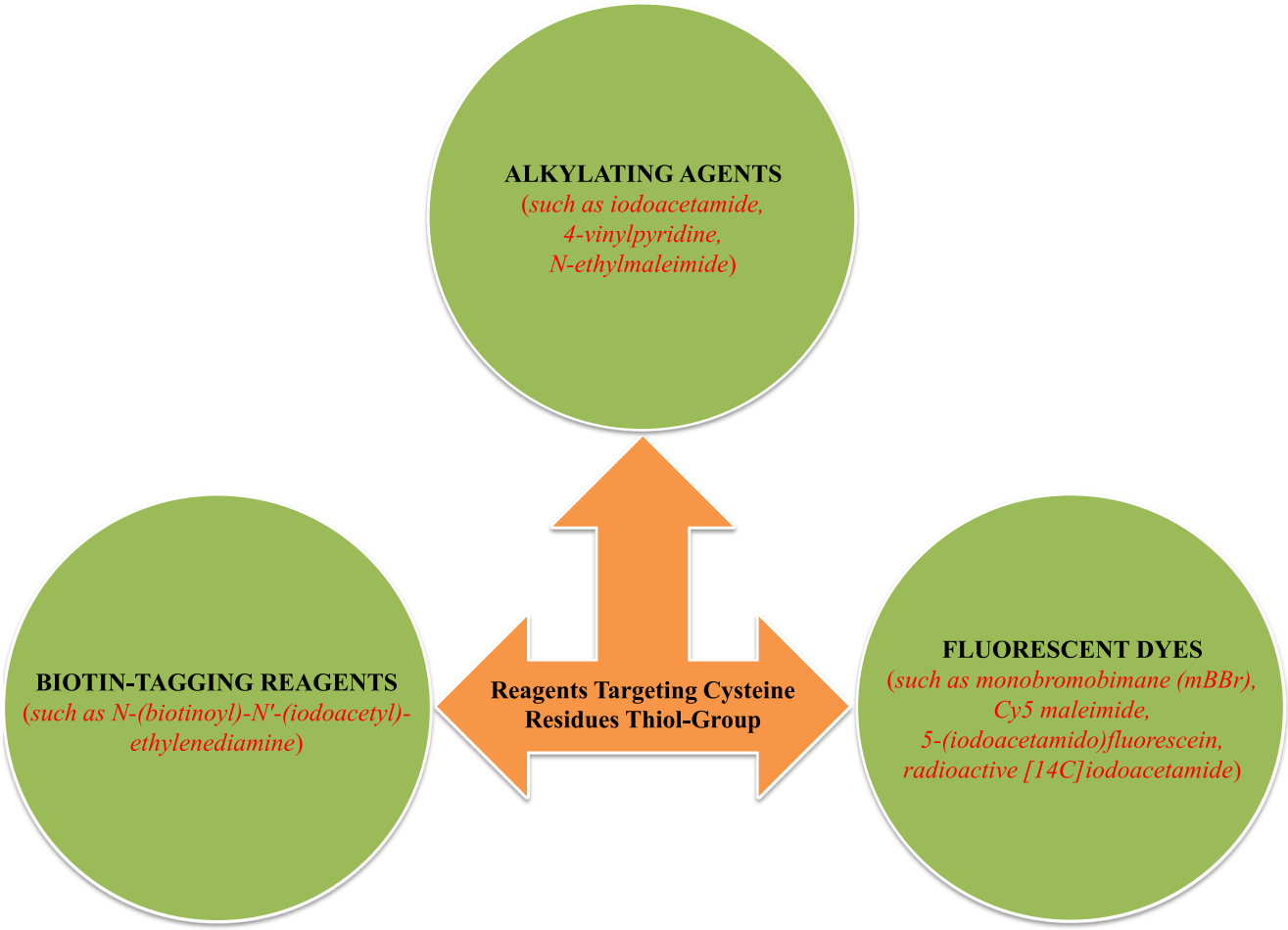

Fig. 8 Major reagents targeting cysteine residues thiol group (based on Bykova and Rampitsch 2013)

molecular mass (namely, IAA and 4-vinylpyridine) was successful in the identification of cysteinyl groups involved in disulfide-bridge formation within the proteins (Maeda et al. 2005). Subunit composition of multi-subunit proteins containing interchain disulfide bonds has also been investigated, employing the diagonal 2DE (Gevaert et al. 2006). Additionally, based on a specific modification reaction between the two subsequent runs on the same reverse-phase column, which alters the retention time of specific peptides, Gevaert et al. (2006) developed a technique, combined fractional diagonal chromatography (COFRADIC), in order to reduce the high complexity of proteome-derived peptide mixtures. Since the charge of a protein is altered in S-glutathiolation, Ghezzi et al. (2001) employed this principle to facilitate the detection of oxidized proteins, using the Western immunoblotting of samples separated by IEF gel electrophoresis.

Protein oxidation vs. abiotic stresses

Extensive reports are available on protein oxidation caused by the abiotic stress via elevated ROS in a range of crop plants (reviewed by Gill and Tuteja 2010; Miller et al. 2010; Foyer and Shigeoka 2011; Sharma et al. 2012). Considering some recent reports on the abiotic stress-mediated protein oxidation, the following subsections critically peruse the experimental results and highlight the basic underlying mechanisms and the major outcomes.

Metals/metalloids

The toxic metal/metalloid-accrued protein oxidation has been reported to constitute a post-translational modification in different plant parts. Several metals, including Cd, Pb, and Hg, have been shown to cause depletion of protein-bound thiol groups (Stohs and Bagchi 1995). Roychoudhury et al. (2012) reported that the progressive increase in $CdCl_2$ concentration increased the carbonylated derivative formation in two rice (*O. sativa*) varieties (IR-29 and Nonabokra), more effectively in the former. The Cd^{2+} treatment raised the carbonylation level from 4 to 5.6 nmol mg^{-1} protein in pea (*P. sativum*) plants, wherein a strong in vitro oxidative treatment generated about 0.3 and 1.7 carbonyl groups per 50 kDa protein molecule. The carbonylation level increased from 6.9 to 16.3 nmol mg^{-1} in the case of peroxisomal protein as a result of Cd^{2+} treatment of the intact plant. These values are higher than those for the whole-plant extracts from control and Cd^{2+}-

treated plants, respectively, which could be the result of a higher local ROS concentration in the peroxisomes (Romero Puertas et al. 2002). The effect of excess Co was also associated with a significant increase in carbonylated proteins in *B. juncea* leaves (Karuppanapandian and Kim 2013), and Al-mediated protein oxidation was reported in *Z. mays* (Boscolo et al. 2003), *H. vulgare* (Song et al. 2011; Achary et al. 2012), *P. algarbiensis* (Martins et al. 2013), and *O. sativa* (Pandey et al. 2013; Bhoomika et al. 2014). Plants differing in metal tolerance may exhibit varied extent of protein oxidation; increase in protein oxidation, expressed by the carbonyl content, was significantly higher in roots of the Al-sensitive *P. algarbiensis* but not in the Al-tolerant *P. almogravensis* (Martins et al. 2013). Recently, a differential variation in the amount of carbonylated proteins in roots as well as shoots was argued as one of the major factors responsible for Al-tolerance in *O. sativa* (Bhoomika et al. 2014). Earlier also, two inbred lines of maize (*Z. mays*), namely, Cat100-6 (Al-tolerant) and S1587-17 (Al-sensitive), were reported to exhibit different levels of reactive carbonyls (Boscolo et al. 2003). The differential protein oxidation in both these lines was argued to involve a site-specific mechanism (Stadtman and Oliver 1991; Stadtman 1992). The potential difference in antioxidant defense has been suggested as another major factor contributing to differences in protein oxidation in the two maize lines. Cu at 2 μM and 5 μM significantly stimulated reactive carbonyl formation in two cyanobacteria, viz., *P. foveolarum* and *N. muscorum*, after 24 h of experiment (Singh et al. 2012). Significant increase in protein oxidation (measured as dityrosine) in leaves with respect to roots was credited to varying Cu-tissue loads as well as activities of antioxidative enzymes in Cu-mine-grown *D. stramonium*, *M. sylvestri*, and *C. ambrosioides* (Boojar and Goodarzi 2007). Differential extent of protein oxidation in two grass species, *E. angustifolium* and *L. perenne*, naturally exposed to soil metals/metalloids (such as As, Cu, Hg, Pb, and Zn) in the vicinity of a chemical industrial complex, was credited to the differential efficiency of ROS-metabolizing system (Anjum et al. 2013). Protein oxidation (in terms of reactive carbonyls) has also been reported recently in environmental-Hg-exposed salt marsh macrophytes *H. portulacoides* (Anjum et al. 2014c) and *J. maritimus* (Anjum et al. 2014d, e).

Drought and salinity

Available literature reflects a differential extent of protein oxidation in plants under drought (Bartoli et al. 2004; Gong et al. 2005; Pyngrope et al. 2013; Tian et al. 2013) and salinity (Melgar et al. 2009; Roychoudhury et al. 2011; Shi et al. 2011; Ferreira-Silva et al. 2012; Tanou et al. 2012). Leaf mitochondria of *T. aestivum* were noted to contain several-folds higher concentrations of oxidatively damaged proteins (protein carbonyls) than the chloroplasts or peroxisomes under drought stress (Bartoli et al. 2004). Gong et al. (2005) reported an increased content of carbonyl groups in the leaves of *T. aestivum* under sodium sulfate (2.11 mmol of kg^{-1} soil)-caused drought stress. Drought-mediated inhibition in the activities of antioxidant enzymes [such as superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR)] and elevated accumulation of H_2O_2 were argued by the authors as a major cause of increased protein decomposition and oxidization. Ferreira-Silva et al. (2012) reported an increased protein oxidation in cashew (*Anacardium occidentale*) leaves under salt stress. When 10-day-old seedlings of a drought-sensitive (Malviya-36) and a drought-tolerant (Brown Gora) cultivars of *O. sativa* cultivars, were subjected to −1.0 and −2.1 MPa water-deficit treatments for 24–72 h, with polyethylene glycol 6000 in the medium, a higher increase in protein carbonyl content, confirmed by the protein gel blot analysis with an antibody against 2,4-dinitrophenylhydrazine, was observed in the seedlings of drought-sensitive cultivar, compared to the tolerant one (Pyngrope et al. 2013).

Drought stress-accrued increase in protein carbonylation was reported in the leaves of mutant (*tasg1*) and wild-type (WT) cultivars of *T. aestivum* (Tian et al. 2013). However, a greater functional stability of thylakoid membrane proteins (measured as protein carbonylation level) in *tasg1* compared with the WT, and the higher antioxidant competence of *tasg1*, was argued to play an important role in the enhanced tolerance of *tasg1* to drought-mediated oxidative stress. The carbonylated derivatives, which accumulated more prominently in M-1–48 and Gobindobhog varieties of *O. sativa* than in the salt-tolerant cultivar Nonabokra during salinity stress, were mitigated by the exogenous application of two polyamines, namely, spermidine and spermine (Roychoudhury et al. 2011). Protein carbonylation, nitration, and S-nitrosylation were involved in acclimation to salinity stress in *Citrus aurantium* (Tanou et al. 2012). In root tips of salt-treated *T. aestivum*, the carbonyl level of total soluble proteins increased together with a gradual increase in activities of the total and 20S proteasome (Shi et al. 2011). In *Olea europaea* cv. Allora, Melgar et al. (2009) evidenced a greater leaf protein oxidation (measured as the total carbonyl content) in shade than in sun leaves; whereas salinity stress (caused by 125 mM NaCl) was reported to enhance the carbonyl-group concentration only in shade leaves. Solar irradiance (15 and 100 %)-mediated differential modulation of antioxidant defense system components [including SOD, ascorbate peroxidase (APX), and CAT] was argued as the major factor for the reported above status of the protein oxidation.

Other abiotic stresses

Information is meager on the protein oxidation in plants under other abiotic stresses such as temperature (low/chilling or high) or elevated CO_2. In a study with maize seedlings,

chilling-induced stress resulted in oxidation of both proteins and lipids (Prasad 1996), and it was subsequently shown that the bundle-sheath-cell proteins had a greater oxidative damage, compared to the mesophyll-cell proteins (Kingston-Smith and Foyer 2000). These studies are consistent with the notion that protein oxidation leads to the loss of function (in this case, impaired photosynthesis and growth) at chilling temperatures. In *A. thaliana* and *G. max*, leaf protein carbonylation often increased when plants were exposed to elevated CO_2 (Qiu et al. 2008); the increased carbonyl content was held to be the cause of the leaf chlorophyll loss and decreased photosynthetic rate under elevated CO_2. A loss of protein function due to O_3 exposure-mediated increased protein carbonylation was reported in *G. max* leaves (Qiu et al. 2008). Reports are also available on the association of visible foliar injuries with enhanced carbonylation of the Rubisco small subunit in bean (*Phaseolus vulgaris*) under exposure to O_3 concentrations (54–108 nL L^{-1} over 7 h day^{-1} for up to 30 days) (Kanoun et al. 2002; Leitao et al. 2003). An association of O_3-mediated decrease in Rubisco activity and net photosynthesis with increased protein carbonylation was evidenced in O_3-sensitive (S156) and O_3-tolerant (R123; R331) *P. vulgaris* cultivars (Booker et al. 2009).

Cross talks, conclusions, and prospects

The peroxidation of bio-membrane PUFAs generates a great diversity of aldehydes, some of which (like MDA) are highly reactive (Esterbauer et al. 1991). Nevertheless, lipid peroxidation may also cause the production of key oxylipins and smaller, lipid-derived reactive electrophile species (Alméras et al. 2003). Lipid peroxidation products may react with and eventually deteriorate proteins, other lipids, and nucleic acids (Rhoads et al. 2006). Furthemore, MDA and HNE can form adducts and damage proteins. Most importantly, the conjugation of aldehyde with protein can bring indirect oxidation of proteins (secondary protein carbonylation) (Yamauchi et al. 2008; Møller et al. 2011). Nevertheless, the oxidative decomposition of PUFA initiates chain reactions that lead to the formation of a variety of carbonyl species (three to nine carbons in length), the most reactive and cytotoxic being the α, β-unsaturated aldehydes (4-hydroxy-trans-2-nonenal and acrolein), di-aldehydes (malondialdehyde and glyoxal), and keto-aldehydes (4-oxo-trans-2-nonenal) (Suzuki et al. 2010). Considering the potential mechanism underpinning the MDA-mediated protein oxidation, it is hypothesized that, owing to its high reactivity, the biological effect of MDA is due to its chemical attachment to proteins. In a recent in vitro experiment, using model proteins (such as BSA and Rubisco) and methylesters of C18 PUFAs (major components of plant biomembrane), the MDA generated from peroxidized linolenic acid was suggested to be the major cause of protein modification in heat-stressed *A. thaliana* and *S. oleracea* (Yamauchi et al. 2008). Peroxidation of the isolated *S. oleracea* thylakoid membrane at 37 °C led to MDA-mediated modification of an oxygen-evolving complex 33 kDa protein (OEC33). The light-harvesting complex protein was modified by MDA under illumination in heat-stressed *Arabidopsis* plants, whereas this modification was not observed in linolenic acid-deficient mutants (*fad3fad7fad8* triple mutant), suggesting that linolenic acid is a major source of protein modification by MDA in the heat-stressed plants. The proteins can be damaged in oxidative conditions by their reactions with other lipid-peroxidation product, such as 4-hydroxy-2-nonenal, that may be formed by a paraquat or cold or drought treatment of plants (Millar and Leaver 2000; Taylor et al. 2005). Nevertheless, oxidative stress-caused elevation in HNE (α, β-unsaturated aldehyde) has been related with modified proteins in the mitochondria (Winger et al. 2005).

The abiotic-stress-induced generation of the ROS and their reaction products upsets the delicate redox balance in plant cells, which in turn causes cellular damage that can be marked by analyzing lipid peroxidation and protein oxidation products. On the one hand, lipid peroxidation is one of the major effects of ROS on cell membranes, where MDA has been extensively used to evaluate the abiotic-stress-induced oxidative damage to lipids (Hou et al. 2007; Upadhyay and Panda 2009, 2010; Giannakoula et al. 2010; Pereira et al. 2010; Wu et al. 2013). On the other hand, the ROS and their reaction products-mediated covalent modification of protein lead to the oxidation of proteins, which in turn may affect almost all important functions of the cell. Protein thiolation involving the participation of sulfur-containing amino acids is a reversible process, whereas most of the other protein-oxidation processes are irreversible (Ghezzi and Bonetto 2003). Protein carbonylation, the most frequent as well as the best-studied irreversible protein oxidation, serves as a good indicator of oxidative stress in stressed plants because more stringent oxidation conditions are required for the formation of carbonyl groups (ketones and aldehydes) than for the reversible oxidation of thiols or lipid peroxidation (Davies 2005; Møller et al. 2007, 2011). Plants exhibiting their differential sensitivity to abiotic stresses exhibit a differential extent of the lipid and protein oxidation. This difference may be due to the difference in an interrelated network of physiological and molecular mechanisms and/or the efficiency of ROS-scavenging via the antioxidant defence system (Gill and Tuteja 2010; Braconi et al. 2011; Foyer and Shigeoka 2011; Anjum et al. 2012, 2013, 2014c, d; Krasensky and Jonak 2012; Matamoros et al. 2013).

Peroxidation of lipids may not always be the cause of cell death; in fact, it is a late event of metal-induced cell death, as enunciated by Pan et al. (2001). These authors considered the loss of integrity of the plasma membrane due to lipid

peroxidation as just one of the morphological characteristics during the cell death. In addition, the fact that lipid peroxidation is the primary cellular target of (metal/Al-accrued) oxidative stress cannot always be true and may vary depending on the plant species. In this context, the absence of lipid peroxidation in *Z. mays* under Al exposure was quite intriguing (Boscolo et al. 2003). In addition, the absence of a significant increase in MDA level in the root tip of Al-tolerant (UFRGS17) oat (*Avena sativa*) genotype in comparison to Al-sensitive (UFRGS930598) genotype is also important (Castilhos et al. 2011). It was argued for the first case that in Al-exposed *Z. mays*, ROS (such as H_2O_2) might not be able to attack the Fe^{2+} bound to the membrane. However, Cakmak and Horst (1991) and Yamamoto et al. (2001) evidenced the exposure of these sites for the catalysis of OH• formation in *G. max* and *P. sativum,* respectively. Furthermore, the absence of lipid peroxidation but the presence of protein oxidation and cell death in the same plant under Al-exposure suggests the involvement of other pathways in the oxidative-stress-mediated induction of cell injury (Boscolo et al. 2003). The reduced level of lipid peroxidation was corroborated by less ROS production (the cause of lipid peroxidation) because of an efficient antioxidant defense system in Al-tolerant *A. sativa* genotypes (Castilhos et al. 2011). It is important to highlight here that, though the non-enzymatic lipid oxidation (mainly via ROS) is usually viewed as deleterious, but recent evidence suggests that during stress, both lipid peroxidation (and also reactive electrophile species) generation occurs frequently in cells and can benefit cells in many ways. The work of Farmer and Mueller (2013) can be consulted for further elaboration.

Both the lipid and protein oxidations due to metal stress can also be influenced by some other factors such as the UV-B radiation-fluence rates. The low UV-B-fluence rate (0.1 μmol m^{-2} s^{-1}) was evidenced to decrease significantly the level of lipid peroxidation (measured as MDA) and protein oxidation (measured as reactive carbonyls), mainly due to a low ROS level (Singh et al. 2012). However, this conclusion needs to be ratified by exhaustive molecular/genetic studies. It may be mentioned that lipid oxidation has been studied more extensively than protein oxidation in abiotic-stressed plants. Additionally, deep molecular insights into the ability of plants to repair ROS and their reaction-products-accrued oxidation of lipids and proteins are still lacking, albeit the impacts of metals/metalloids on lipid and protein oxidation have received greater attention than those of other abiotic stresses. In order to gain further in the understanding of abiotic-stress-accrued oxidative stress in plants, future research should be planned (a) to identify and characterize the ROS-mediated specified oxidized lipids and proteins and elucidate the biochemistry, physiology, and molecular biology of the mechanisms involved, underpinning the potential impact of the products of lipid/protein oxidation on other biomolecules and/or cell organelles, and (b) to unveil the interplay among the oxidation products of lipids and proteins. In addition, most of the available information on "lipid and protein oxidation" has stemmed from studies performed under controlled laboratory conditions. However, environmental stress conditions can have a diverse impact on plant growth, metabolism, and productivity under field conditions, where plants are faced with a simultaneous exposure to more than one abiotic and/or biotic stresses (Cramer et al. 2011; Malecka et al. 2014; Suzuki et al. 2014). Thus, a comparative account of experiments conducted under field condition as well as controlled laboratory condition will be interesting as well as rewarding.

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