Oxidative stress is a complex chemical and physiological phenomenon that accompanies virtually all biotic and abiotic stresses in higher plants and develops as a result of overproduction and accumulation of reactive oxygen species (ROS). This review revises primary mechanisms underlying plant oxidative stress at the cellular level. Recent data have clarified the ‘origins’ of oxidative stress in plants, and show that apart from classical chloroplast, mitochondrial and peroxisome sources, ROS are synthesized by NADPH oxidases and peroxidases. ROS damage all major plant cell bio-polymers, resulting in their dysfunction. They activate plasma membrane Ca2+-permeable and K+-permeable cation channels as well as annexins, catalyzing Ca2+ signaling events, K+ leakage and triggering programed cell death. Downstream ROS-Ca2+-regulated signaling cascades probably include regulatory systems with one (ion channels and transcription factors), two (Ca2+-activated NADPH oxidases and calmodulin) or multiple components (Ca2+-dependent protein kinases and mitogen-activated protein kinases). Intracellular and extracellular antioxidants form sophisticated networks, protecting against oxidation and ‘shaping’ stress signaling. Research into plant oxidative stress has shown great potential for developing stress-tolerant crops. This can be achieved through the use of directed evolution techniques to prevent protein oxidation, bioengineering of antioxidant activities as well as modification of ROS sensing mechanisms.

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1. Introduction

Virtually all environmental and biotic stresses trigger a generalised stress response called an oxidative stress which can damage cell components and cause their dysfunction. This is induced by over-production and accumulation of molecules containing activated oxygen and called ‘reactive oxygen species’ (ROS). The reasons causing an oxidative stress mainly include: (i) an imbalance between ROS generation and detoxification due to disturbance of ‘normal’ cell physiology; (ii) ROS biosynthesis de novo as a constituent part of stress signaling and immunity response needed for defense and adaptation. These mechanisms co-exist, because stress factors directly producing ROS (transition metals, ultraviolet or ozone) additionally stimulate ROS generation by NADPH oxidases and peroxidases (Rao et al., 1996; Ranieri et al., 2001) and a number of other processes that are not primarily related to stress or oxidation.

This review summarises and evaluates classical and some new concepts in the field of plant oxidative stress and ROS metabolism. A particular emphasis of this review is on the chemistry of individual ROS, cell and membrane mechanisms leading to ROS generation, amplification and regulation of ROS-mediated signals and programmed cell death.

2. Definitions

The term ROS embraces substances containing one or more activated atoms of oxygen but are not necessarily radicals (for example H₂O₂ is not a radical). Free radicals are any chemical species that exist independently and contain unpaired electron(s). Some free radicals do not have oxygen atoms (for example, transition metals or carbon-centered radicals). Both ROS and free radicals promote oxidative stress through oxidation of cell compounds. The term ‘oxidative stress’ has several meanings. Firstly, it is the ‘physiological state’ (or conditions) when loss of electrons (oxidation) exceeds gain of electrons (reduction) leading to chemical (oxidative) damage of cell compounds. Oxidative stress is therefore associated with severe and long-term redox (reduction/oxidation) imbalance due to the lack of electrons. Secondly, it is one of ‘stress factors’ (similar to salinity, drought and others) damaging cells and triggering signaling and defence reactions. These definitions are related and can be combined.

In most cases oxidative stress starts from the activation of triplet oxygen (O₂). This makes O₂ more active or ‘reactive’; therefore it is also often defined as the stress caused by ‘reactive oxygen species’, ‘reactive oxygen intermediates’, ‘oxygen-derived species’, ‘free oxygen radicals’ etc. Reactive nitrogen species (RNS) are another important class of substances potentially involved in oxidative stress (in this case, sometimes called ‘nitrosative stress’). However experimental data are insufficient yet to understand the mechanisms of plant nitrosative stress; therefore this will not be discussed here in greater detail.

3. Chemistry of oxidising species

Oxygen is the most abundant element in the Earth’s crust (Guido, 2001). Comprising about 89% of the mass in H₂O, oxygen is also the most abundant (by mass) element in living organisms. It is the second most powerful oxidiser known in chemistry after fluorine, which is far rarer than oxygen (Renda et al., 2004; Dowling and Simmons, 2009). Major atmospheric form of oxygen is O₂. This molecule has two unpaired electrons (O₂*) and can exist as a free molecule; therefore it is a free radical. Both electrons in O₂ have the same spin numbers or ‘parallel spins’ which limit (restrict) the number of O₂ targets to those that have two similar electrons with antiparallel spins. This phenomenon is called a ‘spin restriction’ and decreases the reactivity of O₂. O₂ is not very chemically active and not toxic to aerobic organisms. To ‘acquire’ higher reactivity, O₂ requires an input of energy to remove the spin restriction. This energy comes from a number of chemical and biochemical reactions, highly energised electrons in electron transport chains (ETC), ultraviolet, ionising irradiation etc. Among many ROS found in biological systems, singlet O₂ (¹O₂), superoxide radical (O₂−), hydrogen peroxide (H₂O₂), superoxide radical (O₂−), and nitric oxide (NO) (Fig. 1) are crucially important for induction of oxidative stress (Apel and Hirt, 2004). A number of other ROS could also be involved, such as peroxyl, alkoxyl and hydroperoxyl radicals, peroxinitrite, ozone and hypochlorous acid.

3.1. Superoxide radical

3.1.1. Chemistry of superoxide and measurements of superoxide production in plants

Triplet oxygen (O₂) can lose its ‘spin restriction’ by accepting a single electron, for example, due to the ‘leak’ of electrons in plant ETC or functioning of NADPH oxidase. This leads to formation of O₂− which is more reactive than O₂. It is called ‘superoxide anion radical’, ‘superoxide radical anion’, ‘superoxide radical’ or just ‘superoxide’. The half-life of superoxide is typically from 1 to 1000 µs which only allows it to diffuse for few micrometers from site of the generation (Kavdia, 2006). O₂− participates in a
number of reactions, but the prevalent will be the reaction with H⁺ giving hydroperoxyl radical HO_2⁻. The latter molecule is more reactive, more stable and hypothetically permeable through biological membranes. Two molecules of HO_2⁻ react and give O_2 and H_2O_2 (known as 'superoxide dismutation' reaction). The ratio O_2⁻/HO_2⁻ increases with pH (1:1 at pH 4.8, 10:1 at pH 5.8 and 100:1 at pH 6.8, respectively) (Sawyer and Gibian, 1979; Ross, 1985). This points to a special importance of HO_2⁻ at lower pHs, for example in the apoplastic space, mitochondrial intermembrane space, chloroplast thylakoid lumen, growing cell walls, vacuoles, peroxisomes and lysosomes. O_2⁻ predominantly acts as a reducing agent; however HO_2⁻ more often functions as an oxidant. O_2⁻ cannot chemically modify biological macromolecules but it is a major 'origin' of an oxidative stress. It can reduce other radicals and ROS (Fe^{2+}/Cu^{2+}, NO, phenoxyl radical, Fe-S clusters, etc.), leading to formation of strong oxidants (Sawyer and Gibian, 1979; Ross, 1985; Halliwell and Gutteridge, 1999). O_2⁻ reduces Fe^{3+} and Cu^{2+} to Fe^{2+} and Cu⁺ respectively, which interact with H_2O_2 to produce OH⁻ (this ROS modifies virtually all organic molecules and is central to oxidative stress) (Pryor and Squadrato, 1995). O_2⁻/HO_2⁻ pair also reacts with NO⁺, giving the extremely reactive RNSs, peroxynitrite (ONOO⁻) and alkyl peroxinitrite (ROONOO⁻) respectively (Squadrato and Pryor, 1998). Peroxynitrite decomposes to hydroxyl radicals (Pryor and Squadrato, 1995). Interaction between O_2⁻ and NO⁺ probably happens in plant cells (Delledonne et al., 2001; del Río et al., 2002, 2003, 2006).

Depending on preparations and techniques used (Table 1), O_2⁻ generation varies in kinetics, duration and intensity; but it accompanies plant responses to major stresses, such as salinity, drought, hypo- and hyperthermia, heavy metals, UV and others. O_2⁻ modifies virtually all organic molecules and is central to oxidative stress hormone (Kawano et al., 1998); but sometimes it takes a special importance of HO_2⁻ at lower pHs, for example in the apoplastic space, mitochondrial intermembrane space, chloroplast thylakoid lumen, growing cell walls, vacuoles, peroxisomes and lysosomes. O_2⁻ predominantly acts as a reducing agent; however HO_2⁻ more often functions as an oxidant. O_2⁻ cannot chemically modify biological macromolecules but it is a major 'origin' of an oxidative stress. It can reduce other radicals and ROS (Fe^{2+}/Cu^{2+}, NO, phenoxyl radical, Fe-S clusters, etc.), leading to formation of strong oxidants (Sawyer and Gibian, 1979; Ross, 1985; Halliwell and Gutteridge, 1999). O_2⁻ reduces Fe^{3+} and Cu^{2+} to Fe^{2+} and Cu⁺ respectively, which interact with H_2O_2 to produce OH⁻ (this ROS modifies virtually all organic molecules and is central to oxidative stress) (Pryor and Squadrato, 1995). O_2⁻/HO_2⁻ pair also reacts with NO⁺, giving the extremely reactive RNSs, peroxynitrite (ONOO⁻) and alkyl peroxinitrite (ROONOO⁻) respectively (Squadrato and Pryor, 1998). Peroxynitrite decomposes to hydroxyl radicals (Pryor and Squadrato, 1995). Interaction between O_2⁻ and NO⁺ probably happens in plant cells (Delledonne et al., 2001; del Río et al., 2002, 2003, 2006).

3.1.2. Sources of superoxide in stressed plants

It is widely believed that the major source of O_2⁻ and contributor to oxidative stress in plants is an 'electron leakage' in ETCs of chloroplasts and mitochondria (Smarinoff, 1993; Apel and Hirt, 2004; Lesser, 2006; Möller et al., 2007; Möller, 2001; Rinalducci et al., 2008; Takahashi and Badger, 2011). 1–5% of electrons can be 'lost' in ETCs and some of these electrons seem to target and activate O_2 with formation of O_2⁻ (Möller, 2001). O_2⁻ biosynthesis is a common feature of organelles in non-stressed plants, but this can increase under stress, producing more O_2⁻ than antioxidants can detoxify. Sites for 'electron leakage' are demonstrated on the Fig. 2 and are found in photosystem I (Asada, 2006) and photosystem II (Pospíšil et al., 2004), in addition to mitochondrial complexes I and III (reviewed in Möller, 2001; Rinalducci et al., 2008; Hirst et al., 2008; they are not discussed in details here).

EPR spectroscopy tests have demonstrated that pheophytin (pheo.), primary quinone acceptor (Q_a), and cytochrome b559 are probably all able to reduce O_2 leading to the formation of O_2⁻ at the electron acceptor side of photosystem II (Fig. 2) (Ananyev et al., 1994; Cledan and Grace, 1999; Pospíšil et al., 2004, 2006). O_2⁻ is additionally produced in photosystem II by one-electron oxidation of H_2O_2 at the electron donor side (Chen et al., 1992, 1995). However, photosysytem I is considered to be a major site for O_2⁻ generation in chloroplasts (Genty and Harbinson, 1996; Asada, 2006; Foyer and Noctor, 2009). In photosystem I, O_2⁻ is probably synthesised by the 4Fe–4S complexes (clusters X) on psaA and psaB or A/B on psaC at the electron accepting (stromal) side (Asada, 1999). Specialised antioxidant enzymes function in organelles (as well as everywhere in the cell) to prevent oxidative stress (Smarinoff, 2005; Foyer and Noctor, 2009). In chloroplasts, O_2⁻ undergoes superoxide dismutase-catalysed disproportionation to O_2 and H_2O_2, which is then detoxified by ascorbate peroxidases (ascorbate + H_2O_2 → dehydroascorbate + H_2O) (Asada, 2006). Mitochondrial O_2⁻ is also converted by superoxide dismutase (SOD) to O_2 and H_2O_2 but is mainly detoxified by glutathione peroxidases, however catalase and enzymes of so-called ascorbate–gluthathione cycle could also be involved in some tissues (Foyer and Halliwell, 1976; Möller, 2001).

Under pathogen attack, salinity, Cd²⁺, herbicides and xenobiotics, large amount of O_2⁻ is produced in peroxisomes, although normally these organelles function in photosynthesis (where producing and detoxifying H_2O_2, oxidation of fatty acids, metabolism of nitrogen compounds and ROS detoxification (del Río et al., 2006; Reumann and Weber, 2006). Two mechanisms of O_2⁻ generation have been found in peroxisomes. First mechanism is driven by xanthine oxidase in the cellular matrix. The second one is catalysed by NADH/NADPH-dependent small ETC in the peroxosomal membrane comprising NADH:Ferricyanide reductase, cytochrome b, monodehydroascorbate reductase and NADPH:cytochrome P450 reductase and producing O_2⁻ in the cytosol (del Río et al., 2002, 2006; Lopez-Huertas et al., 2000). Stresses stimulate O_2⁻ generation leading to H_2O_2 accumulation in peroxisomes and cytosol by yet unknown pathway. They also decrease the activity of peroxisomal antioxidative defence systems (del Río et al., 2006).

SOD is a major O_2⁻ – scavenging system in peroxisomes (del Río et al., 2006).
Table 1
Production of superoxide radicals in responses to major stresses.

<table>
<thead>
<tr>
<th>Stress</th>
<th>Object</th>
<th>Treatment</th>
<th>Organ</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>Pisum sativum</td>
<td>Long-term</td>
<td>Leaf</td>
<td>Hernández et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>Nicotiana tabacum</td>
<td>Short-term</td>
<td>Suspension culture</td>
<td>Kawano et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>Arabidopsis thaliana</td>
<td>Short-term</td>
<td>Root</td>
<td>Demidchik et al. (2003)</td>
</tr>
<tr>
<td>Pathogens</td>
<td>Hordeum vulgare</td>
<td>Long-term</td>
<td>Leaf</td>
<td>Huckleboven et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Arabidopsis thaliana</td>
<td>Short-term</td>
<td>Root</td>
<td>Demidchik et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Triticum aestivum</td>
<td>Long-term</td>
<td>Leaf</td>
<td>Giovannini et al. (2006)</td>
</tr>
<tr>
<td>Drought</td>
<td>Triticum aestivum</td>
<td>Short-term</td>
<td>Leaf</td>
<td>Mencioni et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>Helianthus annuus</td>
<td>Long-term</td>
<td>Leaf and root</td>
<td>Selotea et al. (2003)</td>
</tr>
<tr>
<td>High light</td>
<td>Arabidopsis thaliana</td>
<td>Short-term</td>
<td>Leaf</td>
<td>Fryer et al., 2003</td>
</tr>
<tr>
<td>Hyperthermia</td>
<td>Arabidopsis thaliana</td>
<td>Long-term</td>
<td>Leaf</td>
<td>Lee et al. (2002)</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>Phaseolus vulgaris</td>
<td>Long-term</td>
<td>Leaf</td>
<td>Dong et al. (2009)</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>Lycopersicum esculentum</td>
<td>Long-term</td>
<td>Leaf</td>
<td>Sgherri et al. (1996)</td>
</tr>
<tr>
<td>Herbicides</td>
<td>Triticum aestivum</td>
<td>Long-term</td>
<td>Leaf</td>
<td>Wanga et al. (2008)</td>
</tr>
</tbody>
</table>

Long-term and short-term are >1 h and <1 h, respectively.

2002, 2006). H₂O₂ produced during the HO₂•− dismutation reaction is probably detoxified through ascorbate-glutathione cycle (Foyer and Halliwell, 1976), similar to other plant cell compartments (del Rio et al., 2003; Reumann and Weber, 2006). Peroxisomes can also be involved in the control of plant antioxidant levels and enzymes repairing oxidised cell components (Reumann et al., 2007).

High light stress and ultraviolet, many xenobiotics and herbicides directly modify chloroplast, mitochondria or peroxisome ETCs, leading to O₂•− generation. For these stresses, ETC electron leakage is definitely a prime cause of oxidative stress. However, for other stresses, such as salinity, pathogen attack, wounding, drought, hyperthermia, heavy metals, ozone, hypoxia, aluminum and other important stresses, disturbance of ETCs seems to be a secondary process occurring in the later stages of stress response (nevertheless, they may still play very important role). This probably develops as a result of decreased antioxidant pool that is exhausted during early (primary) oxidative burst. A central mechanism for these stresses is O₂•− generation due to increased activities of NADPH oxidases and extra- and intracellular peroxidases (Doke, 1983; Bolwell et al., 2002; Foreman et al., 2003; Torres and Dangl, 2005; Torres et al., 1998, 2005; Bindschedler et al., 2006; Demidchik et al., 2003, 2009; Fluhr, 2009; Chang et al., 2012; Steffens et al., 2013).

NADPH oxidase or peroxidase generated ROS can be distinguished by the sensitivity to the NADPH oxidase inhibitor diphenylene iodonium (DPI) (Foreman et al., 2003; Fluhr, 2009). NADPH oxidase is blocked by 1–10 μM DPI while peroxidases are inhibited by one to two orders higher levels of DPI. Additionally, peroxidases (but not NADPH oxidases) are sensitive to azide and cyanide (Halliwell and Gutteridge, 1999; Bindschedler et al., 2006). One cautionary note should be made about the use of these pharmacological techniques. DPI is dissolved in 1–3% dimethyl sulfoxide, which is a radical scavenger affecting adequate ROS measurements; therefore a proper control tests with dimethyl sulfoxide should always be carried out. It should also be noted that a number of more specific blockers of NADPH oxidase have been developed (Williams and Griendling, 2007).

The function of NADPH oxidase in ROS promoted stress reactions, signaling, survival or death, is conserved among Kingdoms (Kawahara et al., 2007; Fluhr, 2009; Jiang et al., 2011). NADPH oxidase is encoded by the Respiratory Burst Oxidase Homologues (RBOH) gene family. This includes ten genes in Arabidopsis (AtRBOH-A-J) and nine in rice; it also exists in all sequenced plant genomes (Groom et al., 1996; Torres et al., 1998; Torres and Dangl, 2005; Kawahara et al., 2007; Wong et al., 2007; Jiang et al., 2011). A reverse genetics approach and ‘over-expression’ of RBOH genes have demonstrated the relation between specific NADPH oxidase homologues (their activity and expression level) and reactions induced by different stresses (reviewed by Apel and Hirt, 2004; Torres and Dangl, 2005; Fluhr, 2009). For example, increase of AtRbohD and AtRbohF expression is required for oxidative burst induced by pathogenic Pseudomonas syringae or Hyaloperonospora parasitica, (Torres et al., 2002). The mechanism of this specificity is yet to be understood.

![Fig. 2. Hypothetical sites of superoxide generation in photosystems I and II.](image-url)
The relationship between NADPH oxidase structure and function has recently been characterised (Kawahara et al., 2007; Sumimoto, 2008; Fluhr, 2009). The transfer of electrons in NADPH oxidase is catalysed by C-terminal cytoplasmic superdomain that is homologous to the ferredoxin reductase. It includes the NADPH-binding and FAD-binding sites which transfer the electron to the N-terminal six transmembrane segments containing the di-heme system (Kawahara et al., 2007; Sumimoto, 2008). Di-heme reacts with O$_2^-$ producing O$_2$•− at the apoplastic side of the plasma membrane. NADPH oxidase is not functional in the absence of Ca$^{2+}$. The activation by Ca$^{2+}$ is stucturally related to the N-terminus of plant NADPH oxidase containing two Ca$^{2+}$-binding helix-loop-helix structural domains (EF-hands) which are similar to Ca$^{2+}$-binding domains in calmodulin and troponin-C. Binding of Ca$^{2+}$ causes a conformational change and intramolecular interaction of the N-terminal Ca$^{2+}$-binding domain with the C-terminal superdomain, resulting in the activation of electron transfer (Bänfi et al., 2004). Half-maximal activation of model NADPH oxidase (animal Nox5; in vitro) is caused by 1 µM Ca$^{2+}$ (Bänfi et al., 2004). Maximal stress induced [Ca$^{2+}$]$_{cyt}$ increases are in the range from 0.3 to 3 µM, fitting well to this number (Demidchik et al., 2003, 2009; Hetherington and Brownlee, 2004; Demidchik and Maathuis, 2007).

Sensitivity to Ca$^{2+}$ increases after calmodulin binding to the NADPH-binding domain or phosphorylation of serine/threonin residues in the FAD-binding domain by protein kinase C (Kobayashi et al., 2007; Tirone and Cox, 2007). Calmodulin-like domain protein kinases (CDPKs) stimulate NADPH oxidases through phosphorylation (Xing et al., 2001; Wu et al., 2010). For example, an increased CDPK expression causes elevation of plant NADPH oxidase activity, which is abolished by protein phosphate 2A and unaffected by protein phosphatase 1 (Xing et al., 2001). Small G proteins (Rac/Rac GTases) increase NADPH oxidase activity in Ca$^{2+}$-dependent manner (Baxter-Burrell et al., 2002; Wong et al., 2007).

Ca$^{2+}$-activated NADPH oxidase works in concert with ROS-activated Ca$^{2+}$-permeable cation channels to generate and amplify stress-induced Ca$^{2+}$ and ROS signals (reviewed by Demidchik and Maathuis, 2007) (Fig. 3). Elevation of cytosolic [Ca$^{2+}$] causes an increase in O$_2$•− production and vice versa, O$_2$•− activates Ca$^{2+}$ influx through ROS-activated cation channels (Demidchik et al., 2009; Demidchik and Maathuis, 2007; Demidchik, 2010). This self-amplification mechanism, likely being an upstream component for many stresses may catalyse amplification and encode weak stimuli (transducing them into dramatic O$_2$•− –Ca$^{2+}$ alterations). For example, a marker of wounding stress, an extracellular ATP (Dark et al., 2011), acts through O$_2$•− –Ca$^{2+}$ signal amplification cycle (Demidchik et al., 2009). Initial small transient ATP-induced increase in [Ca$^{2+}$]$_{cyt}$ results in the production of ROS, which in turn induces massive activation of Ca$^{2+}$-permeable cation channels (Demidchik et al., 2009).

Systems that control [Ca$^{2+}$]$_{cyt}$, such as Ca$^{2+}$-permeable nonselective cation channels (NSCCs), depolarisation- and hyperpolarisation-activated Ca$^{2+}$ channels, Ca$^{2+}$-ATPase, Na$^+$/Ca$^{2+}$ and Ca$^{2+}$/H$^+$ exchangers, cytosolic Ca$^{2+}$-binding proteins and endomembrane Ca$^{2+}$ transporters can potentially regulate the NADPH oxidase activity. These systems are modulated by a number of regulatory enzymes, for example specialised kinases and phosphatases, as well as chemical/physical regulators (pH, hormones etc.). This maintains fine oxidative balance and generate adequate O$_2$•− – Ca$^{2+}$ responses, which encode information about individual stress factors. The spatial distribution of the O$_2$•− –Ca$^{2+}$ system within a cell is also regulated. For example, SCN1/ArthoGDI1 RhoGTPase GDP dissociation inhibitor is capable of “focusing” O$_2$•− production by ArthoHc to specific zones of the cell (Carol et al., 2005). This explains greater ROS-Ca$^{2+}$ responses in young elongating parts of the cell (such as tips of root hairs) in response to various stimuli (Demidchik et al., 2003, 2007, 2009, 2010; Foreman et al., 2003). Having four-dimensional system (X-Y-Z-time) O$_2$•− –Ca$^{2+}$-mediated signals have high complexity and diversity, which is probably necessary for simultaneous interaction with a multitude of environmental cues.

Although some studies suggest that NADPH oxidase activation is not ubiquitous, virtually all stress-factors tested were able to produce O$_2$•− burst through this mechanism (Fluhr, 2008). This has been shown for a number of plant species, all organs and tissues, calluses, suspensions and protoplasts (Foreman et al., 2003; Apel and Hirt, 2004; Torres and Dangl, 2005; Demidchik et al., 2009; Fluhr, 2009; Dubiella et al., 2013). The analysis of the available data suggests that NADPH oxidase activity during stress is mainly required for the following physiological functions: (a) recognition of stress factor and its intensity (to adjust gene expression and metabolism for adaptation); (b) triggering the programmed cell death (to defend against pathogens and some abiotic stresses); (c) stomatal closure during drought stress response; and (d) simultaneous “processing” stress, immunity, defence and developmental chemical signals (abscisic acid, ethylene, brassinosteroids, auxin, gibberellic acid, methyl jasmonate, salicylic acid, volatiles etc.). NADPH oxidase and Ca$^{2+}$-permeable cation channels form a “regulatory hub”, which is responsible for perception, transduction and encoding of stress stimuli (Demidchik and Maathuis, 2007; Fig. 3).

3.2. Hydorgen peroxide

3.2.1. Chemistry of H$_2$O$_2$ and its role in oxidative stress

Hydrogen peroxide (H$_2$O$_2$, HOOH; Fig. 2) is the most stable ROS with essential physiological functions (Halliwell and Gutteridge, 2007; Apel and Hirt, 2004; Foyer and Noctor, 2009). H$_2$O$_2$ is a weak acid without unpaired electrons (“non-radical”), and it is stable molecule as compared to superoxide, hydroxyl and singlet oxygen. Nevertheless, the lifetime of H$_2$O$_2$ in living tissues is not tremendously long (<1s) due to activities of catalases and peroxidases that decompose this substance (Halliwell and Gutteridge, 2007). In contrast to cytoplasm, which is a highly-reduced and antioxidant-enriched alkaline compartment, extracellular space is acidic and normally does not contain high levels of H$_2$O$_2$ scavenging enzymes, catalases and peroxidases, as well as superoxide dismutase removing its precursor (Hernández et al., 2001; Mhamdi et al., 2012). This low activity of enzymatic ROS scavengers promotes H$_2$O$_2$ accumulation in the apoplasm, promoting oxidative stress.

The cytoplasm is typically a thin layer because the vacuole occupies up to 95% of cell volume; therefore endogenously produced H$_2$O$_2$ may rapidly diffuse outside, presumably crossing the plasma membrane through aquaporins (Dynowski et al., 2008). Another source of H$_2$O$_2$ is directly from the apoplasm, where it is produced through dismutation of O$_2$•−/HO$_2$•− synthetized by NADPH oxidases and extracellular heme-containing Class III peroxidases (Apel and Hirt, 2004; Cosio and Dunand, 2009). A number of other non-radical organic peroxides (ROOR, ROOH or RO2OH) can be produced in the cell, such as lipid peroxides. These compounds promote oxidative stress in different ways, for example in lipid peroxidation chain reactions, and probably also involved in ‘shaping’ the intra- and extracellular redox signal (Møller et al., 2007; Suzuki et al., 2012).

3.2.2. Measurements of H$_2$O$_2$ production in plants

Although quantitative test of unstable oxygen-centered radicals is meaningless, [H$_2$O$_2$] measurement provides useful information on the development of oxidative stress. Standard techniques to test [H$_2$O$_2$] include application of colorimetric, fluorescent and luminescent probes, which, if oxidised by H$_2$O$_2$, change their spectral characteristics or emit photons (Rhee et al., 2010). These probes can be membrane-permeant or -impermeant; hence they allow differentiate with H$_2$O$_2$ content in the cell and apoplasm. They are
Fig. 3. Involvement of plasma membrane in the regulation of oxidative stress in higher plants.

not 100% specific to H$_2$O$_2$ and, in fact, interact with other ROS (Rhee et al., 2010). The analysis of reports dealing with H$_2$O$_2$ shows that H$_2$O$_2$ concentration increases from 0.03–1 μM (“at rest”) to 0.1–10 mM (“under stress”) depending on preparation, stress factor, technique and other variables. Huge variation in the kinetics and amplitude of H$_2$O$_2$ accumulation has also been reported. Detectable [H$_2$O$_2$] can be found from a few seconds to several days after the application of stress factor. This difference in results can be caused by a number of reasons. H$_2$O$_2$ is probably produced in specific areas (hot spots), for example in tips of growing root hairs (Foreman et al., 2003); meaning total tissue/organism H$_2$O$_2$ activity can be very low. Most techniques used for H$_2$O$_2$ measurements show the cumulative effect of H$_2$O$_2$ production and cannot monitor H$_2$O$_2$ dynamics. Chemiluminescent probes report H$_2$O$_2$ faster and they are more sensitive than fluorescent probes. Physical and chemical conditions used for H$_2$O$_2$ detection, such as high pH values (pH 8–9), can alter the cell potency to generate ROS. H$_2$O$_2$ measurements can also be affected by the structure of plant tissues. For example, leaf cuticula is virtually impermeable for H$_2$O$_2$ probes. Finally, the nature, exposure time and intensity of the imposed stress vary dramatically across studies.

More efficient methods for measuring H$_2$O$_2$ have been recently developed (Rhee et al., 2010; Michelet et al., 2013). Boronate-based fluorescent probes (‘peroxysensor family’ of H$_2$O$_2$ probes) fluoresce after the H$_2$O$_2$-induced removal of a boronate group (Miller et al., 2005). They are membrane-permeant and can be combined with SNAP-tag, which targets them to different cell compartments to assess [H$_2$O$_2$] spatially. Although these probes do not interact with other ROS, they react with H$_2$O$_2$ irreversibly and cannot test [H$_2$O$_2$] dynamics (Dickinson et al., 2010). Another group of probes utilizes specially modified green fluorescent proteins (GFP) which include two redox-sensitive cysteine residues (Rhee et al., 2010). H$_2$O$_2$ induces formation of di-sulfide bond and modifies spectral characteristics of protein fluorescence. However, this probe lacks specificity to H$_2$O$_2$. The latest addition to fluorescent H$_2$O$_2$ probes is a genetically encoded and reversible “hydrogen peroxide sensor” (HyPer) (Malinouski et al., 2011). The design of HyPer is based on bacterial OxyR transcription factor (Kim et al., 2002) with a yellow fluorescent protein inserted into the regulatory domain of this molecule (Belousov et al., 2006). H$_2$O$_2$ acts on OxyR regulatory domain and causes the formation of a di-sulfide bond between C199 and C208 which changes the spectral properties of YFP (Kim et al., 2002; Belousov et al., 2006). This di-sulfide bond can be repaired by thiol-disulfide oxidoreductase in vivo; therefore HyPer can react with H$_2$O$_2$ again (Belousov et al., 2006). Formation of oxidised HyPer shows the dynamics of H$_2$O$_2$ in individual cell compartments (Malinouski et al., 2011). Another approach for detection of [H$_2$O$_2$] involves peroxalate nanoparticles, which undergo a three-component chemiluminescent reaction between H$_2$O$_2$, peroxalate esters and fluorescent dyes inside the cell (Lee et al., 2007a,b). This technique is probably sensitive and specific to H$_2$O$_2$, however the delivery of nanoparticles and their potential intracellular redox activity may cause problems. Michelet et al. (2013) have recently developed novel EPR spectroscopy-based techniques to measure extracellular H$_2$O$_2$ content in cells of Chlamydomonas reinhardtii. These authors have used a spin-trapping assay containing 4-POBN/ethanol/Fe-EDTA. Fe-EDTA reacts with H$_2$O$_2$ forming *OH which is trapped by 4-POBN/ethanol (giving stable detectable adduct).

3.2.3. Sites of H$_2$O$_2$ generation and “targets” of H$_2$O$_2$ in plant cells

Sites of superoxide productions are widely considered as “origins” of H$_2$O$_2$ generation. Peroxidases were first proposed to be involved in the generation of H$_2$O$_2$ during biotic stress (Bolwell...
O₂ → O₂⁻ \rightarrow e⁻ + 2H⁺ \rightarrow H₂O₂ → OH⁻ + OH⁺ \text{ (I)}

Me^{2+} + H₂O₂ \rightarrow Me^{3+} + OH⁻ + OH⁺ \text{ (II)}

Me^{2+} + O₂⁻ \rightarrow Me^{3+} + OH⁻ + OH⁺ \text{ (III)}

Fig. 4. Reactions synthesising hydroxyl radical in plants. I. – reduction of oxygen leads to formation of hydroxyl radicals. II. – outer-sphere electron transfer in classical Fenton-like reaction (when transition metals do not bind covalently to H₂O₂). III. – inner-sphere electron transfer that involves strong peroxide binding to a transition metal.

and Wojtaszek, 1997; Bolwell et al., 1998; Bindschedler et al., 2006). Now it is widely accepted that some class II peroxidases, for example Cu-containing amino oxidases and polyamine oxidases, gluthathione and ascorbate oxidases are also important for ROS production induced by salinity, high light, heavy metal and other abiotic stresses (Rodríguez et al., 2002, 2007; Chang et al., 2009). Interestingly, peroxidases are probably regulated by a negative feedback mechanism; some of them are inhibited by H₂O₂ (Kitajima, 2008).

Oxidative stress is widely believed to be induced by H₂O₂. It is not exactly true because *OH biosynthesis is required to accomplish H₂O₂-mediated oxidation. The biosynthesis of H₂O₂ is difficult to relate directly with distinct physiological functions; hence why, in a number of studies, H₂O₂ (0.01–10 mM) is added to plant cells exogenously and the induced reactions are investigated. H₂O₂ is a weak oxidant and cannot chemically modify DNA, amino acids or lipids. It may interact directly with SH groups but its main target is transition metal binding sites converting it to OH⁻ (Fry et al., 2002; Fry, 2004). H₂O₂ has been reported to deactivate some enzymes, for example fructose biphosphate; however, this may be caused by residues of transition metals in experimental solutions (Halliwell and Gutteridge, 1999). Most proteins withstand 100 mM H₂O₂ in transition metal-free solutions (Halliwell and Gutteridge, 1999). A number of H₂O₂ ‘sensors’ have been proposed to exist in plant cells (Apel and Hirt, 2004), however only a few of them have been proven by experiments.

3.3. Hydroxyl radical

The hydroxyl radical (*OH; Fig. 1) is a prime cause of oxidative damage to proteins and nucleic acids as well as lipid peroxidation during oxidative stress. It is directly involved in oxidative stress signaling and PCD (Demidchik et al., 2003, 2010). For example, this ROS induces the greatest activation of Ca²⁺ and K⁺ channels amongst all known ROS and free radicals, leading to Ca²⁺ influx and K⁺ efflux immediately after application of a stress factor (Demidchik, 2010). The estimated ‘in vivo’ half-life of *OH is approximately 1 ns, which allows *OH diffusion over very short distances (<1 nm) (Sies, 1993). Second-order rate constants for reactions of *OH with most organic molecules are extremely high. *OH interacts with cell biopolymers are limited by diffusion-controlled rate (Anbar and Neta, 1967; Sies, 1993). Specific *OH scavengers and antioxidants do not exist and the widely reported effects of mannitol, sorbitol, dimethyl sulfoxide, thiourea or others are, in fact, not due to *OH scavenging. These substances probably interact with *OH ‘longer-living’ precursors or chelate transition metals.

Fenton reaction(s) is central to *OH bio-synthesis (Fig. 4). Study conducted by Fenton in 19th century aimed to establish effect of Fe²⁺ on tartaric acid (Fenton, 1894). However his findings were so important for natural sciences that they have been used for more than a century. Nowadays, “Fenton chemistry” and “Fenton-like reagents” are referred to reactions taking place in the presence of H₂O₂ and transition metals and producing *OH, water and superoxide (Goldstein et al., 1993). Although many intermediates are formed, the net “Fenton reaction” is as follows:

(I) metal reduced + H₂O₂ → metal oxidised + *OH + OH⁻; (II) metal oxidised + H₂O₂ → metal reduced + H₂O₂ + *H₂O₂ + H⁺ (Koppenol, 2001). These equations were proposed by Fritz Haber (Nobel Prize winner in 1918) and his student Joseph Weiss in the 1930s and since called the Haber-Weiss cycle (Haber and Weiss, 1932). Importantly, ascorbic acid is likely to serve as a pro-oxidant reductant for iron and copper in Haber-Weiss cycle in plants, because its concentration in plants (including the apoplastic) is very high (1–20 mM) (Fry et al., 2002; Foyer and Noctor, 2011). This pro-oxidant activity of the ascorbate is poorly studied and sometimes ignored.

*OH can also be generated by homolytic bond fission of H₂O. In this case, electrons in covalent bonds are equally distributed to atoms. This requires significant input of energy by ultraviolet quanta, freezing–drying cycle, heat or ionizing radiation (Halliwell and Gutteridge, 1999). The generation of *OH from H₂O₂ though has a lower energy threshold therefore, under some natural conditions, *OH can probably be directly generated from H₂O₂ (HOOH → *OH + *OH) and hydroperoxides (ROOH → RH + *OH) by sunlight (Downes and Blunt, 1879). This process is called “photo Fenton”. The physiological importance of *OH produced by homolytic fission is still debated, apart from studies on UV stress where it is directly involved in oxidative destruction of cell components (Jain et al., 2004; Kataria et al., 2005).

In classical Fenton-like reactions metals do not bind covalently to H₂O₂ (outer-sphere electron transfer; Fig. 4; reaction II). Another mechanism of *OH generation that involves strong peroxide binding to a metal center has been shown in biological systems over the last two decades (Fig. 4; reaction III) (Sawyer et al., 1993, 1996; Fridovich, 1998; Pospíšil et al., 2004). This is the so-called “inner-sphere electron transfer” process forming a temporary covalent bond between peroxide and metal ion. Heme oxygenases (Ortiz de Montellano, 1998), cytochrome P450 (Sono et al., 1996), bleomycin (Burger, 2000), superoxide reductases (Mathe et al., 2002) and some PSII proteins (Pospíšil et al., 2004) are examples of systems, which 'use' this mechanism of radical generation.

Measurement of *OH is a problem due the extreme reactivity of *OH and its very short lifetime. Nevertheless, several studies have successfully undertaken such measurements. A number of techniques for *OH detection has been developed, including colorimetric, fluorescent, luminescent and radioactively-labeled probes (Halliwell and Gutteridge 1999). However, it seems that EPR spectroscopy is only the technique providing specificity of *OH measurement (Liszkay et al., 2003, 2004; Jain et al., 2004; Renew et al., 2005; Demidchik et al., 2010; Michelet et al., 2013; Šeršen and Kráľová, 2013). EPR spectrometry is also not an ‘ideal’ method because *OH spin traps (DMPO, EMPO, DEPMPO or POBN) decompose at room temperature (so, the signal intensity drops) and react with other radicals, for example with superoxide (Pou et al., 1989). Moreover EPR spectroscopy cannot be used efficiently for studying inner tissues. Nevertheless, EPR-based techniques are still far more sensitive and accurate than widely used imaging approaches.

EPR analysis allowed the assessment of *OH production in a single root of Arabidopsis thaliana (Renew et al., 2005). This study has clearly demonstrated that the root produces *OH without stress that is probably required for ROS-Ca²⁺-driven cell elongation (Demidchik et al., 2003; Foreman et al., 2003) and cell wall remodeling (Liszkay et al., 2004). This production depends on the activity of NADPH oxidase RbohC and the presence of transition metals in the cell wall. Nevertheless, over-production of *OH, for example resulting from UV-B irradiation, has been shown to block elongation growth in plants (Jain et al., 2004; Kataria et al., 2005). Salt-induced *OH production in intact plants has been demonstrated in Arabidopsis thaliana roots (Demidchik et al., 2010). This production results in K⁺ loss through *OH-activated K⁺ efflux channels and leads to PCD via K⁺-dependent cell death proteases and endonu-
cleases (Demidchik et al., 2010) (Fig. 3). Thus *OH over-production can be the source of severe stresses and plant cell death.

The importance of *OH over-production for destructive changes in photosynthetic apparatus is widely acknowledged (Møller et al., 2007; Šeršen and Královičová, 2013), but this has only recently been confirmed by direct EPR spectroscopy tests (Pospíšil et al., 2004; Šnyrčová et al., 2006; Pospíšil, 2008). Under stress conditions, *OH is generated by both photosystems. In PSI, leakage of electrons leads to biosynthesis of superoxide (Fig. 2) that dismutates to $\text{H}_2\text{O}_2$, which in turn accumulates in stroma. $\text{H}_2\text{O}_2$ can be reduced to *OH by free transition metals in stroma (classical Fenton-like reaction; Fig. 4) or by formation of $\text{H}_2\text{O}_2$–Fe complexes of ferredoxin (inner-sphere electron transfer) (Šnyrčová et al., 2006; Pospíšil, 2009). In PSI, three transition metal binding sites are probably involved in *OH production from $\text{H}_2\text{O}_2$ (Pospíšil et al., 2004; Pospíšil, 2009).

Firstly, Fenton-like reaction of $\text{H}_2\text{O}_2$ with free transition metals can occur in stroma; secondly, non-heme iron (the ligand is unknown) could be involved through inner-sphere electron transfer; and, thirdly, heme-iron of cyt b$_{559}$ could also participate in similar reaction and form Fe–$\text{H}_2\text{O}_2$ complexes. Overall, this can result in an oxidative damage of chloroplasts and their dysfunction.

The production of *OH for signaling needs could be related to the presence in the cell of the specific chemical sites (metal pockets), which bind catalytically active transition metal cations, such as Cu$^{2+}$–Fe$^{2+}$3$^+$ or Mn$^{2+}$–3$^{1+}$ (Demidchik et al., 2014) (Fig. 3). These sites usually contain pairs of cysteine and histidine forming a complex with transition metal (Demidchik et al., 2014). Intriguingly, Demidchik et al. (2007) have demonstrated that the activation of single-channel conductances by $\text{H}_2\text{O}_2$ requires the delivery of $\text{H}_2\text{O}_2$ directly to the channel macromolecule at the extracellular side of the plasma membrane. Supporting this hypothesis, Rodrigo-Moreno et al. (2013) have recently found that Cu$^{2+}$ acts on K$^+$ efflux at the cytosolic side of the plasma membrane.

3.4. Singlet oxygen

The activation of $\text{O}_2$ in chloroplasts and mitochondria, for example through absorption of light quanta in P680 (Asada, 2006), can lead to the formation of two types of extremely reactive $\text{O}_2$-derived species: non-radical $1\Sigma^+$ $\text{O}_2$ ($22.4 \text{kcal}$) and the more reactive free radical $1\Sigma^+$ $\text{O}_2$ ($37.5 \text{kcal}$) (Schweitzer and Schmidt, 2003). The term ‘singlet oxygen’ traditionally covers both species. $1\Sigma^+$ $\text{O}_2$ can decay into $1\Delta^+$ $\text{O}_2$, but the significance of this process in the cell is not proven. Singlet oxygen is detoxified rapidly at the cytosolic side of the plasma membrane.

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3.5. Transition metals

Transition metals and their complexes with S-containing amino acids and some other organic ligands, are major redox switches in living systems (Outten and Theil, 2009; Robinson and Winge, 2010). IUPAC defines transition metal as any element with an incomplete d sub-shell, or which can give rise to cations with an incomplete d sub-shell (http://goldbook.iupac.org/). Forty elements (21 to 30, 39 to 48, 71 to 80, and 103 to 112) fit in this definition and can be considered as transition metals (McCleverty, 1999). However quite few transition metals have demonstrated significance for biological systems. From the physiological point of view, the most important transition metals are Cu, Fe and Mn. Although Ni, Hg, Cr and Co can also be involved in some metabolic reactions, they are clearly less important for cell physiology. Cu and Fe are the most abundant transition metals in living systems (Outten and Theil, 2009; Robinson and Winge, 2010). Their role as electron transport components in most redox enzymes is based on their ability to change their valence more easily as compared to other metals found in cells (Bergmann, 1992; Robinson and Winge, 2010). Mn and Ni show very similar properties to Cu and Fe, when coordinated by some organic ligands (for example by His residues); however they almost completely lack electron transfer capacity in free ionic form.

The catalytic activity of Cu and Fe may increase several times during stress conditions (Becana and Klucas, 1992; Moran et al., 1994, 1997; Becana et al., 1998). The toxic and regulatory effects of Cu and Fe are mainly related to *OH generation. Cu is well-known eco-toxicant (Bergmann, 1992). After application of Cu-containing fungicides or in Cu mining areas, Cu directly affects plants, causing an oxidative stress, lesions, inhibition of metabolic reactions, suppression of photosynthesis, mineral disorders, loss of membrane integrity and cell death (Bergmann, 1992; Demidchik et al., 1997, 2001).

Cu is about 60 times more potent as a catalyst of the Haber-Weiss cycle and several billion times more soluble than Fe under biological pH range, but Fe is more abundant in the cell (Bergmann, 1992; Halliwell and Gutteridge 1999; Fry et al., 2002). Taking this into account, Cu as a major catalyst of *OH generation seems to be much more probable (Fry et al., 2002; Demidchik et al., 2003). Cu and Fe are typically bound in organic complexes with carbohydrates and proteins; hence their catalytic activity should be considered instead as a concentration of free ionic forms. Moran et al. (1997) discovered that specific Fe-chelating phenolic compounds can be synthesised in response to stresses that increase Fe catalytic activity, promote *OH generation, triggering DNA damage and lipid peroxidation. Another potential mechanism of Cu and Fe “mobilization” and increase of their catalytic activities during stresses is through formation of complexes with polyamines such as spermine, spermidine and putrescine. These substances have been shown to be synthesised in plant cells in response to stresses (Alčzár et al., 2006; Moschou et al., 2009; Shi and Chan, 2014) and proven to form redox-active complexes with both Cu (Guskos et al., 2007) and Fe (Tadolini, 1988). Thus, $\text{H}_2\text{O}_2$-induced damage and signaling are focused on Cu- and Fe-binding centres that generate *OH (Demidchik et al., 2007, 2014). Cu and Fe may also bind to specific targets in proteins, such as His–pockets or Cys–pockets and cause their oxidation, leading to damages (Demidchik et al., 2014). There is accumulating evidence that oxidative stress would not be possible without Fe and Cu as removal of these metals (using specific chelators or decreasing accumulation) results in termination of oxidative burst (Sayre et al., 2008; Demidchik et al., 2014).
4. ROS-mediated signaling and regulation of plant cell physiology

A number of models for ROS sensing and signaling in plant cells have been proposed (Apel and Hirt, 2004; Asada, 2006; Demidchik and Maathuis, 2007; Foyer and Noctor, 2005; Sierla et al., 2013; Demidchik et al., 2014). Potential ROS receptors include the following: (A) two-component histidine kinases; (B) redox-sensitive transcription factors, such as NPR1 or Heat Shock Factors; (C) ROS-sensitive phosphatases; (D) redox-regulated ion channels (reviewed by Apel and Hirt, 2004; Demidchik and Maathuis, 2007; Sierla et al., 2013; Demidchik et al., 2014). The role of first three mechanisms requires investigation while the direct involvement of ion channels in ROS sensing has recently been studied in details.

The changes of ion activities through modulation of ion channel/receptor conductance are the fastest and most important switch of physiological and biochemical parameters in animal cells responding to external and internal factors including oxidative stress. It involves at least two mechanisms: (A) a change in ionic composition modifying metabolic interactions, as ions are direct regulators of enzymes of osmotic pressure; (B) changing the electric potential difference across the membrane which can modulate activities of active transporters, such as H+-ATPase, and functioning of vesicular transport and cytoskeleton. Evidence obtained in vitro using recombinant protein techniques show that plant G-proteins (modulators of signaling cascades and ion channels) are directly regulated by ROS (20 μM H2O2) via stimulation of alpha subunit dissociation (Wang et al., 2008). Nevertheless, this observation requires confirmation by tests performed in vivo. The activity of several plant cation channels have been shown to be modified in the presence of high [H2O2] showing that ion channels can be involved in the perception of H2O2-encoded messages. Exogenous H2O2 activates Ca2+-permeable non-selective cation channels in protoplasts isolated from Arabidopsis thaliana guard cells (Pei et al., 2000). These channels are probably involved in ABA- and jasmonate-induced stomata closure (Munemasa et al., 2011). Exogenous H2O2 inhibits K+ outwardly rectifying channels in guard cells and root epidermal cells (Demidchik et al., 2003; Kohler et al., 2003), but it does not change the activity of Ca2+--, K+-, Cl--selective channels and NSCCs in green algae Nitella flexilis or Arabidopsis thaliana root plasma membrane NSCCs when applied in the whole cell configuration (Demidchik et al., 1997, 2001, 2003, 2007). Exogenous H2O2 stimulates anion efflux in cultured Arabidopsis thaliana cells, mimicking the ABA effect (Trouverie et al., 2008). However, this action seems to be related to the activation of Ca2+- conductance, which in turn activates Cl--currents. Endogenously applied H2O2 activates Arabidopsis thaliana root Ca2+-permeable NSCCs (Demidchik et al., 2003, 2007). This activation is observed only in outside-out mode when H2O2 was delivered closely to the plasma membrane cytoplasmic side. The direct voltage-dependent activation of Arabidopsis thaliana plasma membrane K+ channel SKOR (heterologously expressed in HEK cells) by H2O2 has been recently discovered (García-Mata et al., 2010). Cys-168 residing in S3 alpha-helix of SKOR voltage sensor complex is responsible for the sensitivity to H2O2.

• OH action on ion channel mediated activities has been investigated by the addition of a •OH-generating mixture (Cu2+, l-ascorbic acid and H2O2) to protoplasts and intact cells (Demidchik et al., 2003, 2007, 2010; Foreman et al., 2003). These experiments have shown that •OH activates Ca2+-permeable NSCCs (Ca2+ influx channels) and K+ outwardly rectifying channels (catalysing K+ efflux) in mature root epidermal cells, elongation zone, pericycle cells, cortex and root hairs of Arabidopsis thaliana. •OH-induced activation of Ca2+ influx and K+ efflux has also been observed in mature and young root epidermis of maize, clover, pea, wheat and spinach (Demidchik et al., 2003). Gork-1 probably encodes •OH-activated K+ efflux conductance while genes encoding •OH-activated Ca2+-permeable channels have not been yet identified. Both •OH-induced Ca2+ and K+ conductances could also be related with activities of annexins, as they are decreased in KO line lacking these systems (Laohavisit et al., 2012). Moreover, Ca2+/K+ transporting annexins might include functional peroxidase domain producing ROS (Laohavisit et al., 2008).

Activation of K+ efflux channels by •OH (for example, during response to salinity) leads to massive loss of K+ from root cells (Demidchik et al., 2003, 2010, 2014). Animal endonucleases and caspases are directly inhibited by cytosolic K+; therefore, cation channel mediated K+ loss stimulates these enzymes and induces apoptosis (Seon and Ja-Eun, 2002). Similar mechanism of programmed cell death induction has been discovered in plants (Demidchik et al., 2010; Fig. 3). Root cell endonucleases and pro-caspase activates in K+-dependent mode. This causes symptoms typical of PCD. K+ channel antagonists or radical scavengers stop or delay K+ loss and the appearance of PCD symptoms (Demidchik et al., 2010). Interestingly, expression of animal anti-apoptotic CED-9 gene can decrease the H2O2-induced K+ efflux from leaf segments (Shabala et al., 2007). This suggests that plant and animals share similar K+-mediated mechanisms of apoptosis-like PCD.

In animal cells, singlet O2 generation leads to inhibition of the mitochondrial inner membrane K+ influx channels which regulate mitochondrial volume (Duprat et al., 1995, Fornazari et al., 2008). This results in cytochrome c release and transporting superoxide anion (produced in mitochondria during stresses) to the cytosol. Whether similar reactions take place in plants is unknown. The involvement of singlet O2 in ion channel activation could be through H2O2, which can accumulate after singlet oxygen detoxification in organelles and diffuse to the cytosol where it activates Ca2+ and triggers signaling cascades, for example sending ROS/Ca2+ messages to the nucleus. The latter is called retrograde signaling and might play an essential role in the regulation of organelle protein biosynthesis under high light and probably other environmental stresses (Fernández and Stranda, 2008; Chang et al., 2009; Karpiński et al., 2013; Kim and Apel, 2013).

Major physiological effect induced by ROS-induced activation of Ca2+-permeable cation channels is a transient elevation of cytosolic free Ca2+ (Demidchik and Maathuis, 2007; McKain and Pittman, 2009; Fig. 4). Elevation of [Ca2+]<sub>i</sub> links H2O2 accumulation and intracellular signaling and gene expression (Demidchik, 2010; Fig. 3). It activates downstream Ca2+-dependent regulatory cascades through Ca2+-binding proteins (CBPs). Approximately 250 CBP genes exist in Arabidopsis thaliana genome (Day et al., 2002). CBPs undergo reversible interaction with Ca2+ that leads to their conformational change and facilitates interactions with a number of cell targets (Gifford et al., 2007; Dubiella et al., 2013). Classical CBPs contain ‘EF-hand’ motifs providing high-affinity binding of Ca2+. Five classes of CBPs include: calmodulins, calmodulin-like proteins, Ca2+-dependent protein kinases (CDPKs), calcineurin B-like proteins and NADPH oxidases. CDPKs directly transduce ROS-Ca2+ signals to catalytic activity while the calmodulins, calmodulin-like proteins, and calcineurin B-like proteins play in intermediate sensors regulating downstream systems, which in turn react with final target or other regulators. NADPH oxidases, as mentioned above, enhance weak Ca2+ signals, amplifying these signals using ROS biosynthesis de novo and ROS-activated Ca2+ influx channels. Apart from calmodulins and NADPH oxidases that exist in animals, the other three CBP classes are only found in plants and probably other bacteria. These three classes of CBPs are found in all plant cell organelles and involved in a multitude of functions (DeFalco et al., 2010).

The activity and expression level of certain CBPs increase in the presence of elevated ROS concentrations, and in response to biotic and abiotic stresses which generate ROS (DeFalco et al., 2010).
2010). For example, H₂O₂ causes fast induction of CDPK3 in Arabidopsis root suspension culture protoplasts, leading to the change of the expression of some stress-responsive genes and activity of 28 target proteins (Mehlmer et al., 2010). Stress hormones (abscisic and jasmonic acids) also stimulate CDPKs (Munemasa et al., 2011). This effect probably relies on ROS-Ca²⁺ signaling induced by these hormones. CDPKs are involved in the regulation of specific NADPH oxidases (such as AtRBOHD, SrRBOH and others) via Ca²⁺-dependent phosphorylation of these proteins (Kobayashi et al., 2007; Dubiella et al., 2013). This is another amplification mechanism of ROS-Ca²⁺ signaling.

ROS have been shown to change the activity of a number of other regulatory enzymes, in particular specific kinases and phosphatases such as MAP kinases and other Ser/Thr kinases, MAPK phosphatases etc. (reviewed by van Breusegem et al., 2008; Pitzschke and Hirt, 2009; Rodriguez et al., 2010). The mechanism by which this regulation occurs is unclear as well as not much being known about the downstream targets. In some cases, researchers do not know the nature of reactions catalysed by proteins involved in the redox regulation. For example it was demonstrated that EXECUTER 1 and 2 control the singlet oxygen induced retrograde signaling from chloroplasts to nucleus. They regulate stress-specific gene expression and this process is dependent upon enzymatic lipid peroxidation. However the mechanism by which these proteins function has yet to be indentified (Lee et al., 2007a,b; Przybyla et al., 2008; Kim and Apel, 2013). It is unlikely that many kinase/phosphatase-triggered cascades form primary components/sensors of ROS-mediated signaling. They probably serve as long-term downstream metabolic and genetic adjustment elements (switches) integrating ROS signaling into the cellular context and helping plants to adapt or make a decision for PCD. Some kinase/phosphatase systems lie upstream of ROS production providing stimulation feedback loop (van Breusegem et al., 2008).

ROS-Ca²⁺ signaling is not restricted to cytoplasm and may exist in the nucleus (Mazars et al., 2010), chloroplasts (Johnson et al., 1995) and mitochondria (Logan and Knight, 2003), where stress induced Ca²⁺ and ROS transients have been measured. These reactions could play the role of regulators of genomes in the nucleus and the organelles, although the exact their functions have not yet been identified (Mazars et al., 2010; Kim and Apel, 2013).

Some plant transcription factors and their regulators can probably play the role of ROS sensors (Despres et al., 2003; Hong et al., 2013). For example, TGA1 transcription has two specific Cys residues (Cys-260 and Cys-266) which enable its binding to NPK1 regulator (Despres et al., 2003). Oxidation of these Cys groups leads to loss of this interaction and so regulates transcription factor binding to DNA. Heat shock transcription factors may also be involved in direct ROS sensing (Miller and Mittler, 2006; Hong et al., 2013). They regulate transcription of various defense-related genes and are active during stresses including oxidative stress (Miller and Mittler, 2006). Similar systems in fungi (Hahn and Thiele, 2004; Hong et al., 2013) and animals (Ahn and Thiele, 2003) sense H₂O₂ by two Cys residues; but this mechanism has yet to be confirmed in plants.

5. Mechanisms of oxidative damage

5.1. Oxidation of lipids

Oxidative stress causes reversible or irreversible (causing complete loss of some physiological activities) modifications of bio-molecules such as proteins, polynucleic acids, carbohydrates and lipids (Sies and Cadenas, 1985; Møller et al., 2007; Farmer and Mueller, 2013). Among these, oxidation of lipids is particularly dangerous because it propogates free radicals through so-called 'chain reactions'. Lipid oxidation (also known as lipid peroxidation) is widely considered as a “hallmark” of oxidative stress (Farmer and Mueller, 2013).

The lipid peroxidation usually includes three sequential stages: initiation, propagation, and termination (Catalá, 2006; Farmer and Mueller, 2013). Initiation of lipid peroxidation (initiation stage) is triggered by hydrogen atom abstraction form the lipid molecule. This can be caused by hydroxyl, alkoxyl, peroxyl radicals as well as peroxyxinitrite but not by hydrogen peroxide or superoxide (Halliwell and Gutteridge, 1999). H⁺ is abstracted from methylene group (-CH₂−) giving –CH⁻ or lipid radical (L•), which is a carbon centered radical. Phospholipids (most abundant membrane lipids) are susceptible to radicals and peroxidation because the double bond in the fatty acid weakens the C–H bond and facilitates H⁺ abstraction. L• can activate O₂ and form an oxygen centered ‘lipid peroxyl radical’ (LOO•), which in turn is capable of abstracting H⁺ from a neighboring fatty acid to produce a lipid hydroperoxide (LOOH) and a second lipid radical (L•) (Catalá, 2006). This gives a rise to the propagation phase. LOOH can undergo ‘reductive cleavage’ by reduced transition metals (mainly Fe²⁺ or Cu⁺) and form lipid alkoxyl radical (LO), which is also reactive and induces further abstraction of H⁺ from neighboring fatty acid. Another important mechanism of lipid peroxidation is via direct reaction of double bonds with singlet oxygen from PSII reaction centre, which gives LOOH (Krieger-Liszka et al., 2008; Przybyla et al., 2008; Farmer and Mueller, 2013). Singlet oxygen is also formed in reaction of two LOO• molecules.

Severe lipid peroxidation leads to the damage of membranes, collapse of their barrier function, followed by disintegration of organelles, oxidation and dysfunction of proteins, DNA and RNA (Halliwell and Gutteridge, 1999; Farmer and Mueller, 2013). Terminal products of lipid peroxidation are ‘aggressive’ substances, such as aldehydic secondary products (malondialdehyde, 4-hydroxy-2-nonenal, 4-hydroxy-2-hexenal and acrolein), which are markers of oxidative stress (Del et al., 2005; Farmer and Mueller, 2013). They are easy to measure, for example using classical thiobarbituric acid assay for malondialdehyde (Hodges et al., 1999) or more modern sensitive mass spectrometry-based techniques that can identify individual lipid species targeted by peroxidation and to study the chemical complexity of oxidative products formed due to this process (reviewed by Shulaev and Oliver, 2006; Farmer and Mueller, 2013).

5.2. Modification of proteins

ROS can oxidise any proteinogenic amino acid (Halliwell and Gutteridge, 1999; Møller et al., 2007; Avery, 2011). Such modification to this key organic component of life results in loss of a given protein-mediated function, such as specific metabolic, structural, transport or regulatory activities. Protein oxidation also results in accumulation of toxic protein aggregates, and in the case of severe damage, induces PCD (Demidchik et al., 2010; Avery, 2011). Major ROS-induced modifications to amino acids are summarised in Table 2.

Products of lipid peroxidation, such as 4-hydroxyxenonenal and malondialdehyde have been shown to react and oxidise a number of amino acids (such as lysine or histidine) (Table 2). Møller et al. (2007) have recently reviewed mechanisms of oxidative damages to the most important plant protein complexes, such as photosystem I, D1 protein of photosystem II, ribulose-1,5-bisphosphate carboxylase/oxygenase and SOD. With an exception of D1 protein, which is probably affected by singlet oxygen, these damages require the presence or increase of activity of catalytically-active transition metals (catalysing biosynthesis of ‘OH).

Reversibility of protein oxidation reactions in vivo is not well understood due to a lack of analytical tools for studying protein
structure inside the cell and hence the existing data is mainly based on in vitro analyses. It is believed that most types of protein oxidation damage are irreversible with an exception of S-containing amino acids, such as Met and Cys (Shacter, 2000; Bechtold et al., 2004; Müller et al., 2007; Hawkins et al., 2009; Onda, 2013). The oxidation of most amino acids is widely considered to be a pathological phenomenon, while the oxidation of S-containing amino acids, such as Met and Cys (Berlett and Stadtman, 1997; Shacter, 2000; Møller et al., 2007; Hawkins et al., 2009; Onda, 2013). The formation (insertion) of carbonyl group called carbonylation is the most commonly occurring oxidative protein modification after modifications to Cys and Met (Lounifi et al., 2013). It requires higher ‘inputs of energy’ than oxidation of Cys and Met, involves most amino acids and results in severe changes in protein structure/function and pathophysiological effects (Berlett and Stadtman, 1997; Shacter, 2000; Tanou et al., 2009; Lounifi et al., 2013). Carbonylation usually refers to a process that forms reactive ketones or aldehydes which can be detected by ‘Brady’s test’ with 2,4-dinitrophenylhydrazine (leading to formation of hydrzones). The oxidation of side chains of lysine, arginine, proline, and threonine is considered to be a primary protein carbonylation reaction, which produces 2,4-dinitrophenylhydrazine detectable products.

### Table 2

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Oxidised form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine</td>
<td>Cysteine → cystine → cysteine sulfinic acid → cysteine sulfonic acid → cystic acid</td>
</tr>
<tr>
<td>Methionine</td>
<td>R- and S-stereoisomers of methionine</td>
</tr>
<tr>
<td>Histidine</td>
<td>2-Oxohistidine, asparagine, aspartate, 4-hydroxyynenal-histidine (HNE-His)</td>
</tr>
<tr>
<td>Glutamyl (glutamine, glutathione, glutamate)</td>
<td>Oxallic acid, pyruvic acid</td>
</tr>
<tr>
<td>Lysine</td>
<td>α-Aminoacidic semialdehyde, chloramines, malondialdehyde-lysine (MDA-Lys), 4-hydroxyynenal-lysine (HNE-Lys), acrolein-lysine, carboxymethyllysine, p-hydroxyphenylacetaldehyde-lysine (pHA-Lys)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>p-Hydroxyphenylacetaldehyde, dihydroxy, nitrotyrosine, chlorotyrosines, L-3,4-dihydroxyphenylalanine (L-DOPA)</td>
</tr>
<tr>
<td>Threonine</td>
<td>2-Amino-3-ketobutyric acid</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Hydroxy- and nitro-tryptophans, kynurenines</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Hydroxyphenylalanines</td>
</tr>
<tr>
<td>Valine, Leucine</td>
<td>Hydroperoxides and hydroxides</td>
</tr>
<tr>
<td>Proline</td>
<td>Hydroxoproline, pyrroolidone, glutamic semialdehyde</td>
</tr>
<tr>
<td>Arginine</td>
<td>Glutamic semialdehyde, chloramines</td>
</tr>
</tbody>
</table>

Formation (insertion) of carbonyl group called carbonylation is the most commonly occurring oxidative protein modification after modifications to Cys and Met (Lounifi et al., 2013). It requires higher ‘inputs of energy’ than oxidation of Cys and Met, involves most amino acids and results in severe changes in protein structure/function and pathophysiological effects (Berlett and Stadtman, 1997; Shacter, 2000; Møller et al., 2007; Hawkins et al., 2009; Onda, 2013). Carbonylation usually refers to a process that forms reactive ketones or aldehydes which can be detected by ‘Brady’s test’ with 2,4-dinitrophenylhydrazine (leading to formation of hydrzones). The oxidation of side chains of lysine, arginine, proline, and threonine is considered to be a primary protein carbonylation reaction, which produces 2,4-dinitrophenylhydrazine detectable products.

Secondary protein carbonylation reaction occurs via the addition of aldehydes which are produced during lipid peroxidation (usually they are aggressive carbonyl species having three to nine carbons in length). Carbonylation leads to the addition of a large and reactive group into the protein chain. It has a number of dangerous effects on protein characteristics, including covalent intermolecular cross-linking, cleavage, or changing the rate of protein degradation. All these significantly modify (usually inhibit) protein enzymatic and physiological activities. Elevated protein carbonylation has been found for a number of plant stresses such as salinity (Tanou et al., 2009), drought (Bartoli et al., 2004) and cadmium toxicity (Romero-Puertas et al., 2002). Protein carbonylation has been always considered as irreversible (Berlett and Stadtman, 1997) but evidence has now appeared that it can be enzymatically reversed for some transcription factors (Wong et al., 2008). Therefore this reaction can potentially be a novel ROS signaling mechanism (Wong et al., 2008; Lounifi et al., 2013).

The proteomic analyses of Arabidopsis thaliana have demonstrated that nitrosative stress (NO* donors) results in protein modification called S-nitrosylation (Lindermayr et al., 2005; Lounifi et al., 2013). It is likely that this reaction is induced by peroxynitrite (the product of NO* reaction with O2−) and leads to severe protein function disturbance or signaling events by the analogy with animal cells (Halliwell and Gutteridge, 1999; Lounifi et al., 2013).

#### 5.3. Effect on carbohydrates

Carbohydrates are the most abundant group of organic molecules in plants (and on the planet), and at the same time, are less studied in terms of oxidative damage and role in stress signaling. They mechanically support and shape plant cells (cellulose, pectin, etc.), store reduced carbon (starch, sucrose etc.), regulate enzyme activities and osmotic pressure (low molecular weight sugars), provide non-enzymatic antioxidant defence (flavonoids, mannitol etc.) and play other key roles. Oxidation of carbohydrates is potentially harmful for plants. Cu2+-generated *OH react non-enzymatically with xyloglucans and pectins, breaking down them into the parts and causing cell wall loosening (Fry et al., 2002). This may facilitate cell expansive growth and promote fruit ripening (Fry et al., 2002; 2004). This reaction is beneficial, but in stress conditions, when catalytic Cu and Fe activities increase several-fold, it could have pathophysiological consequences (Recana and Klucas, 1992; Moran et al., 1994).

Mono and disaccharides probably act as ROS scavengers (Couée et al., 2006). Their ability to scavenge *OH is as follows (EPR and HPLC tests): maltose > sucrose > fructose > glucose > deoxyribose > sorbitol (Morelli et al., 2003). Nevertheless the metabolism of products (apart from formate) synthesised in these reactions is unclear. Accumulation of some carbohydrates (for example mannitol) has been shown to correlate with increased resistance to oxidative stress in a number of species (Shen et al., 1997; Couée et al., 2006). Nevertheless, the direct link between oxidative stress induced modifications to carbohydrates and plant
physiology is still missing. They probably act as structural, osmotic, nutrient and signaling agents rather than redox switches or major targets for ROS (Couée et al., 2006).

5.4. Effect on nucleic acids

Oxidative damage of DNA is a subject of extensive research in animal physiology, because it is a major reason of cancerogenesis. This problem is not relevant to agronomically important plants; therefore it is insufficiently studied in plant physiology. Nevertheless, oxidative DNA damage can be reason of aging of seeds stocks and, sometimes, death of crop plants (Britt, 1996). This is classically subdivided into three types of lesions: mismatched bases, double-strand breaks, and chemically modified bases (Cooke et al., 2003; Yoshiyama et al., 2013). Hydroxyl radicals are a main damaging factor for polynucleic acids, reacting with them to addition to double bonds of nucleotide bases and by abstraction of H+ from each of the C-H bonds of 2′-deoxyribose and methyl group of thymine. 8-oxo-7,8-dihydroguanine (8-oxoG) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) are common detected products of •OH induced DNA/RNA oxidation (Cooke et al., 2003; Wang et al., 2010; Yoshiyama et al., 2013).

Repair systems preventing DNA damage exist in plants, including direct reparation of damaged part of the molecule, in addition to base and nucleotide replacement (Britt, 1996; Tuteja et al., 2001; Yoshiyama et al., 2013). Protection also includes enforcement of antioxidant defence in both cytosol and organelles. Vanderauwera et al. (2011) have shown that nuclear ROS scavenging enzymes (peroxiredoxin and glutathione) are insufficient to protect DNA during oxidative stress. They have demonstrated that catalase and cytosolic ascorbate peroxidase are important for protection of nuclear DNA in stress conditions. Moreover, ROS-induced DNA damage triggers signaling phenomena and activates specific transcription factors, inducing DNA reparation (Yoshiyama et al., 2013).

6. Defense against oxidative stress

The systems of ROS scavenging and cell reparation, which are involved in plant defense against oxidative stress has been the subject of extensive research, and has been the focus of a number of very good recent reviews (some most recent reviews: Smirnoff, 2005; Dietz et al., 2006; Pitzschke et al., 2006; Santos and Rey, 2006; Müller et al., 2007; Foyer and Noctor, 2009, 2011; Gill and Tuteja, 2010; Asensi-Fabado and Munne-Bosch, 2010; Farmer and Muller, 2013; Zagorchev et al., 2013). Therefore these will not be discussed in detail here.

Plants have several strategies to withstand an oxidative stress (reviewed by Alscher et al., 2002; Dietz et al., 2003, 2006; Mittler et al., 2004; Gill and Tuteja, 2010). Firstly, it is an activation and de novo synthesis of antioxidants - enzymes and non-enzymatic substances directly scavenging (removing) ROS and free radicals. Major enzymatic antioxidants, which show high affinity to specific ROS, include cytosolic Cu-Zn-SOD, mitochondrial Mn-SOD, chloroplastal Fe-SOD (all SODs: superoxide + 2H+ → H2O2 + O2), catalases (2H2O2 → 2H2O + O2), peroxidases (R/HOOH + R-H → R + 2H2O/ROH), peroxiredoxins (ROOH → ROH), thioredoxins and glutaredoxins (both: R–S–S–R → 2R–SH). Non-enzymatic antioxidants are non-specific to different ROS and comprise ascorbic acid, glutathione, proline, polyamines, betaine, carotenes, some flavonoids and α-tocopherol. These are most important, but a number of other molecules act as antioxidants. There is also a group of enzymes maintaining ROS scavenger function, such as monodehydroascorbate reductase, dehydroascorbate reductase, thioredoxin reductase, glutathione reductase, glutathione S-transferases and others, which reduce oxidised antioxidants, such as ascorbic acid and glutathione. The first line of defence also includes substances that decrease catalytic activity of transition metals, such as metallothioneineis (small Cys-rich proteins), phytochelatines (oligomers of glutathione), pectins and other cell wall polysaccharides and structural proteins (reviewed by Fry et al., 2002; Cobbett and Goldsbrough, 2002; Hassinen et al., 2011; Zagorchev et al., 2013).

Secondly, plants probably synthesize protein isoforms and lipids which are less sensitive to oxidation. This requires upstream signaling step to activate specific genetic/metabolic pathways (Wang et al., 2004; Myouga et al., 2008). This mechanism is well established for animals but it is still debated whether it is important for plants.

Thirdly, most plants probably protect living tissues from stresses by the layers of dead cells, which die rapidly by programed ROS-induced mechanisms (Ca2+/ influx/K+ efflux; Demidchik and Maathuis, 2007; Demidchik et al., 2010, 2014). These provide a shield against infection or aggressive agents. The enhanced mycorrhization could be another mechanism of this kind (fungal hyphae shields root from aggressive environment), which is critically important for plant defense against heavy metals (Schutzenbueler and Polle, 2002). And finally, plants activate biosynthesis systems for reparation of damaged components (reviewed by Muller et al., 2007; Yoshiyama et al., 2013).

7. Conclusions

The analysis of research into the mechanisms of oxidative stress in plants has shows that ROS with proven importance for oxidative damage and signaling include hydroxyl radical, singlet oxygen, hydrogen peroxide, superoxide radical and nitric oxide (forming peroxynitrite). In most cases, oxidative damage occurs through •OH generation, mainly from H2O2 in the presence of transition metals, such as Cu+/2+ or Fe2+/3+. Apart from exogenous sources (UV, O3, Cu etc.) ROS are generated by photosystems, mitochondrial complexes, electron transport chains of peroxisomal membrane, xanthine oxidase of the peroxisome matrix, NADPH oxidases of the plasma membrane and peroxidases expressed in all cell compartments including cell walls. Accumulating evidence demonstrates that all major stresses trigger the NADPH oxidase mediated oxidative burst. Many stresses also stimulate ROS generation by Class III peroxidases. Novel genetically encoded and reversible H2O2 probes such as HyPer provide advanced tools for testing H2O2 dynamics in planta and can shed light on unknown mechanisms of plants oxidative stress and redox signaling. It has become clear that ROS signals are sensed by cation channels which convert them into physiological responses such as Ca2+ influx and cytosolic Ca2+ elevation as well as K+ efflux. Ca2+ elevation activates various two-component and multi-component signaling systems leading to induction of genetic and metabolic adjustments. K+ efflux through ROS-activated K+ channels results in stimulation of cytosolic cell death proteases and endonucleases, leading to programmed cell death. Other potential ROS sensors include histidine kinases, redox-sensitive transcription factors and ROS-sensitive phosphatases. Oxidative stress can damage all cell components. Lipid peroxidation starts from the interaction of hydroxyl radical or singlet oxygen with double bonds of polyunsaturated fatty acids and results in a chain reaction converting functional lipids into toxic aldehydes and ketones. Reversible oxidation of S-containing amino acids plays the role of regulatory redox switches in plant cells. Major mechanisms of irreversible modification of amino acids include ‘primary’ and ‘secondary’ carbonylation. Plant carbohydrates and nucleic acids are very sensitive to •OH but the role of their oxidative damage in physiology is still debated. Networks of enzymatic (specific) and
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