Oxidative Stress and Salt Tolerance in Plants

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Abstract Salt stress can induce ionic stress and osmotic stress in plant cells. A direct result of these primary effects is the enhanced accumulation of reactive oxygen species (ROS) that are harmful to plant cells at high concentrations. To cope with the oxidative stress resulting from the ROS, higher plants have developed a complex scavenging system including enzymatic and non-enzymatic (antioxidants) system. In plant cells, specific ROS producing and scavenging systems are found in different organelles such as chloroplasts, mitochondria, and peroxisomes; and the ROS-scavenging pathways from different cellular compartments are coordinated. Relatively low levels of ROS can be used for signaling molecules to control abiotic stress responses. Coordinated work of ROS-scavenging pathways from different

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cellular compartments in modulating the level of ROS in cells preventing cellular damage and controlling ROS signaling may play a key role in plant salt tolerance. Here we attempt to summarize the recent researches on ROS and the mechanism of salt tolerance of plants under salt stress, and we also propose some perspectives involved in ROS and plant salt tolerances in the future.

1 Introduction

Salinity is a worldwide problem that limits distribution and production of major crops. One-fifth of irrigated agriculture is adversely affected by soil salinity (Flowers and Yeo 1995). So increasing crop salt tolerance is essential for sustaining food production.

Salinity can induce ionic stress and osmotic stress. As a consequence of these primary effects, secondary stresses such as oxidative stress often occur. Salt stress induces the accumulation of reactive oxygen species (ROS) that are harmful to plant cells at high concentrations. They cause oxidative damage to membrane lipids, proteins, and nucleic acids (Gómez et al. 1999; Hernández et al. 2001). Therefore, it is important to understand how plants respond and adapt to oxidative stress. Antioxidant resistance mechanisms may provide a strategy to enhance plant salt tolerance. In this review, recent progress in research on oxidative stress induced by salinity and salt tolerance is discussed.

Molecular oxygen (O_2) has a relatively low reactivity toward most biological substances. Partially reduced forms of O_2 are extremely reactive and may oxidize biological molecules. Reduction of O_2 to an active oxygen molecule results from the addition of one, two, or three electrons to form a superoxide radical (O_2^{-}), hydrogen peroxide (H_2O_2), or hydroxyl radical ($OH^{\bullet}$), respectively (Asada and Takahashi 1987; Apel and Hirt 2004).

The first step in O_2 reduction produces O_2^{-}. The half-life for O_2^{-} is approximately 2–4 ms. O_2^{-} can oxidize specific amino acids, such as histidine, methionine, and tryptophane. In the cellular environment, O_2^{-} also causes lipid peroxidation, thereby weakening cell membranes. Via the Mehler reaction, more harmful ROS such as $OH^{\bullet}$ are produced if O_2^{-} accumulates in the cells.

The second O_2 reduction generates H_2O_2, a relatively long-lived molecule (1 ms) that can diffuse some distance from its site of production (Levine et. al. 1994; Willekens et. al. 1997). H_2O_2 can oxidize SH groups. The biological toxicity of H_2O_2 can be enhanced in the presence of metal catalysts through Haber–Weiss or Fenton-type reactions.

The third production of reduced oxygen is that of the hydroxyl radical ($OH^{\bullet}$). It is the most reactive ROS causing oxidative damage and has a half-life of <1 ms. As result, it has a very high affinity for biological molecules at its site of production, reacting at almost diffusion-controlled rates. There is no specific antioxidant to scavenging the $OH^{\bullet}$. So the cell should be equipped with a perfect scavenging system for O_2^{-} and H_2O_2 to protect itself from oxidative damage.

Under high salinity conditions, the production of ROS is increased dramatically and the physiological homeostasis of the cell is disrupted. To cope with the

oxidative stress resulting from the ROS, higher plants have developed a complex antioxidant system consisting of low molecular weight antioxidants, including carotenoids, ascorbate, glutathione (GSH), and tocopherol (V_E), as well as antioxidant-enzymes such as superoxide dismutases (SOD), catalase (CAT), and the ascorbate–glutathione cycle (Asada 1999).

2 Production of ROS in Plant Cells Under Salinity

Under normal conditions, the production and removal of ROS are at an equilibrium. Many stresses such as salt stress, drought, chilling, heat shock, and high-light stress disturb the balance and enhance the production of ROS. In plants, ROS are continuously produced predominantly in chloroplasts, mitochondria, and peroxisomes.

2.1 Chloroplasts

The chloroplast is considered to be a focal point of ROS metabolism because, under light, the oxygen pressure in chloroplasts is much higher than in other organelles. Therefore, the chloroplast is considered as a major producer of O_2^- and H_2O_2 (Davletova et al. 2005). In chloroplast thylakoids, the reaction centers of PSI and PSII are the major generation sites of ROS.

Superoxide (O_2^-) is generated by the one-electron reduction of molecular oxygen in the plastoquinone (PQ) pool by plastosemiquinone, by ferredoxin (Fd) or by iron–sulfur redox centers in the electron transport chain within PSI (Dat et al. 2000). The O_2^- formed is rapidly converted to hydrogen peroxide (H_2O_2) either spontaneously or by SOD. Production of ROS by this source is enhanced in plants by conditions limiting CO_2 fixation, such as salt stress, as well as by the combination of these conditions with high-light stress (Mittler et al. 2004). H_2O_2 can also lead to the production of the hydroxyl radical ($OH^\bullet$). Singlet oxygen ($^1O_2$*) is mainly produced by the reaction centre of PSII (P680). Oxygen in the ground (triplet) state ($^3O_2$) is excited to the singlet state ($^1O_2$) by excited triplet chlorophyll molecules in the reaction center (Fryer et al. 2002; Hideg et al. 2002). The yield of $^1O_2$* is increased by high light or UV and this is accompanied by the photoinhibition of PSII (Hideg et al. 2002).

2.2 Mitochondria

The mitochondrial electron transport chain (mtETC) contains four electron transporting complexes (complexes I–IV) and one H^+-translocating ATP synthetic complex (complex V). Two of these complexes were shown to be responsible for much of the O_2^- generated: complex I (the NADH ubiquinone oxidoreductase) and

complex III (the ubiquinol–cytochrome *c* oxidoreductase; Boveris and Chance 1977; Takeshiga and Minakami 1979; Beyer 1991; Mittler 2002). In contrast, O_2^- generation via the mitochondrial respiratory chain in most organisms is probably also produced by a non-enzymatic mechanism. In the course of electron transport reactions in the respiratory chain, ubisemiquinone (UQ10) species donate electrons to oxygen and provide a constant source of O_2^- (Raha and Robinson 2000).

O_2^- generated from the respiratory chain is reduced by dismutation to H_2O_2 and O_2. H_2O_2 is a relatively low-toxic compound. It can react with reduced Fe^{2+} and Cu^+ to produce highly toxic hydroxyl radicals and diffuse from the mitochondrion into other cellular parts (Grene 2002; Sweetlove and Foyer 2004).

In green tissue ROS production in the mitochondrion is very low, compared with mitochondria in mammalian cells. One reason is that plant mitochondria have an alternative oxidase (AOX). AOX competes with the cytochrome bc1 complex for electrons and catalyzes the tetravalent reduction of O_2 by ubiquinone producing H_2O, which reduces ROS production (Apel and Hirt 2004). However, in the dark or in non-green tissues, mitochondria are a major source of ROS (Puntarulo et al. 1988).

Through perturbing mtETC and increasing mtROS, salt stress is at least partly responsible for oxidative stress and plant responses (Hernández et al. 1993; Mittova et al. 2003).

2.3 Peroxisomes

In plant cells, peroxisomes are probably the major intracellular H_2O_2-producing organelle. In recent years, it was demonstrated that O_2^- is also produced in peroxisomes. There are at least two sites of O_2^- generation (del Río et al. 2006). One site occurs in the peroxisomal matrix. Xanthine oxidase catalyzes the oxidation of xanthine and hypoxanthine to uric acid and produces O_2^- radicals (Halliwell and Gutteridge 2000). The other site is the peroxisomal membrane, where a small electron transport chain appears to be involved (del Río et al. 2006). Three integral peroxisomal membrane polypeptides (PMPs) of this electron transport chain with molecular masses of 18, 29, and 32 kDa have been characterized and demonstrated to be responsible for O_2^- generation (López-Huertas et al. 1999).

H_2O_2 production in peroxisomes has at least two pathways: one is the disproportionation of O_2^- generated in this organelle. The other is a direct pathway. During photorespiration glycolate is catalyzed by glycolate oxidase, yielding H_2O_2. Fatty acid β-oxidation, the enzymatic reaction of flavin oxidases, can also produce H_2O_2 (Baker and Graham 2002; del Río et al. 2002).

Salt stress can increase lipid peroxidation of peroxisomes. However, peroxisomal H_2O_2 content is unaffected by salinity (Mittova et al. 2003). This may be correlated with porins in the peroxisomal membranes (Reumann et al. 1997). Further studies are needed in order to find direct evidence for the relationship between peroxisomal ROS production and salt stress.

2.4 Other Sources

Plasma membrane NADPH oxidases contain a multimeric flavocytochrome that forms an electron transport chain capable of reducing O_2 to O_2^-. This enzyme could be the enzymatic source participating in ROS accumulation during NaCl stress (Hernández et al. 2001). In addition to NADPH oxidases, pH-dependent cell wall peroxidases, germin-like oxalate oxidases, and amine oxidases are proposed to generate ROS in the apoplast (Bolwell and Wojtaszek 1997; Hu et al. 2003; Walters 2003). Cell wall peroxidases produce O_2^- at the expense of NADH in a Mn^{2+}-dependent reaction (Elstner and Oßwald 1994).

3 ROS Scavenging Systems and Salt Tolerance in Plants

Salt stress enhances ROS production in plant cells. In order to keep the balance between ROS production and scavenging, plants developed scavenging systems against ROS, involving both enzymatic and non-enzymatic (antioxidants) systems. Major ROS-scavenging enzymes include SOD, ascorbate peroxidase (APX), CAT, glutathione peroxidase (GPX), glutathione reductase (GR), and peroxiredoxins (Prxs). Antioxidants include ascorbic acid (AsA), GSH, carotenoids, and V_E. Interestingly, higher plants also developed specific ROS-scavenging systems in different organelles to efficiently remove the ROS produced in these cellular parts; and, in particular under environmental stress such as salt stress, they coordinately work to provide plant cells with a highly efficient machinery for detoxifying ROS.

3.1 Chloroplasts

Thylakoid SOD (tSOD), thylakoid APX (tAPX), and Fd-dependent reduction of mondehydroascorbate (MDA) form the thylakoidal scavenging system, which functions as the first defense against ROS (Mittler et al. 2004). The O_2^- photogenerated by the PSI complex is in situ disproportionated to H_2O_2 and O_2, catalyzed by tSOD. Then the H_2O_2 is reduced to water by AsA, catalyzed with tAPX, and AsA is oxidized to the MDA radical. All the above reactions make up the water–water cycle. Subsequently, MDA is directly reduced to AsA by either reduced ferredoxin (redFd; Miyake and Asada 1994) or spontaneously disproportionated to dehydroascorbate (DHA). Then MDA or DHA is reduced to AsA by the ascorbate–glutathione cycle (AsA-GSH cycle). Reduction of H_2O_2 by Prx is another mechanism to inactive ROS at PSI (Dietz 2003).

ROS that escape from the thylakoid or are produced in the stroma undergo detoxification by stromal SOD, stromal APX, and the stromal AsA-GSH cycle. PrxR and GPX cycle are also involved in H_2O_2 removal in the stroma (Mittler et al. 2004).

H_2O_2 can convert to $OH^{\bullet}$ by the Haber–Weiss reaction (Imlay and Linn 1988). $OH^{\bullet}$ is the most reactive ROS, causing oxidative damage to chloroplast components. No specific scavenging enzyme is available for $OH^{\bullet}$. In fact, damage by H_2O_2 and O_2^- results primarily from their role in hydroxyl radical production rather than from direct action (Imlay and Linn 1988). So the cell should be equipped with a perfect scavenging system for O_2^- and H_2O_2 to protect itself from oxidative damage.

In the chloroplasts $^1O_2$ is a ROS without unpaired electron. Once formed, $^1O_2$ rapidly reacts with nearby molecules, causing oxidative damage to proteins, lipids, and DNA. In the PSII reaction center two molecules of β-carotene participate in the quenching of $^1O_2$ generated via ^{3}P680*. Tocopherols (V_E) can also quench $^1O_2$, but the rate is two orders of magnitude lower than that with β-carotene (Krasnovsky Jr 1998).

Non-enzymatic antioxidants such as AsA and GSH are the major cellular redox buffers. AsA concentration in chloroplasts is high. It can react directly with hydroxyl radicals, superoxide, and singlet oxygen (Buettner and Jurkiewicz 1996).

Chloroplastic SOD, APX, and GR play a central role in the enzymatic scavenging system. They all have two types: thylakoid-bound and stromal type. CuZn-SOD is the major isoform of SOD in the chloroplast. Several plants such as tobacco also contain Fe-SOD (Kurepa et al. 1997). It is exclusively localized in the chloroplast stroma. Our results showed that thylakoid-bound SOD, APX, GR, and stromal SOD, APX, GR in the chloroplasts of the halophyte *Suaeda salsa* L. are markedly enhanced under high salinity (Pang et al. 2005; Zhang et al. 2005). This is possibly an important mechanism of salt tolerance in halophytes. Overexpression of tAPX (Yabuta et al. 2002; Murgia et al. 2004) can increase tolerance to methyl viologen (MV)-induced oxidative stress. Transgenic plants overexpressing *Escherichia coli* catalase in chloroplasts show enhanced resistance to photooxidative stress by MV (Miyagawa et al. 2000). Plants with cytosolic APX overexpressed in chloroplasts show enhanced tolerance to salt and drought stresses (Badawi et al. 2004). Transgenic tobacco plants expressing both SOD and APX in chloroplasts have enhanced tolerance against MV-mediated oxidative stress (Kwon 2002). Mutants of tAPX are thought to be lethal (Yabuta et al. 2002). Plants with reduced tAPX activity are sensitive to MV stress (Tarantino et al. 2005) or paraquat-induced photooxidative stress (Tarantino et al. 2005). All these results suggest that the ROS enzyme scavenging system in chloroplasts plays a central role to protect the chloroplast from oxidative damage induced by environmental stresses.

3.2 Mitochondria

ROS produced in mitochondria can damage mitochondrial lipids, proteins, and DNA. So the overproduced ROS must be scavenged in time. Mitochondrial Mn-SOD catalyzing the production of H_2O_2 from O_2^- is the first step in scavenging the O_2^- generated from the mitochondrial electron transport chain (Kliebenstein et al. 1998; Møller 2001; Mittler et al. 2004). O_2^- can also be converted to H_2O_2 by spontaneous dismutation. Consequently, H_2O_2 is removed by mitochondrial APX through the AsA-GSH cycle (Jiménez et al. 1997; Chew et al. 2003) or CAT, which was reported in maize

(*Zea mays*) mitochondria (Sweetlove and Foyer 2004). Prxs also could reduce mitochondrial H_2O_2. These enzymes use reduced thioredoxins as reductant sources, which in turn are reduced by thioredoxin reductase (Sweetlove and Foyer 2004).

In addition to directly detoxifying ROS, plant mitochondria also can modulate superoxide production from mtETC. There are two modulation mechanisms. In the first, AOX acts to maintain a basal ubiquinone pool reduction state as initially proposed by Purvis and Shewfelt (1993) and diminishes mtROS production (Popov et al. 1997; Purvis 1997; Maxwell et al. 1999). Second, uncoupling protein (UCP) uncouples by facilitating a proton leak across the membrane, consequently removes inhibition of the mtETC (Hourton-Cabassa et al. 2004; Sluse and Jarmuszkiewicz 2004), and then decreases ROS formation.

When oxidative damage is produced, some enzymes such as GST and the type II Prx /thioredoxin system may be involved in repairing lipid peroxidation and some forms of protein oxidation (Rhoads et al. 2006).

Salinity up-regulates the levels of ASA and GSH and the activities of SOD, APX MDHAR, DHAR, and GPX in root mitochondria of the wild salt-tolerant tomato species *Lycopersicon pennellii* (Mittova et al. 2004). Transcript levels of mitochondrial MnSOD were strongly induced by salt treatment in the salt-tolerant variety but not in the NaCl-sensitive variety (Hernández et al. 2000). Overexpression of mitochondrial Mn-SOD from *Nicotiana plumbagnifolia* in *Nicotiana tabacum* mitochondria protected the latter from oxidative damage (Bowler et al. 1991). A yeast strain deficient in mitochondrial MnSOD regained its resistance to oxidative stress when plant mitochondrial MnSOD was expressed in it (Scandalios 1993; Zhu and Scandalios 1992). Among several potential mechanisms for ROS detoxification in plant mitochondria, only MnSOD is firmly established. Further experiments are needed to testify other ROS detoxification mechanisms in plant mitochondria and their roles in salt tolerance.

3.3 Peroxisomes

Plant peroxisomal SOD has been reported in at least nine different plant species (del Río et al. 2002). Three of them have been purified and characterized (del Río et al. 2002): a CuZn-SOD and a Mn-SOD from watermelon and a Mn-SOD from pea leaves. They convert the O_2^- generated in peroxisomes to H_2O_2 and O_2. Peroxisomal H_2O_2 can be converted to H_2O through CAT and the AsA-GSH cycle in peroxisomes (Mittler et al. 2004). APX, monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), and GR of the cycle in peroxisomes were purified from pea leaves and tomato leaves and roots (del Río et al. 1998; Mittova et al. 2004; Kużniak and Skłodowska 2005). The intraperoxisomal distribution and function of the AsA-GSH cycle have also been identified (del Río et al. 2006). DHAR and GR are found in the matrix of peroxisomes, whereas APX and MDAR are bound to the cytosolic side of the peroxisomal membrane. The presence of APX and MDAR in the leaf peroxisomal membrane suggests a dual complementary function in the peroxisomal metabolism of these

membrane-bound antioxidant enzymes. First, MDAR reoxidizes NADH to maintain a constant supply of NAD^+ for peroxisomal metabolism. Second, the membrane-bound antioxidant enzymes prevent H_2O_2 leaking from the peroxisomes (del Río et al. 2002). GPX has been reported in leaf peroxisomes of tomato plants (Kużniak and Skłodowska 2005) and a putative Prx with a molecular mass of 60 kDa has been localized in the matrix of pea leaf peroxisomes (Corpas et al. 2003). They can also decrease the level of H_2O_2.

Salt-induced peroxisomal oxidative stress probably results from salt-induced stomatal closure. Under these conditions, the photorespiratory peroxisomal glycolate oxidase increases the rate of ROS generation and leads to oxidative stress (Smirnoff 1993). Salinity up-regulates the levels of ASA and GSH and the activities of SOD, APX, CAT, and MDAR in root peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii* (Mittova et al. 2004). Overexpression of an *Arabidopsis* peroxisomal APX gene in tobacco increases protection against oxidative stress caused by aminotriazole which inhibits catalase activity. However, the overexpression cannot protect plants from oxidative damage caused by paraquat, which leads to the production of reactive oxygen species in chloroplasts (Wang et al. 1999). These results indicated that the protection provided by the targeted expression of antioxidant genes may be selective, depending on the nature of oxidative stress.

3.4 Apoplast

The enzymatic components responsible for ROS detoxification in the apoplast and cell wall are only partially known. Previous studies have failed to detect the antioxidant enzymes of the AsA-GSH cycle in the apoplast (Castillo and Greppin 1988; Polle et al. 1990; Luwe 1996; Vanacker et al. 1998a, b). However, recently the presence not only of SOD activity, but also of the AsA-GSH cycle and CAT in the apoplast of both barley (*Hordeum vulgare*) and oat (*Avena sativa*) leaves were identified. Little or no GSH has been found in the apoplast of plant cells (Luwe 1996; Vanacker et al. 1998a, b). Salt stress can change the apoplastic ROS-scavenging components of two pea cultivars (Hernández et al. 2001).

4 Salt Stress, Oxidative Stress, ROS Signal Perception/Sensing and Salt Tolerance in Plants

A high concentration of ROS can lead to phytotoxicity whereas relatively low levels can be used for signaling. Under salt stress, ROS production in plant cells, including extracellular and intracellular spaces, can be sensed by ROS sensors. Salt-induced ROS are predominantly formed as H_2O_2 and this process occurs inside the cell. A possible signal transduction pathway under salt stress is proposed (Fig. 1) which is maybe different from the pathway induced by extracellular H_2O_2

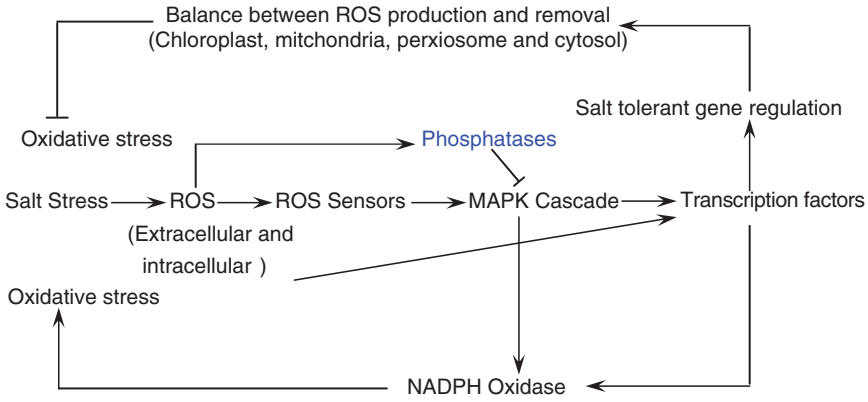

Fig. 1 A possible ROS signal transduction pathway in plant cells under salt stress

(Avsian-Kretchmer et al. 2004). Salt stress rapidly activates the mitogen-actived protein kinase (MAPK) casade. Intracellular ROS can also influence the ROS-induced MAPK signal pathway through inhibition of phosphatases or downstream transcription factors. Then gene expression in response to salt stress can be regulated. In salt-tolerant plants, ROS production and removal was found to be kept in balance and the level of oxidative stress to be low. NADPH oxidases possibly amplify ROS signaling (Mittler et al. 2004).

5 Perspectives

Different plant species have different responses to salt stress and their capacity for resistance to salt stress is also not identical. Salt stress can secondarily induce oxidative stress and lead to the accumulation of ROS. The mechanism of ROS production and scavenging in different organelles has been described in many published papers. There are still many uncertainties in understanding signal transduction under salt stress conditions.

5.1 Sensors of ROS Perception

To date, no ROS receptor has been unambiguously identified in plants. Plant cells sense ROS possibility via at least three different mechanisms:

1. ROS sensors: redox-sensitive transcription factors, such as NPR1(non-expressor of pathogenesis-related genes 1), control the onset of systemic acquired resistance, plant immunity, to a broad spectrum of pathogens that is normally established after a primary exposure to avirulent pathogens.

2. Heat-shock transcription factors (HSFs) are the terminal components of the signal transudation pathway for gene activation in response to heat stress.
3. Direct inhibition of phosphatases by ROS (Mittler et al. 2004).

How the plant cells sense ROS under salt stress is still an open question.

5.2 Key Components in ROS Signal Transduction Cascade

Salt stress rapidly (within 5–10 min) activates *Arabidopsis* mitogen-activated protein kinase kinase kinase (AtMEKK1; Ichimura et al. 1998), mitogen activated protein kinase kinase (AtMKK2; Teige et al. 2004), and MAPKs (ATMPK3, ATMPK4, ATMPK6; Mizoguchi et al. 1996; Ichimura et al. 2000). A role for the MAPK module consisting of MEKK1–MKK2–MPK4/MPK6 has now been confirmed in cold and salt stress (Nakagami et al. 2005). Mutant plants *mkk2*-null are hypersensitive to salt stress (Nakagami et al. 2005). Plants with overexpression of *ANP1* (a MAPKKK; Kovtun et al. 2000) and *MKK2* (Nakagami et al. 2005) are more tolerant to salt stress. Different transcript factors (WRKY, Zat, RAV, GRAS, Myb families) induced or activated by the MAPK casade regulate the ROS-scavenging and ROS-producing pathways (Mittler et. al. 2004).

H_2O_2 is sensed via the modification of thiol groups in certain proteins. The inactivation of *Arabidopsis* protein tyrosine phosphatases (AtPTP1) by H_2O_2 may be mediated by the oxidation state of the active-site cysteine. AtPTP1 can inactivate *Arabidopsis* MPK6 (Gupta and Luan 2003). When oxidative stress occurs, AtPTP1 is inactivated by H_2O_2. Then the MAPK casade is activated and transcription factors are regulated.

Thus, it can be seen that transcription factors and the MAPK casade are key components in ROS signal transduction. In *Arabidopsis*, the role of the MAPK casade has been investigated under salt stress. Nevertheless its role in other plants is still unclear. The expression of different transcription factors is enhanced by ROS and this includes members of the WRKY, Zat, RAV, GRAS, and Myb families (Mittler et al. 2004). To find out which transcription factors could be regulated by salinity is a challenge. Large-scale transcriptome analyses coupled with proteomic and metabolomic analyses of plants perturbed at the levels of individual or multiple components of the salt induced ROS network will be essential for future studies.

5.3 The Mechanism of Salt Tolerance Involved in Antioxidants in Plants

Salt stress does not induce oxidative damage in salt-tolerant plants (Hernández et al. 2000; Mittova et al. 2004; Wang et al. 2004; Pang et al. 2005; Zhang et al. 2005). This is due to an enhancement of the ROS scavenging system. Conversely,

salt-sensitive plants can be damaged by salt stress-induced ROS (Hernández et al. 2000; Mittova et al. 2004). There are perhaps different signal transduction or transcript factors in the salt-tolerant and salt-sensitive plants. It is also possible that there is considerable difference in non-coding regions of antioxidant enzyme genes, such as SOD, between halophytes and nonhalophytes. This needs more direct proof.

5.4 Coordinated Work of the Different Cellular Parts Under Salinity

Different organelles have their special ROS scavenging systems. The function of the components of the different organelles' ROS scavenging systems has been clarified. It is considered that the different compartments are protected by their own ROS-scavenging systems at first, and they then also somehow work coordinately to protect plant cells from oxidative damage. It is reported that the cytosolic H_2O_2–scavenging enzyme ascorbate peroxidase 1 (APX1) can protect chloroplasts during light stress (Davletova et al. 2005). This provides evidence for cross-compartment protection of thylakoid and stromal/mitochondrial APXs by cytosolic APX1. At present there is a new view of the ROS network: the coordinated function of ROS-scavenging pathways from different cellular compartments in modulating the level of ROS in cells prevents cellular damage and controls ROS signaling. In the future, research on the relationship between the different subcellular ROS-scavenging pathways will be a powerful approach for understanding plant salt tolerance at the cellular level.

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References

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–99

Asada K (1999) The water–water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol 50:601–639

Asada K, Takahashi M (1987) Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ, Osmond CB, Arntzen CJ (eds) Photoinhibition. Elsevier, Amsterdam, pp 227–287

Avsian-Kretchmer O, Gueta-Dahan Y, Lev-Yadun S, Gollop R, Ben-Hayyim G (2004) The salt-stress signal transduction pathway that activates the *gpx1* promoter is mediated by intra-cellular H_2O_2, different from the pathway induced by extracellular H_2O_2. Plant Physiol 135:1685–1696

Badawi GH, Kawano N, Yamauchi Y, Shimada E, Sasaki R, Kubo A, Tanaka K (2004) Overexpression of ascorbate peroxidase in tobacco chloroplasts enhances the tolerance to salt stress and water deficit. Physiol Plant 121:231–238

Baker A, Graham I (2002) Plant peroxisomes. Biochemistry, cell biology and biotechnological applications. Kluwer, Dordrecht

Beyer R (1991) An analysis of the role of coenzyme Q in free radical generation and as an antioxidant. Biochem Cell Biol 70:390–343

Bolwell GP, Wojtaszek P (1997) Mechanisms for the generation of reactive oxygen species in plant defence – broad perspective. Physiol Mol Plant Pathol 51:347–366

Boveris A, Chance B (1977) Mitochondrial production of superoxide radical and hydrogen peroxide. In: Reivich M, Coburn R, Lahiri S, Chance B (eds) Tissue hypoxia and ischemia. Plenum, New York, pp 67–82

Bowler C, Slooten L, Vandenbranden S, De Rycke R, Botterman J (1991) Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. EMBO J 10:1723–1732

Buettner GR, Jurkiewicz BA (1996) Chemistry and biochemistry of ascorbic acid. In: Cadenas E, Packer L (eds) Handbook of antioxidants. Dekker, New York, pp 91–115

Castillo FJ, Greppin H (1988) Extracellular ascorbic acid and enzyme activities related to ascorbic acid metabolism in *Sedum album* L. after ozone exposure. Environ Exp Bot 28:231–238

Chew O, Whelan J, Millar AH (2003) Molecular definition of the ascorbate–glutathione cycle in *Arabidopsis* mitochondria reveals dual targeting of antioxidant defenses in plants. J Biol Chem 278:46869–46877

Corpas FJ, Pedrajas JR, Sandalio LM, León AM, Carreras A, Palma JM, Valderrama R, Río LA del, Barroso JB (2003) Localization of peroxiredoxin in peroxisomes from pea leaves. Free Radical Res 37[Suppl 2]:19

Dat J, Vandenabeele S, Vranová E, Montagu MV, Inzé D, Breusegem FV (2000) Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 57:779–795

Davletova S, Rizhsky L, Liang HJ, Zhong SQ, Oliver DJ, Coutu J, Shulaev V, Schlauch K, Mittler R (2005) Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of *Arabidopsis*. Plant Cell 17:268–281

Dietz KJ (2003) Plant peroxiredoxins. Annu Rev Plant Biol 54:93–107

Elstner EF, Oßwald W (1994) Mechanisms of oxygen activation during plant stress. Proc R Soc Edin B 102:131–154

Flowers TJ, Yeo AR (1995) Breeding for salinity resistance in crop plants. Where next? Aust J Plant Physiol 22:875–884

Fryer MJ, Oxborough K, Mullineaux PM, Baker NR (2002) Imaging of photo-oxidative stress responses in leaves. J Exp Bot 53:1249–1254

Gómez JM, Hernández JA, Jiménez A, Río LA del, Sevilla F (1999) Differential response of antioxidative enzymes of chloroplast and mitochondria to long term NaCl stress of pea plants. Free Radical Res 31:S11–S18

Grene R (2002) Oxidative stress and acclimation mechanisms in plants. In: Somerville CR, Myerowitz EM (eds) The *Arabidopsis* book. American Society of Plant Biologists, Rockville. Available at: www.aspb.org/publications/Arabidopsis/

Gupta R, Luan S (2003) Redox control of protein tyrosine phosphatases and mitogen-activated protein kinases in plants. Plant Physiol 132:1149–1152

Halliwell B, Gutteridge JMC (2000) Free radicals in biology and medicine. Oxford University Press, Oxford

Hernández JA, Corpas FJ, Gómez M, Río LA del, Sevilla F (1993) Salt induced oxidative stress mediated by activated oxygen species in pea leaf mitochondria. Physiol Plant 89:103–110

Hernández JA, Jiménez A, Mullineaux P, Sevilla F (2000) Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defenses. Plant Cell Environ 23:853–862

Hernández JA, Ferrer MA, Jiménez A, Barcelo AR, Sevilla F (2001) Antioxidant systems and $O_2^{\cdot-}/H_2O_2$ production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. Plant Physiol 127:817–831

Hideg E, Barta C, Kalai T, Vass I, Hideg K, Asada K (2002) Detection of singlet oxygen and superoxide with fluorescent sensors in leaves under stress by photoinhibition or UV radiation. Plant Cell Physiol 43:1154–1164

Hourton-Cabassa C, Matos AR, Zachowski A, Moreau F (2004) The plant uncoupling protein homologues: a new family of energy-dissipating proteins in plant mitochondria. Plant Physiol Biochem 42:283–290

Hu X, Bidney DL, Yalpani N, Duvick JP, Crasta O, Folkerts O, Lu GH (2003) Overexpression of a gene encoding hydrogen peroxide-generating oxalate oxidase evokes defense responses in sunflower. Plant Physiol 133:170–181

Ichimura K, Mizoguchi T, Irie K, Morris P, Giraudat J, Matsumoto K, Shinozaki K (1998) Isolation of ATMEKK1 (a MAP kinase kinase kinase)-interacting proteins and analysis of a MAP kinase cascade in *Arabidopsis*. Biochem Biophys Res Commun 253:532–543

Ichimura K, Mizoguchi T, Yoshida R, Yuasa T, Shinozaki K (2000) Various abiotic stresses rapidly activate *Arabidopsis* MAP kinases ATMPK4 and ATMPK6. Plant J 24:655–665

Imlay JA, Linn S (1988) DNA damage and oxygen radical toxicity. Science 240:1302–1309

Jiménez A, Hernández JA, Río LA del, Sevilla F (1997) Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol 114:275–284

Kliebenstein DJ, Monde RA, Last RL (1998) Superoxide dismutase in *Arabidopsis*: an eclectic enzyme family with disparate regulation and protein localization. Plant Physiol 118:637–650

Kovtun Y, Chiu WL, Tena G, Sheen J (2000) Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc Natl Acad Sci USA 97:2940–2945

Krasnovsky AA Jr (1998) Singlet molecular oxygen in photobiochemical systems: IR phosphorescence studies. Membr Cell Biol 12:665–690

Kurepa J, Hérouart D, Van Montagu M, Inzé D (1997) Differential expression of CuZn- and Fe-superoxide dismutase genes of tobacco during development, oxidative stress and hormonal treatments. Plant Cell Physiol 38:463–470

Kużniak E, Skłodowska M (2005) Fungal pathogen-induced changes in the antioxidant systems of leaf peroxisomes from infected tomato plants. Planta 222:192–200

Kwon SY, Jeong YJ, Lee HS, Kim JS, Cho KY, Allen RD, Kwak SS (2002) Enhanced tolerances of transgenic tobacco plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against methyl viologen-mediated oxidative stress. Plant Cell Environ 25:873–882

Levine A, Tenhaken R, Dixon R, Lamb C (1994) H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79:583–593

López-Huertas E, Corpas FJ, Sandalio LM, Río LA del (1999) Characterization of membrane polypeptides from pea leaf peroxisomes involved in superoxide radical generation. Biochem J 337:531–536

Luwe M (1996) Antioxidants in the apoplast and symplast of beech (*Fagus sylvatica* L.) leaves: seasonal variations and responses to changing ozone concentration in air. Plant Cell Environ 19:321–328

Maxwell DP, Wang Y, McIntosh L (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc Natl Acad Sci USA 96:8271–8276

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Mittler R, Vanderauwera S, Gollery M, Breusegem FV (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490–498

Mittova V, Tal M, Volokita M, Guy M (2003) Up-regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species. Plant Cell Environ 26:845–856

Mittova V, Guy M, Tal M, Volokita M (2004) Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. J Exp Bot 55:1105–1113

Miyagawa Y, Tamoi M, Shigeoka S (2000) Evaluation of the defense system in chloroplasts to photooxidative stress caused by paraquat using transgenic tobacco plants expressing catalase from *Escherichia coli*. Plant Cell Physiol 41:311–320

Miyake C, Asada K (1994) Ferredoxin-dependent photoreduction of monodehydroascorbate radicals in spinach thylakoids. Plant Cell Physiol 35:539–549

Mizoguchi T, Irie K, Hirayama T, Hayashida N, Yamaguchi-Shinozaki K, Matsumoto K, Shinozaki K (1996) A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously

with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 93:765–769

Murgia I, Tarantino D, Vannini C, Bracale M, Carrabvieri S, Soave C (2004) *Arabidopsis thaliana* plants overexpressing thylakoidal ascorbate peroxidase show resistance to paraquat-induced photooxidative stress and to nitric oxide-induced cell death. Plant J 38:940–995

Møller IM (2001) Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annu Rev Plant Physiol Plant Mol Biol 52:561–591

Nakagami H, Pitzschke A, Hirt H (2005) Emerging MAP kinase pathways in plant stress signalling. Trends Plant Sci 10:339–346

Pang CH, Zhang SJ, Gong ZZ, Wang BS (2005) NaCl treatment markedly enhances H_2O_2-scavenging system in leaves of halophyte *Suaeda salsa*. Physiol Plant 125:490–499

Polle A, Chakrabarti K, Schümann W, Rennenberg H (1990) Composition and properties of hydrogen peroxide decomposing systems in extracellular and total extracts from needles of Norway Spruce (*Picea abies* L., Karst.). Plant Physiol 94:312–319

Popov VN, Simonina RA, Skulachev VP, Starkov AA (1997) Inhibition of the alternative oxidase stimulates H_2O_2 production in plant mitochondria. FEBS Lett 415:87–90

Puntarulo S, Sánchez RA, Boveris A (1988) Hydrogen peroxide metabolism in soybean embryonic axes at the onset of germination. Plant Physiol 86:626–630

Purvis AC (1997) Role of the alternative oxidase in limiting superoxide production in plant mitochondria. Physiol Plant 100:165–170

Purvis AC, Shewfelt RL (1993) Does the alternative pathway ameliorate chilling injury in sensitive plant tissues? Physiol Plant 88:712–718

Raha S, Robinson BH (2000) Mitochondria, oxygen free radicals, disease and ageing. Trends Biochem Sci 25:502–508

Reumann S, Bettermann M, Benz R, Heldt HW (1997) Evidence for the presence of a porin in the membrane of glyoxysomes of castor bean. Plant Physiol 115:891–899

Rhoads DM, Umbach AL, Subbaiah CC, Siedow JN (2006) Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. Plant Physiol 141:357–366

Río LA del, Pastori GM, Palma JM, Sandalio LM, Sevilla F, Corpas FJ, Jiménez A, López-Huertas E, Hernández JA (1998) The activated oxygen role of peroxisomes in senescence. Plant Physiol 116:1195–1200

Río LA del, Corpas FJ, Sandalio LM, Palma JM, Gómez M, Barroso JB (2002) Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. J Exp Bot 53:1255–1272

Río LA del, Sandalio LM, Corpas FJ, Palma JM, Barroso JB (2006) Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. Plant Physiol 141:330–335

Scandalios JC (1993) Oxygen stress and superoxide dismutases. Plant Physiol 101:7–12

Sluse FE, Jarmuszkiewicz W (2004) Regulation of electron transport in the respiratory chain of plant mitochondria. In: Day DA, Millar AH, Whelan J (eds) Plant mitochondria: from genome to function, vol 1. (Advances in photosynthesis and respiration) Kluwer, Dordrecht, pp 229–245

Smirnoff N (1993) The role of active oxygen in response of plants to water deficit and desiccation. New Phytol 125:27–58

Sweetlove LJ, Foyer CH (2004) Roles for reactive oxygen species and antioxidants in plant-mitochondria. In: Day DA, Millar AH, Whelan J (eds) Plant mitochondria: from genome to function, vol 1. (Advances in photosynthesis and respiration) Kluwer, Dordrecht, pp 307–320

Takeshiga K, Minakami S (1979) NADH and NADPH dependent formation of superoxide anions by bovine heart submitochondrial particles and NADH-ubiquinone reductase preparation. Biochem J 180:129–135

Tarantino D, Vannini C, Bracale M, Camp M, Soave C, Murgia I (2005) Antisense reduction of thylakoidal ascorbate peroxidase in *Arabidopsis* enhances paraquat-induced photooxidative stress and nitric oxide induced cell death. Planta 221:757–765

Teige M, Scheikl E, Eulgem T, Doczi R, Ichimura K, Shinozaki K, Dangl JL, Hirt H (2004) The MKK2 pathway mediates cold and salt stress signaling in *Arabidopsis*. Mol Cell 15:141–152

Vanacker H, Carver TLW, Foyer CH (1998a) Pathogen induced changes in the antioxidant status of the apoplast in barley leaves. Plant Physiol 117:1103–1114

Vanacker H, Harbinson J, Carver TLW, Foyer CH (1998b) Antioxidant defenses of the apoplast. Protoplasma 205:129–140

Wang BS, Lüttge U, Ratajczak R (2004) Specific regulation of SOD isoforms by NaCl and osmotic stress in leaves of the C3 halophyte *Suaeda salsa* L. J Plant Physiol 161:285–293

Wang J, Zhang H, Allen RD (1999) Overexpression of an *Arabidopsis* peroxisomal ascorbate peroxidase gene in tobacco increases protection against oxidative stress. Plant Cell Physiol 40:725–732

Walters DR (2003) Polyamines and plant disease. Phytochemistry 64:97–107

Willekens H, Chamnongpol S, Davey M, Schraudner M, Langebartels C, Van Montagu M (1997) Catalase is a sink for H_2O_2 and is indispensable for stress defence in C_3 plants. EMBO J 16:4806–4816

Yabuta Y, Motoki T, Yoshimura K, Takada T, Ishikawa T, Shigeoka S (2002) Thylakoid membrane-bound ascorbate peroxidase is a limiting factor of antioxidative systems under photo-oxidative stress. Plant J 32:912–925

Zhang QF, Li YY, Pang CH, Lu CM, Wang BS (2005) NaCl enhances thylakoid-bound SOD activity in the leaves of C3 halophyte *Suaeda salsa L.* Plant Sci 168:423–430

Zhu D, Scandalios JG (1992) Expression of the maize *MnSod* (*Sod3*) gene in MnSOD-deficient yeast rescues the mutant yeast under oxidative stress. Genetics 131:803–809