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Editors

Nitric Oxide Action in Abiotic Stress Responses in Plants

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Foreword

Plants are essential to life on Earth, and they have been harnessed by humans for food, fuel, and many other purposes. The need to increase crop production is becoming more urgent due to increasing population and diversion of crops to biofuels production. Furthermore, this increase in production needs to be done sustainably, with reduced inputs, and in the face of global environmental change. It is also notable that at least one-third of the world's food production is grown under irrigation—much of this irrigation is unsustainable, using water supplies that are both overexploited and under threat from changing weather patterns resulting from global climate change. To meet the consensus targets for increased food production by 2050, significant increases in historical annual increases in production are required, which requires substantial innovations in agricultural production. Many of these innovations must ultimately come from, among other sources, plant science research. One important and relevant area of this plant science research is covered in-depth in this book.

The gap between potential yield and actual yield is primarily due to the effects of abiotic stresses on crop production. It is therefore an imperative to improve our ability to maintain crop production in environments with suboptimal conditions such as low water or nutrient supplies, or high salinity. One of the key components of plant responses to these abiotic stresses is nitric oxide (NO). Understanding the role of NO in the control of plant reactions provides fundamental information to underpin the much-needed innovations to come in global food production.

As such, the book edited by Drs. Khan, Mobin, Mohammad, and Corpas provides a useful and timely compilation of up-to-date overviews of advances in the important area of plant sciences, "Nitric Oxide Action in Abiotic Stress Responses in Plants." In this volume, a range of papers have been brought together which address the multifarious roles of NO in plant function and the responses of plants to many abiotic stresses. The breadth of areas covered in this volume highlights well the importance of this simple molecule and thus the utility of this compilation. The highly international set of contributors, including many from developing countries, is also both valuable and encouraging.

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Chapter 13
Role of Nitric Oxide in Salt Stress-induced Programmed Cell Death and Defense Mechanisms

Péter Poór, Gábor Laskay and Irma Tari

Abstract During the last decade, it has been shown by several authors and in several plant species that nitric oxide (NO) accumulates in tissues exposed to high salinity. This gaseous-free radical and signaling compound can attenuate the ionic component of salt stress by enhancing Na^+ extrusion from the cells via Na^+/H^+ exchange through the activation of plasma membrane and vacuolar H^+-ATPases and H^+-pyrophosphatase. NO alleviates the osmotic stress caused by high salt concentrations by stimulating the biosynthesis of compatible osmolytes such as proline, glycine betaine, and soluble sugars, and it also protects the cells from the oxidative damage by enhancing non-enzymatic and enzymatic antioxidants. However, NO may promote programmed cell death (PCD) depending on the NO scavenging capacity of the cells and on the cellular redox status as well as on the flux and dose of local reactive nitrogen and oxygen forms generated in various cell compartments. Particular attention is paid to the role of NO and NO-induced protein modifications in the activation of specific steps of PCD during salt stress.

Keywords High salinity · Nitric oxide · Programmed cell death · Reactive oxygen species · Salt stress

13.1 Introduction

It is estimated that more than 6 % of the total land area and about 30 % of the world's irrigated area are salt affected and the increasing secondary salinization of soil and groundwater is one of the most important environmental factors that decrease the productivity of crop plants (Munns 2002; Lambers 2003).

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High salinity imposes osmotic stress on plants by preventing water uptake of root tissues and causes a rapid reduction in stomatal closure, transpiration, photosynthesis, and eventually in shoot growth rate. The salt-specific ionic effects appear later and the excessive amount of NaCl in leaf tissues results in premature senescence of older leaves, decreases the biomass production, and finally it may induce programmed cell death (PCD) in root or leaf tissues of salt-sensitive species or genotypes (Munns and Tester 2008; Shabala 2009). Understanding the mechanisms that regulate salt tolerance and the salt-induced PCD at the molecular, cellular, tissue, or whole-plant levels is an important problem in plant biology and molecular biological technics speeded up the elucidation of the distinct and relevant responses in salt-tolerant and sensitive plants. Nitric oxide (NO) is a biologically active gaseous-free radical that transmits the environmental or hormonal signals during different biotic and abiotic stresses including salt stress (Corpas et al. 2011). Salt-induced NO generation in parallel with hydrogen peroxide (H_2O_2) accumulation can act both independently and synergistically, and they are involved in several downstream signal transduction pathways determining cell fate (Siddiqui et al. 2011). In the past ten years, the role of NO in salt tolerance and salt toxicity has been studied using exogenously applied NO generators, NO scavengers, and mutant plants with higher or lower activities in NO production. These plants have generally but not exclusively mutations in the genes encoding the most important enzymes participating in NO biosynthesis or NO levels may be controlled by specific inhibitors.

In this review, we highlight the current state of the art of the physiological and molecular aspects of NaCl-induced NO signaling in plants and discuss the roles of endogenous and exogenous NO in NaCl toxicity and salt tolerance mechanisms.

13.2 NaCl Tolerance in Plants

Plant responses to salt stress can be divided into two phases: A rapid, osmotic phase that inhibits the growth of young leaves, and a slower, ionic phase that elicits ion-specific effects and accelerates senescence of mature leaves (Munns and Tester 2008). It can be concluded, however, that the effects of salt stress depend not only on the concentration of NaCl but also on the duration of the stress and on the age and sensitivity of the plants. Salt stress-induced osmotic, ionic, and oxidative stresses cause strong toxicity and finally induce PCD in sensitive species (Zhu 2002) while salt-tolerant plants, especially the halophytes, might be growing at their optimum rate at the same salt concentration.

Munns and Tester (2008) defined three distinct types of plant adaptation to highsalinity: osmotic stress tolerance, the exclusion of Na^+ or Cl^- ions from the cells, and tissue tolerance that is the tolerance of Na^+ after vacuolar sequestration inside the cells.

Salt stress reduces water uptake by the roots leading to a reduction in relative water content (RWC) of the tissues and to lower osmotic potential which results

in growth inhibition, stomatal closure, and a decrease in photosynthesis (Munns and Tester 2008). These short-term changes are solely due to osmotic stress evoked by high salinity. The balance of osmotic potential between the soil solution and cell compartments can be achieved by the accumulation of compatible solutes such as proline (Pro), glycine betaine, mannitol, or sugars. They can also act as osmoprotectants because their high concentration in the cytosol and organelles is beneficial for the maintenance of the tertiary structure of proteins (Rhodes et al. 2004). Halophytes or salt-tolerant glycophytes with high tissue tolerance can use Na^+ for osmotic adjustment after its sequestration into the vacuole (Munns and Tester 2008).

Plants have multiple Na^+ transport systems to circumvent salt toxicity. The maintenance of the optimal K^+/Na^+ ratio, the high K^+, and low Na^+ concentrations in the cytoplasm are of primary importance to protect cellular functions (Zhu 2003). In barley high-affinity Na^+ uptake trough, a uniporter was inhibited by external K^+ in long-term experiments (Haro et al. 2005), suggesting that the uptake of sodium depends on the K^+/Na^+ discrimination of the transporter system. The removal of the excess Na^+ from the cytoplasm occurs by secondary active transport through Na^+/H^+ antiporters which can be localized to the plasma membrane (PM) and tonoplast, the former is known as SOS1 in *Arabidopsis* and the latter is the member of NHX vacuolar exchanger family (Blumwald 2000, Hasegawa et al. 2000). The importance of these antiporters to improve salt tolerance was confirmed by the overexpression of vacuolar Na^+/H^+ (AtNHX1) or PM (SOS1) antiporter genes in *Arabidopsis* (Zhu 2002; Pardo et al. 2006). SOS1 is preferentially expressed in the xylem parenchyma cells, suggesting a role of this antiporter in loading Na^+ into the xylem (Pardo et al. 2006). Na^+/H^+ antiporter activity is driven by the electrochemical proton gradient generated by H^+ pumps, such as PM and vacuolar H^+-ATPases and H^+-pyrophosphatase (H^+-PPase) (Hasegawa et al. 2000). Zhang et al. (2006) found that the salt stress-induced increase in vacuolar H^+-ATPase and H^+-PPase activities resulted in an increased H^+ translocation, Na^+/H^+ exchange, and enhanced salt tolerance. Recent research has demonstrated that the members of HKT transporter family play an essential role in Na^+ exclusion from the leaves under salt stress. *Arabidopsis* HKT1;1 was found to localize to the plasma membrane of xylem parenchyma cells and this transporter mediates the removal of Na^+ from the xylem sap via Na^+ uptake into xylem parenchyma cell (Horie et al. 2009). Comparing the salt stress-induced gene expression of the salt-sensitive *Arabidopsis thaliana* with its salt-tolerant relative *Thellungiella salsuginea* (previously *Thellungiella halophyla*), the salt-tolerant species showed stress-specific and stress intensity-dependent responses and a fundamental difference was found in the steady-state amount of SOS1 and other salt stress-related mRNAs and in the level of their induction during salt stress (Dassanayake et al. 2011).

Various sub-cellular organelles or cell compartments such as chloroplasts, mitochondria, peroxisomes, and the apoplast are common sites of reactive oxygen species (ROS) production under high salinity (Ashraf 2009). The survival of plants under

ROS by various antioxidant systems (Foyer and Noctor 2005; Miller et al. 2010). These enzymes act as ROS scavengers to alleviate the salt-induced oxidative damage in distinct cell compartments. Superoxide dismutase (SOD) catalyzes the dismutation of superoxide radical into molecular oxygen and hydrogen peroxide (H_2O_2). H_2O_2 can be scavenged by peroxidases (POX), especially by ascorbate- or glutathione peroxidase (APX or GPX) and by catalase (CAT). Glutathione reductase (GR) recycles oxidized glutathione (GSSG) to glutathione (GSH) using NADPH to re-establish the reduced GSH pool (Miller et al. 2010). These enzymes, in accordance with non-enzymatic antioxidants such as ascorbate (ASA), GSH, tocopherol, and flavonoids (Foyer and Noctor 2003), maintain appropriate H_2O_2 levels required for signal transduction processes under salt stress. The association of high levels of non-enzymatic antioxidants and the high activity of ascorbate–glutathione cycle with salt tolerance can be observed in several species (Ashraf 2009), but the activities of antioxidant enzymes were not a reliable criterion for salt tolerance in some species from the Brassicaceae family (Siegal et al. 1982).

Similarly, the accumulation of polyamines, putrescine (Put), spermidine (Spd), and spermine (Spm) is associated with salt stress (Gill and Tuteja 2010) because they have a positive effect on the maintenance of ion balance, membrane stabilization, and ROS scavenging in plants and inhibit both lipid peroxidation and stress-induced oxidative reactions (Ashraf and Harris 2004; Groppa and Benavides 2008). Alternatively, polyamines can act as prooxidants because their catabolism by the activities of diamine oxidase (DAO) and polyamine oxidases (PAO) produces H_2O_2, which can induce PCD (Cona et al. 2006; Yoda et al. 2006; Moschou et al. 2008; Wimalasekera et al. 2011).

Photosynthesis is among the primary processes affected by salinity. The osmotic component of salt stress restricts CO_2 availability by limitation of its diffusion through stomata and mesophyll. Ionic effects can seriously inhibit the photosynthetic metabolism; excessive Na^+ or Cl^- accumulation in chloroplasts leads to the degradation of chlorophyll, damage to light-harvesting complexes, suppression of PS II activity (Mehta et al. 2010), and reduced efficiency of ribulose-1,5-bisphosphate carboxylase (Rubisco) (Abdel-kader et al. 2007; Chaves et al. 2009). Genes or proteins associated with photosynthesis are not among the most altered genes in plants exposed to salt stress. It was found that larger alterations can be observed at transcriptomic level (5–10 %) than at protein level (<1 %) (Chaves et al. 2009). Several genes encoding proteins of the photosynthetic apparatus (subunits of ATP synthase, proteins from PS II and PS I) or enzymes of the Calvin cycle and photorespiration such as fructose-*bis*phosphatase, aldolase, phosphoribulokinase, glycine hydroxymethyltrasferase, or transketolase were downregulated by salt stress; however, the members of multigene families may be differently affected (Kilian et al. 2007).

The biomass production of plants is largely determined by the balance between photosynthesis and respiration. The respiration of tissues exposed to salt stress may increase or decrease or there are no consistent changes in respiratory rate in about one-third of the studies. Depending on salt tolerance strategy, a higher res-

determines the carbon allocation from shoot to roots and thus the growth capacity of plant parts and it fuels the ATP requirement for the ion exclusion mechanisms in root tissues (Jakoby et al. 2011).

Respiratory rate of isolated plant mitochondria may be reduced by high salt concentrations. Mitochondrial electron transport produces ATP by utilizing the H^+ electrochemical gradient generated by Complex I, Complex III, and Complex IV localized in the inner mitochondrial membrane. The activity of electron transport chain complexes, the NADH dehydrogenases (Complex I), and succinate dehydrogenase (Complex II) is inhibited at toxic Na^+ concentrations owing to protein denaturation and complex disassembly (Flowers 1974; Hamilton and Heckathorn 2001). Salt stress decreased the capacity of the cytochrome pathway, whereas the capacity of alternative oxidase (AOX) increased, which prevented the high reduction level of the ubiquinone pool. Since the major site of superoxide production along the respiratory chain is the ubiquinone pool, higher AOX activity plays a pivotal role in lowering mitochondrial ROS formation in plant cells (Jolivet et al. 1990; Smith et al. 2009). Mitochondria generate a large portion of cellular ROS, and a strong link exists between the mitochondrial antioxidant capacity and the salt tolerance of plants. ROS signals emanating from plant mitochondria determine the survival or death signals under salt stress (Dutilleul et al. 2003). Although nuclear-encoded mitochondrial proteins were over-represented among salinity responsive proteins, there was no strict correlation between transcript level and protein abundance. In contrast, they showed an inverse relationship (reduced mRNS/increased protein abundance) in the cases of NADH dehydrogenase 39-kDa subunit and malate dehydrogenase while there was a decrease in both transcript and protein abundances of cytochrome *c* oxidase subunit 6b and fumarase in short-term experiments. Glutathione peroxidase and mitochondrial heat shock protein 70-2 were up-regulated at mRNS level while salt stress enhanced the protein abundance of Mn-SOD (Jiang et al. 2007; Taylor et al. 2009). This suggests that the protection of mitochondrial function under abiotic stress takes place by fast changes at transcriptional and protein levels.

13.3 NaCl Toxicity and Salt-induced Cell Death in Plants

PCD is an organized and controlled active cell suicide during the normal life cycle of plants and in response to changing environment. It can be triggered by the developmental program or by abiotic and biotic stressors and it is associated with specific biochemical and molecular hallmarks (van Doorn 2005; van Doorn and Woltering 2005; Gunawardena 2008; van Doorn 2011). Although the three main forms of animal PCD, the apoptosis, autophagy, and necrosis-like cell death, were compared with various types of plant PCD, there is no clear correspondence between these main categories due to the presence of cell wall and the lack of phagocytes in plants. Cytoplasmic shrinkage, nuclear chromatin condensation, and finally the fragmentation of DNA, the hallmarks of animal apoptosis, are found in

In animal cells, DNA fragmentation during apoptosis is catalyzed by a cysteinyl-aspartate specific proteinase, "caspase-"activated DNase which cleaves DNA into internucleosomal fragments. These cysteine proteases and caspases are central players of animal apoptosis. They exist as inactive proenzymes that are activated by proteolytic processing catalyzed by initiator caspases. They work in a cascade and cleave the inactive proenzyme of the down-stream effector caspases to form active heterodimers (Enari et al. 1998) which then degrade specific protein substrates. However, up to now, no homolog of animal caspases has been found in plants, but there are other enzymes, such as vacuolar processing enzyme (VPE) which exhibit caspase-like activities (Hatsugai et al. 2004). In plants, other cell-death-specific cysteine proteases and metacaspases show structural similarities to caspases, but they have proteolytic activity adjacent to arginine and lysine residues of the substrates (Woltering 2010). Metacaspases are involved in various types of cell death induced by abiotic stressors, e.g., oxidative stress (He et al. 2008). However, it was found that type I metacaspases suppress and type II metacaspases stimulate cell death program in plants (Woltering 2010).

Release of apoptogenic factors such as cytochrome *c* (cyt *c*) from the intermembrane space of mithocondria is a common feature of animal and plant PCD which drives the assembly of the apoptosome, a caspase-activating complex in the cytoplasm of animal cells (Kroemer et al. 2007). The permeabilization of mitochondrial membrane and cyt *c* release was associated with plant PCD as well, following death stimuli, such as heat shock, menadione, or ceramide treatments (reviewed by Reape and McCabe 2010).

In plants, the generation of excess amount of ROS was proposed as a key inducer of various types of PCD (De Pinto et al. 2012). Increased ROS, especially H_2O_2, may activate PM-Ca^{2+} channels and increase $[Ca^{2+}]_{cyt}$ which is an inducer of the permeabilization of mitochondrial membranes. In animal cells, the integrity of the outer mitochondrial membrane is maintained through the balance of pro-apoptotic (Bax, Bak, Bad, Bid) and anti-apoptotic (Bcl-2 and Bcl-xL) proteins. These proteins can control the release of proteinaceous factors from the intermembrane space by controlling the formation of permeability transition pore (PTP) on the mitochondrial envelope through the interaction of voltage-dependent anion channel, the adenine nucleotide transporter, and cyclophilin D (Jones 2000). Although the pro-apoptotic Bax protein operates in PTP formation in animal systems and Bcl-2 and Bcl-xL antiapoptotic factors inhibit Bax action and can confer resistance to death, their plant homologs have not been identified yet. However, a cell death suppressor, Bax-inhibitor-1, has been reported in *Arabidopsis* plants that localizes to the endoplasmatic reticulum and exhibits a pH-dependent Ca^{2+} channel-regulating activity (Ihara-Ohori et al. 2007; Kim et al. 2008).

The formation of PTP following stress-induced increase in $[Ca^{2+}]_{cyt}$ and ROS production leads to the loss of mitochondrial membrane potential ($\Delta\psi_m$) and to the release of cyt *c* into the cytoplasm during PCD of plant cells, too. Although cyt *c* does not appear to directly activate PCD in plants (Balk et al. 2003), its absence from the mitochondrial electron transport chain may lead to enhanced generation of ROS (Vianello et al. 2007). However, in rice, an increased ROS production

preceded the cyt *c* release from root mitochondria under salt stress. The analysis of the rice salt proteome by two-dimensional IEF/SDS-PAGE revealed that four mitochondrial proteins, glycoside hydrolase, mitochondrial heat shock protein 70, 20S proteasome subunit, and Cu/Zn-SOD, were up-regulated during PCD induction (Chen et al. 2009).

Autophagic cell death, also known as type II cell death in animals, was characterized by a lack of chromatin condensation and by the appearance of autophagosomes, the double membrane-possessing structures. Autophagosomes contain hydrolytic enzymes and digest the engulfed cellular components, but their role as executioners of PCD has been debated in plants (Cacas and Diamond 2009). During micro-, macro-, and megaautophagy, the disappearance of organelles and cytoplasmic constituents occurs through the activity of lytic compartments (autophygosomes or vacuole) (van Doorn and Woltering 2005), which contributes to the recycling of cellular components, thus the outcome of authophagy is dependent on the plant fitness and may have a pro-life and/or pro-death function.

Salt stress-induced PCD is also accompanied by the retraction of the plasma membrane from the cell wall, condensation of the cytoplasm and the nucleus, DNA laddering, loss of membrane integrity, release of cytochrome *c* from mitochondria, increase in caspase 3-like protease activity, and changes in the ion homeostasis and K^+ efflux (Fig. 13.1) (Wang et al. 2010a; Poór et al. 2013).

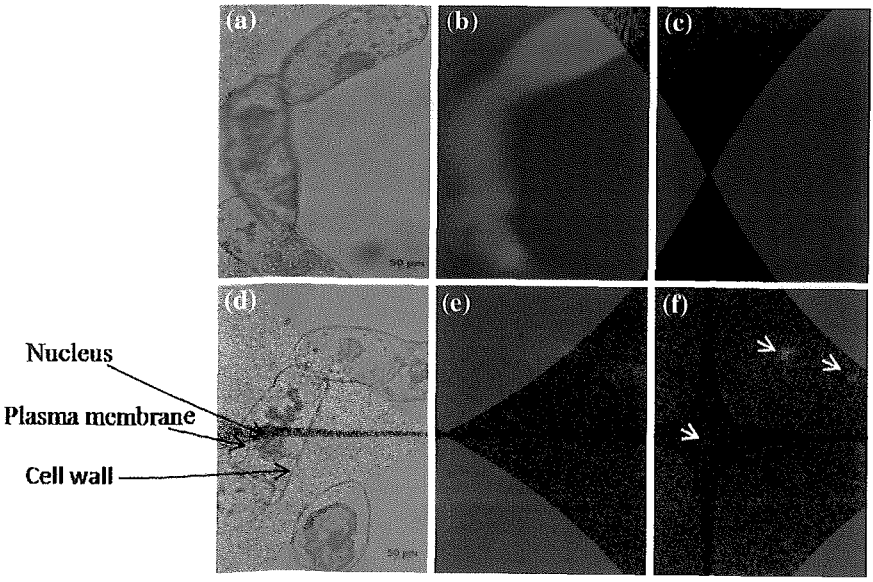

Fig. 13.1 Detection of DNA fragmentation by TUNEL staining in tomato cell suspension treated with 250 mM NaCl for 6 h. *Part A* Control (**a**, **b**, **c**), 250 mM NaCl (**d**, **e**, **f**), bright field microscopy (**a**, **d**); Hoechst 33258 staining (**b**, **e**); TUNEL-positive nuclei (*f*) (reproduced from Poór et al. 2013)

NaCl has multiple ways to induce toxicity and cell death in plants. First, the NaCl-induced osmotic stress reduces cell expansion in root tips and young leaves; however, the osmotic component of salt stress does not lead to the activation of the endonuclease involved in DNA degradation and it is not enough to induce PCD in plant cells. Affenzeller et al. (2009) reported that the exogenous treatment with sorbitol (an agent which induces only osmotic but not ionic stress) did not result in DNA laddering on agarose gel, which is one of the hallmarks of the apoptosis-like PCD.

Secondly, NaCl treatment induces ion disequilibrium in plant cells (Huh et al. 2002). Under saline conditions, Na^+ enters the cell cytoplasm through the non-selective cation channels (NSCC) causing membrane depolarization and resulting in K^+ leakage from the cell through depolarization-activated outward-rectifying K^+ (KOR) channels (Shabala 2009). The accumulation of toxic Na^+ can induce a loss of enzyme activities in the cells. The release of K^+ from the cytoplasm causes K^+ deficiency, which can activate cysteine proteases, the effectors of PCD (Shabala 2009; Demidchik et al. 2010). Potassium is required in many physiological processes of plants, including protein and nucleic acid synthesis. It can be concluded that the decrease in the cytoplasmic K^+/Na^+ ratio is crucial for triggering PCD in living cells (Joseph and Jini 2010).

Finally, salt stress also causes strong oxidative stress-generating ROS such as singlet oxygen ($^1O_2$), superoxide radical (O_2^-), hydroxyl radical (OH), and H_2O_2, which can react with lipids, proteins, and nucleic acids, inactivate enzymes, and enhance lipid peroxidation, membrane leakage, and DNA breakdown; thus, they can induce PCD. ROS are also involved in various signaling pathways such as those of plant hormones, e.g., abscisic acid, jasmonic acid, or ethylene during the plant stress responses (Van Breusegem and Dat 2006; De Pinto et al. 2012). Salt stress increases cytosolic $[Ca^{2+}]$ and activates PM-bound NADPH oxidase, which generates superoxide in the apoplast. At the same time, ROS production causes K^+ efflux via ROS-activated NSCC channels, which also induces PCD (Shabala 2009). Other sources of ROS are the photosynthetic and respiratory electron transport chains or the peroxisomes (Foyer and Noctor 2003). In addition, the interaction of ROS and NO and the balance between antioxidants and hormone levels can adjust the induction of PCD or tolerance mechanisms in plants under high salinity.

13.4 NO Production in Plants Exposed to NaCl

NO is a gaseous signaling molecule, which regulates a wide range of physiological and biochemical processes, and the growth and development of plants as well as their responses to biotic and abiotic stresses (Neill et al. 2003; Wendehenne et al. 2004; Delledone 2005; Corpas et al. 2011; Siddiqui et al. 2011).

In plant kingdom, NO can be generated non-enzymatically via reduction of nitrite to NO by the mitochondrial electron transport chain and by the reduction of apoplastic nitrite under acidic conditions. The enzymatic reduction of nitrite to NO is a function of nitrate reductase (NR) in plant tissues (Yamasaki and Sakihama 2000).

Arabidopsis NR is encoded by two nitrate reductase genes, *NIA1* and *NIA2*, and *nia1/nia2* double mutant plants show low NO accumulation (Desikan et al. 2002; Bright et al. 2006). In this case, NO production can be reduced by specific NR inhibitors such as sodium azide (NaN_3) or tungstate (Bright et al. 2006; Sang et al. 2008).

Arginine (Arg)-dependent NO production by nitric oxide synthase (NOS)-like activities suggests the presence of oxidative pathway of NO synthesis in plants (del Rio et al. 2004; Corpas et al. 2006). Plant NOS activity can be inhibited by animal NOS inhibitors that act as Arg analogs, such as NG-nitro-L-arginine-methyl ester (L-NAME) and NG-monomethyl-L-arginine (L-NMMA) (Guo and Crawford 2005). Xanthin oxidoreductase and polyamine/hydroxylamine-dependent NO production has also been reported in plants (reviewed by Mur et al. 2013). NO content of tissues can be elevated by the application of exogenous NO donors (e.g., diethylamine nitric oxide, DEANO; 2,2'-(hydroxynitrosohydrazono)*bis* ethane DETA/NO; *S*-nitrosoglutathione, GSNO; sodium nitroprusside, SNP) which cause different concentration and time-dependent increases in tissue NO level (Mur et al. 2013).

Various salt treatments enhanced NO production in many plant species and plant organs (Fig. 13.2). NO accumulation was observed in roots (Xie et al. 2008, Xu et al. 2011a), leaves (Tanou et al. 2012), calli (Zhao et al. 2004, 2007; Vital

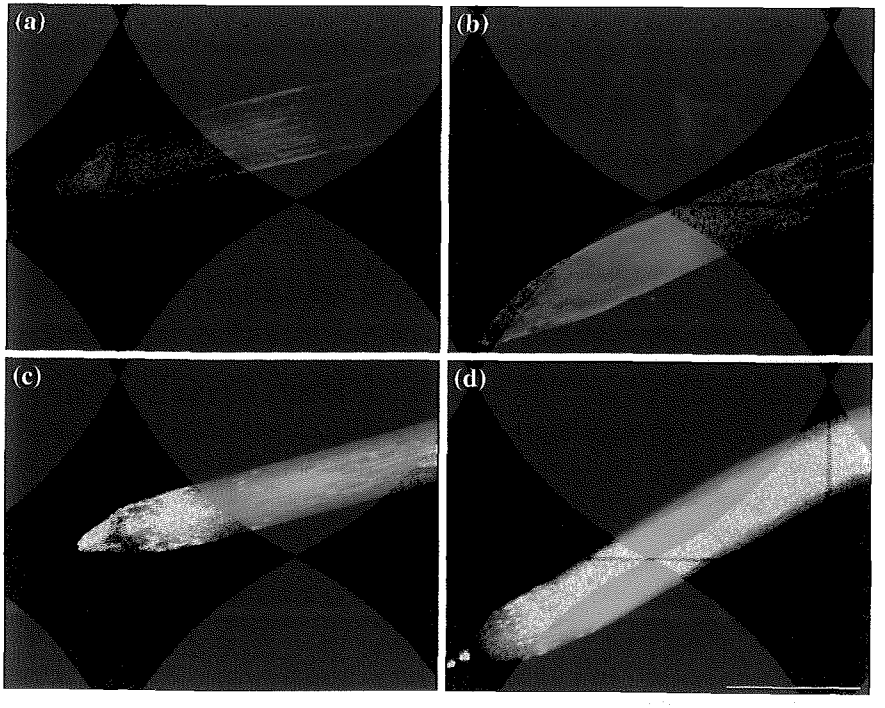

Fig. 13.2 Changes in NO levels in the apical segments of tomato roots treated with 250 mM NaCl for 0 (**a**), 1 (**b**), 3 (**c**), and 6 (**d**) hours. NO was visualized using 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM DA). White bars represent 1 mm (Poór et al. unpublished results)

et al. 2008; Wang et al. 2009; Sun et al. 2010; Yang et al. 2010), cell suspension cultures (Banu et al. 2010; Poór and Tari 2011), and protoplasts (Gémes et al. 2011) after NaCl treatment. Zhang et al. (2006) observed time-dependent changes in NO production in *Zea mays* leaves after treatment with 100 mM NaCl. NO accumulation displayed a maximum after 2 h of NaCl exposure and after 8 h it decreased back to the control level. A similar tendency was observed in *Arabidopsis thaliana* calli (Wang et al. 2009) and *Solanum lycopersicum* cell suspension culture (Poór et al. 2013). Rapid NO and ROS productions were detected in calli of a highly resistant species, *Populus euphratica* during salt stress(Sun et al. 2010). These results suggested that the NaCl-induced NO production was transient and NO levels increased significantly in parallel with ROS production in the first minutes and hours of salt treatment. Unfortunately, in most of the studies, the production of NO was determined only at one time-point after the salt exposure and there was no time-dependent analysis of NO and other reactive nitrogen species (RNS) such as peroxinitrite ($ONOO^-$) during salt stress in parallel with the determination of various types of reactive oxygen forms, which can determine the fate of the cells later.

Numerous studies reported that NO could be synthesized by NOS-like activity as well as by NR after salt treatment. NaCl-induced NO synthesis was inhibited by the NOS inhibitor L-NAME in *Atriplex centralasiatica* (Xu et al. 2011a), *Olea europaea* (Valderrama et al. 2007), *Phragmites communis* (Zhao et al. 2004), and *Populus euphratica* (Zhang et al. 2007). In contrast, Liu et al. (2007) reported that NO production, induced by 100 mM NaCl, was not affected by L-NAME in *Phaseolus vulgaris*. In *Arabidopsis thaliana*, Zhao et al. (2007a) observed a reduced quantity of the NOA1 protein and decreased NO levels after being exposed to 100 mM NaCl for 2 h. *AtNOA1* (*AtNOS1*) was identified as a putative *Arabidopsis* NOS gene, because these *Arabidopsis* mutants were defective in NO accumulation in the roots (Guo et al. 2003), but later it was found that *AtNOS1* had no NOS activity in vitro. Thus, *AtNOS1* was renamed as NO-associated protein 1 (*AtNOA1*), which is a chloroplast-targeted GTPase essential for ribosome assembly (Moreau et al. 2008). On the other hand, the NR-mediated NO production was involved in salt stress response of *Nicotiana tabacum* (Charrier et al. 2013) and *Olea europaea* (Valderrama et al. 2007). These discrepancies could be explained by the differences in the salt concentrations applied, the duration of salt exposure, the plant species and tissues, and the age of plants in the experiments. It can be concluded that the origin of NO may vary from species to species and from tissue to tissue that show different sensitivities to increased Na^+ concentrations.

13.5 NO in Signal Transduction

The role of NO in the regulation of gene expression was revealed first in large-scale transcriptional analysis of *Arabidopsis thaliana* by DNA-microarrays (Grün et al. 2006). Using NO donor, SNP, 342 genes were up-regulated and 80 were

down-regulated in *Arabidopsis* roots (Parani et al. 2004). These genes are involved in signal transduction, cellular transport, photosynthesis, abiotic stress response, and disease resistance. The transcript levels of signal transduction components such as defence-related *MAP* kinases, *WRKY* transcription factors, and *ERE* (ethylene response element) binding proteins, dehydration responsive element binding proteins (*DREB*1 and *DREB2*), oxidative stress-related proteins (glutathione transferases, *ABC* transporters), mitochondrial (*AOX1*) or chloroplastic proteins, e.g., *SOD* and *APX*, and genes participating in iron homeostasis (ferritin) were enhanced by SNP (reviewed by Grün et al. 2006).

NO participates in multiple signaling pathways in plants (Lamattina et al. 2003; Corpas et al. 2004; Lamotte et al. 2005; Besson-Bard et al. 2008; Distefano et al. 2008; Moreau et al. 2010; del Rio 2011). It interacts with the iron atom of the hem moiety in guanylate cyclase (GC), which, in this activated form, produces the second messenger cyclic GMP (cGMP) and interferes with the activity of protein kinases (PKs). A novel soluble GC that binds NO and generates cGMP has been recently described in *Arabidopsis* (Mulaudzi et al. 2011). NO increases cytosolic Ca^{2+} levels, which modulate the activity of Ca^{2+}-dependent protein kinases (CDPKs) or mitogen-activated protein kinases (MAPKs). Phosphatidic acid (PA), a lipid-derived second messenger is also involved in NO signaling. NO interferes with various steps of ROS-induced signaling and acts as an important mediator of H_2O_2-induced cell death (Wang et al. 2013).

NO can modulate protein structure or activity through *S*-nitrosylation of specific cysteine residues, through nitration of specific tyrosines or through binding to metal cofactors of the enzymes. *S*-nitrosylation of proteins, a reversible post-translational modification, occurs when a cysteine thiol in special position of the protein reacts with NO in the presence of an electron acceptor. The formation of *S*-NO bonds affects the function of a wide range of proteins, e.g., of those participating in signal transduction, such as tyrosine phosphatase 1B (Li and Whorton 2003), nuclear factor-κB kinase (Reynaert et al. 2004), or R2R3-MYB class transcription factors (Palmieri et al. 2008). *S*-nitrosylation of proteins participating in auxin and salicylic acid signaling has been excellently reviewed recently by Astier et al. (2012). NO in the presence of O_2 can react with GSH to form *S-nitrosoglutathione (GSNO)*, a reactive nitrogen form, which is a long-distance signaling molecule and a natural reservoir of NO.

NO is able to react with various forms of ROS, e.g., with superoxide generating $ONOO^-$ and thus can regulate the redox status of the cell. Peroxynitrite is a strong oxidant, thus may target and inhibit cysteine-containing thiols, such as tyrosine phosphatases (Spoel et al. 2010). Peroxynitrite can react with tyrosine and tryptophan residues yielding 3-nitrotyrozine and nitrotryptophane (Vandelle and Delledonne 2011). Tyrosine nitration can change the function of proteins, and it may promote or inhibit the activity of enzymes. Tyrosine nitration may also interfere with signal transduction because the nitration of specific tyrosines prevents the phosphorylation of the regulatory proteins (Corpas et al. 2013).

Salt stress disrupts the equilibrium between NO production and elimination resulting in changes in NO signaling and in physiological responses. NO

as a messenger and effector molecule can induce both cell death and salt stress tolerance, which depends on a variety of factors, such as cell type, cellular redox status, and the flux and dose of the local NO concentration (Wang et al. 2010b).

13.6 NO and Salt Tolerance

The NO donor SNP enhanced the salt tolerance in many plant species. SNP increased germination, and root- and shoot growth under salinity stress as compared to the NaCl-treated controls in various plant species.

Osmotic stress tolerance induced by the application of an NO donor is associated with an enhanced RWC in several species under high salinity (Sheokand et al. 2010; Zeng et al. 2011; Khan et al. 2012).

NO application significantly enhanced the NaCl-induced osmotic stress tolerance via the accumulation of osmoprotectants such as glycine betaine, soluble sugars, or proline (Pro). Khan et al. (2012) found that SNP treatment increased the glycine betaine content in *Brassica juncea* leaves under salt stress. Enhanced soluble sugar content was found in *Triticum aestivum* seedlings (Zheng et al. 2009) and *Solanum lycopersicum* (Wu et al. 2011) after pre-treatment with the NO donor SNP under salt stress, which ameliorated the NaCl-induced osmotic stress component.

The NO donor induced the accumulation of Pro by enhancing the activities of Δ^1-pyrroline-5-carboxylate synthetase (P5CS), the enzyme catalyzing the rate limiting step of Pro biosynthesis, and by inhibiting the activity of the catabolic enzyme, Pro dehydrogenase (ProDH) in *Triticum aestivum* (Ruan et al. 2004b). Similarly, Pro was found to accumulate after SNP treatment in *Arabidopsis thaliana* (Zhang et al. 2010), *Brassica juncea* (Khan et al. 2012), *Kosteletzkya virginica* (Guo et al. 2009), and *Solanum lycopersicum* (Wu et al. 2011) under salt stress that enhanced tolerance to high salinity. In contrast, SNP decreased the Pro content in *Brassica juncea* (Zeng et al. 2011) and in *Brassica rapa* (López-Carrión et al. 2008). In the latter case, the NO donor did not change the activity of the biosynthetic enzyme, Δ-1-pyrroline-5-carboxylate reductase (P5CR), but increased the activity of ornithine-δ-aminotransferase (δ-OAT) implicated also in Pro biosynthesis under salt stress. SNP together with 100 mM NaCl induced a significant rise in the activity of ProDH as compared to salt treatment suggesting that an increased Pro degradation contributed to low proline level in this species.

Salt stress increased the permeability of membranes, which can be partially prevented by the use of an NO donor leading to increased viability of cells. Accordingly, SNP decreased the membrane leakage in *Cicer arietinum* (Sheokand et al. 2010), *Phragmites communis* (Zhao et al. 2004), *Triticum aestivum* (Ruan et al. 2002), *Zea mays* (Zhang et al. 2006), and *Brassica juncea* (Khan et al. 2012).

The ionic effect of salt stress and the decrease in optimal K^+/Na^+ ratio can also be mitigated by the use of NO donors. Higher NO levels correlated with higher K^+/Na^+ ratios in *Arabidopsis thaliana* (Wang et al. 2009), *Brassica juncea* (Khan

et al. 2012), *Brassica rapa* (López-Carrión et al. 2008), *Kosteletzkya virginica* (Guo et al. 2009), *Phragmites communis* (Zhao et al. 2004), *Populus euphratica* (Zhang et al. 2007), and *Triticum aestivum* (Ruan et al. 2004a; Zheng et al. 2009). Zhao et al. (2007a, b) found that due to the reduced NO levels, *Atnoa1* mutants displayed lower K^+/Na^+ ratios in their shoots than wild-type plants under salt stress. These results confirm the positive effect of NO in maintaining the optimal K^+/Na^+ ratios. In contrast, SNP had no effect on the osmotic potential and on the concentrations of Na^+, K^+, Cl^-, and NO_3^- in the halophyte *Suaeda salsa* shoots (Song et al. 2009). The NO donor SNP enhanced the activities of PM and vacuolar H^+-ATPases as well as that of vacuolar H^+-PPase in *Arabidopsis thaliana* calli (Wang et al. 2009), *cucumber* (Shi et al. 2007), reed (Zhao et al. 2004), wheat (Ruan et al. 2004a; Xie et al. 2008), and maize tissues (Zhang et al. 2006) under salt stress. SNP treatment also enhanced the contents of other ions such as Ca^{2+} in *Brassica juncea* (Khan et al. 2012) and *Phragmites communis* (Zhao et al. 2004) under salinity. In wheat leaves, Ca^{2+} accumulation has been associated with increased Pro content on NO donor treatment (Ruan et al. 2004b).

Application of exogenous NO donor can also enhance salt tolerance by alleviating oxidative damage. SNP decreased the salt stress-induced harmful lipid peroxidation, malondialdehyde (MDA) content or the level of thiobarbituric acid reactive substances (TBARS) in *Atriplex centralasiatica* (Xu et al. 2011a), *Brassica juncea* (Zeng et al. 2011; Khan et al. 2012), *Brassica rapa* (López-Carrión et al. 2008), *Cicer arietinum* (Sheokand et al. 2008, 2010), *Cucumis sativus* (Shi et al. 2007; Lin et al. 2012a, b), *Glycine max* (Simaei et al. 2011), *Hordeum vulgare* (Li et al. 2008), *Kosteletzkya virginica* (Guo et al. 2009), *Solanum lycopersicum* (Wu et al. 2011), and *Triticum aestivum* (Ruan et al. 2002; Zheng et al. 2009; Hasanuzzaman et al. 2011; Xu et al. 2011b).

NO can scavenge H_2O_2 and can protect plant cells from oxidative damage under salt stress by increasing the activity of antioxidative enzymes although their activities can be affected differently. NO donors decreased the salt-induced ROS production and activated SOD, peroxidases such as APX and GPX, CAT, and GR in various plant species. SNP promoted SOD-, POD-, and APX activities in *Brassica juncea* (Zeng et al. 2011; Khan et al. 2012), *Populus euphratica* (Sun et al. 2010), and *Cicer arietinum* plants (Sheokand et al. 2010). In contrast, higher activities of POX, APX, and GR were observed in the NO-deficient *Atnoa1* plants, and these mutants also showed lower activities of SOD and CAT than wild-type plants under NaCl stress (Zhang et al. 2010). 0.05 mM of SNP increased the activity of APX, whereas it decreased that of SOD and POD, and did not affect the activity of CAT in the roots of *Phaseolus vulgaris* plants exposed to high salinity (Liu et al. 2007). The NO donor SNP can also control the level of non-enzymatic antioxidants, such as those of ascorbate or glutathione. SNP treatments increased the reduced form of both metabolites and elevated the ratios of GSH/GSSG and ASC/DHA (Sheokand et al. 2010; Hasanuzzaman et al. 2011; Wu et al. 2011; Lin et al. 2012a). In other species such as *Gossypium hirsutum*, the NO donor decreased APX- and GR activity under salt stress, but CAT activity was not different from the salt-treated controls (Vital et al. 2008).

The positive effect of exogenous NO on photosynthesis is well demonstrated in rice (Uchida et al. 2002). The maximal photochemical efficiency of photosystem II (Fv/Fm) decreased in WT and *Atnoa1* mutant of *Arabidopsis* under salt stress, but the extent was higher in the mutant (Zhao et al. 2007a, b). Wu et al. (2010) observed that in salt-stressed plants, the NO donor attenuated the decrease in stomatal conductance (g_s), transpiration rate (E), leaf chlorophyll content, net CO_2 fixation rate (P_N), the ratio of variable to maximum fluorescence (Fv/Fm), relative electron transport rate (ETR), the effective quantum efficiency of photosystem II (PS II) reaction centers (Fv′/Fm′), the photochemical quenching coefficient (qP), and counteracted the increase in non-photochemical quenching coefficient (qN) in tomato plants. SNP decreased the salt-induced loss of chlorophyll content in *Arabidopsis thaliana* (Zhang et al. 2010), *Brassica juncea* (Khan et al. 2012), *Citrus aurantium* (Tanou et al. 2012), *Triticum aestivum* (Ruan et al. 2002, 2004a) and *Zea mays* (Zhang et al. 2006).

Moreover, exogenous NO treatment affects the stress hormone levels under salt stress. The concentration of the stress hormone abscisic acid (ABA) increased after SNP treatment in the leaves of wheat seedlings (Ruan et al. 2004b) but ethylene production decreased in the wild type but not in ethylene receptor mutant, *etr1-3 Arabidopsis* callus cultures (Wang et al. 2010c).

Protein nitration and *S*-nitrosylation are also involved in acclimation to salinity stress (Valderrama et al. 2007; Fares et al. 2011; Tanou et al. 2012). Tanou et al. (2009) reported that some proteins undergo a post-translational regulation through either oxidation and/or *S*-nitrosylation in citrus plants exposed to salinity. These authors found that both H_2O_2 and SNP pretreatments before salt stress alleviated the salinity-induced protein carbonylation and caused the accumulation of *S*-nitrosylated proteins in the leaves. These results indicate an overlap between H_2O_2- and NO-mediated protein modifications in citrus plants during the acclimation to salinity.

Not only Pro but also polyamine contents changed significantly under salt stress. NO donor caused an increase in Spm content, (Spd + Spm)/Put ratio, and PAO activity in cucumber plants (Fan et al. 2010). This increase in Spm content may play a role in the protection against high salinity-induced damage (Yamaguchi et al. 2006).

13.7 NO- and Salt-induced Programmed Cell Death

NO is involved in plant PCD induction as a messenger and an effector molecule (Wang et al. 2010b) but in contrast to the H_2O_2-triggered PCD (Dat et al. 2003; Gechev et al. 2006), NO alone is not able to kill the cells (Zago et al. 2006). NO also plays a fundamental role in senescence and HR (Lin et al. 2012a, b), and it acts in strong partnership with H_2O_2 during the induction of PCD (Delledonne et al. 2001; De Pinto et al. 2002). Moreover, it can react with superoxide generating toxic $ONOO^-$ (Delledonne et al. 2001), which at high concentration initiates

cell death (Gupta and Igamberdiev 2011). Time-course experiments indicated that O_2^- rather than H_2O_2 functions in synergism with NO to trigger the PCD program (Blokhina and Fagerstedt 2010; Arasimowicz-Jelonek et al. 2012).

In salt-tolerant *Populus euphratica* callus cells, the antioxidant enzymes were induced by the rapid increase in NO and ROS production after salt treatment, thus the cells could maintain high K^+/Na^+ ratios. NO and ROS productions exhibited only small changes in salt-sensitive *Populus popularis,* therefore the cells displayed low K^+/Na^+ ratio and cell viability (Sun et al. 2010). Zhao et al. (2007a, b) demonstrated that a NOS inhibitor or a NO scavenger reduced endogenous NO levels and enhanced NaCl-induced decrease in K^+/Na^+ ratio in *Arabidopsis.* Moreover, the decrease in the K^+ concentration and the increase in that of Na^+ were greater in *Atnoa1* mutant than in wild-type plants under salt stress due to the reduced endogenous NO levels. *Atnoa1* plants exhibited a much higher increase in H_2O_2 contents than wild types in response to NaCl treatments, which indicates that endogenous NO can effectively attenuate oxidative stress. The ion disequilibrium was the primary cause of cell death in the apical region of primary roots in wild-type and *sos1 Arabidopsis* mutant but the effect was much more pronounced in the mutant plants. Salt-induced PCD displayed the hallmarks of both apoptosis- and lysigenous-type PCD, the nuclear fragmentation, and DNA ladder as well as high vacuolation of cells in the elongation/differentiation zones. The authors suggested that the elimination of primary roots and the differentiation of the secondary roots in plants exposed to salt shock appear to be an adaptive mechanism (Huh et al. 2002).

Both enhanced and reduced Ca^{2+} levels were reported in plants exposed to high salinity (Halperin et al. 1997; Rabie and Almadini 2005; Yang et al. 2007), which can alleviate salt injury or may be involved in signal transduction. Gao et al. (2004) found an elevated steady-state level of the cytoplasmic Ca^{2+} and an increased apoplastic Ca^{2+} concentration in *Arabidopsis* roots during salt stress. Other authors found a significantly increased Ca^{2+} content on dry mass basis in tomato suspension cells when exposed to lethal concentrations of NaCl (Poór et al. 2012).

Overproduction of NO or the other RNS can cause toxic symptoms, called "nitrosative stress." This, in combination with oxidative stress due to overproduction of ROS, can lead to a "point of no return" in PCD initiation. The generation of RNS and ROS takes place in various cell compartments and depending on the half-life, transport properties, and concentrations of molecular species, they can act synergistically or can scavenge one another.

PM NADPH oxidase, the enzyme that catalyzes one electron reduction of O_2 to O_2^-, has been described as an important source of apoplastic H_2O_2 after dismutation of superoxide during salt stress (Yang et al. 2007). Treatment with 150 mM NaCl or the NO donor SNP increased the activity of the PM NADPH oxidase and the generation of H_2O_2 in *Populus euphratica* callus cultures. Moreover, it was observed that the application of NMMA, a NOS inhibitor, strongly blocked the plasma membrane NADPH oxidase activity and the production of H_2O_2 under salt stress in these tissues (Zhang et al. 2007). It was found by other authors that

S-nitrosylation of the Cys-890 of the protein reduced the activity of NADPH oxidase in the leaves of *Arabidopsis* in response to an avirulent pathogen (Yun et al. 2011), suggesting that NO might regulate ROS production by this enzyme in various ways.

Chloroplasts, the important sources of ROS under salt stress, are capable of both Arg- and nitrite-dependent NO generation (Jasid et al. 2006) suggesting that they contribute to nitrosative and oxidative stresses very effectively. Chloroplastic proteins such as oxygen-evolving enhancer protein 1 and 2, small and large subunits of Rubisco, Rubisco activase, plastocyanin, and phosphoribulokinase are targets of *S*-nitrosylation in potato (Kato et al. 2012). Photosynthesis-related proteins involved in the Calvin–Benson cycle were largely targeted by carbonylation, nitration, and nitrosylation in citrus plants primed with H_2O_2 or SNP prior to salt stress (Tanou et al. 2012). The authors discussed the importance of these post-translational modifications in the acclimation to high salinity. In contrast, nitration of specific tyrosine of D1 protein (PSBA) of photosystem II leads to the dissociation of the PS II dimers and PS II-LHCII supercomplexes during high light stress (Galetskiy et al. 2011). Carbonic anhydrase in sunflower (Chaki et al. 2013) and chloroplastic glycerinaldehyde-3-phosphate dehydrogenase in *Arabidopsis* (Lozano-Juste et al. 2011) were also among the Tyr-nitrated proteins under stress conditions that showed reduced activity.

Peroxisomes are sites of the L-Arg-dependent NO production and contain CAT- and H_2O_2-producing flavin oxidase that participates in photorespiration (del Río 2011). Peroxisomes in *Arabidopsis* were also shown to be required for the accumulation of NO in the cytosol during salt stress (Corpas et al. 2009).

The peroxisomal enzyme, CAT, and APX activities were inhibited by NO (Clark et al. 2000). Since glycine hydroxymethyltrasferase, a mitochondrial enzyme participating in photorespiration, was also down-regulated by salt stress and NO (Kilian et al. 2007), it can be assumed that nitrosative stress contributes to salt stress-induced inhibition of photorespiration. The catabolism of PAs by DAO or PAO enhanced H_2O_2 production which was suggested to be a signal leading to PCD in salt-stressed tobacco tissues. In these experiments, the authors demonstrated that spermidine was secreted and oxidized by PAO in the apoplast upon salt stress (Moschou et al. 2008), but other PAO isoenzymes (AtPAO2, AtPAO3, and AtPAO4) were localized to peroxisomes (Kamada-Nobusada et al. 2008) or to the vacuole (Cervelli et al. 2004).

Salt stress-induced PCD is mediated by the status of the mitochondrial PTP and ROS production in tobacco protoplasts. The cells displayed several morphological hallmarks of apoptosis, such as nuclear DNA degradation, formation of PTP on mitochondrial membrane, and a decrease in the mitochondrial membrane potential ($\Delta\psi_m$). Externally applied ascorbic acid prevented the decrease in $\Delta\psi_m$ and the increase in ROS production, suggesting that ROS formation plays a key regulatory role in PCD initiation. ATP synthesis was reduced in plant mitochondria by the inhibition of cytochrome oxidase activity (Yamasaki et al. 2001). In *Arabidopsis* plants, NO induced the de-polarization of ψ_m and the release of cyt *c* from the intermembrane space and resulted in increased ROS production, suggesting that the

NO effect depends on the physiological status of plant cells (Zottini et al. 2002). Since the role of cyt *c* may be different in plant and animal PCDs, other apoptosis-inducing factors can also be released from the mitochondrial intermembrane space (reviewed by Reape and McCabe 2010).

Rice *nitric oxide excess* (*noe1*) mutant was identified due to high NO and *S*-nitrosothiol contents of plants. The map-based cloning revealed that *NOE1* encoded *OsCATC*, so the plants were catalase deficient (Lin et al. 2012b). These plants had higher H_2O_2 levels under high light, which consequently promoted NO production via the activation of NR. The plants exhibited a cell death phenotype under high light intensity and the accumulation of NO promoted the *S*-nitrosylaton of glyceraldehyde-3-phosphate (GAPDH) and thioredoxin (TRX), the two enzymes, which are involved in *S*-nitrosylation-regulated cell death in animal cells (Wang et al. 2013). TRX is presumed to be *S*-nitrosylated in the active site, which inhibits its oxido-reductase function (Sumbayev 2003). Since TRX is a key modulator of cell redox status, this post-translational modification promotes apoptosis. GAPDH has also been shown to be *S*-nitrosylated, which decreases its enzymatic activity (reviewed by Astier et al. 2012). The NtGAPCb isoform of this protein constitutively interacts with NtOSAK (*Nicotiana tabacum* osmotic stress-activated protein kinase) both in the cytosol and the nucleus. This kinase was found to be phosphorylated and thus activated in plant tissues during salt stress. The *S*-nitrosylation of NtGAPCb had no influence on the interaction with NtOSAK or on its kinase activity but prevented the nuclear localization of the complex (Burza et al. 2006). In *Arabidopsis* peroxiredoxin IIE (PerIIE), an enzyme involved in the maintenance of redox status of the cells possesses a peroxynitrite reductase activity, thus it can control $ONOO^-$ levels. PerIIE activity is inhibited by *S*-nitrosylation, suggesting a regulatory effect of NO on the strength of nitrosative stress (Romero-Puertas et al. 2007).

It has been shown recently that short-term salt stress induces apoptosis-like, whereas long-term salt stress non-apoptotic PCD (Andronis and Roubelakis-Angelakis 2010). In both cases, the execution of cell death is mediated by the induction of cysteine proteases, some of them showing caspase-like activities. Caspase-like activity seems to be activated by K^+ deficiency in plant cells (Demidchik et al. 2010), which is in good correlation with the induction of cysteine protease activity in salt-stressed plants (Kovács et al. 2012).

Long-term salt stress increased H_2O_2 and NO productions, resulted in elevated lipid peroxidation, caspase-like activity, and cell death in maize roots. However, an exogenously applied NO donor, DETA/NO, reversed the detrimental effect of salt stress and reduced the salt-induced oxidative stress and caspase-like activity in maize tissues (Keyster et al. 2012). Plant metacaspases are considered to be ancestors of metazoan caspases, their maturation involves an autocatalytic processing of the zymogen form and their active participation in PCD. The zymogen form of AtMC9, a member of *Arabidopsis* metacaspase family, was constitutively *S*-nitrosylated in vivo which kept the enzyme in an inactive form. However, the active, processed enzyme was no longer a target of *S*-nitrosylation (Belenghi et al. 2007).

Poór et al. (2013) found that NO production by tomato cell suspension increased in the first hour, but did not change later at the lethal concentration of NaCl. In contrast, ROS production increased significantly for 6 h during the experimental period. Salt-induced ROS production was controlled by ethylene, which enhanced the percentage of dead cells. The salt stress caused a loss of the membrane semipermeability, chromatin condensation, cell shrinkage, and DNA fragmentation in the cells. The effect of specific inhibitors confirmed that the Ca^{2+} signaling, MAPKs, and cysteine proteases are involved in salt stress-induced PCD in tomato suspension cultures.

13.8 Conclusion and Perspectives

Despite the fact that the knowledge concerning the role of NO in higher plants under salt stress has increased considerably in the recent ten years, there are still many gaps in comprehending the mode of NO action that controls the salt stress-induced cell death or the acclimation of plants to saline environments.

Salt stress enhanced the NO production of tissues in many plant species. Unfortunately, most of the authors determined NO accumulation only at one time-point after salt exposure although the rise is transient and may vary from some minutes to few hours or days (Keyster et al. 2012). This NaCl-generated NO can be inhibited by NOS and NR inhibitors, suggesting that several NO-producing mechanisms can be activated in a time- and tissue-specific manner.

It can be concluded from several reports that the treatment with exogenous NO donors could alleviate the toxic effect of salt stress. The application of SNP increased the biomass, the contents of osmoprotectants such as glycine betaine, proline, and soluble sugars, and increased RWC and K^+/Na^+ ratios under salt stress. SNP decreased the salt stress-induced lipid peroxidation, ROS production, activated the antioxidant enzymes such as SOD, POX, GPX, APX, CAT, GR, and DHAR, and enhanced the non-enzymatic antioxidant pools (ASA and GSH). The NO donor has also a positive effect on photosynthesis under high salinity. Unfortunately, in most of the experiments, the NO levels of the tissues have not been determined during the acclimation period after SNP treatment. Moreover, SNP can generate not only NO but highly toxic cyanide thus treatment with 0.5 mM SNP caused PCD within 12 h in tobacco BY-2 cell suspensions (Vitecek et al. 2007).

NO-mediated *S*-nitrosylation and nitration of proteins may be involved in acclimation to salinity stress, but the enhancement of peroxynitrite-dependent Tyr nitration may shift the cells to the initiation of PCD (Corpas et al. 2013).

In plant tissues, NO and ROS produced during salt stress cooperate to trigger PCD (Fig. 13.3). The reaction of NO with superoxide radical leads to peroxynitrite, which is not essential in NO-mediated PCD. Moreover, NO and H_2O_2 can chemically react with each other producing singlet oxygen and hydroxyl radicals. These very reactive radicals can induce fast cell death (Wang et al. 2013).

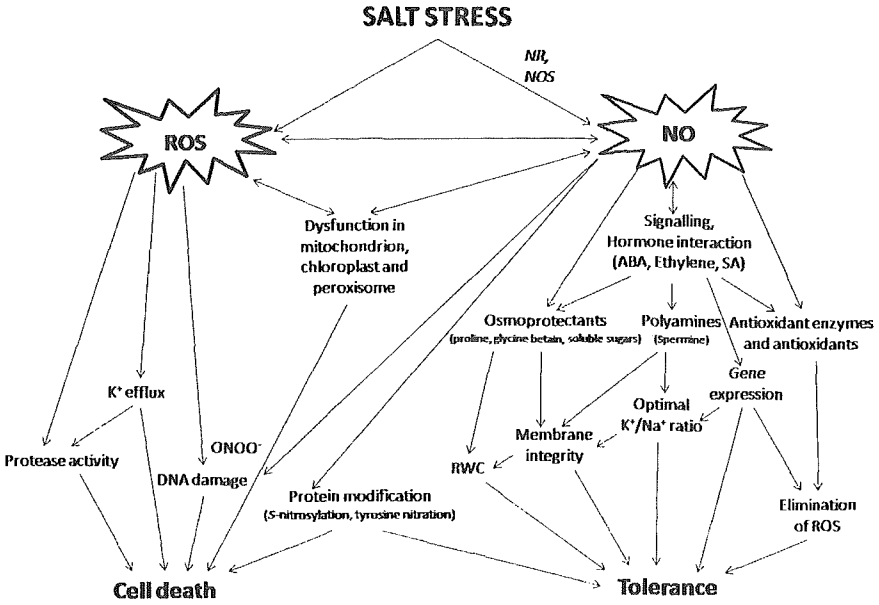

Fig. 13.3 The role of NO in salt-induced cell death and salt salt tolerance

Although there are a number of experimental data on the physiological aspects of NO-mediated alleviation of NaCl-induced stress, our knowledge is imperfect concerning the changes in NO contents in parallel with those of individual species of RNS and ROS.

The NO-mediated signal transduction, the detailed investigation of NO-mediated post-translational protein modifications, and gene expression changes have been performed only in few species under lethal salt concentrations. To see whether these changes were due to the NO-dependent changes in the activity of the related enzymes or due to the NO-induced control of gene expression, further analyses have to be done.

The description of the specificity of the individual reactive nitrogen forms in the signal transduction of plant hormones accumulating under salt stress would also provide new insights into converging and diverging signaling pathways.

Finally, the comparison of salt stress-dependent changes in the transcriptome, proteome, and metabolome and the evaluation of the data by systems biology tools would be particularly useful in defining differences between salt-sensitive and salt-tolerant genotypes or between glycophytes and halophytes.

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