REVIEW

Role of Nitric oxide in regulation of H_2O_2 mediating tolerance of plants to abiotic stress: A synergistic signalling approach

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The relationship between abiotic stress, nitric oxide (NO) and Hydrogen peroxide (H_2O_2) is a challenging one. It is now clear that H_2O_2 and NO function as signalling molecules in plants. A wide range of abiotic stresses results in H_2O_2 generation, from a variety of sources and it has many essential roles in plant metabolism but at the same time, accumulation related to virtually any environmental stress is potentially damaging. NO is gaining increasing attention as a regulator of diverse patho-physiological processes in plant science, mainly due to its properties (free radicals, small size, no charge, short-lived, and highly diffusible across biological membranes) and multifunctional roles in plant growth, development and regulation of remarkably broad myriad of plant cellular mechanisms. Various abiotic stresses can induce NO synthesis, but its origin and mode of action in plants have not yet been completely resolved. Recent studies on NO production have tended to high light the questions that still remain unanswered rather than telling us more about NO metabolism. But regarding NO-H_2O_2 signalling and functions, new findings have given an impression of the intricacy of NO-H_2O_2 related signalling networks against abiotic stresses. Cellular responses to NO-H_2O_2 are complex, with considerable cross-talk between responses to several abiotic stresses. In last few years, the role of NO in H_2O_2 mediating tolerance in plants to abiotic stress has established much consideration.

Key words: antioxidant / H_2O_2 / NO / oxidative stress / salinity / signalling molecules / UV-radiation

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Key words: antioxidant / H_2O_2 / NO / oxidative stress / salinity / signalling molecules / UV-radiation

There is now compelling evidence that H_2O_2 and NO function as signalling molecules in plants (Foyer et al., 1997; Neil et al., 1999; Bolwell, 1999; Durner and Klessing, 1999; Dat et al., 2000).

Previous studies also have shown that both NO and H_2O_2 function as stress signals in plants, mediating a range of resistance mechanisms in plants under stress conditions (Neill et al., 2002a; Wendehenne et al., 2004; Delledonne, 2005). During the last few years, much attention has been paid to NO research since its discovery as a crucial signalling molecule in mammals that plays an number of diverse functions i.e. vasodilatation, neurotransmission, smooth muscle contraction and relaxation, innate immune response, egg fertilization, defence against pathogenic microorganisms and apoptosis (Schmidt and Walter, 1994; Jeffrey and Synder, 1995; Stamler, 1994; Lloyd-Jones and Bloch, 1996; Wink and Mitchell, 1998; Ignarro, 2000; Hess et al., 2005). It has been considered that NO also plays a vital role in diverse physiological functions in plants, induction of seed germination, reduction of seed dormancy (Beligni and Lamattina, 2000; Bethke et al., 2006, 2007; Libourel et al., 2006; Zheng et al., 2009) regulation of plant metabolism and senescence (Leshem et al., 1998; Guo and Cramford, 2005) induction in cell death (Pedroso and Durzan, 2000) regulation of stomatal movement (Garcia-Mata and Lamattina, 2001; Neill et al., 2003; Guo et al., 2003; Sakihama et al., 2003; Bright et al., 2006; Garcia-Mata and Lamattina, 2007) Photosynthesis regulation (Takahashi and Yamasaki, 2002) mitochondrial functionality (Zottini et al., 2002) and gravitopism (Hu et al., 2005) floral regulation (He et al., 2004). In spite of its potential role in a series of studies, NO proved to be capable of regulating the multiple plant responses towards a variety of biotic and abiotic stresses and alleviating some consequences provoked by oxidative stresses (Delledonne, 2005; Bligni and Lamatttima, 1999a; Crawford and Guo, 2005). In many research, high levels of NO has the capacity to damage membranes and DNA fragementation

(Pedroso et al., 2000; Yamasaki, 2000; Romero-Puertas et al., 2004) and to reduce photosynthesis in Oat and alfalfa (Hill and Bennet, 1970) and reproduction in carrot cell suspensions (Zottini et al., 2002) are inhibited by NO exposure.

Moreover, NO behaves as a plant hormone equivalent to ethylene (Fig. 1) that is, as a gaseous signal transmitter (Leshem, 2000; Guo et al., 2003; Yamasaki, 2005). There are several enzymatic systems that have been shown to produce NO, mainly nitrate reductase (NR) (Rockel et al., 2002) and L-Arg-dependent nitric oxide synthase (Corpas et al., 2004). However, the gene for the plant NOS has not been identified yet (Zemojtel et al., 2006; Neill et al., 2008). Interestingly, NO signalling is based on interactions with plant hormones. Furthermore, CTK stimulated more NO signal formation, probably mainly via a NR source under the conditions of drought stress in *Zea mays*. High NO signal intensity alleviated drought-induced Reactive oxygen species (ROS) damage to plants. Thus, the signal probably played a direct role in eliciting CTK regulation to energy absorption and excitation energy trapped in response to drought (Shao et al., 2010).

There are rapid increasing evidence that NO plays an important role in cyto-protection by regulating the level and toxicity of ROS (Lamattina et al., 2003) and by inducing transcriptional changes which permitted identification of genes involved in different functional processes such as signal transduction, defense and cell death, transport, biotic metabolism and ROS production and degradation (Palmieri et al., 2008). NO inhibits the plants from oxidative damage by the regulation of general mechanisms for cellular redox homeostasis and also promoting the transformation of O_2 to H_2O_2 and O^{-I} and also by enhancing the H_2O_2-scavenging enzyme

Table 1. Electrochemical potential (mv) of heavy metals in aqueous media (pH 7, 25 °C, after Weast, 1984)

Metal Cation	Redox potential (mv)
Zn^{2+}	-1.18
Cd^{2+}	-0.82
Ni^{2+}	0.65
Pb^{2+}	0.55
Cu^{2+}	0.26
Fe^{2+}	+0.35
Hg^{2+}	+0.43
Ag^{2+}	+0.1.57

Table 2. The inducible effect of heavy metal stress on the expression of some signalling molecules (eg. H_2O_2) in plants.

signalling molecule	Heavy Metal	References
H_2O_2	Cd	Romero-Puertas et al.,1999
	Cd	Cho and Seo (2005)
	Cd	Schutzendubel et al.(2001)
	Cu	Drazkiewicz et al.,(2004)
	Cd, Cu	Maksymice and Krupa (2006b)
	Hg	Cho and Park (2000)
	Mn	Demirerska-Kepova et al (2004)

Table 3. Genes & Proteins responding to different environmental stresses that are also induced in plants by heavy metals.

Genes & proteins induced	Metal	References
Os MSRMK2-multiple-responsive MAPK gene, induced by high salt drought, UV, H_2O_2.	Cu , Cd & Hg	Agrawal et al.(2003 a, c)
Os BWMK1-MAPK gene induced by drought heating, H_2O_2	Cu, Cd & Hg	Agrawal et al (2003 b)
OS EDR1-MAPKK gene induced by H_2O_2, drought ,ozone.	Cu, Cd & Hg	Kim et al (2003)
16 KDa protein related to PR-10 protein induced by H_2O_2, ethylene.	Pb &Cu	Przymusinski and Gwozdz (1999)

activities (Lamattina et al., 2003; Shi et al., 2007; Zheng et al., 2009). Although, the NO molecule itself possesses antioxidant properties (Karplus et al., 1991). NO rapidly reacts with, oxygen species, hemes, thiols and proteins to produce biochemical signals that directly and indirectly regulate enzymatic activities. The effects of NO depend on its location and concentration. Beneficial reactions counteract oxidative and nitrosative stresses, while damaging reactions, due to high levels of NO, cause oxidative and nitrosative damage and cell death (Apoptosis). NO contributes to structural and functional adoptive plasticity and to the habituation of stress to their sites. The use of NO donors and enzyme inhibitors offers a new experimental approach and counter measures to control stress signals throughout conifers life histories. The most crucial role of NO in plant growth and development, starting from germination to flowering, ripening of fruits, senescence and biotic and abiotic stresses has

been nicely reviewed previously by many researcher (Lamattina et al., 2003; Arasimowicz and Floryszak-Wieczrek, 2007; Beligni and Lamattina, 1999b; Garcia-mata and Lamattina, 2001; Bethke et al.,

2004; Stohr and Ullrich, 2002; Beligni and lamattina, 2001; Yamasaki, 2005; Romero-Puertas et al., 2004; Wendehenne et al., 2004; Bolwell, 1999; Neill et al., 2003).
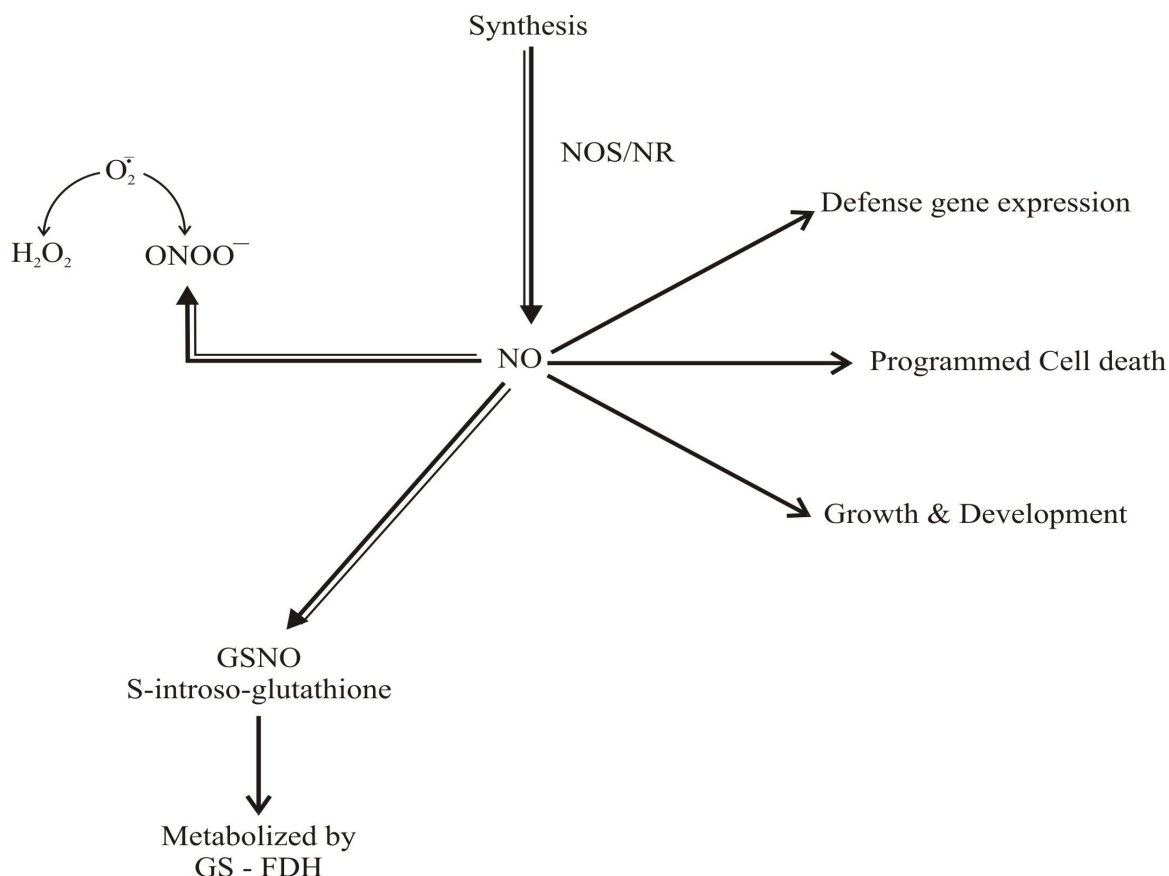
Synthesis

NOS/NR

O_2^-

H_2O_2 $ONOO^-$

NO

Defense gene expression

Programmed Cell death

Growth & Development

GSNO
S-introso-glutathione

Metabolized by
GS - FDH

Fig. 1 NO signalling in plants. Double lined arrows represent potential synthesis and biochemical interactions while single lined arrows represent potential cellular effects and target sites for NO action

H_2O_2 is potentially reactive oxygen (reduction product of O_2), but not a free radical (Halliwell et al., 2000), generated as a result of oxidative stress via superoxide (O_2^-), presumably in a non-controlled manner during electron transport processes such as photosynthesis and mitochondrial respiration. Ribulose bisphosphate carboxylase oxygenase (RuBisCO), an enzyme best known for incorporating CO_2 into organic substrates in plants also has an ancient oxygenase function, which plays a key role in regulating peroxide balance in cells (Perry et al., 2010). Potential sources include

NADPH oxidase, cell wall peroxidases, amine oxidases, Oxalate oxidase, flavin-containing oxidases, Type III oxidases and misfires in the electron transport chains of chloroplasts and mitochondria (Mehler reaction) (Bolwell and Wojtaszek, 1997; Bolwell et al., 2002; Halliwell and Gutteridge, 1999). Broadly, these events are enhanced by stresses (Alscher et al., 1997; Bol-well, 1999) although they occur as an integral part of many facets of plant development. Whatever, the source of ROS, it is now apparent that H_2O_2 acts as a signal to induce a range of molecular, biochemical

and physiological responses with in cells and plants (Fig. 2). Also, H_2O_2 participant in implication of ROS mediated control of the K^+ channel resulting in mineral nutrient partitioning with in the plants by identifying a critical target Cys (168) to be essential for sensitivity of H_2O_2 (Garcia-Mata et al., 2010). H_2O_2 is produced in response to various stimuli and mediates cross-talk between signalling pathways and is an attractive signalling molecule contributing to the phenomenon of cross-tolerance in which exposure of plants to one stress offers protection towards another (Cheng and Song, 2006; Bowler and Fluhr, 2000). In addition, exposure to low levels of one stress can induce tolerance towards subsequent higher levels of exposure to the same stress, a phenomenon termed acclimation tolerance (Prasad et al., 1994; Bhattacharjee, 2005). Therefore, H_2O_2 is considered as a fundamental fact of life in anaerobic environment (Moller, 2001).
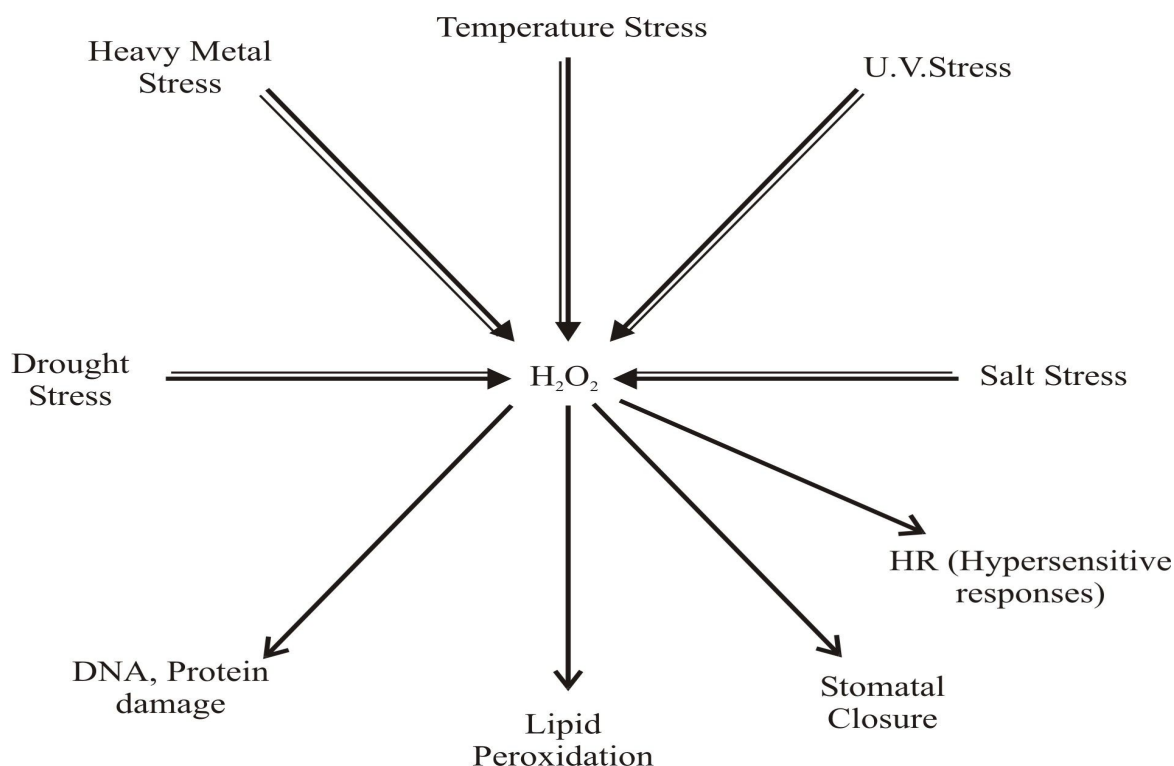

Fig. 2 H_2O_2 signalling in plants. Double lined arrows represent potential synthesis and single lined arrows represent major cellular effects of H_2O_2

The cellular responses of H_2O_2 differ according to its site of synthesis or perception for example, whether the H_2O_2 is synthesized in plastids or at the plasma membrane. The genetically controlled production of H_2O_2 (e.g. by NADPH oxidases) is apparently used by plants to release an intracellular signal that, often together with NO, controls a variety of processes (Guo et al., 2003; Neil et al., 2002). H_2O_2 might induce a general stress response, but it does not have the required specificity to selectively regulate nuclear genes required for dealing with localized stress, e.g. in chloroplasts or mitochondria (Moller and sweetlove, 2010). During normal metabolism in a plant cell, H_2O_2 is generated in chloroplast, mitochondria and peroxisome and is kept in homeostasis by complicated and effective scavenging systems that have developed over the course of evolution (Zhao and Blumwald, 1998). In

particular, H_2O_2 that is produced by cytosolic membrane-bound NADPH oxidases has been implicated as a signal in a wide range of biotic and abiotic stress responses. These responses include defense reactions against pathogens and herbivores (Mittler, 2002) the closure of stomata (Kwak et al., 2003) and the regulation of cell expansion and plant development (Kwak et al., 2003; Foreman et al., 2003). Its generation via electron transport is increased in response to environmental stresses such as excess excitation energy, drought and cold (Bartosz, 1997; Dat et al., 2000) and also induced in plants following exposure to a wide variety of abiotic and biotic stimuli.

It has a long lifespan, is able to cross biological membranes, and rapidly diffuses from cell to cell or can be transported long distances from its sites of origin in plants. Thus, H_2O_2 has all of the characteristic features of an intercellular signalling molecule and, for this reason, has received increasing attention in recent years (Levine et al., 1994; Alvarez et al., 1998; Neill et al., 2002).In addition, H_2O_2 is an evolutionarily ancient signalling molecule that not only played a key role in inducing evolution of oxygenic photosynthesis but also modulates many physiological events, such as stomatal movement, hypersensitive responses, and programmed cell death (Cheng and Song, 2006). As a response to oxidative stress and particularly H_2O_2 is involved in a variety of reactions against abiotic stresses and signalling cascades necessary for all aspects of plant growth and the integration of activity, ranging from the development of individual root hair to xylem differentiation, lignifications to wall loosening and wall cross-linking to root/shoot coordination and stomatal control. Understanding the role of H_2O_2 in plant growth or stress responses requires models that accommodate the large number of ways in which it can be formed and degraded at any given time, and that ROS produced by one source may be the drivers or substrates for a second (Allan and Fluhr, 1997).

Moreover, increasing evidence now indicates that H_2O_2 acts as a local and systemic signal that directly regulates expression of numerous genes. Some of these are involved in plant pathogen defense responses, while others are invoked during adaptation of plants to abiotic stress (Desikan et al., 2001; Wang et al., 2006). Major abiotic stress includes extremes of temperatures, UV irradiation, O_3 exposure, heavy metal accumulation and drought (Prasad et al., 1994; Lamb and Dixon, 1997; Orozco-Cardenas and Ryan, 1993). Thus, while the involvement of H_2O_2 in stress responses is of particular interest, it really must be considered in the context of and even as a special case of H_2O_2 involvement in normal growth and metabolism. Several recent reviews have described the biological activities of ROS, placing special emphasis on the signalling role of H_2O_2 (Mittler, 2002; Neil et al., 2002; Overmyer et al., 2003).

Abiotic stress

Because plants lack the capability of locomotion as a means of responding to change in their environment, they are exposed to various environmental stresses and must adapt to them in other ways. It is now well established that virtually all biotic and abiotic stresses induce or involve oxidative stress to some degree and the ability of plants to control oxidant levels is highly correlated with stress tolerance. For this purpose, they are equipped with complex processes such as perception, transduction and transmission of stress stimuli (Turner et al., 2002; Xiong et al., 2002; Kopyra and Gwozdz, 2004).

Abiotic stresses are major constraint to agricultural production worldwide. The plants have an inbuilt to respond to fluctuations in circadian and seasonal environmental conditions. Abiotic stresses disrupt the cellular redox homeostasis which leads to the oxidative stress or generation of ROS (Asada, 2006). The generation of ROS in different location of the plants cell (Mitochondria, Chloroplast, Peroxisome and Nucleus) causes injury and cell death (Mano, 2002). On the other hand, ROS play a vital role in intracellular redox signalling, activating antioxidant resistance mechanisms. Thus, it is a surviving response for plants to control the concentration of ROS. However, H_2O_2 and NO both are important bioactive signalling molecules with multiple physiological functions in plants, synthesized in various plants under different environmental conditions (stresses).

NO is believed to be involve in two respiratory electron transport pathways in mitochondria (Yamasaki et al., 2001; Zottini et al., 2002) where it mediates the modulation of ROS and enhances anti oxidant defense systems in plants exposed to various abiotic stresses. The exogenous supply of NO protect plant from damage by eliminating the (O_2^-) and lipid radical R, activates the antioxidant enzymes-activities especially superoxide dismutase (SOD) (Shi et al., 2007). However, in many studies, there have been increasing evidence that NO acts as a chain breaking antioxidant arresting lipid peroxidative reactions and activates gene expression of antioxidant enzymes (Nunoshiba et al., 1993; Ramamurthi and Lewis, 1997). Also, NO is an endogenous modulator of gibberellins-induced programme cell death in barley aleurone cells by inducing cytoprotective proteins and the synthesis of catalase (CAT), SOD, gluthione (GSH), S-transferase and alternative oxidase (Beligni et al.,

2002; Huang et al., 2002; Polverari et al., 2003). Furthermore, the properties of NO (Free radical, small size, no-charge, short-lived and highly diffusible across biological membranes) makes it is a very good agent to act as a signalling messenger in response of environmental stresses. NO generated at the same time as H_2O_2 in response to abiotic stresses was found to mediate defense responses similar to those seen following H_2O_2 generation. It is now clear that NO is a major signal molecule in plants (Durner and Klessing, 1999) and involve in signalling against abiotic stresses are discussed here because it seems likely that NO can be synthesized during stress responses at the same time as H_2O_2 and may be that cellular effects reflects the responses to both H_2O_2 and NO.

H_2O_2 is a form of ROS generated as a result of oxidative stress, arises from an imbalance in the generation and metabolism of ROS, with more ROS (such as H_2O_2) being produced than are metabolized. It is generated via O_2^-, presumably in a non–controlled manner, during electron transport processes such as photosynthesis and mitochondrial respiration in response to environmental stresses such as excess excitation energy, drought and cold (Bartosz, 1977; Dat et al., 2000). H_2O_2 is believed to mediate cross-tolerance and acclimation tolerance as well (Bowler and Fluhr, 2000; Prasad et al., 1994). Moreover, it may be that cellular responses to H_2O_2 differ according to its site of synthesis or perception, for example whether the H_2O_2 is synthesized in plastids or at the plasma membrane. Now it is apparent that H_2O_2 acts as a signal to induce a range of molecular, biochemical and physiological responses with in cells and plants. H_2O_2 can induce the expression of genes potentially involved in its synthesis such as NADPH oxidase (Desikan et al., 1998) and also of those encoding proteins involved

in its degradation, implying a complex mechanism for cellular regulation of oxidative status. H_2O_2 induced the expression of genes encoding ascorbate peroxidase in germinating rice embryos (Morita et al., 1999) and in Arabidopsis leaves (Karpinski et al., 1999), and wounding induced the expression of gene encoding a CAT via H_2O_2 in embryos and leaves of maize (Guan and Scandalios, 2000). Systemic responses to excess excitation energy stress were found to be mediated by H_2O_2, indicating that it can also function as a signal during abiotic stresses (Mullineaux et al., 2000).

Recent work has shown that H_2O_2 induces the expression of genes encoding protein required for Peroxisome biogenesis (lopez-Huertas et al., 2000). Peroxisomes are important sources of ROS, as well as antioxidants and NO and are thus important regulator of the cellular redox state. Induction of peroxisome biogenesis genes by various abiotic stresses like temperature, UV and mechanical injury (also generates H_2O_2) and exogenous H_2O_2 (Lopez-Huertas et al., 2000), places H_2O_2 as a key signal molecule mediating various cellular responses to biotic and abiotic stresses. Plants pre-treated with low concentrations of H_2O_2 have shown significantly greater tolerance to abiotic stress than untreated control plants (Gechev et al., 2002). This acquisition of abiotic stress tolerance usually occurs concomitantly with enhanced antioxidative status, as reflected by higher activities and or protein levels of CAT. Results of (Asada, 1999; Kuzniak and Sklodouska, 1999; Nagalakshmi and Prasad, 2001; Wang et al., 2010) suggest that glutathione peroxidase (GPx) activites were significantly enhanced by H_2O_2 pre-treated from initiation to the end of chilling as found in *Zoysia cultivars* (Wang et al., 2010). In yeast, a GPx, GPx3, can function as a receptor for H_2O_2 and a transducer of redox signals, to activate gene expression (Delaunay et al., 2002). Further studies is needed to understand the actual function of GPx in chilling stress during H_2O_2 pretreated as observed during the chilling resistance. However, the fact that oxidative stress is a common facet of many cellular stress responses means that elucidating those intracellular signalling processes mediating H_2O_2 signalling is of potential significance to any programme aimed at improving crop tolerance of abiotic stresses in order to enhance crop productivity. It is probable that additional H_2O_2 responses will be characterized in the near future.

NO and interactions with H_2O_2 under abiotic stresses

It is commonly observed that NO and ROS such O_2^- and H_2O_2 are generated in response to similar abiotic stress stimuli and with similar kinetics. NO and ROS can interact in various ways under stress conditions. For example, they can interact chemically as in the formation of compounds such as peroxynitrite. NO may also affect the activities of enzymes that alter ROS levels and vice versa. Thus, they could both impact either negatively or positively on the same or related signalling pathways and thereby lead to additive and passively synergistic responses.

Lum et al., (2002) observed that exogenous H_2O_2 induced NO generation in the guard and other cells of *Phaseolus aureus* leaves. In a chemiluminescence-based assay, the H_2O_2 induced a substantial increase in an apparent NOS-like activity. This increase was reduced by using a potential NOS inhibitor. Interestingly, the H_2O_2-induced NO generation was inhibited by the calcium channel blocker verapamil. Thus, it is possible that Ca^{2+} ions may mediate this effect of H_2O_2. She et al., (2004) and He et al., (2005) also reported that H_2O_2 induced NO synthesis in *vicia faba* guard cells and

that again this accumulation could be reduced by a NOS-inhibitor. Moreover, they found that both NO generation and stomatal closure stimulated by darkness or UV-B were dependent on guard cell synthesis of H_2O_2. Similarly, in Arabidopsis, H_2O_2 induces guard cells to synthesize NO, required if the induction of stomatal closure is to follow (Bright et al., 2006). Removal of the H_2O_2 with antioxidant or inhibition of its synthesis by inhibiting NADPH oxidase activity prevents NO generation and stomatal closure. However, H_2O_2 stimulates NO accumulation of guard cells in the At noa1 (At noa1) mutant was as in the wild type (Bright et al., 2006). Thus, the requirement of At NOS1 for NO synthesis must be epistatiscally upstream of H_2O_2 and its signalling effects.

Studies of Wi et al., (2010) suggest that H_2O_2 is important signalling molecule involves in response to biotic and abiotic stresses and in developmental and physiological processes and stress tolerance of H_2O_2 treated transgenic plants resulted from reduced ethylene biosynthesis, which decreased ROS accumulation via increased gene expression and activity of ROS-detoxifying enzymes, including MnSOD, CuZnSOD, and CAT. Regardless, the current evidence would suggest that H_2O_2 induces guard cell NO synthesis through the activity of NR. It also appears that the ABA-H_2O_2-NO cascade is not restricted to guard cells. Zhang et al., (2007) have now demonstrated that it also operates in the mesophyll cells of *Zea mays* leaves and that the effects of ABA and H_2O_2 on the induction of antioxidant defenses dependent on NO generation and could be reduced using cPTIO or L-NAME. While it is well established that H_2O_2 induces NO synthesis and accumulation, there has also been some suggestions that NO may modulate H_2O_2 levels. H_2O_2 accumulation can either to enhanced

expression of antioxidant enzymes and increased expression of other defense proteins such as Glutathione S-transferase or it can initiate PCD depending on the intensity of the oxidative signal or oxidative load on the tissues. Both She et al., (2004) and He et al., (2005) have reported that this was the case in *Vicia faba* guard cells. However, Dong et al., (2005), also working with *Vicia faba* guard cells, did not observe this phenomenon. NO does not appear to induce H_2O_2 generation in Arabidopsis guard cells (Bright et al., 2006) or in maize mesophyll cells (Zhang et al., 2007). Thus, there is argument as to whether or not this occurs but feedback mechanisms and autocatalysis are part and parcel of signalling pathways, and it may be that such discrepancies simply reflect differences in the physiological states of the tissues examined.

Function of NO in H_2O_2 mediating tolerance to:

Salinity stress

Soil salinity is one of the major abiotic stresses that adversely affect crop productivity and quality. About 20% of irrigated agricultural land is adversely affected by salinity (Flowers and Yeo, 1995). Salt tolerance is a complex trait involving the coordinated action of many gene families that perform a variety of functions such as control of water loss through stomata, ion sequestration, metabolic adjustment, osmotic adjustment and antioxidative defense (Abogadallah, 2010). In sodic soils, Na^+ binds to negatively charged clay particles, causing swelling and dispersal, thus making the soil less unfit for crop growth (Chinnusamy et al., 2005). High salt concentration causes osmotic and ionic stress in plants (Zhu, 2002). It limits growth and development of plants by affecting several key metabolic processes (Hasegawa et al., 2000; Marschner, 2002; Siddiqui et al., 2008, 2009a ; Khan et al., 2010). Further, salinity alters the

activities of many enzymes involve in nitrate and sulphate assimilation pathways in plants which lowers their energy status and increase the demand for nitrogen and sulphur (Siddiqui et al., 2009b). Much of the injury at cellular level caused by salinity stress is associated with oxidative damage due to ROS. Plants appear to possess wide arrays of defense strategies to protect from oxidative damage. However, less is known about NO involvement in tolerance of plants to salt stress. The exogenous sodium nitripruside (SNP), a NO donor significantly alleviated the oxidative damage of salinity to seedlings of rice (Uchida et al., 2002), lupin (Kopyra and Gwozdz, 2003) and cucumber (Fan et al., 2007; Yu-qing et al., 2007) enhanced seedlings growth (Song et al., 2009) and increased the dry weight of maize and *Kodtetzkya virginica* seedlings (Zhang et al., 2007; Guo et al., 2009) under salt stress. Pre-treatment of NO effectively contributed to better balance between C and N metabolism by increasing total soluble protein and by enhancing the activities of endopeptidase and carboxypeptidase in plant under salt stress (Zheng et al., 2010).

In previous studies, it was established that exogenous NO induced the expression of plasma membrane (PM) H^+-ATPase in plants under salt stress. Zhao et al., (2004) in *Phragmities communis* and Zhang et al., (2007) in *Populus euphratica* reported that NO enhanced salt tolerance of calluses under salinity also by increasing K^+/Na^+ ratio , and this process was due to H_2O_2 dependent increase in the (PM) H^+-ATPase activity. Exogenous H_2O_2 possibly functioned directly or may have induced intracellular H_2O_2 generation to act as a signalling molecule under stress. Pretreatment of wheat seeds with H_2O_2 has been shown to improve the subsequent salt tolerance of the seedlings (Wahid et al., 2007). The control of Na^+ involvement across

the PM and tonoplast to maintain a low Na^+ concentration in adaptation to salt stress (Rausch et al., 1996). Liu et al., (2007) showed that glucose-6-phosphate dehydrogenase played an important role in NR-dependent NO production, and in establishing to tolerance of red kidney been root to salt stress.

In previous studies, NO decreases membrane permeability, rate of ROS production, malondialdehyde (MDA) and H_2O_2 and intracellular CO_2 concentration under salt stress by inducing ROS scavenging enzymes activities CAT, peroxidises (POD), SOD, ascorbate peroxidise (APX) and proline accumulation (Kopyra and Gwozdz, 2003; Fan et al., 2007; Yu-quing et al., 2007; Shi et al., 2007; Sheokand et al., 2008; Lopez-Carrion et al., 2008; Guo et al., 2009). Moreover, NO induces not only ROS-scavenging enzymes activities, but also expression of transcripts for stress related genes encoding sucrose-phosphate synthase, Δ-pyroline-5-carboxylate synthase (Uchida et al., 2002). Furthermore, NO participate in enhancement of photosynthesis by inducing the photosynthetic pigments under salt stress (Ruan et al., 2002; Fan et al., 2007) and also in ATP synthesis and two respiratory electron transport pathways in mitochondria (Yamasaki et al., 2001; Zottini et al., 2002) where, it mediates the modulation of ROS and enhances the antioxidant defense system in plants subjected to salinity (Zheng et al., 2009). It has been initially hypothesized that H_2O_2 might be downstream signal molecules to regulate the activity of PM H^+-ATPase. Avsian et al., (2004) have shown that salt stress induced oxidative stress in the form of H_2O_2, the production of which occurred in intracellular spaces. Further results indicated that H_2O_2 content increased greatly under salt stress. Since H_2O_2 might be the candidate downstream signal molecule, we tested PM H^+-ATPase activity

and K and Na ratio in calluses by adding H_2O_2. The results suggested that H_2O_2 inducing an increased K to Na ratio. Therefore, it is clear from summing up this new assay that NO may be regulate the H_2O_2 generation. Since H_2O_2 is involved in downstream signal molecule of NO, PM-NADPH oxidase, the main source of H_2O_2 production might be the regulated target of NO. The results indicated that PM-NADPH oxidase is required for H_2O_2 accumulation and PM-NADPH oxidase activity could attribute to NO in calluses under salt stress (Fig. 3).
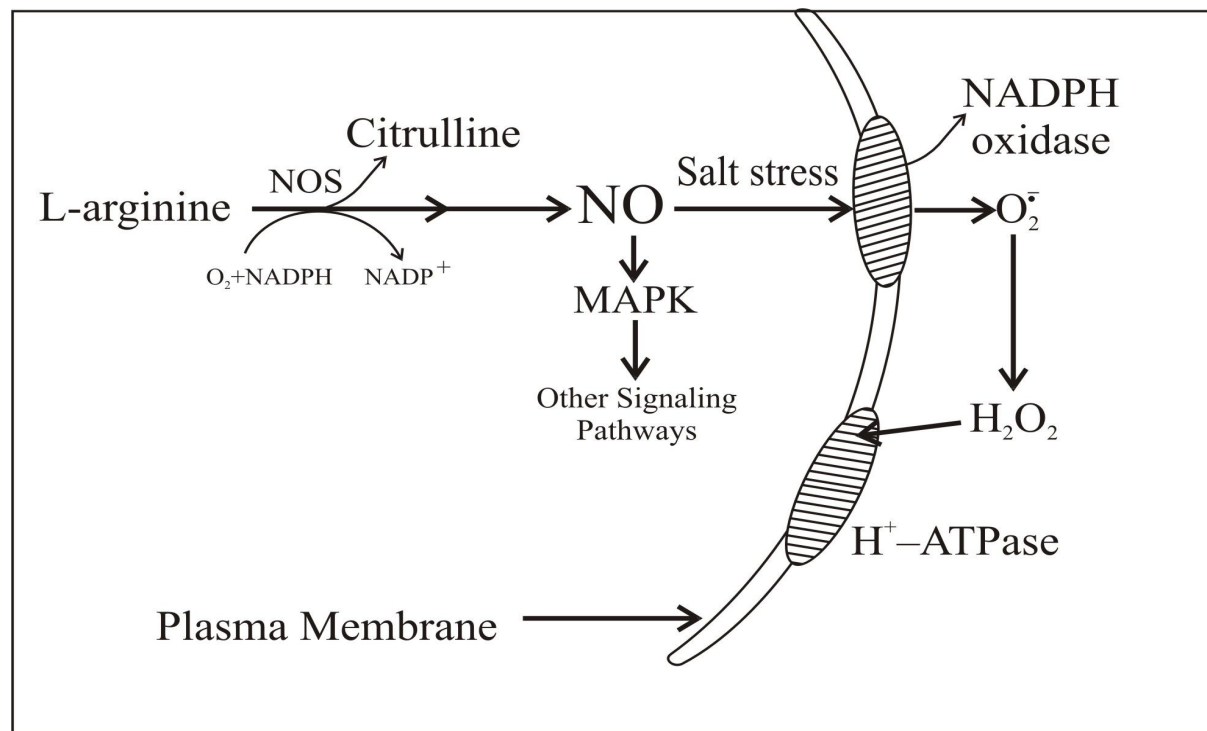

Fig. 3 A model for functioning of NO and H_2O_2 as signalling molecules in inducing salt resistance.

In fig. 3, it clear that H_2O_2 rather than NO is the major signalling molecule that mediate directly the activity of PM H^+-ATPase under salt stress. Normally, NO generated from NOS, acts as a signal molecule to regulate other mechanisms. Under salt stress, accumulated NO activates PM-NADPH oxidase activity. Then, a number of H_2O_2 produced from PM-NADPH oxidase. The PM H^+-ATPase is activated greatly by the accumulated H_2O_2. Research on role of NO and H_2O_2 under stress conditions in plant is advancing rapidly. Further research work is required to decipher the mechanisms through which NO and H_2O_2 acts and how K and Na elements uptake might be connected with salt resistance.

Heavy metal stress

As we know that heavy metals contamination affects the biosphere in many places worldwide. Heavy metal toxicity is one of the major abiotic stresses leading to hazardous health effects in animals and plants. Because of their high reactivity they can directly influence growth, senescence and energy synthesizing processes (Maksymiec, 2007). Tolerance to heavy metals in plants may be defined as the ability to survive in a soil that is toxic to other plants, and is manifested by an interaction between a genotype and its environment (Macnair et al., 2000). Plants possess a range of potential cellular mechanisms that may be involved in the

detoxification of heavy metals and thus tolerance to metal stress (Hall, 2002). The effects of their toxic influence on plants is largely a strong and fast inhibition of growth processes of the above and underground parts, as well as the activity decrease of the photosynthetic apparatus, often correlated with progressing senescence processes (Krupa et al., 1993; Maksymiec et al., 1994; Ouzounidou et al., 1995; Maksymiec and Baszynski, 1996b; Skorzynska-Polit and Baszynski, 1997; Weckx and clijsters, 1997; Molas, 2002; Sobkowiak and Dekert, 2003; Alaoui-Sosse et al., 2004; Lin et al., 2005). Growth inhibition and senescence stimulation caused by heavy metals in excess are intriguing effects, more so, as the knowledge of their mechanisms can have a great significance in eco-physiology and medicine.

Like other stresses, NO also plays the vital role in enhancement of antioxidant enzymes activities and alleviates the toxicity of heavy metals. Exogenous application of SNP reduced cupper (Cu) toxicity and NH_4^+ accumulation in rice leaves. Moreover, studies of Wang et al., (2010) also suggest that application of the NO donor SNP efficiently alleviated the copper toxicity effect, as shown by increases in chlorophyll content and the biomass of fresh/dry leaves in *Lycopersicon esculentum*. SNP treatment also induced the transcription and increased activities of antioxidant enzymes, including CAT, peroxidase, SOD and ascorbate peroxidase led to reduction in H_2O_2 accumulation in the leaves. Special inhibitors or scavengers of NO synthesis is diminished the ameliorating effect of NO on copper (Cu) toxicity (Wang et al., 2010). The protective effect of SNP on the toxicity and $NH4^+$ accumulation can be reversed by PTIO, a NO scavenger, suggesting that the protective effect of SNP is attributable to NO

released. These results also suggest that reduction of Cu induced toxicity and $NH4^+$ accumulation by SNP is most likely mediated through its ability to scavenge active oxygen species (Yu et al., 2005). Kopyra and Gwozdz (2003) also found that SNP pretreatment significantly reduced $O_2^{.-}$ induced specific fluorescence in *Lupinus luteus* roots under heavy metals treatment. Results obtained in this study suggest that antioxidant function of NO may be traced by a scavenging O_2^-, resulting in a decrease of its intracellular concentration. The detoxification and antioxidative properties of NO also found in soyabean cell cultures under Cd and Cu (Singh et al., 2008). Moreover, NO decreased the Al^{3+} toxicity in root elongation of *Hibiscus moschetuos* (Tian et al., 2006). Application of SNP promoted ROS-scavenging enzymes reduced accumulation of H_2O_2 and induced the activity of H^+-ATPase and H^+-PPase in plasma membrane or tonoplast, also significantly alleviated the growth inhibition induced by $CuCl_2$ in tomato plants. These results suggested that exogenous NO could effectively induced tomato seedlings to adjust physiological and biochemical mechanisms against Cu toxicity, and maintain fundamentally metabolic capacity and normal growth under heavy metal stress (Cui et al., 2009). Hu et al., (2007) also found that pre-treatment of NO improved wheat seeds germination and alleviated oxidative stress against Cu toxicity by enhancing the activity of SOD and CAT and by decreasing the lipoxygenase activity and malondialdehyde synthesis.

NO was reported to have the ability to reduce Cu-induced toxicity in tomato through antioxidant enzyme activity and metallothionein accumulation, and that metallothionein acts downstream of NO signalling (Wang et al., 2010). NO is most likely mediated through the modulation in the activities of

antioxidant enzymes (CAT, POD and APX) involved in H_2O_2 detoxification and in the maintenance of cellular redox couples (GR), and contents of molecular antioxidants (Particularly non-protein thiol, ascorbate and its redox status) (Tewari et al., 2008). Zhang et al., (2008a) reported that pre-treatment with SNP increased the accumulation in Cu treated cells by about 1.5 fold, which this effect could be blocked by addition of cPTIO. Cu and NO were able to stimulate the Δ-pyroline-5-carboxylate synthetase (P5CS) activity, the key enzyme of proline biosynthesis and up regulate the expression of P5CS in the Cu treated algae. These results indicate that Cu-responsive proline biosynthesis is closely related to NO generation in *Chlamydomonas reinhardtii*, suggesting the regulatory function of NO in proline metabolism under heavy metal stress. NO protected the plants against Al^{3+} induced oxidative stress and increased root elongation was correlated with a decreased in Al^{3+} accumulation in root apexes (Wang and Yang, 2005). SNP-exposed plants of Wheat showed enhanced activities of SOD, CAT, APX and protein content, whereas decreased H_2O_2 and Malondialdehyde under Al stress (Zhang et al., 2008b).

Furthermore, a recent study showed that SNP alleviated Cd-toxicity, atomic absorption spectrometry and fluorescence localization showed that treatment with SNP decreased Cd accumulation in both cell wall and soluble fraction of leaves; although SNP increased Cd accumulation in the rice roots obviously. SNP in nutrient solution had little effect on the transpiration rate of rice leaves, but this treatment increased pectin and hemicellulose content and decreased cellulose content significantly in the cell wall of rice roots. Based on these results, we conclude that decreased distribution of Cd in the soluble fraction of leaves and roots and increased distribution of Cd in the cell wall of roots are responsible for the NO induced increase of Cd tolerance in rice. It seems that exogenous application of NO enhances Cd tolerance of rice by increasing pectin and hemicelluloses content in the cell wall of roots, increasing Cd accumulation in root cell wall and decreasing Cd accumulation in soluble fraction of leaves (Xiong et al., 2009). The production of H_2O_2 in rice leaves enhanced under Cd treatment, in the case Cd alone, H_2O_2 content induced significantly with increase in the concentration of Cd (Fig. 4). Cd toxicity resulted in reduced length, biomass, protein content and activities of antioxidant enzymes (Sharma et al., 2010).

As a consequence of a general stress response, cytotoxic H_2O_2 get accumulated in the cells (Levine et al., 1994) and can act as a secondary messenger (Dietz et al., 1999). High H_2O_2 and O_2^- had been reported earlier in the case of various other plants under Cr Zn Pb etc. (Dietz et al., 1999; Panda, 2003; Panda et al., 2003a, b; Choudhury and Panda, 2004). The increase in SOD activity indicated higher H_2O_2 level seen by the increase in total peroxide content in leaves, which tallies with those observed in the case of *Brassica juncea* and *Vigna radiata* under Zn and Al treatment (Prasad et al., 1999; Panda et al., 2003b).

Moreover, H_2O_2 increase usually occurred after Cu, Cd (Drazkiewicz et al., 2004; Romero-Puertas et al., 2004; Maksymiec and Krupa, 2006b) and Hg (Cho and Park, 2000) treatment of *Arabidopsis Thaliana* and tomato plants, respectively. However, in barley plants only Mn increased the H_2O_2 content after 5 days but not Cu (Demirevska-Kepova et al., 2004). This difference may indicate that H_2O_2 accumulation developed differently during a larger

stress action. After a long time of Cd action, SOD activity decrease was observed (Sandalio et al., 2001). However, this effect was connected with

attenuation of the enzymic antioxidative system, and increased per oxidation of lipids may have not resulted in H_2O_2 level decrease.
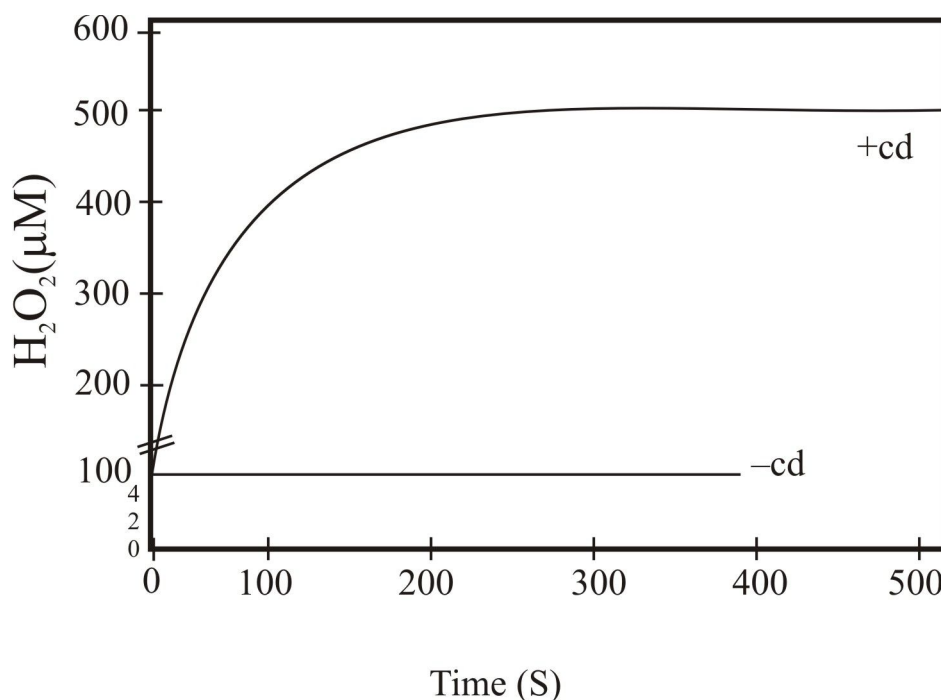

Fig. 4 Stimulated H_2O_2 concentration in the absence and presence of 50 µM Cadmium

More recently, Lin et al., (2005) have shown that Cu can act through changes in H_2O_2-dependent peroxidase activity followed by cell wall stiffening due to the formation of cross-linking among the cell wall polymers. Also Cd (Non-reducing ions) enhances H_2O_2 accumulation (Schutzendubel et al., 2001; Romero-Puertas et al., 2004; Cho and Seo, 2005; Maksymiec and Krupa, 2006b). NADPH oxidase (a source of H_2O_2 production) is involved in plant growth (Papadakis and Roubelakis-Angelakis, 1999; Liszkay et al., 2003) and plant response to Cu (Lamb and Dixon, 1997; Orozco-Cardenas et al., 2001; Quartacci et al., 2001; Maksymiec and Krupa, 2006b). Increased accumulation of H_2O_2, usually connected with changes in the cellular redox status, alerts the plant cell against environmental stresses (Lamb and Dixon, 1997; Ghoshory et al., 1998; Orozco-Cardenas et al., 2001; Foyer and Noctor,

2003; Rentel and Knight, 2004) and may enhance the plants antioxidant response through calcium signalling in the expression of glutathione transeferase gene (Rentel and Knight, 2004).

In fact, accumulation of H_2O_2 has been observed in Cd-exposed roots (Schutzendubel et al., 2001) and in Cd exposed *Nicotiana tabacum* suspension cultures (Piqueras et al., 1999). It was suggested that Cd triggered an oxidant burst as in pathogenesis because they detected H_2O_2 in the culture medium (Piqueras et al., 1999). Taking all these observation together, a hypothetical framework may be suggested that Cd induces a transient loss in antioxidative capacity perhaps accompanied by a stimulation of oxidant producing enzymes, which results in intrinsic H_2O_2 accumulation. H_2O_2, then, would act as a signalling molecule triggering secondary defenses. These in turn, would cause an

ultimately cell wall rigidification and lignifications, thereby, decreasing cellular viability and finally resulting in cell death (Fig. 5).

Drought stress

Drought stress induced free radicals cause lipid per oxidation and membrane deterioration in plants and it, also leads to an imbalance between antioxidant defenses and the amount of AOS resulting in oxidative stress (Van Breusegem et al., 2001). AOS are necessary for inter and intracellular signalling, but at high concentration can cause damage at various levels of organization including chloroplast (Smirnoff, 1993). Apart from morphological structures contributing to drought stress tolerance, plants have evolved a variety of physiological and biochemical processes, which act as components of drought tolerance (Ren et al., 2007; Wang et al., 2007). Plants have also developed enzymatic antioxidant system to cope with drought stress and to avoid oxidative damage (Shvaelva et al., 2006; Horvath et al., 2007).
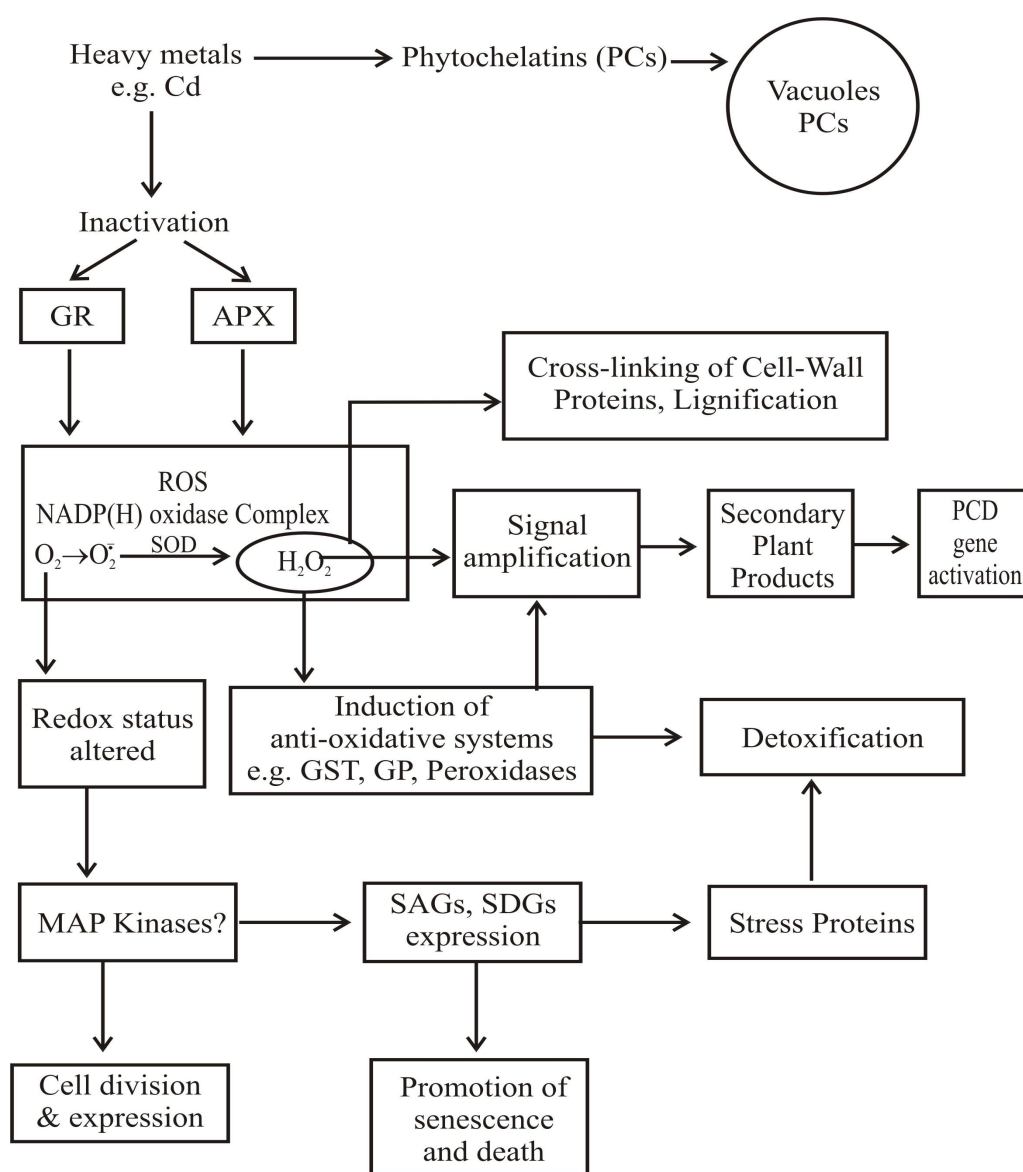
Fig. 5 Hypothetical view of heavy metal action on the cellular redox control and related for antioxidant signalling (SAGs, Senescence-associated genes; SDGs, Senescence-down-regulated genes)

Arasimowicz-Jelonek et al., (2009) reported in their study that roots subjected to mild (5 to10 h) water deficit showed slightly enhanced NO synthesis in cells of root tips and in the surrounding elongation zone as compared to severe (17 h) water stress resulted in an intensive NO production in cucumber roots. Also drought promoted NO production in pea, wheat and *Nicotiana tabacum* was reported by many researchers (Gould et al., 2003; Leshem and Haramaty, 1996; Kolbert et al., 2005).

Application of SNP enhanced plant tolerance to drought stress by reducing water stress, in leakage and transpiration rate, and inducing stomatal closure (Garcia-Lamattina, 2001). Exogenous SNP alleviated oxidative damage, accelerated protein synthesis and enhanced photosynthesis rate, and increased the activities of SOD and CAT and also maintained higher relative water content (RWC) and lower leaf water loss in leaves of wheat seedlings exposed to polyethylene glycol (PEG), termed was drought stress. Interestingly, such effects of SNP were reversed by the addition of carboxy-PTIO [2-(4-carboxyphenyl-4, 4, 5, 5-tetramethylimidazoline-1-oxyl-3-oxide], a specific NO scavenger (Tan et al., 2008). These results suggested that application of SNP might confer an enhanced resistance to drought stress in plants. Sang et al., (2008) demonstrated that water stress induced the synthesis of NO in the maize mesophyll cells and activity of NOS in cytosolic and microsomal fractions of maize leaves and this NO production was blocked by the pre-treatment with inhibitors of NOS and NR, suggesting that NO is produced from NOS and NR in leaves of maize plants exposed to water stress. The pre-treatment of NOS and NR inhibitors inhibited activities of chloroplast and cytosolic antioxidant enzymes i.e. SOD, APX and GR and

decreased activities of these enzymes were enhanced by the exogenous application of NOS, thereby, decreasing the accumulation of H_2O_2 induced by water stress. The potential ability of NO to scavenger H_2O_2 is at least in part due to the induction of sub-cellular antioxidant defense. Hao et al., (2008) reported that NO dependence on NOS-like activity in participated in the signalling of drought induced protective responses in maize seedlings. Both NOS activity and rate of NO release increased substantially under dehydration stress. The high NOS activity induced by c-PTIO as NO scavenger and NO accumulation blocked by NOS inhibitor- NG-nitro-L-arginine methyl-ester (L-NAME) in dehydration-treated maize seedlings indicated that most NO production under water deficit stress may be generated from NOS-like activity. After dehydration stress, detached maize leaves pre-treated with SNP maintained more water content by decreasing transpiration rate. Exogenous application of SNP alleviated the membrane permeability, a cell injury index when c-PTIO as a specific NO scavenger was applied, the effects of applied SNP was counteracted. Treatment L-NAME on leaves also led to higher membrane permeability, higher transpiration rate and lower SOD activity than those of control leaves indicating that NOS-like activity was involved in the antioxidative defense under water stress. These results suggested that NO dependence on NO-like activity serves as a signalling component in the induction of protective response and is associated with drought tolerance in maize seedlings. The protective effect of NO in osmotic stress was recently confirmed in two ecotypes of reed suspension cultures. The findings of Zhao et al., (2008) suggest that poly ethylene glycol (PEG-60000) induced NO release and activities of antioxidant enzymes in stress tolerant, but not

sensitive, ecotype reed can effectively protect against oxidative damage and confers an increased tolerance to osmotic stress.

Moreover, studies of Li et al., (2010) suggest that the treatment with the ROS scavenger DMSO dramatically reduced the effects of localized root irradiation on the induction of HR and expression of the AtRAD54 gene in bystander tissues, suggesting that ROS play a critical role in mediating the bystander mutagenic effects in plants. Sergi et al., (2003) reported that accumulation of H_2O_2 in the walls of mesophyll cells of *Cistus clusii and Cistus albidus* plants increased at the onset of drought and also recently have shown that H_2O_2 accumulates in senescing leaves of drought-susceptible plants (Munne-Boch et al., 2001) and conclude that drought stress was the cause of H_2O_2 accumulation in test species. The accumulation of H_2O_2 observed at the onset of drought in the mesophyll cell walls of *C .albidus* and *C.clusii* may be associated with its function in cellular signalling at the first stage of drought or with drought-induced changes in cell wall structure (Scandalios et al., 1997) or both. Accumulation of H_2O_2 in mesophyll cell walls occurred at the first symptoms of drought in both species, indicating that H_2O_2 may play a role in inter or intracellular signalling or both (Doke, 1997; Foyer and Noctor, 1999). In addition, H_2O_2 is necessary for the peroxidase-mediated oxidative polymerization of cynnamil alcohols to form lignin (Potikha et al., 1999). Furthermore, diurnal variations in H_2O_2 suggest a particular role for this ROS in the response of plants to a combination of stresses. After a general introduction to the concept of drought and oxidative stress and its relationship, Jubany-Mari et al., (2010) describe the role of H_2O_2 in drought stress responses, emphasizing the importance of studies in H_2O_2 subcellular

localization, needed for a better understanding of its role in plant responses to stress. Drought, oxidative stress and H_2O_2, in particular can enhance the expression of several genes (Bray, 1993; Dat et al., 2000; Desikan et al., 2000; Neale et al., 2000) and H_2O_2 can play a role in inter-and intracellular signalling (Doke, 1997; Foyer and Noctor, 1999). Thus, the possibility of an H_2O_2 -dependent regulation of stress response genes cannot be excluded, but further research is needed to conform it. Moreover, Sergi et al., (2003) reported also that the accumulation of H_2O_2 was associated with enhanced formation of lipophilic antioxidants indicating efficient scavenging of ROS in the chloroplasts of drought-stresssed *C.clusii and C.albidus* plants. The role of H_2O_2 in intracellular signalling in drought-stressed plants and in the putative regulation of antioxidant synthesis needs to be investigated (Fig. 6).

Temperature stress

Like other stresses, heat and cold stress have been a major limitation to crop productivity. Today, one of the biggest challenges for plant growth and productivity is to cope with the abrupt and often unpredictable temperature fluctuations. High temperature induces oxidative stress, lipid peroxidation, membrane injury, protein degradation, enzyme inactivation, pigment bleaching and DNA strands disruption in plants (Suzuki and Mitter, 2006).Similarly low temperature (cold stress) caused many changes in biochemical and physiological processes and ROS-homoeostasis in plants (Zhao et al., 2009; Xin and Browse, 1998; Suzuki and Mittler, 2006). The ability of plants to acquire tolerance to freezing temperature is a consequence of numerous changes in plant cells during a period called hardening (Browse and Xin, 2001). The initial perturbations in plant metabolism are

followed by alteration in plant development and morphology with a final result achievement of maximum freezing tolerance (Huner et al., 1998). Potentially harmful to plant cells when in high levels, the ROS production during low temperature stress could have a role in stress perception and protection (Suzuki and Mittler, 2006). The tight control of ROS levels under optional growth conditions and especially during stress events is attained by a network of antioxidant enzymes and small molecules found in almost all cellular compartments (Mittler et al., 2004).
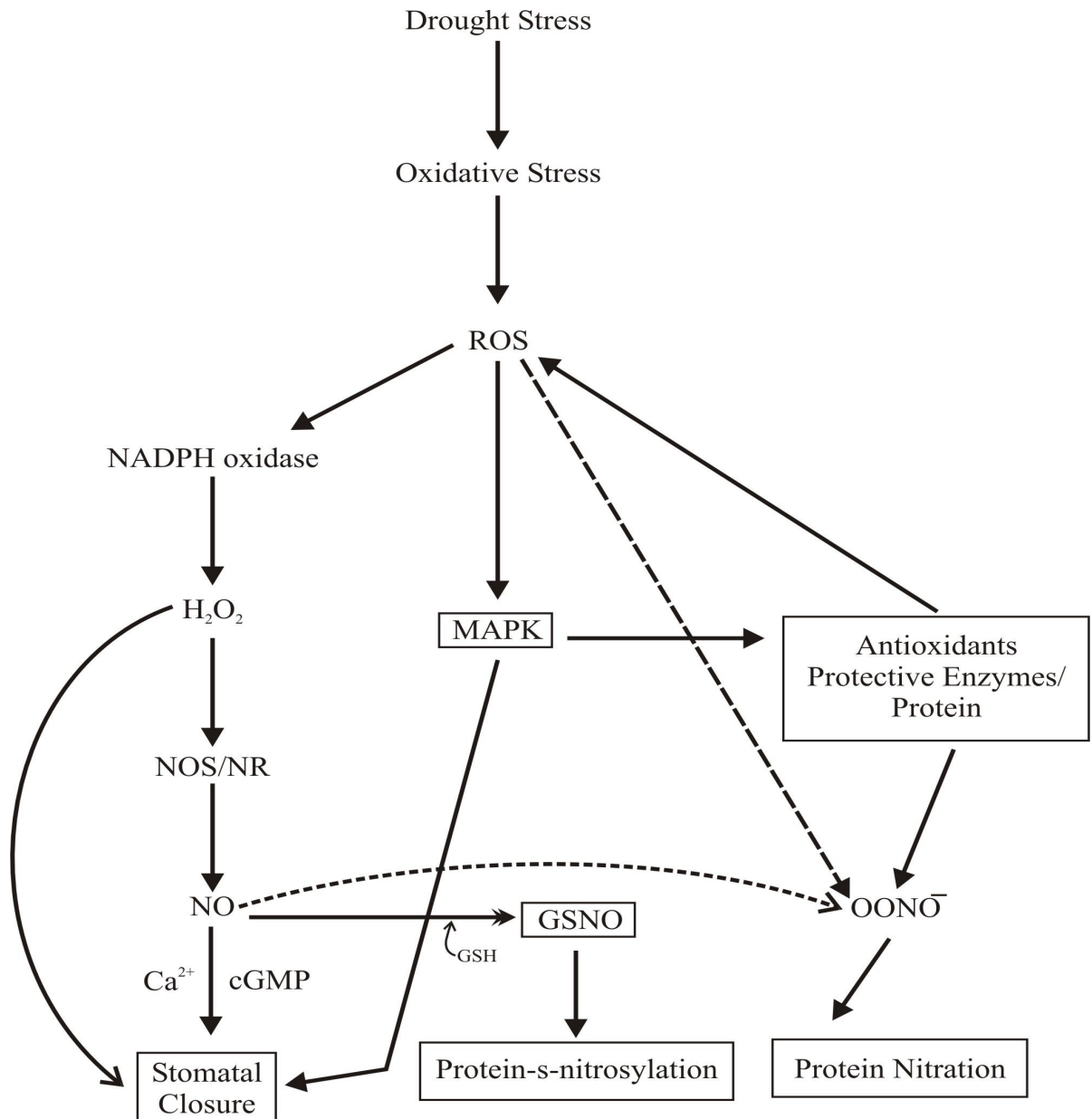
Fig. 6 Drought stress under signalling interaction between H_2O_2 and NO in order to mediate plant survival under condition of water scarcity. H_2O_2 induces NO generation by NR and NOS-like enzymes via as an yet to be fully characterized signalling pathway that may include the OX11 protein kinase and involve Ca^{2+}. NO also enhances antioxidant gene and enzymes activity via MAPK and other signalling pathways. NO may also signal by inducing conformational changes in protein as a result of S-nitroslation/nitration

In many studies, researchers tried to find out the role of NO in alleviation of H_2O_2 mediating heat and cold stress. However, high temperature treatment of lucerne cells resulted in an increase of NO synthesis, whereas, the application of exogenous NO induced cold tolerance in tomato, wheat and maize (Neil et al., 2003). NO production was increased by short time heat stress in alfalfa (Leshem, 2000). It is possible that this effect was related with antioxidative action of NO, which elevates adverse affects imposed by the intensification of peroxidative metabolism in heat and cold stress (Neil et al., 2002). Both heat and chilling caused reduction in membrane protein thiol level and increased accumulation of thiobarbituric acid reactive substances in 72 hr old germinating tissues (indicators of oxidative stress) and reduced germination and early growth performances (Bhattacharjee, 2009). Bouchard and Yamasaki (2008, 2009) reported that the increase in production of NO at high temperature and suggested that heat stress stimulated the NO production that could play a role in the induction of cell death in *symbiodinium microadriaticum* by mediating an increase in caspase like activity. Zhao et al., (2009) reported that cold acclimation induced an increase in endogenous NO production in wild type (*Arabidopsis thaliana*) and At noa1/rif1 (for nitric oxide associated1 resistant to inhibition by fosmidomycin1) leaves, while endogenous NO level in nia1 nia2 (NR-defective double mutant) leaves was lower than that is wild-type ones and it was little changed during acclimation. Cold acclimation stimulated NR activity and induced up-regulation of NIA1 gene expression.

In contrast, cold acclimation reduced quantity of NOA1/RIF1 protein, and inhibited NOS activity. These results indicate the up-regulation of NR

dependent NO synthesis. Moreover, seedlings of nia1nia2 were less tolerant to freezing than wild type plants. Pharmacological studies using NR inhibitor, NO scavenger and NO donor showed that NR-dependent NO level was positively correlated with freezing tolerance. Further, cold acclimation up and down regulated expression of P5CS1 and ProDH genes, respectively, resulting in enhanced accumulation of Pro and wild-type plants. The stimulation of Pro accumulation by cold acclimation was reduced by NR inhibitor and NO scavenger, while pro accumulation by cold acclimation was not affected by NOS inhibitor. In contrast to wild type plants, cold acclimation up-regulated Pro DH gene expression in nia1nia2 plants than wild type plants. These findings demonstrate that NR-dependent NO production plays an important role in cold acclimation-induced increase in freezing tolerance by modulating Pro accumulation in Arabidopsis.

Furthermore, Uchida et al., (2002) reported that rice seedlings treated with low levels of H_2O_2 or NO allowed the survival of more green leaf tissue and resulted in higher quantum yield for photosynthesis II than in non-treated controls under salinity and heat stress. Apostolova et al., (2008) reported that levels of endogenous peroxides were strongly increased in the spring wheat cultivar in response to cold hardening, and to a lesser extent in the winter wheat. However, H_2O_2 pre-treatment reduced production of H_2O_2 under further chilling stress, postponing oxidative damage. Results of Wang et al., (2010) showed that pre-treatment with H_2O_2 at appropriate concentration may improve the tolerance of warm-season Zoysia grasses to chilling stress and that manila grass had better tolerance to chilling, as evaluated by lower Malondialdehyde (MDA) and EL, and better turf grass quality, regardless of the pre-treatment applied. Pre-treatment with H_2O_2 has
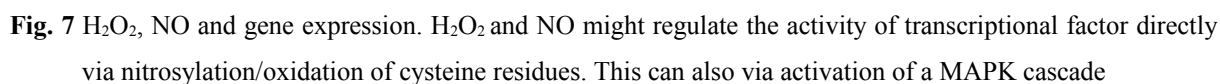
been shown to induce chilling tolerance in normally chill-sensitive maize seedlings (Neto et al., 2005). Similarly regenerated potato nodal explants treated with H_2O_2 became significantly more thermo tolerant compared with untreated control (Lopez-Delgodo et al., 1998). Apel and Hirt (2004) reported that H_2O_2 presumably provide an atmosphere in which peroxidases can act in two different catalytic modes. In the presence of H_2O_2 and phenolic substrates peroxidases operate in the peroxidatic cycle and are engaged in the synthesis of lignin and other phenolic polymers.

However, if the phenolic substrates are replaced by NADPH or related reduced compounds, a chain reaction starts that provides the basis for the H_2O_2 producing NADPH-oxidase activity of peroxidases (Chen and Schopfer, 1999). Peroxidase H_2O_2 production is distinguished from that by the phagocyte-type NADPH-oxidase by different Km values for oxygen, different requirements for NADH and NADPH, and different sensitivities of the two enzymes to inhibitors such as cyanide, azide and Diphenyleneiodnium (DPI). Because H_2O_2 is a mild oxidant that can oxidize thiol residues, it has been speculated that H_2O_2 is sensed via modification of thiol groups in certain proteins. So, therefore, H_2O_2 activates several MAPK cascades have important functions in plant stress responses and development and are key players in ROS signalling and in innate immunity. In Arabidopsis, the transmission of ROS and pathogen signalling by MAPKs involves the coordinated activation of MPK6 and MPK3. Genetic analysis studies of Lumbreras et al., (2010) showing that MAPK phosphatase 2 (MKP2) regulates oxidative stress and pathogen defence responses and functionally interacts with MPK3 and MPK6 as well as MKP2 is a key regulator of MPK3 and MPK6

networks controlling both abiotic and specific pathogen responses in plants.

In Arabidopsis, H_2O_2 activates the MAPKs, MAK3 and MAK6 via MAPKKKANP1 (Kovtun et al., 2000). Over-expression of ANP1 in transgenic plants resulted in increased tolerance to heat shock, freezing and oxidative stress (Kovtun et al., 2000). H_2O_2 also increases expression of the Arabidopsis nucleotide diphosphate (NDP) kinase 2 (Moon et al., 2003). Over expression of AtNDPK2 reduced accumulation of H_2O_2 and enhanced tolerance to multiple stresses including cold, salt and oxidative stress. The effect of NDPK2 might be mediated by the MAPKs, MAK3 and MPK6 because NDPK2 can interact and activate the MAPKs. These data suggest a scenario in which various stresses induce ROS generation that in turn activate MAPK signalling cascades.

It was also exhibited that pre-treatment of H_2O_2 induces not only ROS-scavenging enzyme activities but also expression of transcripts for oxidative stress related gene encoding sucrose phosphate synthase, D-pyrroline-5-carboxylate synthase and small heat shock protein 26 (HSP26). These findings demonstrate that H_2O_2 and NO plays an important role in tolerance of rice seedlings to both salt and heat stress by acting as signal molecules for the response. Song et al., (2006) found that application of SNP and S-nitrose-N-acetyl penicillamine (SNAP) (both are NO doner) dramatically alleviated heat stress induced ion leakage increase, growth suppression and cell viability decrease in callus of reed under heat stress and also elevated the activities of SOD, APX, CAT and POD. This result suggest that NO can effectively protect callus from oxidative stress induced by heat stress and that NO might act as a signal in activating active oxygen scavenging enzymes under heat stress (Fig. 7).

Fig. 7 H_2O_2, NO and gene expression. H_2O_2 and NO might regulate the activity of transcriptional factor directly via nitrosylation/oxidation of cysteine residues. This can also via activation of a MAPK cascade

Microarray analysis of H_2O_2 induced gene expression in Arabidopsis indicates potential H_2O_2-responsive cis-elements in genes regulated by H_2O_2 (Desikan et al., 2001). One of these elements, the as-1 promoter element, also has high homology with redox-sensitive mammalian AP-1 cis–element (Karin et al., 1997). However, recent analysis of transgenic plants indicates that ROS other than H_2O_2 activates this as-1 element (Garreton et al., 2002). Further analysis will reveal whether similarity exists among plant, animal and fungal regulatory cis-

elements of ROS-responsive genes. In contrast to O_2^-, H_2O_2 can diffuse in to cells and activate many of the plant defenses, including PCD (Dangl and Jones, 2001). The suppression of ROS detoxifying mechanisms is crucial for the onset of PCD. ROS production at the apoplast alone without suppression of ROS detoxification does not result in the induction in of PCD (Delledonne et al., 2001; Mittler et al., 1999). These data indicate an absolute requirement for the co-ordinated production of ROS and down-regulation of ROS scavenging

mechanisms. The initial and very rapid accumulation of H$_2$O$_2$ is followed by a second and prolonged burst of H$_2$O$_2$ production. Recent work shows that H$_2$O$_2$ function as a second messenger mediating the systematic expression of various defense related genes in tomato plants (Orozco et al., 2001). Although oxidative stress is a primary response to any kind of stress (like temperature, drought and pathogen challenge) that leads to PCD (Bolwell, 1999) and H$_2$O$_2$ induces PCD in various system (Desikan et al., 1998; Levine et al., 1994; Solomon et al., 1999), in some cases H$_2$O$_2$ is not signal for PCD induction (Glazener et al., 1996; Ichinose et al., 2001). A study shows that a threshold exposure time of cells to H$_2$O$_2$ is required during which period transcription and translation are necessary (Desikan et al., 1998; Solomon et al.,1999). A transient increase in H$_2$O$_2$ was suggested to signal activation of protective mechanism for acclimation to chilling. Exogenous application of H$_2$O$_2$ can induce tolerance to chilling, high temperature and biotic stress, all of which cause elevated endogenous H$_2$O$_2$ production. A transient increase in H$_2$O$_2$ was suggested to signal activation of protective mechanisms for acclimation to chilling. These results suggests surprisingly that large number of genes that respond to an increase in H$_2$O$_2$ concentration would be in time with the proposed role of H$_2$O$_2$ as an ubiquitous signal for oxidative stress.

Ultra-violet Radiation stress

The decrease of stratospheric ozone layer from anthropogenic inputs of chlorinated fluorocarbons has resulted an increase of radiation of UV-B (280-315n.m) that leads to an increase of ion leakage, loss of chlorophyll, and decreases the maximum efficiency of PS-II photochemistry (Fv/Fm) and the quantum yield of PS-II electron transport (PS-II)

and increase H$_2$O$_2$ and thylakoid membrane protein oxidation. Protein oxidation is defined here as covalent modification of a protein induced by ROS or by-products of oxidative stress. Most types of protein oxidations are especially irreversible, whereas, a few involving sulphur containing amino acids are reversible (Ghezzi et al., 2003). Protein oxidation is wide spread and often used as a diagnostic marker for oxidative stress. Concerns about potential impacts of stratospheric ozone depletion contributed to spark-interest in studies of plant responses to enhanced UV-B levels during the last two decades (Caldwell et al., 2003). Ambient UV-B appears to have a measurable (generally modest) effect reducing plant growth, particularly in the case of herbaceous plants (Ballare et al., 1996; Krizek et al., 1998; Mazza et al., 1999a). However, its modification may have large impacts on the interactions between plants and phytophagous insects (Ballare et al., 2001; Paul and Gwynn-Jones, 2003). The most common effect of solar UV-B is increased plant resistance to insects, measured in terms of leaf area consumed and /or insect growth is standard feeding bioassays (Ballare et al., 1996; Rousseaux et al., 1998; Mazza et al., 1999b; Zavala et al., 2001).

Progress in the understanding of the mechanisms that mediate these effects of UV-B has been slow for various reasons. First, no specific UV-B receptors have yet been identified in plants, and no UV-B perception mutants are available for comparative studies. Second, most of the information on UV-B effects at the molecular level has been obtained in indoor-exposure experiments with heavily-unbalanced UV-B sources. Third, UV-B has the potential to damage key macro-molecules and cellular structures, particularly when high doses are used in laboratory studies, therefore specific

UV-B responses are difficult to separate from secondary consequences of generalized damage under these conditions.

UV-B radiation resulted in the increase in NO and ROS in Arabidopsis (Mackerness et al., 2001). The results of Wang et al., (2006) supported that NO generated from NOS-like activity appeared to act in the same direction or synergistically with ROS to induce ethylene synthesis in defence response under UV-B radiation in maize leaves. Zhang et al., (2003) and An et al., (2005) reported that UV-B induced increase of NOS activity in maize hypocotyls, indicating that NO may act as a second messenger and perform anti-oxidant responses to UV-B radiation, and SNP-exposed maize plants exhibited increase activity of glucosidase and protein synthesis. Moreover, UV-B induced stomatal closure, which was mediated by NO and H_2O_2 and generation of NO was caused by a NOS-like activity (He et al., 2005). However, other authors reported that NO generated in guard cells were produced by NR activity (Bright et al., 2006). A study of Santa-Cruz et al., (2010) shows that NO protects against oxidative damage and that NOS-like activity is also required for HO-1 induction under UV-B radiation. Pre-treatments with SNP, a NO-donor, prevented chlorophyll loss, H_2O_2 and O_2^- accumulation and ion leakage in UV-B-treated plants. In addition, experiments carried out in the presence of L-NAME, well-known inhibitor of NOS and nitrate reductase, showed that NOS is the endogenous source of NO that mediates HO-1 (has antioxidant properties and is up-regulated by ROS in ultraviolet-B-irradiated soybean plants) expression.

Furthermore, Shi et al., (2005) reported that addition of SNP can partially alleviated UV-B induced decrease of chlorophyll content, Fv/Fm of ΦPS-I and oxidative damage to the thylakoid membrane in bean leaves. The potassium salt of cPTIO, arrested NO mediated protective effects against UV-B induced oxidative damage. Incubation of thylakoid membrane with increasing H_2O_2 concentration showed a progressive enhancement in carbonyl contents. H_2O_2 contents were suppressed in the presence of NO under UV-B radiation through increasing activities of CAT, SOD and APX and these results suggest that NO can effectively protect plants from UV-B destruction mostly mediated by enhanced activities of oxidative enzymes. As might be expected from previous work, some of H_2O_2-induced genes in Arabidopsis suspension cultures-encode anti-oxidant enzymes, defence and stress-related proteins. Interestingly, genes encoding signalling proteins such as transcription factors, protein kinases and protein phosphatases were also up-regulated by H_2O_2. These genes were similarly induced by other stresses such as wilting, UV-challenges. For example UV-B induced genes expression has been shown to occur via H_2O_2, as exposure of Arabidopsis plants to UV-B in the presence of antioxidants led to down-regulation of the UV-B induced gene (A-H-Mackerness et al., 1996). Moreover, activation of a NADPH oxidase by UV has been demonstrated in laboratory studies in Arabidopsis (Rao et al., 1996) and it is most interesting to note that the results of Casati and Walbot (2003) show clear up-regulation of a NADPH oxidase gene by solar UV-B in field grown plants. Moreover, UV-B may activate a variety of molecular sensitizers and give rise to enhanced H_2O_2 levels, which may lead to convergence with the wound induced cascade downstream of NADPH oxidase. Arabidopsis leaves pre-treated with H_2O_2 have been shown to develop increased tolerance to excess light (Karpinski et al., 1999). Finally, it has been concluded that consistent with a signalling role

for this compound, some reports also have shown that endogenously synthesized H_2O_2 in response to abiotic stresses, can protect plants against UV-B stress and can induce stress response genes.

Conclusion and Future Prospects

From being molecules of somewhat novelty interest, in the last few years, H_2O_2 and NO have emerged to be central players in the world of plant cell signalling, particularly under various stressful situations. The full range of biological functions for these two signalling molecules remain to be catalogued and determining the ways in which they interact, both together and with ever-increasing array of signals known to be recognized by plants, will need to be elucidated. Other research priorities must include full characterization of the enzymes through which the intracellular concentrations of H_2O_2 and NO are regulated and where these enzymes are located in different cells and tissues. The intracellular signalling cascades that transduce H_2O_2 and NO perception in to cellular responses have so far been characterized only superficially. Finally, they raise the question of how H_2O_2 and NO are detected by cells. Such perception could conceivably involve direct interaction of H_2O_2 and NO with cellular proteins, such as transcription factors, ion channels or enzymes.

Currently, research data show that H_2O_2 can play a dual role in the cells. During oxidative stress, H_2O_2 is a strong toxic oxidant causing cell damage or even cell death. At the same time it serves conversely as a signalling molecule to activate a defense system for restoring the redox homeostasis in plant cells. So far it is still under research whether H_2O_2 is situated at a common centre for the signalling pathways providing responses to various signals triggered by abiotic stresses like production of NO. The data available to date show that NO is a key factor involved in responses to a number of abiotic stimuli. They indicate that endogenous NO is a key factor in the tolerance of cells to the oxidative stress induced by a range of abiotic conditions, and that this probably involves the enhanced expression of genes encoding antioxidant enzymes. Of course, there are numerous unanswered questions and important areas for further research in future. The mechanisms by which NO is generated are still largely unresolved and elucidation of how it is made by different plant cells in different situations is clearly a research priority. No doubt, NO a ubiquitous bioactive molecule, plays an important role in a broad spectrum of multiple H_2O_2 mediating physiological processes in plants. However, most of the work has still to be done. In relevance of (1) NO functions as a signalling molecule in interaction with plant hormones, nutrients and metals, (2) NO mediated defense gene regulator in plants, (3) Functions of endogenous NO in different cells or organs of plants and (4) NO biosynthesis pathways in plant and its regulation to environmental stimulus and cellular redox homeostasis regulation. From above discussion, it is clear that both NO and H_2O_2 have now been shown to function as synergistic signals in plants mediating a range of responses to abiotic stresses. Given that such stresses impose considerable constraints on crop production, there is a real need for continued research in this field.

Acknowledgements

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