Functional Plant Science and Biotechnology ©2012 Global Science Books

Role of NO-Mediated H_2O_2 Signaling under Abiotic Stress (Heavy Metal)-Induced Oxidative Stress in Plants: An Overview

Taqi Ahmed Khan[1] • Mohd Mazid[2*] • Jaime A. Teixeira da Silva[3] • Firoz Mohammad[2] • Mohd Nasir Khan[4]

[1] Department of Biochemistry, Faculty of Life Sciences, AMU, Aligarh, 202002 India
[2] Plant Physiology Division, Department of Botany, Faculty of Life Sciences, AMU, Aligarh, 202002 India
[3] Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki cho, Kita gun, Ikenobe, 761-0795, Japan
[4] Department of Biology, College of Science, University of Tabuk, Tabuk, 74191 Kingdom of Saudi Arabia

Corresponding author: * **mazidmohd699@gmail.com**

ABSTRACT

Environmental stress, like heavy metal, constitutes the most significant factor leading to a substantial and unpredictable decrease in crop yield in agriculture. Hydrogen peroxide (H_2O_2) and nitrogen oxide (NO) are produced as primary signals in a stress signal cascade. H_2O_2 accumulation can lead to either enhanced expression of antioxidant enzymes or increased expression of other defense proteins. Alternatively, it can initiate programmed cell death, particularly when NO is also produced, depending on the intensity of the oxidative signal or oxidative load exerted by heavy metal toxicity in tissues. In this review, we examine the regulatory role of H_2O_2 and NO signaling in oxidative stress induced by heavy metals in plants, exemplified by a number of research studies and show how this signaling in response to heavy metals may provide some clues to improving crop productivity.

Keywords: antioxidant, signaling molecules
Abbreviations: APX, Ascorbate peroxidase; **CAT**, Catalase; **ET**, ethylene; **GPX**, Glutathione peroxidase; **H_2O_2**, hydrogen peroxide; **HM**, heavy metal; **L-NAME**, NG-nitro-L-arginine methyl-ester; **LPX**, lipid peroxidation; **MAPK**, mitogen-activated protein kinase; **MDA**, malondialdehyde; **NO**, nitrogen oxide; **NR**, Nitrate reductase; **PCD**, programmed cell death; **ROS**, reactive oxygen species; **SNP**, sodium nitropruside

CONTENTS

INTRODUCTION: HISTORICAL BACKGROUND OF H_2O_2 AND NO

Plants frequently suffer stressful interactions with their environment. These include biotic stresses such as pathogens and pests, and abiotic stresses such as temperature, drought or chemical pollution (e.g. enhancement of heavy metal (HM) concentration in soil). During the 1990's to the present, important discoveries have been made regarding the biochemical mechanisms by which plant cells signal to their neighbours that they are suffering from a stress. Enhanced generation of reactive oxygen species (ROS) by abiotic stresses such as HMs is common in plants and is referred to as oxidative stress because of their potential to damage cells (Prasad *et al*. 1994). Many stresses limit CO_2 assimilation more than electron transport capacity leading to modulation of the latter by photosynthetic control. This favours the increased oxidation of photosystem I (PS-I) and an increased reduction of PS-II (Makeno *et al*. 2002). In these circumstances, electron flow to oxygen increases the yield of superoxide radical ($O_2^{\cdot-}$) in the Mehler reaction (oxygen produced in PS-II during photolysis of water is further reduced in PS-I) (Upham *et al*. 1990). Among them, the production of a signaling molecule such as hydrogen peroxide (H_2O_2) or nitrogen oxide (NO) has gained increasing attention although the precise mechanisms of these interactions and their significance in stress signaling are not completely understood.

Injury to plants exposed to stress is related to oxidative damage at the cellular level (Foyer and Noctor 2003). Under normal metabolic processes, the low amount of ROS are metabolic by-products of plant cells (Hung *et al*. 2005), although the release of radicals into the cytosol can be enhanced under certain stress conditions (del Río *et al*. 1996),

Received: 27 August, 2011. Accepted: 9 February, 2012.

Review

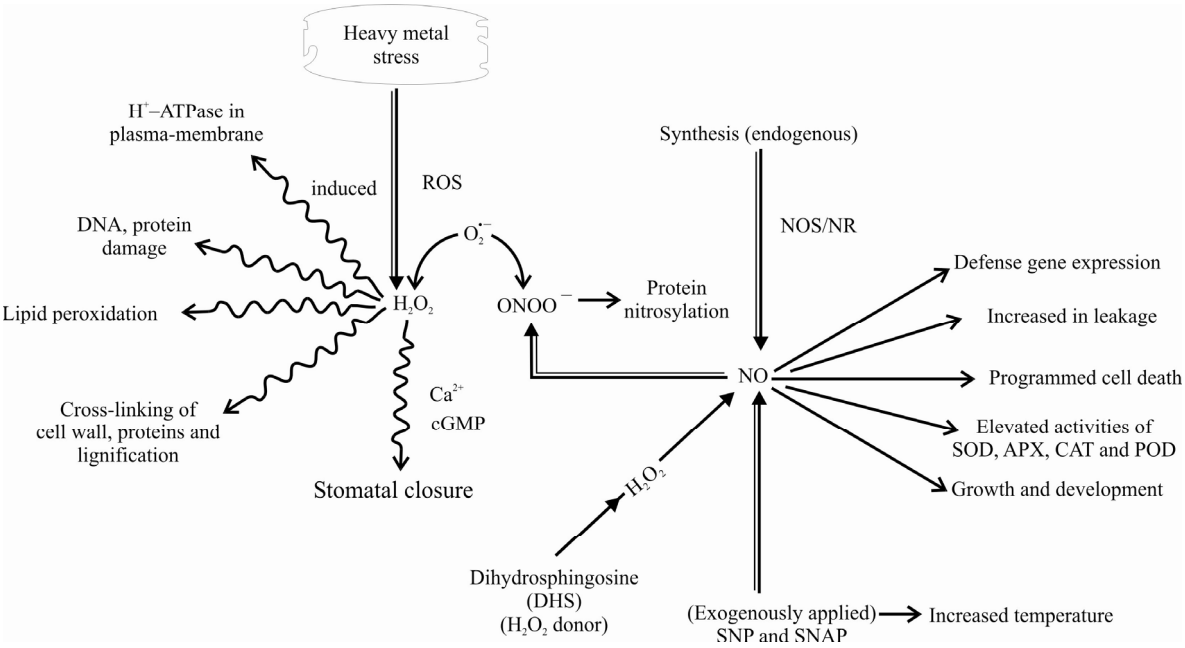

Fig. 1 A schematic representation of NO-H_2O_2 signaling and responses induced by their synergistic interaction. APX, Ascorbate peroxidase; **CAT**, Catalase; **cGMP**, cyclic guanosine-monophosphate; **H_2O_2**, hydrogen peroxide; **NR**, Nitrate reductase; **ROS**, reactive oxygen species; **SNP**, sodium nitropruside; **SOD**, superoxide dismutase.

producing oxidative stress in the cells. $O_2^{\cdot-}$ is immediately converted to O_2 and H_2O_2 ($O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2$). Therefore, the production of these radicals results in an increase of H_2O_2 in the cell (del Río *et al*. 1996), although the precise intracellular concentrations of H_2O_2 that are likely to be toxic will vary (rate of H_2O_2 synthesis in a cell) with the rate of H_2O_2 production and are normally balanced by very efficient antioxidant systems (Noctor and Foyer 1998). For example, the aqueous leaf extract of *Lagerstroemia speciosa* has the potential to inhibit lipid peroxidation and effectively neutralize ROS such as H_2O_2- and NO-based free radicals (Saumya and Basha 2011). The plant cell wall expresses monoamine oxidases (MAOs) that catalyze oxidation of secreted amines and produce H_2O_2 used by cell wall peroxidases for lignification of cell wall or for plant defense. Similarly, Verma and Sharma (2010) proposed that H_2O_2 generated within cell walls of seeds serves as a signaling molecule guiding germination events, including protein reserve mobilization. Gas spaces (aerenchyma) form as an adaptation to submergence to facilitate gas exchange and their formation is mediated by H_2O_2 (Steffens *et al*. 2011).

H_2O_2 and NO both function as stress signaling molecules in plants (Neill *et al*. 1999; Dat *et al*. 2000; Mazid *et al*. 2011a, 2011b), mediating a range of defensive mechanisms in plants under stressful conditions (Wendehenne *et al*. 2004; Delledonne 2005). The concept of H_2O_2 as a signal molecule is realistic because this molecule is relatively stable and diffusible (Foyer *et al*. 1997). The effects of the exogenous application of H_2O_2 are constant with such a signaling role in abiotic stresses, although the number of studies is still small. The basis of long-lived effects of H_2O_2 on growth and tolerance against abiotic stresses is not well known. However, one possibility is the influence of ROS on DNA methylation (Creda and Weitzman 1997). Despite this, H_2O_2 has a short lifespan (16 h) and is able to cross biological membranes and rapidly diffuse from cell to cell or can be transported long distances from its sites of origin (chloroplasts, mitochondria and peroxisomes) to the site of action in plants. Thus, H_2O_2 has all of the characteristic features of an intercellular signaling molecule and, for this reason, has received more attention (Alvarez *et al*. 1998; Neill *et al*. 2002a, 2002b). H_2O_2 is produced in response to various stimuli and mediates cross-talk between signaling pathways and is an attractive signaling molecule contributing to the phenomenon of cross-tolerance (Bowler and Fluhr 2000; Cheng and Song 2006). Similarly, NO is an important signaling molecule in plants and can induce a decrease in ROS accumulation to an extent varying with the degree of abiotic stress tolerance in plants (Yang *et al*. 2006; Tian *et al*. 2007; Wu *et al*. 2010). Also, NO is a widespread intra- and intercellular messenger with a broad spectrum of regulatory functions in many physiological processes (del Río *et al*. 2004; Grun *et al*. 2006). In plants, NO is involved in ethylene (ET) emission (Lesham and Haramaty 1996), in response to drought (Lesham 1996), growth and cell proliferation (Ribeiro *et al*. 1999), metabolism and senescence (Corpas *et al*. 2004), programmed cell death (PCD) (Clark *et al*. 2000) and stomatal closure (Garcia-Mata and Lamattina 2002) (**Fig. 1**).

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. In addition, exposure to low levels of one stress can induce tolerance towards subsequently higher levels of exposure to the same stress, termed acclimation tolerance (Bhattacharjee 2005). Therefore, H_2O_2 is considered as a fundamental fact of life in an anaerobic environment (Moller 2001). In addition, H_2O_2 is an evolutionarily ancient signaling molecule that not only played a key role in inducing the evolution of oxygenic photosynthesis, but also modulates many physiological events, such as stomatal movement, hypersensitive responses, and PCD (Cheng and Song 2006).

NO also plays a vital role in diverse physiological processes in plants such as the induction of seed germination, reduction of seed dormancy (Bethke *et al*. 2007; Zheng *et al*. 2009), regulation of plant metabolism and senescence (Leshem *et al*. 1998; Guo and Crawford 2005) induction of PCD (Pedroso and Durzan 2000), regulation of stomatal movement (Bright *et al*. 2006), regulation of photosynthesis (Takahashi and Yamasaki 2002), and floral regulation (He *et al*. 2004). A high level of NO has the capacity to damage membranes and to fragment DNA (Yamasaki 2000; Romero-Puertas *et al*. 2004), and to reduce photosynthesis in oats (*Avena sativa*) and alfalfa (*Medicago sativa*) (Hill and Bennet 1970), as well as regulating the multiple plant responses towards a variety of abiotic stresses (Crawford and Guo 2005; Delledonne 2005; Neill *et al*. 2008; Zheng *et al*. 2009). NO is involved in various physiological processes and prolongs the storage life of fruit. However, little attention has been paid to the effects of NO on the storage metabolism in fruit during

storage. Sun *et al.* (2011) showed that the decrease of firmness and accumulation of sugar and acid: sugar ratio in peach (*Prunus persica*) fruit during storage is significantly inhibited by treatment with NO (10 µmol/L). Treatment with NO could promote fructose and glucose metabolism during the first few days of storage and increase the content of sucrose and the activities of sorbitol dehydrogenase, sorbitol oxidase and sucrose phosphate synthase in fruit during storage. NO signaling is based on interactions with plant hormones like auxin, jasmonic acid and others. NO inhibits plants from oxidative damage by regulating general mechanisms for cellular redox homeostasis and also promoting the transformation of O_2 to H_2O_2 and O^- and also by enhancing H_2O_2-scavenging enzyme activities It is well known that the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) play a significant role in scavenging ROS. The activities of both SOD and POD are significantly increased by exogenous NO (Shi *et al*. 2007; Zheng *et al*. 2009), although the NO molecule itself possesses antioxidant properties (Karplus *et al*. 1991) and reacts with ROS, hemes, thiols and proteins to produce biochemical signals that directly and indirectly regulate enzymatic activities. Also, beneficial reactions counteract oxidative and nitrosative stresses, while damaging reactions due to high levels of NO ($>10^{-3}$ $mol.L^{-1}$) cause oxidative and nitrosative damage and cell death (Mastrocola *et al*. 2005). The most crucial role of NO in plant growth and development, starting from germination to flowering, ripening of fruits, senescence and abiotic stresses has been extensively reviewed (Arasimowicz and Floryszak-Wieczrek 2007; Wendehenne *et al*. 2004; Siddiqui *et al*. 2011). However, the molecular proof of plant responses to abiotic stresses mediated by NO is still scarce.

H_2O_2 AND NO: SOURCES AND SIGNALING IN PLANT SYSTEMS

However, H_2O_2 and NO both have proved to be important bioactive signaling molecules with multiple physiological functions in plants. It seems likely that NO can be synthesized during a stress response at the same time as H_2O_2 and may be that cellular effects reflect the responses to both H_2O_2 and NO. Furthermore, the properties of NO makes it is a very good agent to act as a signaling messenger in response of environmental stresses like HM action. The exogenous supply of NO protects plants from damage by eliminating the $O_2^{\cdot-}$ and lipid radical, activating the activities of antioxidant enzymes, especially SOD (Shi *et al*. 2007). However, in many studies, there has been increasing evidence that NO acts in chain-breaking antioxidant-arresting lipid peroxidation reactions and activates gene expression of antioxidant enzymes (Ramamurthi and Lewis 1997). NO can also trigger the defense system of plants in response to abiotic stresses. For instance, it has been reported to exert some protective effects on drought stress, heat stress, HM stress and UV radiation stress (Tian *et al*. 2007; Yang *et al*. 2006; Baranek *et al*. 2010; Wu *et al*. 2010; Mazid *et al*. 2011a, 2011b).

Autoxidation of redox active metals such as Fe^{2+} or Cu^+ result in $O_2^{\cdot-}$ formation and subsequently in H_2O_2 and $OH^{\cdot}$ production via Fenton-type reactions. Cellular injury by this type of mechanism is well-documented for iron (Halliwell and Gutteridge 1986) copper (Li and Trush 1993b) as well as other metals (Shi *et al*. 1993). To assess whether the observed decreases in antioxidative defences would be sufficient to explain H_2O_2 accumulation, quantitative estimates of antioxidative capacity are necessary. In fact, accumulation of H_2O_2 has been observed in Cd-expressed roots (Schützendübel *et al*. 2001) and in Cd-expressed tobacco suspension cultures (Piqueras *et al*. 1999). It was suggested that Cd triggered an oxidative burst because it detected H_2O_2 in the culture medium (Piqueras *et al*. 1999). Schützendübel and Polle (2002) suggested that a significant intracellular H_2O_2 accumulation could be explained after Cd exposure because of the Cd-induced depletion of GSH and inhibition of antioxidative enzymes. In pine roots, H_2O_2 was detected at an early stage (6 h after the addition of Cd) when the roots still appeared visibly fully viable and no lipid peroxidation was found (Schützendübel *et al*. 2001). Moreover, H_2O_2 disappeared within some hours but thereafter the differentiation of protoxylem elements became apparent in unusual places of the previous elongation zone of root tips (Schützendübel *et al*. 2001). Therefore, it may be suggested that heavy metals induced a transient loss in antioxidative capacity, perhaps accompanied by a stimulation of oxidant-producing enzymes, which results in intrinsic molecules that trigger secondary defences.

Redox signals can then be transmitted via H_2O_2 locally in the apoplast or via long distance through the vascular system where this oxidant has a longer life time than in antioxidant-rich compartments or tissues (Foyer 2005). Other evidence also points to an important role for the generation of H_2O_2 during photosynthesis in chloroplasts and through photorespiration in PCD (Mateo *et al*. 2004). During acclimation, H_2O_2 levels could be a signal that induces antioxidant enzymes, such as CAT, that subsequently protect the plant from excess H_2O_2 production at chilling temperature (Inzé and Montagu 1995). The H_2O_2 produced from other oxidative bursts functions as a local trigger of PCD of challenged cells and causes the rapid cross-linking of cell wall proteins (Bradly *et al*. 1992; Prasad *et al*. 1994). Furthermore, H_2O_2 appears to act as a signal molecule, inducing transcription of defence genes, such as GST and GPX in surrounding cells (Bradly *et al*. 1992).

On the other hand, NO plays a crucial role in regulating plant growth and development, including germination, flowering, fruit ripening and organ senescence (Arasimowicz and Floryszak-wieczorek 2007). In plant mitochondria, electron transfer along the respiration chain is coupled to the formation of ATP (Maxwell *et al*. 1999; Affourtit *et al*. 2001), and the redundant electron leads to the formation of ROS if the ATP synthesis were to be blocked (Petrussa *et al*. 2008).

Mittova *et al*. (2003) reported that salt stress increased the content of H_2O_2 and MDA in tomato mitochondria, and was probably due to a salt-induced increase in the rate of $O_2^{\cdot-}$ production (Moller 2001). In addition, Zheng *et al*. (2009) observed that exogenous NO treatment significantly decreased MDA and H_2O_2 contents and the $O_2^{\cdot-}$ production rate in wheat seed mitochondria during germination under salt stress. Moreover, earlier studies demonstrated that exogenous NO protects leaves against oxidative damage in reed (*Phragmites australis*. L) under heat (Song *et al*. 2006) and in wheat under drought (Tian and Lei 2006), ascribed mainly to the increased activities of SOD, CAT and POD. In accordance with their findings, Zheng *et al*. (2009) observed that the activities of SOD and CAT in the seed mitochondria were significantly increased by exogenous NO treatment in winter wheat, which might have contributed to alleviating oxidative stress in the mitochondria of germinating wheat seed and thereby improving germination under salt stress.

In healthy plants, nitrite is rapidly converted to ammonium by nitrite reductase (NiR) in the chloroplast. Translocation of nitrite from the cytosol into the chloroplast requires a ΔpH across the envelope (chloroplast/stromal membrane) and the activity of the photosynthetic active transport mechanism at the thylakoid membranes. Therefore, nitrite accumulation followed by NO production could be detected when the redox potential generated by the chloroplast is altered. There are reports that show high NO emissions from plants when in the dark (Yamasaki and Sakihama 2000) or when treated with photosynthetic electron transport inhibitors (Huber *et al*. 1996; Yamasaki and Sakihama 2000). In this sense, two facts must be considered when discussing the apparent contradiction between a high diurnal NR activity and a nocturnal stomatal closure. High and transient NO production was detected in sunflower, tobacco and spinach leaves when the light was switch off

(Rockel *et al*. 2002) and when epidermal *Vicia fava* strips (Neill *et al*. 2002a, 2002b) were placed in the dark, stomatal closure could be partially prevented by treating them with a specific NO scavenger (2-phenyl-4,4,5,5-tetramethylimidazoline-l-oxyl-3-oxide (PTIO) (Garcia Mata and Lamattina 2001). These results also support the idea that NO has an role during nocturnal induction of stomatal closure, in addition to NO-mediated stomatal closure in ABA treated epidermal strips.

Potential sources of H_2O_2 production in the cell include NADPH oxidase, cell wall peroxidases, amine oxidases, Oxalate oxidase, flavin-containing oxidases, Type III oxidases and misfires (photorespiration and Mehler reaction) in the electron transport chains of chloroplasts and mitochondria (Mehler reaction) (Halliwell and Gutteridge 1999; Bolwell *et al*. 2002). Broadly, these events are enhanced by both biotic and abiotic stresses (Alscher *et al*. 1997; Bolwell 1999), although they occur as an integral part of many facets of plant development.

Energy imbalances

ROS metabolism necessarily links with all aspects of plant life. The effect of oxidative stress on photosynthesis is a major threat at any time but especially when the capacity for electron transport chain (ETS) exceeds the capacity for recycling NADPH and ATP. This is reduced by the presence of the xanthophyll cycle (Demming-Adams *et al*. 1999), but its effectiveness is not satisfactory. In this case, the water-water cycle and the Mehler-ascorbate peroxidase cycle prevent this damaging effect. Both of these cycles are important in field conditions (Logan *et al*. 1998a). The generation of toxic O_2 species is increased under abiotic stress conditions. Ascorbate is present in the chloroplast, cytosol, vacuole and apoplastic space of leaf cells at high concentrations (Foyer *et al*. 1991; Polle *et al*. 1990). Oxidation of ascorbate occurs in two sequential steps, first by producing mono-dehydroascorbate, and if not rapidly re-reduced to ascorbate, the mono-dehydroascorbate dispropionate to ascorbate and dehydroascorbate. The regulation of ascorbate within the chloroplast provides a putative mechanism for the regulation of electron transport (Neubauer and Schreiber 1989). Ascorbate is not only a potent antioxidant, but is implicated in the pH-mediated modulation of PS-II activity and its down-regulation associated with zeaxanthin formation (Neubauer and Yamamoto 1992). This is a potent mechanism for preventing damage that occurs by generation of ROS under the influence of abiotic stress. Moreover, this cycle helps to generate the low luman pH values removed for the formation of zeaxanthin (Hager 1969; Pfundel and Dilley 1993), involved in the mechanism of thermal energy dissipation in the field (Demming-Adams 1992). In addition, in the chloroplast, SOD and ascorbate peroxidase exist in both soluble and thylakoid-bound forms. Superoxide generated at the membrane surface can thus be trapped and converted immediately to H_2O_2 to be scavenged by the membrane-bound ascorbate peroxidase (Neubauer and Schreiber 1989; Nakano and Asada 1980; Anderson *et al*. 1983). Moreover, isolated intact chloroplasts rapidly metabolize exogenously added H_2O_2 (Nakano and Asada 1980; Anderson *et al*. 1983; Neubauer and Schreiber 1989), indicating that *in situ* the chloroplasts may eliminate H_2O_2 generated both internally and externally.

In plant systems, abiotic stress results from the presence of elevated levels of oxidizing agents that are able to abstract electrons from essential organic molecules and disturb cellular functions. The most commonly encountered oxidising molecules in the cell are ROS that derive from the abundantly available and rather inert dioxygen (O_2) (Elstner 1999). Direct transfer of one electron to O_2 produces the $O_2^{\cdot-}$ that subsequently acts as an oxidant in single electron transfer reactions to form of H_2O_2, $OH^{\cdot}$ and finally H_2O. Due to its exceedingly high reacting ability, $OH^{\cdot}$ will react within diffusion distance with any organic molecule and is unlikely to serve as a signaling molecule whereas such a function is assumed for $O_2^{\cdot-}$, H_2O_2, O_2 as well as reactive nitrogen species such as peroxynitrite ($ONOO^-$) (Neill *et al*. 2003). In this cycle, because SOD dismutates $O_2^{\cdot-}$ to H_2O_2, little doubt exists that the overproduction of a H_2O_2-scavenging enzyme alongside SOD will have a cooperatively protective effect on oxidative stress-induced damage. Further work is required to test which combination of SOD and H_2O_2-scavenging enzymes provides the best protection.

Moreover, intracellular ROS is scavenged in both a highly efficient and adaptable H_2O_2-related stress or its prevention, resulting from activity in the chloroplasts, mitochondria or other organelles, reflecting the integration of all cellular activities. In mitochondria, alternative oxidase (AOX) provides an additional way of reducing ROS production which is too often overlooked in stress response studies (Wagner 1995; Popov *et al*. 1997). AOX is induced by a number of stresses (Farrar and Rayan 1987; Xie and Chen 1999; Popov *et al*. 2001; Robson and Vanlerberghe 2002; Fiorani *et al*. 2005; Hanquing *et al*. 2010). Antisense suppression of AOX also leads to significantly higher ROS production while AOX over-expression has the opposite effect (Parsons *et al*. 1999). The decrease of ROS accumulation could alleviate oxidative damage and enhance the adaptive capacity for plants.

Substrate oxidase

Glycolate oxidase of peroxidase (1.1.3.1) and urate oxidase (1.7.3.3) in glyoxysomes, are flavin-containing enzymes directly producing H_2O_2 (del Río *et al*. 1992; Hanna *et al*. 2009). In addition, another H_2O_2 producing sulphite oxidase has also been identified in peroxisome (Hansch *et al*. 2006). Other flavin containing oxidases important in specific compartments or tissues include a variety of monoamine (MAO) and polyamine (PAO) oxidases and germin-like proteins. Production of polyamines under water or low temperature stress has also been correlated with protection against oxidative stress (Nayyar and Chandler 2004). Similarly, diamine oxidases (DAO) using putrescine and cadaverine or spermidine in the apoplast as their substrates, are also important in H_2O_2 production for identification, as well as being induced in response to HM stress and in wounding and in cells destined for PCD (Langebartels *et al*. 2002). However, amine oxidase is also constitutively present in the apoplast (Liu *et al*. 1995) and different enzymes (e.g. polyamine oxidases) show different patterns of constitutive and abiotic stress (e.g. HMs) induced expression (Asthir *et al*. 2004). In *Mesembryanthemum crystallinum*, HM shock activated both diamine oxidase and guaiacol peroxidase, as did exogenous cadaverine (Shevyakova *et al*. 2006).

Peroxidases (Type III)

They are more frequently secreted into the apoplast and involved in phenolic metabolism using H_2O_2 as a substrate. They are largely intracellular (in contrast to type I and II, type I has rich diversity) and are involved in control of the cellular H_2O_2 level (Veitch 2004). In addition, they can generate H_2O_2 with oxidation of NADH (Sukalovic *et al*. 2005; Cheeseman 2007; Bonifacio *et al*. 2011). They are also important in the responses of plants to abiotic stress. The complexity of plant responses and H_2O_2 metabolism is especially clear with respect to apoplastic concentration of HMs intimately associated with this in a signaling cascade (Davies *et al*. 2006). In bean (*phaseolus bulgaris*) for example, Malusa *et al.* (2002) reported that induction of oxidative stress, lipid-per oxidation and an increase in phenolic production reflecting a redirection of carbon metabolism (Cakmak 1994).

NADPH oxidases

All plant NADPH oxidases are membrane proteins that oxidize NADPH at the cytosolic surface of the plasma membrane, and reduce O_2 to $O_2^{\cdot-}$ at the outer surface (Sagi

and Fluhr 2006; Wong *et al*. 2007; Miller *et al*. 2009; Marino *et al*. 2012). They were first identified spontaneously from cauliflower (*Brassica oleracea*) (Askerlund *et al*. 1987), and the activity was attributed to a membrane-bound peroxidase. However, some enzymes with these characteristics were found to lack flavin cofactor, suggesting that they were mechanistically different from the mammalian enzymes (Murphy *et al*. 2000). Plant homologs to neutrophyll NADPH oxidase make up a gene family identified as respiratory burst oxidase homologs (RHOB) of which there are 10 members in *Arabidopsis*. All have significant similarity to one subunit of the neutrophyll enzyme (Keller *et al*. 1998), but plant transcripts are larger and have a hydrophilic N-terminal domain with binding sites suggesting Ca and proteins stimulation of $O_2^{\cdot-}$ production. The activity of RHOB proteins as integrates agents between ROS production are plant responses to stress is suggested by evidence linking them to Ca-dependent signaling associated with such diverse activities as root hair growth (Carol and Dolan 2006), ABA-induced Ca-channel activation in guard cells (Desikan *et al*. 2006) and activation of a MAPK cascade (Mittler *et al*. 2004; Zhang *et al*. 2006). In some cases, NADPH oxidase has been implicated in response to abiotic stresses like HM stress.

H_2O_2 and NO signaling

In addition, the peroxisome is a small cell organelle, is an important source of ROS as well as antioxidants and NO, and is thus an important regulator of the cellular redox state. Induction of peroxisome biogenesis genes by various abiotic stresses like HM action and mechanical injury (which 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 abiotic stresses. Plants pre-treated with low concentrations of H_2O_2 have shown significantly greater tolerance to abiotic stress than untreated controls (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. In yeast, a glutathione peroxidase, GPX3, can function as a receptor for H_2O_2 and a transducer of redox signals, to activate gene expression (Delaunay *et al*. 2002). In addition, the fact that oxidative stress is a common facet of many cellular stress responses means that elucidating those intracellular signaling processes mediating H_2O_2 signaling is of potential significance to any programme aimed at improving crop tolerance of abiotic stresses like HM stress, etc.

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 APX in germinating rice embryos (Morita *et al*. 1999) and in *Arabidopsis* leaves (Karpinski *et al*. 1999).

There are several enzymatic systems that have been shown to produce NO, mainly NR (Rockel *et al*. 2002) and L-Arg-dependent nitric oxide synthase (NOS) (Corpas *et al*. 2004). However, the gene for plant NOS has not been identified yet (Zemojtel *et al*. 2006; Neill *et al*. 2008). Confocal laser scanning microscopy (CLSM) has been widely used to study fluorescent probe distribution in fixed and living plant tissues (Sandalio *et al*. 2008). To imagine H_2O_2 and NO accumulation in leaves from pea plants treated with Cd, specific fluorescent probes were used. Cross-sections of control and Cd-treated leaves incubated with DCF-DA (2′,7′-dichlorofluorescin diacetate) showed a very bright green fluorescent due to peroxides, mainly to H_2O_2, in xylem vessels from vascular tissues and epidermis. However, in Cd-treated leaves, the fluorescence increased considerably in palisade mesophyll cells and to a lesser extent, in sclerenchyma cells. The analysis of H_2O_2 by cytochemistry with $CeCl_3$ and electron microscopy showed that in xylem vessels from control plants, the H_2O_2-dependent precipitates were derived DAF-2DA (2,7-dichloro-fluorescein diacetate) green fluorescence was found in xylem vessels, schlerenchyma and epidermal cells of control plants; however, in contrast with H_2O_2 generation, Cd treatment produced a significant reduction of NO-dependent fluorescence observed in control leaves. As a putative control Cd-treated pea plants were incubated with 10 μM SNP (NO donor), and the NO-dependent fluorescence was observed by CLSM. The constitutive L-Arg-dependent NOS activity previously described in pea plants is dependent on Ca and CaM (Corpas *et al*. 2004). The reduction of constitutive NO production by Cd was reversed by supplying Ca levels of NO to those observed in control leaves.

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). RuBisCO (ribulose-bi-phosphate carboxylase oxygenase, a photosynthetic enzyme) 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). 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 (Neil *et al*. 2002; Guo *et al*. 2003). Results of Verma *et al.* (2011) highlight the powerful selective de-lignifying capability of the H_2O_2-activated ammonium molybdate system energized by microwave radiation. Ammonium molybdate pre-treatment effects are due to transformation to a peroxometal complex on reacting with H_2O_2.

Moreover, 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 of rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) (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). H_2O_2 participate in implication of ROS-mediated control of the K^+ channel resulting in mineral nutrient partitioning within the plants by identifying a critical target Cys (168) to be essential for sensitivity of H_2O_2 (Garcia-Mata *et al*. 2010). Also, 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). Babosha and Komarova (2010) investigated on a background of modelled oxidative outbreak caused by the stress from exogenous H_2O_2. Exogenous zeatin inhibited growth in different treatments with H_2O_2 bringing about both acceleration and inhibition of root and hypocotyl growth. The involvement of H_2O_2 and NO 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.

ROLE OF CA^{2+}, ABA IN H_2O_2 AND NO SIGNALING AGAINST HEAVY METAL STRESS

Our knowledge about the scope of stress-induced signaling in plants leading to modification of gene expression has increased in recent years. A number of signaling molecules have been demonstrated to be involved in the stress signaling pathway. A myriad array of evidences suggests that

H_2O_2 serves as a messenger to pass the stress signal to downstream responses in plants. However, our present understanding about the stress signaling in plants is that the changes of GSH/GSSG, following the synthesis of H_2O_2 constitute an early stress signal leading to the physiological response, usually activation of antioxidant mechanisms or medication of gene expression (Trewavas and Malhó 1997; Hung *et al*. 2005; Usha *et al*. 2009; Xiong *et al*. 2012). Using cDNA microarray technology to carry out a transcription analysis, Desikan *et al.* (2001a) provided further evidence of H_2O_2 as a central signaling player. Numerous studies show that the expression of some genes is up-regulated by H_2O_2. In addition, some are repressed and classified by their potential biological functions. These genes include heat shock proteins, heat shock transcription factors, mediators for calcium signaling and enzyme proteins tyrosine phosphatises (PTPs), a blue Cu-binding protein which is a signal catalyst for redox reactions mitochondrial uncoupling proteins, pyruvate dehydrogenase and a myb-related transcription factors. In addition, some of the H_2O_2-sensitive genes could also be involved in plant hormone signaling. It is interesting to note that various genes encoding potential transcription factors were induced by H_2O_2 suggesting that these transcription factors mediate further downstream H_2O_2 responses and finally induce physiological changes to promote adaptation to stresses.

More line of evidence concerning the relationship between H_2O_2, NO and Ca^{2+} signals were provided by a study of H_2O_2 homeostasis in *Arabidopsis* (Yang and Poovaiah 2002) in which a close interaction between intracellular H_2O_2 and cytosolic Ca^{2+} was found in response to abiotic stress, indicating that an increase in cytosolic Ca^{2+} boosted the generation of H_2O_2. These findings suggested that Ca^{2+} influx plays a major role to HM response induce signaling and also that an intracellular Ca^{2+} source might be involved. Further evidence indicated that H_2O_2 and Ca^{2+} are both involves in a signaling cascade leading to the closure of stomata in *Arabidopsis* (Pei *et al*. 2000). In this study, H_2O_2-activated Ca^{2+}-channel mediated both the influx of Ca^{2+} in protoplasts and increases in $[Ca^{2-}{}_{cyt}]$ in intact guard cells (Pei *et al*. 2000). In addition to serving as a link in a signaling cascade, the fluctuation of $[Ca^{2-}{}_{cyt}]$ could be one of the mechanisms that lead plants to remember what they have suffered (Knight *et al*. 1996). The treatment with H_2O_2 could activate Ca^{2+} channels to elevate $[Ca^{2-}{}_{cyt}]$ level (Pei *et al*. 2000). For a certain period of time, this could be autocatalytic because H_2O_2 production in the oxidative burst requires a continuous Ca^{2+} influxes which activate the PM

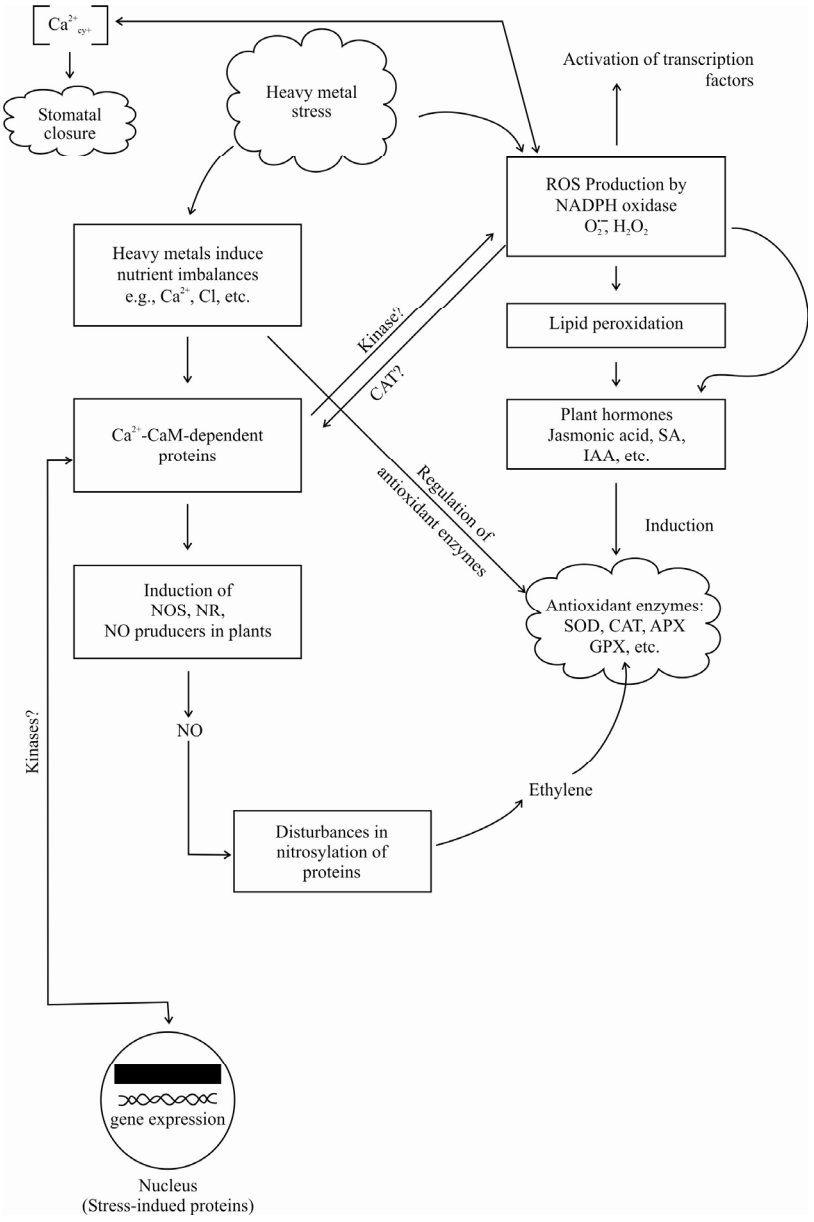

Fig. 2 A generalized model for H_2O_2- and NO-mediated signaling pathway in plant tolerance against heavy metal stress. APX, Ascorbate peroxidase; **CAT**, Catalase; **GPX**, glutathione peroxidise; **H_2O_2**, hydrogen peroxide; **IAA**, indole-3-acetic acid; **NR**, Nitrate reductase; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase.

Table 1 Production of H_2O_2 induced by heavy metal stress in plants.

Heavy metal	Reference
Cd	Cho and Seo 2005
Cd	Schutzendubel *et al*. 2001; Chao *et al*. 2008; Liu *et al*. 2011
Cu	Drazkiewicz *et al*. 2004
Cd, Cu	Maksymice and Krupa 2006b
Hg	Cho and Park 2000; Shiyab *et al*. 2009
Mn	Demirerska-Kepova *et al*. 2004

located NADPH oxidase (PM-NADPH) (Lamb and Dixon 1997; Yang and Pooviah 2002). Later, the [$Ca^{2-}{}_{cyt}$] elevation activates the calcium sensor, calmodulin and subsequently passes the signal to a down-stream target, CAT. This finally down-regulates H_2O_2 levels by stimulating the plant CAT activity. Together, these results provide evidence indicating that Ca^{2+} has dual functions in regulating H_2O_2 homeostasis, which in turn, influences redox signaling in response to environmental stress signal in plants (**Fig. 2; Table 1**).

Moreover, H_2O_2 modulates the expression of various genes, including those encoding antioxidant enzymes and modulators of H_2O_2 production (Neill *et al*. 2002a, 2002b). The H_2O_2-induced transcripts encoded proteins (genes) with functions such as metabolism, energy, proteins destination and transport, cellular organization and biogenesis, cell rescue or defence and transcription (Desikan *et al*. 2001a). Among these genes, the genes encoding potential transcription factors should be emphasized due to their capacity for activating the expression of downstream target genes. In Arabidopsis, a transcription factors CBF1 (C-repeat binding factor) binding to C-repeat and dehydration-responsive element (CRT/DRE), was first identified by Stockinger *et al.* (1997). CBF1 belongs to the APETALAA2/EREBP family of transcription factors. According to results of Hung *et al.* (2005), CBF seems to be able to indirectly regulate the cytosolic redox status by activating downstream genes encoding antioxidant enzymes for example, CAT. Nevertheless, there are still is no evidence connecting the H_2O_2 signal with CBF accumulation or activation. The strongest evidence showing H_2O_2 it activates transcription factors is provided by the study on yeast transcription factors YAP1 (Coleman *et al*. 1999). However, till date, the direct influence of H_2O_2 on transcription factor activity is far from clarity.

Previous studies showed that ABA-induced NO production is dependent on H_2O_2 generation in *Arabidopsis* guard cells (Bright *et al*. 2006) and in maize (*Zea mays* L.) mesophyll cells (Zhang *et al*. 2007). However, how H_2O_2 regulates NO production in ABA signaling is not clear. It has been shown that ABA-induced H_2O_2 can induce increase in the concentration of Ca^{+2} and the expression of CaM1 and the content of CaM in leaves of maize plants (Hu *et al*. 2007), and that Ca^{+2} is required for H_2O_2-induced NO production in guard cells of mung bean (Lum *et al*. 2002). The Ca^{+2}-dependent NO production was also observed in tobacco (*Nicotiana tabacum* L.) cells challenged by the elicitor crytogein (Lamotte *et al*. 2004) and in the endopoly-galacturonase1 (BcPG1) elicited grapevine (*Vitis vinifera* L.) cells (Vandelle *et al*. 2006). In addition, the results of Sang (2008) provide evidence that both Ca^{+2} and CaM are involved in ABA and H_2O_2 induced NO production in leaves of maize plants and also their data showed that ABA and H_2O_2 induced increase in the generation of NO was substantially blocked by pre-treatments with the Ca^{+2} chelator EGTA, the Ca^{+2} channel blockers $LaCl_3$ and verapamil and the CaM antagonists TFP and W7. However, pre-treatment with W5, an inactive structural analogue of W7 had very little effect on the ABA and H_2O_2 induced NO production in mesophyll cells of maize leaves. Moreover, exogenous Ca^{+2} also induced an increase in the production of NO and the increase is presented by pre-treatments with the CaM antagonists TFP and W7 (**Fig. 2**).

In plants, pharmacological, biochemical, physiological and immunological evidence indicates the presence of NOS-like activity similar to a certain extent to mammalian NOS (Corpas *et al*. 2004; Liu *et al*. 2007), although no plant NOS similar to the mammalian one has been characterized (Crawford *et al*. 2006; Neill 2007). It has been shown that NOS is localized in the cytoplasm, chloroplasts, mitochondria peroxisomes and nucleus in plant cells (Liu *et al*. 2007). The occurrence of NOS activity in pea leaf peroxisome was demonstrated by biochemical methods and electron paramagnetic resonance (ESP) spectroscopy (Corpas *et al*. 2004). In previous studies, however, it was reported that NR was the main source of NO production in response to ABA and H_2O_2 in Arabidopsis guard cells (Bright *et al*. 2006). The results described above suggest that there are different sources of NO in response to ABA and H_2O_2 in guard cells and mesophyll cells. However, NR does not exclude NR as a source and could also participate in the ABA and H_2O_2 induced NO production in the mesophyll cells of maize leaves. Therefore, it is clear from all results taken together that Ca^{+2}-CaM factors both upstream and downstream of NO production in ABA and H_2O_2 signaling. Recent studies suggests that ABA-induced H_2O_2 production mediates NO generation, which in turn, activates a MAPK and results in the up-regulated expression and activities of antioxidant enzymes in ABA signaling in leaves of maize plants (Zhang *et al*. 2007).

In addition, studies have shown that NADPH oxidase, H_2O_2, NO, Ca^{2+}-calmodulin (CaM) and MAPK are required for ABA-induced up-regulation in the activities of antioxidant enzymes such as SOD, CAT, APX and GR in leaves of maize plants (Zhang *et al*. 2007). It has been shown that ABA-induced H_2O_2 production mediates NO generation, which, in turn, activates MAPK and results in the up regulation in the expression and the activities of antioxidant enzymes in ABA signaling (Zhang *et al*. 2007). Ca-CaM has been also shown to be necessary for ABA-induced antioxidant defense and function both upstream and downstream of H_2O_2 production in leaves of maize plants (Hu *et al*. 2007). However, whether Ca^{2+}-CaM is involved in NO induced antioxidant defense in plants and if so what the relationship between Ca^{2+}-CaM and NO in the H_2O_2 signaling is remains to be determined (**Table 2**).

Moreover, Sang *et al.* (2008) results suggests that Ca^{2+}-CaM functions both upstream and downstream of NO production, which is mainly from NOS in ABA and H_2O_2 induced antioxidant defense in leaves of maize plants. An increasing body of evidence indicates that NO which was first identified as a unique diffusible molecular messenger in animals, plays important roles in various physiological processes in plants including defences responses and PCD, hormones responses, abiotic stress, root and xylem differentiation and development, germination, iron homoeostasis and flowering (Neill *et al*. 2003; Crawford 2006). There are several potential sources of NO including NOs-like enzyme, NR and non-enzymatic sources (Crawford 2006). In case of animals, NO is formed exclusively by the enzyme NOS (nitric oxide synthase), catalyses the conversion of L-arginine in to L-citrulline and NO in the presence of molecular oxygen (Crawford 2006). Although no plant NOS similar to the mammalian one has been identified (Crawford *et al*. 2006; Neill 2007), several lines of evidence have demonstrated the presence of NOS-like activity in pea leaf peroxisomes has been biochemically characterized which is strictly dependent on L-arginine, NADPH, tetrahydrobiopterin (BH4) and CaM and requires Ca^{+2} (Corpas *et al*. 2004). Similarly, several studies showed that pre-treatment with NG-nitro-L-Arg methyl ester (L-NAME), a well known competitive inhibitor of mammalian NOS, produced ABA-induced increases in the production of NO in pea guard cells (Neill *et al*. 2002a, 2002b) and maize mesophyll cells (Zhang *et al*. 2007), suggesting that NOS is involved in ABA and H_2O_2 signaling. However, more evidence is required for the involvement of NOS in H_2O_2-induced antioxidant defense against HMs in plants (**Fig. 1**).

Furthermore, results of Sang *et al.* (2008) showed that

Table 2 Stress-related genes modulated by NO.

Plant system	Treatment	Gene expression	Reference
Nicotiana tabaccum cell suspension cultures	Cryptogein	Cryptogein-induced and NO-dependent expression of ACC synthase and SHSP	Lamotte *et al*. 2004
NO and other kinds of oxidative stress, *Arabidopsis thaliana*-Col-O-plants: AFLP analysis	SNP infiltrated in leaves	2500 cDNA	Polverari *et al*. 2003
Arabidosis thaliana Col-O-plants microarray analysis	SNP applied via the roots	2400 genes	Parani 2004
Glycine max cell suspension cultures	SNP	Pr-1; Pal; CHS	Delldonne *et al*. 1998
Arabidosis thaliana Col-O-plants: WT mutants overexpression at APx	SNP	HP only in the absence of SA	Murgia *et al*. 2004b
Arabidosis thaliana	SNP	HY5 and MYB1	Tossi *et al*. 2011
Arabidosis thaliana	SNP	Atnoa1/rif1	Ahlfors *et al*. 2009
Kosteletzkya virginica	SNP	Catalase and peroxidase	Guo *et al*. 2009

SNP, sodium nitropruside; **NO**, nitric oxide; **APx**, ascorbate peroxidise; **SHSP**, small heat shock protein; **ACC**, 1-aminocyclopropane-1-carboxylate; **cDNA**, complementary deoxyribonucleic acid

Ca^{+2}-CaM is required for ABA-H_2O_2 induced increase in the production of NO and the activity of NOS in leaves of maize plants, and the increase the production of NO are mainly from NOS and their data also suggest that there are a cross-talk exists between Ca^{+2}-CaM and NO in H_2O_2 signaling and HM stress as well as CaM is necessary for the generation of NO and the activity of NOS induced by exogenous Ca^{+2} in leaves of maize plants. Also, studies showed that both ABA and H_2O_2 induce NO generation in maize mesophyll cells (Zhang *et al*. 2007). To investigate whether Ca^{+2}-CaM is involved in H_2O_2 induced growth of NO, the Ca^{+2}-chelator, ethylene glycol-bis (2-amino ethyl ether)-*N*,*N*,*N*,*N*-tetra acetic acid (EGTA), the Ca^{+2} channel blockers $LaCl_3$ and verapamil, and the CaM antagonists TFP and W7 were used. Pre-treatment with these inhibitors significantly reduced the increase in the production of NO induced by ABA or H_2O_2 in mesophyll cells of maize leaves but these pre-treatment's alone did not affect the production of NO in the untreated leaves.

ABIOTIC STRESS: H_2O_2 AND ANTIOXIDANT SYSTEM

Plants constantly monitor their surroundings and make appropriate metabolic, structural and physiological adjustments to accommodate environmental changes. Within frame work of genetic background, plant productivity is dependent on this constant adjustment of gene expression in response to environmental cues. The genome-environment interaction is an essential focus for the elucidation of the nature of the phenotypic variation leading to successful stress tolerance responses (Pastori and Foyer 2002). Moreover, this interaction is also a key determinant to plant tissue composition related to crop quality factors, as well as plant anatomy, morphology and development. Plant integrates a diverse range of environmental and metabolic signals via a network of interacting signal transduction pathways that together regulate gene expression during stress (Tuteja and Sopory 2008). Plants make use of an interacting network of common pathway and components to optimize the stress tolerance responses called cross tolerance. A common signaling system involving hormones, oxidant and antioxidants has evolved to provide adequate defense and protection against hazardous amounts like HMs (Maksymiec 2007).

Also, plants are autotrophic organisms powered by photosynthesis. It is therefore no surprise that redox signals from the light reactions of photosynthesis initiate profound changes in gene function. It is now widely accepted that redox signal exerts control on nearly every aspect of plant biology from chemistry to development, growth and eventual death (Foyer and Noctor 2003). It is most probable that redox signaling was the first type of sensory regulation that evolved in nature, since it prevented uncontrolled changes in energy production, utilization, and exchange. The system in the chloroplast that sense redox changes and control redox homeostasis has been intensively studied; these include post-translational modification of assimilatory reactions, and control of gene transcription and translation. Moreover, redox controls and signals also ensure that the expression of plastid encoded photosynthetic proteins is precisely co-ordinated with that of nuclear encoded chloroplast components (Surpin *et al*. 2002). However, about two billion years ago, molecular oxygen became intimately involved with the essential energy exchange reactions on which life based (photosynthesis and respiration), allowing use of the very high electrochemical potential of the O_2/H_2O_2 redox couple. However, many processes in plants catalyses only partial reduction of oxygen, and so generate ROS as $O_2^{\cdot-}$ and H_2O_2.

Abiotic stresses disrupt the cellular redox homeostasis which leads to the oxidative stress or generation of ROS (Asada 2006). It is now well established that virtually all 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; Kopyra and Gwozdz 2004). ROS play a vital role in intracellular redox signaling, activating antioxidant resistance mechanisms. Thus, it is a surviving response for plants to control the concentration of ROS. Moreover, a plethora of evidence indicates that ROS are involved in the DNA damage, cell death and signal transduction. ROS generated under HM stress especially $OH^{\cdot}$, $^1O_2$, H_2O_2 react with sugars, purines and pyrimidines. However, no direct evidence is observed in study of He and Hader (2002) in respect to lipid peroxidaton and DNA damage.

As already stated, O_2^1 is an extremely reactive molecule, which can cause extensive damage to proteins and membranes (Gill and Tuteja 2010). Similarly, H_2O_2 participates widely in plant metabolism it can be converted via Fenton-type reactions to the $OH^{\cdot}$. This extremely toxic radical can initiate LPX, cause protein degradation or modification, and mediate DNA damage. In addition to formation through processes intrinsic to photosynthesis, $O_2^{\cdot-}$ and/or H_2O_2 are generated at significant rates by photorespiration, oxidative phosphorylation, fatty acid oxidation and also by many types of oxidative activity (Dutilleul *et al*. 2003). Despite the fact that the generation of $O_2^{\cdot-}$ and H_2O_2 by the plant mitochondrial ETS is well known, the production of these ROS by leaf mitochondria had until recently, been considered to be of only secondary importance. However, several recent reports have suggested that, in plants as in animals, mitochondria are involved in the tolerance to oxidative stresses. $O_2^{\cdot-}$ and H_2O_2 are produced as primary signals in the stress signal cascade (Mittler *et al*. 2004).

Moreover, they are also produced as second messengers during growth, tropic and movement responses as results of hormone action. Rapid components-specific differences in redox state can be of achieved either by modifying the rates of $O_2^{\cdot-}$ and H_2O_2 production or by repression or activation of the antioxidants defence or both. For example, H_2O_2 accumulation in the apoplast, which is important in defence and growth responses, is controlled by regulation of the activity of enzymes that generate H_2O_2 potential NADPH

Table 3 Sources of heavy metal toxicity in soil (Lone *et al*. 2008; Yadav 2010; Karimi *et al*. 2011).

Heavy metal	Sources
Cd	Geogenic sources, metal smelting and refining, fossil fuel, sewage sludge and application of phosphate fertilizers
Ni	Land fill, forest fire, bubble bursting, gas exchange, weathering of soils and geological materials
Pb	Mining and smelting of metalliferous ores, burning of loaded gasoline and municipal sewage
Hg	Forest fire, coal and wood burning
Se	Coal burning, oil refining, combustion of fossil fuels, chemical synthesis (e.g. pigment formulation)
Cu	Smelting, refining and biosolids
Cr	Sludge, solid waste and tanneries
Zn	Electroplating industry, smelting and biosolids

oxidases (Cheeseman 2007). This allows very precise control of the generation of H_2O_2 either in localized microenvironments or in more global bursts. Much remains to be resolved concerning the components of the H_2O_2-induced signaling cascade and the mechanisms by which information on redox status is used to modify gene expression (Pastori and Foyer 2002). H_2O_2-responsive elements are present in the promoters of a number of plants genes. One of the most important developments in the next few years will be the elucidation of transcription factors that are involved in the H_2O_2 signaling cascade. This will important as a signal transcription factor can orchestrate the expression of many genes to improve tolerance against oxidative stress especially induced by HMs (Marceau 2004).

Moreover, mitogen-activated protein kinases (MAPKs) have been found to modulate protein phosphorylation as a result of H_2O_2 signaling. H_2O_2 activates MAPKs, which repress auxin-demonstrated the presence of intensive metabolites and hormone cross-talk between oxidative stress tolerance and plant growth responses. Overall, plants are much more tolerant to H_2O_2 than animals, and their antioxidant systems appear cellular redox state rather than to facilitate the complete elimination of H_2O_2 (Foyer and Noctor 2003). Together with a number of antioxidant enzymes, to hydrophilic low molecular weight antioxidant, ascorbate and GSH, determine the life time of the H_2O_2 in plant cells. Therefore, an appropriate intracellular balance between ROS generation and scavenging exists in all cells. This redox homeostasis requires the efficient co-ordination of reactions in different cell components and is governed by complex networks of proteins and antioxidant systems. These enzymes include SOD which catalyze the dismutation of $O_2^{\cdot-}$ to H_2O_2 and O_2. Till date, three classes of SOD have been identified in plants on the basis of their metal cofactors content. FeSOD are found only in chloroplasts, MnSOD are found mainly in mitochondria, while Cu/ZnSOD is located in the chloroplast, cytosol, apoplast and peroxisomes (Dutilleul *et al*. 2003). The main enzymatic H_2O_2 scavenger of photosynthetic cells are CAT converted H_2O_2 to H_2O and O_2, and APX use ascorbate as the electron donor for H_2O_2 reaction. CAT isoforms are distinguished on the basis of organ specificity and responses to environmental stresses. Chloroplasts contain stromal thylakoid-bound, and luminal APX forms. APX reduced ascorbate as an electron donor. At the thylakoid membrane, the oxidized form of MDA is reduced back to ascorbate via reduced ferredoxin, generated as a result of peroxidase cycle, chloroplast contain a second reducing power while producing ATP called ascorbate-glutathione cycle (Asada 2006) (**Fig. 2**).

Furthermore, it is not only important H_2O_2-detoxifying system in the chloroplast but it also serves a similar function in the cytosol, peroxisome and mitochondria. There are a vast array of other antioxidant components that function in synchrony with the Mehler-peroxidase and ascorbate-glutathione cycles to maintain low levels of H_2O_2 and other oxidants in cells. Of particular note are the peroxiredoxins, GPXs and GST that fulfil essential roles in the elimination of lipids and other lipid derived oxidants in cellular membranes (Pelicano and Carney 2004). Together, the antioxidants form a formidable of $O_2^{\cdot-}$ and H_2O_2 in order to minimize the probability of $OH^{\cdot}$ function and consequent oxidative stress. The amount and redox states of ascorbate and GSH determine cellular H_2O_2 concentrations and therefore influence the known roles of H_2O_2 in signal transduction changes in antioxidant redox state and concentration can be much more pronounced than changes in leaf H_2O_2 contents (Noctor *et al*. 2000).

HEAVY METAL STRESS: A CROSS TALK SIGNALING BETWEEN NO AND H_2O_2

The effects of HMs on plants resulted in growth inhibition, structural damage, and decline in physiological and biochemical activities as well as the function of plants (Mazid *et al*. 2010). Also, toxicity causes the blocking of functional groups of important molecules, e.g. enzymes, polynucleotides, transport systems for essential nutrients and ions, displacement and/or substitution of essential ions from cellular sites, denaturation and inactivation of enzymes, and disruption of cell and organelle membrane integrity. Moreover, elevated levels of HMs not only decrease soil microbial activity and crop production, but also threaten human health through the food chain (McLaughlin *et al*. 1999). Plants have a remarkable ability to take up HMs (**Table 3**).

HM 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). In addition, HMs toxicity is considered to induce the production of ROS and may result in significant damage to cellular constituents (Weckx and Clijsters 1997) but, however, phenomenon of ROS formation found to be least explored (Smirnoff 1995). The ROS are strong oxidizing agents that cause oxidative damage to biomolecules such as lipids and proteins and eventually lead to cell death. Growth inhibition and senescence stimulation caused by HMs in excess are intriguing effects, more so, as the knowledge of their mechanisms can have a great significance in eco-physiology and medicine. Plants possess a range of potential cellular mechanisms that may be involved in the detoxification of HMs 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 decrease of activity of the photosynthetic apparatus (Molas 2002; Sobkowiak and Dekert 2003; Alaoui-Sosse *et al*. 2004; Lin *et al*. 2005).

There are a regulated balance between ROS production and destruction is required if metabolic efficiency and function are to be maintained in both optimal and stress conditions (Foyer *et al*. 1994). Mostly contamination of the aquatic environment occurs as a result of human activities and affects organisms at the biochemical, cellular, population and community level. There are two aspects on the interaction of plants and HMs. On the one hand, HM shows negative effects on plants and on other hand, plants have their own resistance mechanisms against toxic effects and for detoxifying HM pollution. These are originally thought to function as osmotic buffers. However, apart from the osmotic adjustment, they also seem to play a key role in maintaining the natural state of macromolecules, probably by scavenging ROS (Xiong *et al*. 2002). The presence of elevated concentrations of HMs in the growth medium of the germinating seeds suppresses mobilization and translo-

cation of reserve materials from the reserve tissue to the growing regions and their subsequent utilization there (Mishra and Choudhuri 1997). There is good evidence that the alleviation of oxidative damage and increased resistance to environmental stresses is often correlated with an efficient antioxidant system (Inzé and Montagu 1995).

Moreover, HMs toxicity can elicit a variety of adoptive responses in plants. Cells are normally protected against free oxy-radicals by the operation of intricate antioxidant systems, comprising both enzymatic systems such as SOD, CAT and APXs and no-enzymic systems, acting as free radical scavengers such as AA and GSH and phenolic compounds (Bowler *et al*. 1992; Foyer *et al*. 1994; Michalak 2006; Gill and Tuteja 2010). The oxidative damage to different cellular components by H_2O_2 could be minimized either by CAT and APX activities or by a reaction sequence known as ascorbate-glutathione cycle involving the redox pairs of ascorbate-dehydroascorbate and glutathione-glutathione disulfide (Foyer *et al*. 1994; Wang *et al*. 2009; Gill and Tuteja 2010). Enzymatic antioxidants such as selenium-dependent GPX, GST, GR and SOD, as well as the concentration of H_2O_2 and MDA, an indicator of LPX determined to identify which antioxidant enzymes participate in the efficient scavenging of ROS generated upon exposure to high doses of Cd^{2+} exposure (Zhang *et al*. 2011).

Maharana *et al.* (2010) stated that lipid peroxidase (LPX) and H_2O_2 are measured as oxidative stress markers while antioxidant defences are measured as CAT, Glutathione-*S*-transferase and ascorbic acid in order to understand their dissimilarity with respect to pollution levels. They also reported that variations of oxidative stress indices in response to accumulation of HMs within *Padina tetrastromatica* commonly found in tropics could be used as molecular biomarkers in assessment and monitoring environmental quality of ecologically sensitive marine habitats. In case of several plants such as *Phytolacca americana* L. responsiveness to Cd stress is unknown but, however, 6-week-old seedlings of *P. americana* exposed to half strength Hoagland solution with 200 or 400 µmol/L $CdCl_2$ for 4 days, the content of H_2O_2 and MDA, and electrolyte leakage increased, while the photosynthetic rate decreased, indicated that the oxidative damage induced by Cd stress in *P. americana* is one of the metal toxicity mechanism. Moreover, the activities of SOD and POD increased rapidly with elevated Cd concentration and exposure time, CAT activity was stable in response to 200 µmol/L $CdCl_2$ stress, and increased only at 3 d later upon 400 µmol/L $CdCl_2$, treatment (Zhang *et al*. 2011). Thus, the enzymatic ant-oxidation capacity played important role in Cd tolerance of hyper accumulator plant. Therefore, much information is available on the effect of redox HMs on various antioxidant processes in plants (Mazhoudi *et al*. 1997; Schützendübel and Polle 2002; Michalak 2006; Shao *et al*. 2008). But, however, the effect of excess concentrations of some metals (e.g. Zn and Ni) on anti-oxidative processes are rare (Weckx and Clijsters 1997), but found to be useful to plant at lower concentration and affect drastically at elevated concentrations. In addition, the symptoms of Zn and Ni toxicity appeared as reduction in seedling growth (Ozdener and Aydin 2010). The growth of the main root is affected much and as a result, it exhibiting the function of fibrous roots.

Since, water pH is an important factor directly affecting the toxicity of metals in algae, for example unicellular *Chlorella* species. It is known that HM toxicity decreases with decreasing pH. Acidity or alkalinity of one medium can, in turn, moderate the toxicity of the HMs (Cu, Pb, Cd or Zn). Lower PH may increase the bio-availability of metal ions resulting in increased toxicity. Although the responses of several antioxidant enzymes, such as CAT, SOD and APX to environmental stress have been studied in some detail (Bowler *et al*. 1992; Allen 1995; Allen *et al*. 1997), little work has been done to show how the enzymes and genes responsible for GSH synthesis respond to metal induced oxidative stress. The ascorbate/glutathione cycle reduces H_2O_2 to water (Foyer and Halliwell 1976; Noctor 1998; Apel and Hirt 2004; Kachout *et al*. 2010). An Important field for further research would be the tolerance mechanisms of plants exhibiting metal hyper accumulation. The knowledge gained in such investigations could facilitate both selection and the breeding of HM tolerant plants. Therefore, there an additional research is necessary to provide further insight concerning the specific relationship between H_2O_2-NO signaling response and expression of antioxidant system under HM stress (Gill and Tuteja 2010).

Moreover, the adverse action of Cu accumulation in tissues of higher plants has been related to interference of Cu with several key physiological processes (Woolhouse 1983; Fernandes and Henriques 1999). However, plant cell membranes are generally considered as primary sites of Cu injury (de Vos *et al*. 1989; Kahle 1993; Srivastava *et al*. 2005; Nouairi *et al*. 2009). Membrane destabilization is frequently attributed to LPX, due to an enhanced production of toxic oxygen species. The Cu effects on plant growth observed under experimental conditions could be associated with the increase of LPX, measured as MDA production. Cu^{+2} ions themselves are able to initiate LPX in plants (Weckx and Clijters 1996). This phenomenon can be also initiated by the Fe-containing enzyme-lipoxygenase (Thompson *et al*. 1987). This membrane-bound enzyme known to oxidize polyunsaturated fatty acids and to produce ROS, might mediate LPX in Cu-treated plants. Studies of Mazhoudi *et al.* (1997) stated that this increase in LPX is an indicator of increased oxidative damage caused by Cu in all plant parts. On other hand, the involvement of a particular group of enzyme is thought to play an important role in the cellular defense strategy against oxidative stress imposed by HMs (Van Assche and Clijsters 1990; Solanki *et al*. 2011).

In addition, there is a decrease of CAT activity in roots of Cu-treated plants (Mazhoudi *et al*. 1997; Dixit *et al*. 2001; Pourakbar *et al*. 2007; Wang *et al*. 2011). Probably, this might be due to direct effects of Cu-treated ROS on the enzyme proteins, as suggested by Luna *et al.* (1994). The HMs induced decrease in CAT activity can also be attributed to inhibition of protein synthesis; as an example, Ni at higher concentration inhibits the synthesis of CAT and other oxidase proteins during rice seed germination (Das *et al*. 1978). However, the inhibition of CAT activity recorded in roots could not be associated with the decrease of soluble protein content, since apparent protein synthesis in leaves severely impaired while CAT activity in these organs is not affected by excess Cu. Van-Assche *et al.* (1986) and Vangronsveld *et al.* (1993) reported that excessive uptake of toxic Cu induces a significant increase of GPX activity and strong qualitative Cu-specific changes in GPX isozyme pattern of bean (*Phaseolus lunatus*) roots.

Moreover, APX activity strongly diminished by Cu probably as a consequence of the inhibitory effect on the enzyme protein, since the soluble protein content was reduced by Cu. However, in contrast to results of Mazhoudi *et al.* (1997), increased CAT and APX activities protect bean leaves from Cu oxidative stress (Weckx and Clijsters 1996; Dixit *et al*. 2001; Gill and Tuteja 2010; Sevengor *et al*. 2011). However, stem GPX showed both quantitative and qualitative changes in Cu-treated plants. This markedly increased level of stem GPX could be considered as a response to great damage provoked by excess Cu. Peroxidases are known to be HM stressed enzymes (Karataglis *et al*. 1991). A key role of peroxidases has been postulated in stiffening of the cell wall (Sanchez *et al*. 1995; Ye *et al*. 2008). Peroxidases-catalysed lignifications decrease the cell wall plasticity and therefore reduce cell elongation which might represent a mechanical adaptation to stress conditions (Castillo 1986). Also, the findings of Mazhoudi *et al.* (1997) stated that toxicity cause oxidative damage in tomato seedlings which indicated by the accumulation of LPX products as well as only root and stem GPX levels, but not CAT and APX, increased in response to stress (**Fig. 2**).

NO also plays the vital role in enhancement of antioxidant enzymes activities and alleviates the toxicity of HMs

(Kopyra and Gwóźdź 2003; Flora *et al*. 2008; Brahim and Mohamed 2011). Exogenous application of SNP (No donor) reduced Cu toxicity and NH_4^+ accumulation in rice leaves. Moreover, studies of Wang *et al.* (2010) also suggest that application of the SNP efficiently alleviated the copper toxicity effect, as shown by increase 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, APX, SOD and GPX led to reduction in H_2O_2 accumulation in the leaves. Furthermore, the exogenous NO treatment could induce the transcription of defense-related genes, such as *pal*, *pr-1*, *gst* and *chs*, when the plant suffer various stresses (Wu *et al*. 2010). However, little is known about the relationship between exogenous NO treatment and DNA metabolism. Several recent studies demonstrated that the exogenous NO could induce the polymorphism of genomic DNA methylation. Special inhibitors or scavengers of NO synthesis is diminished the ameliorating effect of NO on 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. 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 are also able to stimulate Δ-pyroline-5-carboxylate synthetase (P5CS) activity.

Similarly, Zhang *et al.* (2010) reported that under the stress SNP promoted the activities of plant SOD and CAT significantly, increased the leaf and root Ca and Fe contents and the leaf chlorophyll content, net photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (Gs), and decreased the contents of H_2O_2 and MDA and the concentration of intercellular CO_2 (Ci). They also stated exogenous NO could promote the scavenging of ROS, keep the mineral nutrition in balance, and alleviate the damage of Cd stress to the leaf photosynthetic apparatus, making the tomato seedlings preserve their photosynthetic efficiency. 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 HM stress. These results also suggest that reduction of Cu induced toxicity and NH_4^+ accumulation by SNP is most likely mediated through its ability to scavenge ROS (Yu *et al*. 2005).

Kopyra and Gwozdz (2003) also found that SNP pretreatment significantly reduced $O_2^{\cdot -}$ induced specific fluorescence in *Lupinus luteus* roots under HMs treatment. The detoxification and antioxidative properties of NO also found in soyabean (*Glycine max*) 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). 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 HM 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 MDA synthesis.

Since, NO has reported to have the ability to reduce Cu-induced toxicity in tomato through antioxidant enzyme activity and MTs (metallothionins) accumulation, and that MTs acts downstream of NO signaling (Wang *et al*. 2010). NO is responsive regulatory mechanisms 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). Also, NO protected the plants against Al^{3+} induced oxidative stress and increased root elongation is 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 MDA under Al stress (Zhang *et al*. 2008b).

Moreover, 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. Cd toxicity resulted in reduced length, biomass, protein content and activities of antioxidant enzymes (Sharma *et al*. 2010). Similarly, the Zn and Ni also induced oxidative stress in pigeon pea cultivars is evident from the increased lipid per oxidation in their roots and shoots and affected the dry matter accumulation in roots and shoots of the pigeon pea cultivars (Madhava and Sresty 2000). A similar pattern of response together with an elevation in the photosynthesis is observed in the plants of mustard exposed to Cd through nutrient medium. 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. 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).

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 $O_2^{\cdot -}$ had been reported earlier in the case of various other plants under Cr, Zn, Pb etc. (Dietz *et al*. 1999; Panda *et al*. 2003b; Choudhury and Panda 2004). The increase in SOD activity indicated higher H_2O_2 level seen by the increase in total H_2O_2 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; 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; Ember *et al*. 2009; Watanabe *et al*. 2012). 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 is observed (Sandalio *et al*. 2001). However, this effect is connected with attenuation of the enzymatic antioxidant system, and increased LPX may have not resulted in decrease the H_2O_2 level decrease. 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 (Romero-Puertas *et al*. 2004; Cho and Seo 2005; Maksymiec and Krupa 2006b; Domínguez *et al*. 2010) (**Fig. 2**).

Perhaps, a constitutively high antioxidant capacity or increase in the levels of one or more antioxidants could prevent the oxidative damage and improve resistance to oxidative stress. NADPH oxidase (a source of H_2O_2 production) is involved in plant growth (Liszkay *et al*. 2003) and plant response to Cu (Quartacci *et al*. 2001; Maksymiec and Krupa 2006b; Maksymiec 2007; Remans *et al*. 2010; Keunen *et al*. 2011). Increased accumulation of H_2O_2, usually connected with changes in the cellular redox status, alerts the plant cell against environmental stresses (Foyer and Noctor 2003; Rentel and Knight 2004) and may enhance the plants antioxidant response through calcium signaling 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). Taking all these observation together, a hypothetical framework may be suggested that H_2O_2, then, would act as a signaling molecule triggering secondary defense along with NO involvement under stressful conditions.

CONCLUSION AND FUTURE PROSPECTS

Presently, NO and H_2O_2 have emerged as central players in field of abiotic stress induced signaling in plant system. Since, it is also clear that both signaling molecules can mediate the transcription of number of stress responsive genes. However, some most interesting questions remain to be elucidated till date is as follows; (1) To identify probable mechanisms through which H_2O_2 and NO have direct/indirect effects on transcription factors. (2) The recognition of full range of biological functions for them H_2O_2 and NO remain to be catalogued. (3) Determining the ways in which they interacts. So far, it still under research whether H_2O_2 is situated at a common centre for the signaling pathways providing responses to various signals triggered by HM stress like production of NO.

ACKNOWLEDGEMENTS

The authors are highly thankful for the facilities obtained at AMU Aligarh. Taqi Ahmed Khan is Senior Research fellow on project (SR/FT/LS-087/2007) from the Department of Science and Technology, New Delhi, is gratefully acknowledged.

REFERENCES

Affourtit C, Krab K, Moore AL (2001) Control of plant mitochondrial respiration. *Biochimica et Biophysica Acta* **1504**, 58-69

Agrawal GK, Iwahashi H, Rakwal R (2003a) Rice MAPKs. *Biochemistry and Biophysical Research Communication* **302**, 171-180

Agrawal GK, Jwa N-S, Agrawal SK, Tamogami S, Iwahashi H, Rakwal R (2003b) Cloning of novel rice allene oxide cyclise (OsAOC): mRNA expression and comparative analysis with allene oxide synthase (OsAOS) gene provide insight into the transcriptional regulation of octadecanoid pathway biosynthetic genes in rice. *Plant Science* **164**, 979-992

Agrawal GK, Tamogami S, Iwahashi H, Agrawal VP, Rakwal R (2003c) Transient regulation of jasmonic acid-inducible rice MAP kinase gene (OsBWMK1) by diverse biotic and abiotic stresses. *Plant Physiology and Biochemistry* **41**, 355-361

Ahlfors R, Brosché M, Kollist H, Kangasjärvi J (2009) Nitric oxide modulates ozone-induced cell death hormone biosynthesis and gene expression in *Arabidopsis thaliana*. *Plant Journal* **58**, 1-12

Alaoui-Sosse B, Genet P, Vinit-Dunand F, Toussaint ML, Epron D, Badot PM (2004) Effect of copper on growth in cucumber plants (*Cucumis sativus*) and its relationships with carbohydrate accumulation and changes in ion contents. *Plant Science* **166**, 1213-1218

Allan AC, Fluhr R (1997) Two distinct sources of elicited reactive oxygen species in tobacco epidermal cells. *Plant Cell* **9**, 1559-1572

Allen GJ, Sanders D (1997) Vacuolar ion channel of higher plants. *Advances in Botanical Research and Advances in Plant Pathology* **25**, 217-252

Allen R (1995) Dissection of oxidative stress tolerance using transgenic plants. *Plant Physiology* **107**, 1049-1054

Alscher R, Donahue J, Cramer CL (1997) Reactive oxygen species and antioxidants: Relationships in green cells. *Physiologia Plantarum* **100**, 224-233

Alvarez ME, Pennell RI, Meijer PJ, Ishkawa A, Dixon RA, Lamb C (1998) Reactive oxygen species intermediates mediate a systemic signal network in the establishment of plant immunity. *Cell* **92**, 773-784

Anderson JW, Foyer CH, Walker DA (1983) Light-dependent reduction of hydrogen peroxide by intact spinach chloroplasts. *Biochimica et Biophysica Acta* **724**, 69-74

Apel K, Hirt H (2004) Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annual Reviews in Plant Biology* **55**, 373-399

Arasimowicz M, Floryszak-Wieczorek J (2007) Nitric oxide as a bioactive signaling molecule in plant stress responses. *Plant Science* **172**, 876-887

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology* **141**, 391-396

Askerlund P, Larsson C, Widell S, Moller IM (1987) NAD(P)H oxidase and peroxidase activities in purified plasma membranes from cauliflower inflorescences. *Physiologia Plantarum* **71**, 9-19

Asthir B, Spoor W, Duffus CM (2004) Involvement of polyamines diamine oxidase and polyamine oxidase in resistance of barley to *Blumeria graminis* f sp. *Hordei Euphytica* **136**, 307-312

Babosha AV, Komarova GI (2010) Combined effect of zeatin and hydrogen peroxide on growth of rape and tomato germs as applied to the conditions of biological life support systems. *Aviakosmicia Ekologisickia Medicitina* **44 (3)**, 46-50

Baranek M, Krizan B, Ondrusikova E, Pidra M (2010) DNA-methylation changes in grapevine somaclones following *in vitro* cultures and thermotherapy. *Plant Cell, Tissue and Organ Culture* **101**, 11-22

Bethke PC, Libourel IGL, Aoyama N, Chung Y, Still DW, Jones RL (2007) The *Arabidopsis thaliana* aleurone layer responds to nitric oxide gibberellin and abscisic acid and is sufficient and necessary for seed dormancy. *Plant Physiology* **143**, 1173-1188

Bhattacharjee S (2005) Reactive oxygen species and oxidative burst: Roles in stress senescence and signal transduction in plants. *Current Science* **89**, 7-15

Bolwell GP (1999) Role of reactive oxygen species and NO in plant defence responses. *Current Opinion in Plant Biology* **2**, 287-294

Bolwell GP, Bindschedler LV, Blee KA, Butt VS, Davies DR, Gardner SL, Gerrish C, Minibayeva F (2002) The apoplastic oxidative burst in response to biotic stress in plants: A three component system. *Journal of Experimental Botany* **53**, 1367-1376

Bonifacio A, Martins MO, Ribeiro C, Fontenele AV, Carvalho FEL, Margis-Pinheiro M, Silveira JG (2011) Role of peroxidases in the compensation of cytosolic ascorbate peroxidase knockdown in rice plants under abiotic stress. *Plant, Cell and Environment* **34**, 1705-1722

Bowler C, Fluhr R (2000) The role of calcium and activated oxygen as signals for controlling cross-tolerance. *Trends in Plant Science* **5,** 241-246

Bowler C, Van Montagu M, Inzé D (1992) Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**, 83-116

Bradley DJ, Kjelborn P, Lamb CJ (1992) Elicitor and wound induced oxidative cross-linking of a proline-rich plants cell wall proteins: A novel rapid defense response. *Cell* **70**, 21-30

Brahim L, Mohamed M (2011) Effects of copper stress on antioxidative enzymes, chlorophyll and protein content in *Atriplex halimus*. *African Journal of Biotechnology* **10 (50)**, 10143-10148

Bright J, Desikan R, Hancock JT, Weir IS, Neill SJ (2006) ABA-induced NO generation and stomatal closure in *Arabidopsis* are dependent on H_2O_2 synthesis. *Plant Journal* **45**, 113-122

Cakmak I (1994) Activity of ascorbate-dependent H_2O_2-scavenging enzymes and leaf chlorosis are enhanced in magnesium- and potassium-deficient leaves but not in phosphorus-deficient leaves. *Journal of Experimental Botany* **45**, 1259-1266

Carol R, Dolan L (2006) The role of reactive oxygen species in cell growth: lessons from root hairs. *Journal of Experimental Botany* **57**, 1829-1834

Castillo FJ (1986) Extracellular peroxidases as markers of stress? In: Greppin H, Penel C, Gaspar T (Eds) *Molecular and Physiological Aspects of Plant Peroxidases*, University of Geneva Press, Geneva, pp 419-426

Cerda S, Weitzman SA (1997) Influence of oxygen radical injury on DNA methylation. *Mutation Research* **386**, 141-152

Chao YE, Zhang M, Tian SK, Lu LL, Yang XE (2008) Differential generation of hydrogen peroxide upon exposure to zinc and cadmium in the hyperaccumulating plant species (*Sedum alfredii Hance*). *Journal of Zhejiang University of Science B* **9 (3)**, 243-249

Chaudhury S, Panda SK (2004) Induction of oxidative stress and ultrastructural changes in moss *Taxithelium napalense* (Schwaegr) Broth under lead (Pb) and arsenic (As) phytotoxicity. *Current Science* **86**, 444-446

Cheeseman JM (2007) Hydrogen peroxide and plant stress: A challenging relationship. *Plant Stress* **1 (1)**, 4-15

Cheng YL, Song CP (2006) Hydrogen peroxide homeostasis and signaling in plant cells. *Sciences in China Series C: Life Sciences* **49 (1)**, 1-11

Cho UH, Park JO (2000) Mercury-induced oxidative stress in tomato seedlings. *Plant Science* **156**, 1-9

Cho UH, Seo NH (2005) Oxidative stress in *Arabidopsis thaliana* exposed to cadmium is due to hydrogen peroxide accumulation. *Plant Science* **168**, 113-120

Clark D, Durner J, Navarre DA, Klessig DF (2000) Nitric oxide inhibition of tobacco catalase and ascorbate peroxidase. *Molecular Plant Microbe Interactions* **13**, 1380-1384

Coleman ST, Epping EA, Steggerda SM, Moye-Rowley WS (1999) Yap1p activates gene transcription in an oxidant-specific fashion. *Molecular Cell Biology* **19**, 8302-8313

Corpas FJ, Barroso JB, Carreras A, Quiros M, Leon A, Romero-Puertas MC, Esteban FJ, Valderrama R, Palma JM, Sandalio LM, Gómez M, del Río LA (2004) Cellular and subcellular localization of endogenous nitric oxide in young and senescent pea plants. *Plant Physiology* **136**, 2722-2733

Crawford NM (2006) Mechanism for nitric oxide synthesis in plants. *Journal of Experimental Botany* **57**, 471-478

Crawford NM, Guo FQ (2005) New insights into nitric oxide metabolism and regulatory functions. *Trends in Plant Science* **10**, 195-200

Cui X, Zhang Y, Chen X, Jin H, Wu X (2009) Effects of exogenous nitric oxide protects tomato plants under copper stress. In: *Bioinformatics and Biomedical Engineering*, 3rd International Conference, 11-13 June, 2009, Beijing, pp 1-7

Das PK, Kar M, Mishra D (1978) Nickel nutrition of plants: I. Effects of nickel on some oxidase activities during rice (*Oryza sativa* L.) seed germina-

tion. *Pflanzenphysiologie* **90**, 225-233
Dat JF, Vandenabeele S, Vranova E, Van MM, Inzé D, VanBreusegem F (2000) Dual action of the active oxygen species during plant stress responses. *Cell and Molecular Life Sciences* **57**, 779-795
Davies DR, Bindschedler LV, Strickland TS, Bolwell GP (2006) Production of reactive oxygen species in *Arabidopsis thaliana* cell suspension cultures in response to an elicitor from *Fusarium oxysporum*: Implications for basal resistance. *Journal of Experimental Botany* **57**, 1817-1827
De Vos CHR, Schat H, Vooijs R, Ernst WHO (1989) Copper-induced damage to the permeability barrier in roots of *Silene cucubalus*. *Journal of Plant Physiology* **135**, 164-169
del Río LA, Corpas FJ, Barroso JB (2004) Nitric oxide and nitric oxide synthase activity in plants. *Phytochemistry* **65**, 783-792
del Río LA, Palma JM, Sandalio LM, Corpas FJ, Pastori GM, Bueno P, López-Huertas E (1996) Peroxisomes as a source of superoxide and hydrogen peroxide in stressed plants. *Biochemical Society T* **24**, 434-438
del Río LA, Sandalio LM, Palma JM, Bueno P, Corpas FJ (1992) Metabolism of oxygen radicals in peroxisomes and cellular implications. *Free Radical Biology and Medicine* **13**, 557-580
Delaunay A, Pflieger D, Barrault MB, Vinh J, Toledano MB (2002) A thiol peroxidase is an H_2O_2 receptor and redox-transducer in gene activation. *Cell* **111**, 471-481
Delledonne M (2005) NO news is good news for plants. *Current Opinion in Plant Biology* **8**, 390-396
Delledonne M, Xia Y, Dixon RA, Lamb C (1998) Nitric oxide functions as a signal in plant disease resistance. *Nature* **394**, 585-588
Demirevska-Kepova K, Simova-Stoilova L, Stoyanova Z, Holzer R, Feller U (2004) Biochemical changes in barley plants after excessive supply of copper and manganese. *Environmental and Experimental Botany* **52**, 253-266
Demmig-Adams B, Adams III WW (1992) Photoprotection and other responses of plants to high light stress. *Annual Reviews in Plant Physiology and Plant Molecular Biology* **43**, 599-626
Demmig-Adams B, Adams III WW, Ebbert V, Logan BA (1999) Ecophysiology of the xanthophyll cycle. In: Frank H, Young A, Cogdell R (Eds) *The Photochemistry of Carotenoids*, Kluwer Academic Publishers, Dordrecht, pp 245-269
Desikan R, Last K, Harrett-Williams R, Tagliavia C, Harter K, Hooley R, Hancock JT, Neill SJ (2006) Ethylene-induced stomatal closure in *Arabidopsis* occurs via AtrbohF-mediated hydrogen peroxide synthesis. *Plant Journal* **47**, 907-916
Desikan R, Mackerness S, Hancock JT, Neill SJ (2001) Regulation of the *Arabidopsis* transcriptome by oxidative stress. *Plant Physiology* **127**, 159-172
Desikan R, Reynolds A, Hancock JT, Neill SJ (1998) Harpin and hydrogen peroxide both initiate programmed cell death but have differential effects on gene expression in Arabidopsis suspension cultures. *Biochemistry Journal* **330**, 115-120
Didierjean L, Frendo P, Nasser W, Genot G, Marivet J, Burkard G (1996) Heavy metal-responsive genes in maize: Identification and comparison of their expression upon various forms of abiotic stress. *Planta* **199**, 1-8
Dietz KJ, Baier M, Kramer U (1999) Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. In: Prasad MNV, Hagemeyer J (Ed) *Heavy Metal Stress in Plants: From Molecules to Ecosystems*, Springer-Verlag, Berlin, pp 73-97
Dixit V, Pandey V, Shyam R (2001) Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). *Journal of Experimental Botany* **52 (358)**, 1101-1109
Domínguez DM, García CF, Raya CA, Santiago TR (2010) Cadmium-induced oxidative stress and the response of the antioxidative defense system in *Spartina densiflora*. *Physiologia Plantarum* **139 (3)**, 289-302
Drazkiewicz M, Skorzynska-Polit E, Krupa Z (2004) Copper induced oxidative stress and antioxidant defence in *Arabidopsis thaliana*. *BioMetals* **17**, 379-387
Dutilleu C, Garmier M, Noctor G, Mathieu C, Chétrit P, Foyer CH, de Paepe R (2003) Leaf mitochondria modulate whole cell redox homeostasis, set antioxidant capacity, and determine stress resistance through altered signaling and diurnal regulation. *The Plant Cell* **(15)**, 1212-1226
Elstner E (1990) *Der Sauerstoff*, Wissenschaftsverlag, Mannheim
Ember E, Rothbart S, Puchta R, Eldik RV (2009) Metal ion-catalyzed oxidative degradation of Orange II by H_2O_2. High catalytic activity of simple manganese salts. *New Journal of Chemistry* **33**, 34-49
Farrar JF, Rayns FW (1987) Respiration of leaves of barley infected with powdery mildew: Increased engagement of the alternative oxidase. *New Phytologist* **107**, 119-126
Feng H-Q, Sun K, Li MQ, Li H-Y, Li X, Li Y, Wang Y-F (2010) The expression, function and regulation of mitochondrial alternative oxidase under biotic stresses. *Molecular Plant Pathology* **11 (3)**, 429-440
Fernandes JC, Henriques FS (1991) Biochemical physiological and structural effects of excess copper in plants. *Botanical Reviews* **57**, 246-273
Fiorani F, Umbach AL, Siedow JN (2005) The alternative oxidase of plant mitochondria is involved in the acclimation of shoot growth at low temperature. A study of *Arabidopsis AOX1a* transgenic plants. *Plant Physiology* **139**, 1795-1805
Flora SJS, Mittal M, Mehta A (2008) Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian Journal of Medical Research* **128**, 501-523
Foyer CH, Descourvieres P, Kunert KJ (1994) Protection against oxygen radicals: An important defence mechanism studied in transgenic plants. *Plant Cell and Environment* **17**, 507-523
Foyer CH, Halliwell B (1976) Presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. *Planta* **133**, 21-25
Foyer CH, Lelandais M, Edwards EA, Mullineaux PM (1991) Active oxygen, oxidative stress and plant metabolism. In: Pell E, Steffen K (Eds) *Current Topics in Plant Physiology*, American Society of Plant Physiologists, Rockville, MD, pp 131-144
Foyer CH, Lopez-Delgado H, Dat JF, Scott IM (1997) Hydrogen peroxide and glutathione-associated mechanisms of acclamatory stress tolerance and signaling. *Physiologia Plantarum* **100**, 241-254
Foyer CH, Noctor G (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts peroxisomes and mitochondria. *Physiologia Plantarum* **119**, 355-364
García-Mata C, Lamattina L (2001) Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. *Plant Physiology* **126**, 1196-1204
García-Mata C, Lamattina L (2002) Nitric oxide and abscisic acid cross talk in guard cells. *Plant Physiology* **128**, 790-792
García-Mata C, Wang J, Gajdanowicz P, Gonzalez W, Hills A, Donald N, Riedelsberger J, Amtmann A, Dreyer I, Blatt MR (2010) A minimal cysteine motif required to activate the SKOR K^+ channel of *Arabidopsis* by the reactive oxygen species H_2O_2. *Journal of Biological Chemistry* **285 (38)**, 29286-29294
Gechev T, Gadjev I, Van BF, Inzé D, Dukiandjiev S, Toneva V, Minkov I (2002) Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes. *Cell and Molecular Life Sciences* **59 (4)**, 708-714
Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* **48**, 909-930
Grun S, Lindermayr C, Sell S, Durner J (2006) Nitric oxide and gene regulation in plants. *Journal of Experimental Botany* **57**, 507-516
Guo FQ, Crawford NM (2005) Arabidopsis nitric oxide synthase1 is targeted to mitochondria and protects against oxidative damage and dark-induced senescence. *Plant Cell* **17**, 3436-3450
Guo FQ, Okamoto M, Crawford NM (2003) Identification of a plant nitric oxide synthase gene involved in hormonal signaling. *Science* **302**, 100-103
Guo Y, Tian Z, Yan D, Zhang J, Qin P (2009) Effects of nitric oxide on salt stress tolerance in *Kosteletzkya virginica*. *Life Science Journal* **6 (1)**, 67-75
Hager A (1969) Lichtbedingte pH-Emiedrigung in einem Chloro-plastenkompartiment als Ursache der enzymatischen Violaxanthindzeaxanthin-Umwandlung: Beziehungen zur Photophosphorylierung. *Planta* **89**, 224-243
Hall JL (2002) Cellular mechanism for heavy metal detoxification and tolerance. *Journal of Experimental Botany* **53 (366)**, 1-11
Halliwell B, Gutteridge J (1999) *Free Radicals in Biology and Medicine* (3rd Edn), Oxford University Press, Oxford, 936 pp
Halliwell B, Gutteridge JMC (1986) Iron and free radical reactions: Two aspects of antioxidant protection. *Trends in Biochemical Science* **11**, 375-381
Hanaa H, Abd El-Baky FK, El B El-Baroty, Gamal S (2009) Enhancement of antioxidant production in *Spirulina platensis* under oxidative stress. *Acta Physiologia Plantarum* **31**, 623-631
Hansch R, Lang C, Riebeseel E, Lindigkeit R, Gessler A, Rennenberg H, Mendel RR (2006) Plant sulfite oxidase as novel producer of H_2O_2 – Combination of enzyme catalysis with a subsequent non-enzymatic reaction step. *The Journal of Biological Chemistry* **281**, 6884-6888
He JM, Xu H, She XP, Song XG, Zhao WM (2004) The role and the interrelationship of hydrogen peroxide and nitric oxide in the UV-B-induced stomatal closure in broad bean. *Functional Plant Biology* **32**, 237-247
He YY, Hader DP (2002) UV-B-induced formation of reactive oxygen species and oxidative damage of the cyanobacterium *Anabaena* sp: Protective effects of ascorbic acid and *N*-acetyl-L-cysteine. *Journal of Photochemistry and Photobiology B: Biology* **66**, 115-124
Hill AC, Bennett JH (1970) Inhibition of apparent photosynthesis by nitrogen oxides. *Atmospheric Environment* **4**, 341-348
Hong PC, Jun ZS, Zhong GZ, Shan WB (2005) NaCl treatment markedly enhances H_2O_2 scavenging system in leaves of halophyte *Suaeda salsa*. *Physiologia Plantarum* **125**, 490-499
Hu KD, Hu LY, Li YH, Zhang FQ, Zhang H (2007) Protective roles of nitric oxide on germination and antioxidant metabolism in wheat seeds under copper stress. *Plant Growth Regulation* **53**, 173-183
Huber SC, Bachmann M, Huber JL (1996) Post-translational regulation of nitrate reductase activity: A role for Ca2þ and 14-3-3 proteins. *Trends in Plant Science* **1**, 432-437
Hung SH, Yu CW, Lin CH (2005) Hydrogen peroxide functions as a stress signal in plants. *Botanical Bulletin of Academia Sinica* **46**, 1-10
Inzé D, Van Montague M (1995) Oxidative stress in plants. *Current Opinion in Biotechnology* **6**, 153-158

Iwakiri Y, Satoh A, Chatterjee S, Doomre DK, Chalouni CM, Fulton D, Groszmann RJ, Shah VH, Sessa WC (2006) Nitric oxide synthase generates nitric oxide locally to regulate compartmentalized protein S-nitrosylation and protein trafficking. *Proceedings of the National Academy of Sciences USA* **103 (52)**, 19777-19782

Kachout SS, Mansoura AB, Leclerc JC, Mechergui R, Rejeb MN, Ouerghi Z (2010) Effects of heavy metals on antioxidant activities of *Atriplex hortensis* and *A. rosea*. *Electronic Journal of Environmental, Agricultural and Food Chemistry* **9 (3)**, 444-457

Kahle H (1993) Response of roots of trees to heavy metals. *Environmental and Experimental Botany* **33 (1)**, 99-119

Karataglis S, Moustakas M, Symeonidis L (1991) Effects of heavy metals on isoperoxidases of wheat. *Biologia Plantarum* **33**, 3-9

Karimi A, Khodaverdiloo H, Sepehri M, Sadaghiani MR (2011) Arbuscular mycorrhizal fungi and heavy metal contaminated soils. *African Journal of Microbiology Research* **5 (13)**, 1571-1576

Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux P (1999) Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. *Science* **284**, 654-657

Karplus PA, Daniels MJ, Herriot JR (1991) Atomic structure of ferredoxin-NadpC reductase prototype for a structurally novel flavoenzyme family. *Science* **251**, 60-66

Keller T, Damude HG, Werner D, Doerner P, Dixon RA, Lamb C (1998) A plant homolog of the neutrophil NADPH oxidase gp91(phox) subunit gene encodes a plasma membrane protein with Ca^{2+} binding motifs. *Plant Cell* **10**, 255-266

Keunen E, Remans T, Bohler S, Vangronsveld J, Cuypers A (2011) Metal-induced oxidative stress and plant mitochondria. *International Journal of Molecular Sciences* **12**, 6894-6918

Kim J-A, Agrawal GK, Rakwal R, Han K-S, Kim K-N, Yun Ch-H, Heu S, Park S-Y, Lee Y-H, Jwa N-S (2003) Molecular cloning and mRNA expression analysis of a novel rice (*Oryza sativa* L.) MAPK kinase kinase, OsEDR1, an ortholog of *Arabidopsis* AtEDR1, reveal its role in defense/stress signaling pathways and development. *Biochemical and Biophysical Research Communication* **300**, 868-876

Knight H, Trewavas AJ, Knight MR (1996) Cold calcium signaling in *Arabidopsis* involves two cellular pools and a change in calcium signature after acclimation. *Plant Cell* **8**, 489-503

Kopyra M, Gwozdz EA (2003) Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. *Plant Physiology and Biochemistry* **41**, 1011-1017

Kopyra M, Gwozdz EA (2004) The role of nitric oxide in plant growth regulation and responses to abiotic stresses. *Acta Physiologia Plantarum* **26**, 459-472

Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. *Annual Reviews in Plant Physiology and Plant Molecular Biology* **48**, 251-275

Lamotte O, Gould K, Lecourieux D, Sequeira-Legrand A, Lebrun-Garcia A, Durner J, Pugin A, Wendehenne D (2004) Analysis of nitric oxide signaling functions in tobacco cells challenged by the elicitor cryptogein. *Plant Physiology* **135**, 516-529

Langebartels C, Wohlgemuth H, Kschieschan S, Grun S, Sandermann H (2002) Oxidative burst and cell death in ozone-exposed plants. *Plant Physiology and Biochemistry* **40**, 567-575

Leshem Y, Wills RBH, Ku V (1998) Evidence for the function of the free radical gas-nitric oxide (NO) as an endogenous maturation and senescence regulating factor in higher plants. *Plant Physiology and Biochemistry* **36**, 825-833

Leshem YY (1996) Nitric oxide in biological systems. *Plant Growth Regulators* **18**, 155-159

Leshem YY, Haramaty E (1996) The characterization and contrasting effects of the nitric-oxide free radical in vegetative stress and senescence of *Pisum sativum* Linn foliage. *Journal of Plant Physiology* **148**, 258-263

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

Li Y, Trush MA (1993b) Oxidation of hydroquinone by copper: Chemical mechanism and biological effects. *Biochimica et Biophysica Acta* **300**, 346-355

Lin CH, Chen LM, Liu ZH (2005) Rapid effect of copper on lignin biosynthesis in soybean roots. *Plant Science* **168**, 855-861

Liszkay A, Kenk B, Schopfer P (2003) Evidence for the involvement of cell wall peroxidase in the generation of hydroxyl radicals mediating extension growth. *Planta* **217**, 658-667

Liu L, Eriksson KEL, Dean JFD (1995) Localization of hydrogen-peroxide production in *Pisum sativum* L. using epi-polarization microscopy to follow cerium perhydroxide deposition. *Plant Physiology* **107**, 501-506

Liu X, Wang YQ, Jia WS, Lou CH, Zhang SQ (2007) Localization of NOS-like protein in guard cells of *Vicia faba* L and its possible function. *Chinese Science Bulletin* **52**, 84-90

Liu Z, Chen W, He X (2011) Cadmium-induced changes in growth and antioxidative mechanisms of a medicine plant (*Lonicera japonica* Thunb.). *Journal of Medicinal Plants Research* **5 (8)**, 1411-1417

Logan BA, Demmig-Adams B, Adams III WW, Grace SC (1998a) Antioxidants and xanthophylls cycle-dependent energy dissipation in *Cucurbita pepo* L and *Vinca major* L. upon a sudden increase in growth PPFD in the field. *Journal of Experimental Botany* **49**, 1881-1888

Lone MI, Zhen L-H, Stoffella PJ, Xiao EY (2008) Phytoremediation of heavy metal polluted soils and water. Progress and perspectives. *Journal of Zhejiang University of Science B* **9 (3)**, 210-220

Lopez-Huertas E, Charlton WL, Johnson B, Graham IA, Baker A (2000) Stress induces peroxisome biogenesis genes. *EMBO Journal* **19**, 6770-6777

Lum HK, Butt YK, Lo SC (2002) Hydrogen peroxide induces a rapid production of nitric oxide in mung bean (*Phaseolus aureus*). *Nitric Oxide* **6**, 205-213

Luna CM, Gonzalez CA, Trippi VS (1994) Oxidative damage caused by excess of copper in oat leaves. *Plant Cell Physiology* **35**, 11-15

Madhava Rao KV, Sresty TVS (2000) Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Science* **157**, 113-128

Maharana D, Jena K, Pise NM, Jagtap TG (2010) Assessment of oxidative stress indices in a marine macro brown alga *Padina tetrastromatica* (Hauck) from comparable polluted coastal regions of the Arabian Sea west coast of India. *Journal of Environmental Science (China)* **22 (9)**, 1413-1417

Makino A, Miyake C, Yokota A (2002) Physiological functions of the water-water cycle (Mehler reaction) and the cyclic electron flow around PSI in rice leaves. *Plant and Cell Physiology* **43 (9)**, 1017-1026

Maksymiec W (2007) Signaling responses in plants to heavy metal stress. *Acta Physiologia Plantarum* **29**, 177-187

Maksymiec W, Krupa Z (2006b) The effects of short-term exposition to Cd excess Cu ions and jasmonate on oxidative stress appearing in *Arabidopsis thaliana*. *Environmental and Experimental Botany* **57**, 187-194

Malusa E, Laurenti E, Juszczuk I, Ferrari RP, Rychter AM (2002) Free radical production in roots of *Phaseolus vulgaris* subjected to phosphate deficiency stress. *Plant Physiology and Biochemistry* **40**, 963-967

Marceau M (2004) Transcriptional regulation in *Yesinia*: An update. *Current Issues in Molecular Biology* **7**, 151-178

Marino D, Dunand C, Puppo A, Pauly N (2012) A burst of plant NADPH oxidases. *Trends in Plant Science* **17 (1)**, 9-15

Mastrocola R, Restivo F, Vercellinatto I, Danni O, Brignardello1 E, Aragno M, Boccuzzi1 G (2005) Oxidative and nitrosative stress in brain mitochondria of diabetic rats. *Journal of Endocrinology* **187**, 37-44

Mateo A, Mühlenbock P, Rustérucci C, Chang CCC, Miszalski Z, Karpinska B, Parker JE, Mullineaux PM, Karpinski S (2004) LESION SIMULATING DISEASE 1 is required for acclimation to conditions that promote excess excitation energy. *Plant Physiology* **136**, 2818-2830

Maxwell DP, Wang Y, McIntosh L (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. *Proceedings of the National Academy of Sciences USA* **96**, 8271-8276

Mazhoudi S, Chaoui A, Ghorbal MH, El-Ferjani E (1997) Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum* Mill). *Plant Science* **127**, 129-137

Mazid M, Ali B, Hayat S, Ahmad A (2010) The effect of 4-chloroindole-3-acetic acid on some growth parameters in mung bean under cadmium stress. *Turkish Journal of Biology* **34**, 9-13

Mazid M, Khan TA, Mohammad F (2011a) Potential of NO and H_2O_2 as signaling molecules in tolerance to abiotic stress in plants. *Journal of Industrial Research and Technology* **1 (1)**, 56-68

Mazid M, Khan TA, Mohammad F (2011b) Role of nitric oxide in regulation of H_2O_2 mediating tolerance of plants to abiotic stress: A synergistic signaling approach. *Journal of Stress Physiology and Biochemistry* **7 (2)**, 34-74

McLaughlin MJ, Parker DR, Clarke JR (1999) Metals and micronutrients food safety issues. *Fields Crop Research* **60**, 143-163

Michalak A (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies* **15 (4)**, 523-530

Miller G, Schlauch K, Tam R, Cortes D, Torres MA, Shulaev V, Dangl JL, Mittler R (2009) The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. *Science Signaling* **2 (84)**, ra45

Mishra A, Choudhuri MA (1997) Differential effect of Pb2 and Hg2 on inhibition of germination of seeds of two rice cultivars. *Indian Journal of Plant Physiology* **2**, 41-44

Mittler R, Vanderauwera S, Gollery M, van Breusegem F (2004) Reactive oxygen gene network of plants. *Trends in Plant Science* **9**, 490-498

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

Molas J (2002) Changes of chloroplast ultra structure and total chlorophyll concentration in cabbage leaves caused by excess of organic Ni (II) complexes. *Environmental and Experimental Botany* **47**, 115-126

Moller IM (2001) Plant mitochondria and oxidative stress: Electron transport NADPH turnover and metabolism of reactive oxygen species. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 561-591

Moller IM, Sweetlove LJ (2010) ROS signalling-specificity is required. *Trends in Plant Science* **7**, 370-374

Morita S, Kaminaka H, Masumura T, Tanaka K (1999) Induction of rice cytosolic ascorbate peroxidase mRNA by oxidative stress; the involvement of

hydrogen peroxide in oxidative stress signalling. *Plant and Cell Physiology* **40**, 417-422

Murgia I, Tarantino D, Vannini C, Bracale M, Carravieri S, Soave C (2004b) *Arabidopsis thaliana* plants overexpressing thylakoidal ascorbate peroxidase show increased resistance to Paraquat-induced photo-oxidative stress and to nitric oxide induced cell death. *The Plant Journal* **38**, 940-953

Murphy TM, Vu H, Nguyen T, Woo CH (2000) Diphenylene iodonium sensitivity of a solubilized membrane enzyme from rose cells. *Protoplasma* **213**, 228-234

Nagalakshmi N, Prasad MNV (2001) Responses of glutathione cycle enzymes and glutathione metabolism to copper stress in *Scenedesmus bijugatus*. *Plant Science* **160**, 291-299

Nakano Y, Asada K (1980) Spinach chloroplasts scavenge hydrogen peroxide on illumination. *Plant Cell Physiology* **21**, 1295-1307

Nayyar H, Chander S (2004) Protective effects of polyamines against oxidative stress induced by water and cold stress in chickpea. *Journal of Agronomy and Crop Science* **190**, 355-365

Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, Morris P, Ribeiro D, Wilson I (2008) Nitric oxide, stomatal closure, and abiotic stress. *Journal of Experimental Botany* **59 (2)**, 165-176

Neill S, Bright J, Desikan R, Hancock J, Harrison J, Wilson I (2008) Nitric oxide evolution and perception. *Journal of Experimental Botany* **59**, 25-35

Neill S, Desikan R, Claarke A, Hancock J (1999) H_2O_2 signalling in plant cells. In: Clavert C, Smallwood M, Bowles D (Ed) *Plant Responses to Environmental Stress*, Bios Scientific Publishers, Oxford, pp 59-64

Neill SJ (2007) Interactions between abscisic acid hydrogen peroxide and nitric oxide mediate survival response during water stress. *New Phytologist* **175**, 4-6

Neill SJ, Desikan R, Clarke A, Hancock JT (2002a) Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells. *Plant Physiology* **128**, 13-16

Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT (2002b) Hydrogen peroxide and nitric oxide as signalling molecules in plants. *Journal of Experimental Botany* **53**, 1237-1247

Neill SJ, Desikan R, Hancock JT (2003) Nitric oxide signaling in plants. *New Phytologist* **159**, 11-35

Neubauer C, Schreiber U (1989) Photochemical and non-photo-chemical quenching of chlorophyll fluorescence induced by hydrogen peroxide. *Naturforschung C* **44**, 262-270

Neubauer C, Yamamoto HY (1992) Mehler-peroxidase reaction mediates zeaxanthin formation and zeaxanthin-related fluorescence quenching in intact chloroplasts. *Plant Physiology* **99**, 1354-1361

Noctor G, Foyer CH (1998) Ascorbate and glutathione; keeping active oxygen control. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**, 249-279

Noctor G, Veljovic-Jovanovic S, Foyer CH (2000) Peroxide processing in photosynthesis: antioxidant coupling and redox signalling. *Philosophical Transactions of the Royal Society of London B* **355**, 1465-1475

Nouairi I, Ammar WB, Youssef NB, Miled DDB, Ghorbal MH, Zarrouk M (2009) Antioxidant defense system in leaves of Indian mustard (*Brassica juncea*) and rape (*Brassica napus*) under cadmium stress. *Acta Physiologia Plantarum* **31**, 237-247

Ozdener Y, Aydin BK (2010) The effect of zinc on the growth and physiological and biochemical parameters in seedlings of *Eruca sativa* (L.) (Rocket). *Acta Physiologia Plantarum* **32**, 469-476

Panda SK, Singa LB, Khan MH (2003b) Does aluminium phytotoxicity induce oxidative stress in green gram (*Vigna radiata*)? *Bulgarian Journal of Plant Physiology* **29**, 77-86

Parani MR, Myers R, Weirich H, Smith B, Leaman DW, Goldman SL (2004) Microarray analysis of nitric oxide responsive transcripts in *Arabidopsis*. *Plant Biotechnology Journal* **2**, 359-366

Parsons HL, Yip JYH, Vanlerberghe GC (1999) Increased respiratory restriction during phosphate-limited growth in transgenic tobacco cells lacking alternative oxidase. *Plant Physiology* **121**, 1309-1320

Pastori GM, Foyer CH (2002) Common components, networks, and pathways of cross-tolerance to stress. The central role of "redox" and abscisic acid-mediated controls. *Plant Physiology* **129**, 460-468

Pedroso MC, Durzan DJ (2000) Effect of different gravity environments on DNA fragmentation and cell death in *Kalanchoe* leaves. *Annals of Botany* **86**, 983-994

Pei Z-M, Murata Y, Benning G, Thomine S, Klusener B, Allen GJ, Grill E, Schroeder JI (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature* **406**, 731-734

Pelicano H, Carney D, Huang P (2004) ROS stress in cancer cells and therapeutic implications. *Drug Resistance Updates* **7 (2)**, 97-110

Perry SF, Spinelli OE (2010) Respiration in a changing environment. *Respiratory Physiology and Neurobiology* **173**, 20-25

Petrussa E, Casolo V, Peresson C, Krajˇnakova MF, Vianello A (2008) Activity of a KATP+ channel in *Arum* spadix mitochondria during thermogenesis. *Journal of Plant Physiology* **165**, 1360-1369

Pfundel EE, Dilley RA (1993) The pH dependence of violaxanthin deepoxidation in isolated pea chloroplasts. *Plant Physiology* **101**, 65-71

Piqueras A, Olmos E, Martínez-Soalano JR, Hellin E (1999) Cd-induced oxidative burst in tobacco BY2 cells: Time-course subcellular location and antioxidant response. *Free Radical Research* **31**, 33-38

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

Polverar A, Molesini B, Pezzotti M, Buonaurio R, Marte M, Delledonne M (2003) Nitric oxide mediated transcriptional changes in *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions* **16**, 1094-1105

Popov VN, Purvis AC, Skulachev VP, Wagner AM (2001) Stress-induced changes in ubiquinone concentration and alternative oxidase in plant mitochondria. *Bioscience Reports* **21 (3)**, 369-379

Popov VN, Simonian RA, Skulachev VP, Starkov AA (1997) Inhibition of the alternative oxidase stimulates H_2O_2 production in plant mitochondria. *FEBS Letters* **415**, 87-90

Pourakbar L, Khayami M, Khara J, Farbodnia T (2007) Copper-induce change in antioxidative system in maize (*Zea mays* L.). *Pakistan Journal of Biological Sciences* **10 (20)**, 3662-3667

Prasad KVSK, Pardha Saradhi P, Sharmila P (1999) Concerted action antioxidant enzyme and curtailed growth under zinc toxicity in *Brassica napus*. *Environmental and Experimental Botany* **42**, 1-10

Prasad TK, Anderson MD, Martin BA, Stewart CR (1994) Evidence for chilling induced oxidative stress in maize seedlings and a regulatory role of H_2O_2. *Plant Cell* **6**, 65-74

Quartacci MF, Cosi E, Navari-Izzo F (2001) Lipids and NADPH-dependent superoxide production in plasma membrane vesicles from roots of wheat grown under copper deficiency or excess. *Journal of Experimental Botany* **52**, 77-84

Ramamurthi A, Lewis RS (1997) Measurement and modeling of nitric oxide release rates for nitric oxide donors. *Chemical Research in Toxicology* **10**, 408-413

Remans T, Opdenakker K, Smeets K, Mathijsen D, Vangronsveld J, Cuypers A (2010) Metal-specific and NADPH oxidase dependent changes in lipoxygenase and NADPH oxidase gene expression in *Arabidopsis thaliana* exposed to cadmium or excess copper. *Functional Plant Biology* **37 (6)**, 532-544

Rentel M, Knight MR (2004) Oxidative stress-induced calcium signaling in *Arabidopsis*. *Plant Physiology* **135**, 1471-1479

Ribeiro EA, Cunha FQ, Tamashiro WMSC, Martins IS (1999) Growth phase-dependent subcellular localization of nitric oxide synthase in maize cells. *FEBS Letters* **445**, 283-286

Robson CA, Vanlerberghe GC (2002) Transgenic plant cells lacking mitochondrial alternative oxidase have increased susceptibility to mitochondria-dependent and independent pathways of programmed cell death. *Plant Physiology* **129**, 1908-1920

Rockel P, Strube F, Rockel A, Wildt J, Kaiser WM (2002) Regulation of nitric oxide (NO) production by plant nitrate reductase *in vivo* and *in vitro*. *Journal of Experimental Botany* **53**, 103-110

Romero-Puertas MC, Perazzolli M, Zago ED, Delledonne M (2004) Nitric oxide signalling functions in plant–pathogen interactions. *Cellular Microbiology* **6**, 795-803

Sagi M, Fluhr R (2006) Production of reactive oxygen species by plant NADPH oxidases. *Plant Physiology* **141**, 336-340

Sanchez M, Revilla G, Zarra I (1995) Changes in peroxidase activity associated with cell walls during pine hypocotyls growth. *Annals of Botany* **75**, 415-419

Sandalio LM, Dalurzo HC, Gomez M, Romero-Puertas MC, del Río LA (2001) Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *Journal of Experimental Botany* **52**, 2115-2126

Sandalio LM, Rodríguez-Serrano M, Romero-Puertas M, del Río LA (2008) Imaging of reactive oxygen species and NO *in vivo* in plant tissues. *Methods in Enzymology* **440**, 399-409

Sang J, Zhang A, Lin F, Tan M, Jiang M (2008) Cross-talk between calcium-calmodulin and nitric oxide in abscisic acid signaling in leaves of maize plants. *Cell Research* **18**, 577-588

Saumya SM, Basha PM (2011) Antioxidant effect of *Lagerstroemia speciosa* Pers (banaba) leaf extract in streptozotocin-induced diabetic mice. *Indian Journal of Experimental Biology* **49 (2)**, 125-31

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany* **53 (372)**, 1351-1365

Schützendübel A, Schwanz P, Teichmann T, Gross K, Langenfeld- Heyser R, Goldbold DL, Polle A (2001) Cadmium induced changes in antioxidant systems hydrogen peroxide content and differentiation in Scot pine roots. *Plant Physiology* **127**, 887-898

Sevengor S, Yasar F, Kusvuran S, Ellialtioglu S (2011) The effect of salt stress on growth, chlorophyll lipid peroxidation and antioxidative enzymes of pumpkin seedling. *African Journal of Agricultural Research* **6 (21)**, 4920-4924

Shao HB, Chu LY, Lu ZH, Kang CM (2008) Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. *International Journal of Biological Science* **4 (1)**, 8-14

Sharma I, Pati PK, Bhardwaj R (2010) Regulation of growth and antioxidant

enzyme activities by 28-homobrassinolide in seedlings of *Raphanus sativus* L under cadmium stress. *Indian Journal of Biochemistry and Biophysics* **47 (3)**, 172-177

Shevyakova NI, Rakitin VY, Stetsenko LA, Aronova EE, Kuznetsov VV (2006) Oxidative stress and fluctuations of free and conjugated polyamines in the halophyte *Mesembryanthemum crystallinum* L. under NaCl salinity. *Plant Growth Regulation* **50**, 69-78

Shi Q, Ding F, Wang X, Wei M (2007) Exogenous nitric oxide protect cucumber roots against oxidative stress induced by salt stress. *Plant Physiology and Biochemistry* **45**, 542-550

Shi X, Dalal NS, Kasprzak KS (1993) Generation of free radicals from hydrogen peroxide and lipid hydroperoxides in the presence of Cr(III). *Biochimica et Biophysica Acta* **302**, 294-299

Shiyab S, Chen J, Han FX, Monts DL, Matta FB, Gu M, Su Y, Masad MA (2009) Mercury-induced oxidative stress in Indian mustard (*Brassica juncea* L.). *Environmental Toxicology* **24 (5)**, 462-471

Siddiqui MH, Al-Whaibi MH, Basalah MO (2011) Interactive effect of calcium and gibberellins on nickel tolerance in relation to antioxidant system in *Tritium aestivum* L. *Protoplasma* **248 (3)**, 503-511

Singh HP, Batish DR, Kaur G, Arora K, Kohli RK (2008) Nitric oxide (as sodium nitroprusside) supplementation ameliorates Cd toxicity in hydroponically grown wheat roots. *Environmental and Experimental Botany* **63**, 158-167

Smirnoff N (1995) *Environment and Plant Metabolism: Flexibility and Acclimation*, BIOS Scientific Publishers Ltd., Oxford, 270 pp

Sobkowiak R, Deckert J (2003) Cadmium-induced changes in growth and cell cycle gene expression in suspension-culture cells of soybean. *Plant Physiology and Biochemistry* **41**, 767-772

Solanki R, Anju P, Dhankhar R (2011) Zinc and copper induced changes in physiological characteristics of *Vigna mungo* (L.). *Journal of Environmental Biology* **32**, 747-751

Srivastava M, Ma LQ, Singh N, Singh S (2005) Antioxidant responses of hyper-accumulator and sensitive fern species to arsenic. *Journal of Experimental Botany* **56 (415)**, 1335-1342

Steffens B, Geske T, Sauter M (2010) Aerenchyma formation in the rice stem and its promotion by H_2O_2. *New Phytologist* **190 (2)**, 369-378

Stockinger EJ, Gilmour SJ, Thomashow MF (1997) *Arabidopsis thaliana CBF1* encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/ DRE a *cis*-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. *Proceedings of the National Academy of Sciences of USA* **94**, 1035-1040

Sukalovic VHT, Vuletic M, Vucinic Z (2005) The role of *p*-coumaric acid in oxidative and peroxidative cycle of the ionically bound peroxidase of the maize root cell wall. *Plant Science* **168**, 931-938

Sun Z, Li Y, Zhou J, Zhu SH (2011) Effects of exogenous nitric oxide on contents of soluble sugars and related enzyme activities in 'Feicheng' peach fruit. *Journal of Science Food and Agriculture* **91 (10)**, 1795-800

Surpin M, Larkin RM, Chory J (2002) Signal transduction between the chloroplast and the nucleus. *The Plant Cell* **14**, 327-338

Takahashi S, Yamasaki H (2002) Reversible inhibition of photophosphorylation in chloroplasts by nitric oxide. *FEBS Letters* **512**, 145-148

Tewari RK, Hahn EJ, Paek KY (2008) Modulation of copper toxicityinduced oxidative damage by nitric oxide supply in the adventitious roots of *Panax ginseng*. *Plant Cell Reports* **27**, 171-181

Thompson JE, Paliyath G, Brown JH, Duxbury CL (1987) The involvement of active oxygen in membrane deterioration during senescence. In: Thomson WW, Nothnogel EA, Huffaker RC (Eds) *Plant Senescence: Its Biochemistry and Physiology*, American Society of Plant Physiologists, Rockville, pp 146-155

Tian QY, Sun DH, Zhao MG, Zhang WH (2007) Inhibition of nitric oxide synthase (NOS) underlies aluminum-induced inhibition of root elongation in *Hibiscus moscheutos*. *New Phytologist* **174**, 322-331

Tian X, Lei Y (2006) Nitric oxide treatment alleviates drought stress in wheat seedlings. *Biologia Plantarum* **50**, 775-778

Tossi V, Amenta M, Lamattina L, Cassia R (2011) Nitric oxide enhances plant ultraviolet-B protection up-regulating gene expression of the phenylpropanoid biosynthetic pathway. *Plant, Cell and Environment* **34 (6)**, 909-921

Trewavas AJ, Malhó R (1997) Signal perception and transduction: The origin of the phenotype. *Plant Cell* **9**, 1181-1195

Turner JG, Ellis CH, Devoto A (2002) The jasmonate signal pathway. *Plant Cell* **14**, 153-164

Tuteja N, Sopory SK (2008) Chemical signaling under abiotic stress environment in plants. *Plant Signaling and Behaviour* **3 (8)**, 525-536

Upham BL, Jahnke LS, Foster JG (1990) Interaction of ascorbate and chelated iron in a methylviologen-mediated Mehler reaction. *Photochemistry and Photobiology* **52 (4)**, 691-696

Usha B, Venkataraman G, Parida A (2009) Heavy metal and abiotic stress inducible metallothionein isoforms from *Prosopis julixora* (SW) D.C. show diverences in binding to heavy metals *in vitro*. *Molecular Genetics and Genomics* **281**, 99-108

Van Assche F, Clijsters H (1990) Effects of metals on enzyme activity in plants. *Plant Cell and Environment* **13**, 195-206

Van Assche H, Put C, Clijsters H (1986) Heavy metals induce specific isozyme patterns of peroxidase in *Phaseolus vulgaris* L. *Archieves Internationale de Physiologie et de Biochimie* **94**, 60-68

Vandelle E, Poinssot B, Wendehenne D, Bentejac M, Pugin A (2006) Integrated signaling network involving calcium nitric oxide and active oxygen species but not mitogen-activated protein kinases in BcPG1-elicited grapevine defenses. *Molecular Plant-Microbe Interactions* **19**, 429-440

Vangronsveld J, Weckx J, Kubacka-Zebalska M, Clijsters H (1993) Heavy metal induction of ethylene production and stress enzymes: II. Is ethylene involved in the signal transduction from stress perception to stress responses? In: Pech JC, Latache A, Balague C (Eds) *Cellular and Molecular Aspects of the Plant Hormone Ethylene*, Kluwer Academic Publishers, Dordrecht, pp 240-246

Veitch N (2004) Horseradish peroxidase: A modern view of a classical enzyme. *Phytochemistry* **65**, 249-259

Verma G, Sharma S (2010) Role of H_2O_2 and cell wall monoamine oxidases in germination of *Vigna radiata* seeds. *Indian Journal of Biochemistry and Biophysics* **47 (4)**, 249-53

Verma P, Watanabe T, Honda Y, Watanabe T (2011) Microwave-assisted pretreatment of woody biomass with ammonium molybdate activated by H_2O_2. *Bioresource Technology* **102 (4)**, 3941-3945

Wagner AM (1995) A role for active oxygen species as second messengers in the induction of alternative oxidase gene expression in *Petunia hybrida* cells. *FEBS Letters* **368**, 339-342

Wang L, Yang L, Yang F, Li X, Song Y, Wang X, Hu X (2010) Involvement of H_2O_2 and metallothionein in NO-mediated tomato tolerance to copper toxicity. *Journal of Plant Physiology* **167 (15)**, 1298-1306

Wang SH, Zhang H, Zhang Q, Jin GM, Jiang SJ, Jiang D, He QY, Li ZP (2011) Copper-induced oxidative stress and responses of the antioxidant system in roots of *Medicago sativa*. *Journal of Agronomy and Crop Science* **197 (6)**, 418-429

Wang Y, Feng H, Qu Y, Cheng J, Zhao Z, Zhang M, Wang X, An L (2006) The relationship between reactive oxygen species and nitric oxide in ultraviolet-B-induced ethylene production in leaves of maize seedlings. *Environmental and Experimental Botany* **57**, 51-61

Wang Y, He W, Huang H, An L, Wang D, Zhang F (2009) Antioxidative responses to different altitudes in leaves of alpine plant *Polygonum viviparum* in summer. *Acta Physiologia Plantarum* **31**, 839-848

Wang YS, Yang ZM (2005) Nitric oxide reduces aluminum toxicity by preventing oxidative stress in the roots of *Cassia tora* L. *Plant and Cell Physiology* **46**, 1915-1923

Watanabe M, Veen SVD, Nakajima H, Abee T (2012) Effect of respiration and manganese on oxidative stress resistance of *Lactobacillus plantarum* WCFS1. *Microbiology* **158**, 293-300

Weckx J, Clijsters H (1996) Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. *Physiologia Plantarum* **96**, 506-512

Weckx JEJ, Clijsters HMM (1997) Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. *Plant Physiology and Biochemistry* **35**, 405-410

Wendehenne D, Durner J, Klessig DF (2004) Nitric oxide: A new player in plant signalling and defence responses. *Current Opinion in Plant Biology* **7**, 449-455

Wong HL, Pinontoan R, Hayashi K, Tabata R, Yaeno T, Hasegawa K, Kojima C, Yoshioka H, Iba K, Kawasaki T, Shimamoto K (2007) Regulation of rice NADPH oxidase by binding of Rac GTPase to its N-terminal extension. *Plant Cell* **19 (12)**, 4022-4034

Woolhouse HW (1983) Toxicity and tolerance in the responses of plants to metals. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (Eds) *Encyclopedia of Plant Physiology* (New Series Vol 12C), Springer, Berlin, pp 245-300

Wu XX, Zhu WM, Zhang H, Ding HD, Zhang HJ (2011) Exogenous nitric oxide protects against salt-induced oxidative stress in the leaves from two genotypes of tomato (*Lycopersicom esculentum Mill*). *Acta Physiologia Plantarum* **33**, 1199-1209

Xie ZX, Chen ZX (1999) Salicylic acid induces rapid inhibition of mitochondrial electron transport and oxidative phosphorylation in tobacco cells. *Plant Physiology* **120**, 217-225

Xiong J, An L, Lu H, Zhu C (2009) Exogenous nitric oxide enhances cadmium tolerance of rice by increasing pectin and hemicelluloses contents in root cell wall. *Planta* **230**, 755-765

Xiong J, Fu G, Yang Y, Zhu C, Tao L (2012) Tungstate: Is it really a specific nitrate reductase inhibitor in plant nitric oxide research? *Journal of Experimental Botany* **63 (1)**, 33-41

Xiong L, Schumaker KS, Zhu JK (2002) Cell signaling during cold drought and salt stress. *Plant Cell* **14**, 165-183

Yadav SK (2010) Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African Journal of Botany* **76**, 167-179

Yamasaki H (2000) Nitrite-dependent nitric oxide production pathway: Implications for involvement of active nitrogen species in photoinhibition *in vivo*. *Philosophical Transactions of the Royal Society of London: Biological Sciences* **355**, 1477-1488

Yamasaki H, Sakihama Y (2000) Simultaneous production of nitric oxide and

peroxynitrite by plant nitrate reductase: *In vitro* evidence for the NR-dependent formation of active nitrogen species. *FEBS Letters* **468**, 89-92

Yang JD, Yun JY, Zhang TH, Zhao HL (2006) Presoaking with nitric oxide donor SNP alleviates heat shock damages in mung bean leaf discs. *Botanical Studies* **47**, 129-136

Yang T, Poovaiah BW (2002) Hydrogen peroxide homeostasis: Activation of plant catalase by calcium/calmodulin. *Proceedings of the National Academy of Sciences USA* **99**, 4097-4102

Ye Y, Wang L, Huang X, Lu T, Ding X, Zhou Q, Guo S (2008) Subcellular location of horseradish peroxidase leaves treated with La(III), Ce(III) and Tb(III). *Ecotoxicology and Environmental Safety* **71 (3)**, 677-684

Yu CC, Hung KT, Kao CH (2005) Nitric oxide reduces Cu toxicity and Cu-induced NH^{4+} accumulation in rice leaves. *Journal of Plant Physiology* **162**, 1319-1330

Zemojtel T, Frohlich A, Palmieri MC, Kolanczyk M, Mikula I, Wyrwicz LS, Wanker EE, Mundlos S, Vingron M, Martasek P, Durner J (2006) Plant nitric oxide synthase: A never-ending story? *Trends in Plant Science* **11**, 524-528

Zhang A, Jiang M, Zhang J, Ding H, Xu S, Hu X, Tan M (2007) Nitric oxide induced by hydrogen peroxide mediates abscisic acid-induced activation of mitogen-activated protein kinase cascade involved in antioxidant defense in maize leaves. *New Phytologist* **175**, 36-50

Zhang AY, Jiang MY, Zhang JH, Tan MP, Hu XL (2006) Mitogen-activated protein kinase is involved in abscisic acid-induced antioxidant defense and acts downstream of reactive oxygen species production in leaves of maize plants. *Plant Physiology* **141**, 475-487

Zhang F, Wang Y, Wang D (2007) Role of nitric oxide and hydrogen peroxide during the salt resistance response plant. *Signaling and Behavior* **2 (6)**, 473-474

Zhang F, Wang Y, Yang Y, Wu H, Wang Di, Liu J (2007) Involvement of hydrogen peroxide and nitric oxide in salt resistance in the calluses from *Populus euphratica*. *Plant Cell and Environment* **30**, 775-785

Zhang H, Li YH, Hu LY, Wang SH, Zhang FQ, Hu KD (2008b) Effects of exogenous nitric oxide donor on antioxidant metabolism in wheat leaves under aluminum stress. *Russian Journal of Plant Physiology* **55**, 469-474

Zhang LP, Mehta SK, Liu ZP, Yang ZM (2008a) Copper-induced proline synthesis is associated with nitric oxide generation in *Chlamydomonas reinhardtii*. *Plant and Cell Physiology* **49**, 411-419

Zhang YK, Cui XM, Yang SX, Chen XL (2010) Effects of exogenous nitric oxide on active oxygen metabolism and photosynthetic characteristics of tomato seedlings under cadmium stress. *Ying Yong Sheng Tai Xue Bao* **21 (6)**, 1432-1438

Zhang YX, Zhang HM, Huang ZB, Li LF, Liu JG, Li X (2011) Antioxidative enzymes play key roles in cadmium tolerance of *Phytolacca*. *Americana Huan Jing Ke Xue* **32 (3)**, 896-900

Zhao SY, Blumwald E (1998) Changes in oxidation-reduction state and antioxidant enzymes in the roots of jack pine seedlings during cold acclimation. *Physiologia Plantarum* **104**, 134-142

Zheng C, Jiang D, Liu F, Dai T, Liu W, Jing Q, Cao W (2009) Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. *Environmental and Experimental Botany* **67**, 222-227