Biochemical defence mechanisms of plants to increased levels of ozone and other atmospheric pollutants

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Some of the atmospheric gases at their supra optimum level become pollutants and evoke various types of 'visible' and 'hidden' plant responses which ultimately lead to reduced plant growth and productivity. The biochemical mechanism of the action of the pollutants is better understood now than it was about a decade ago. Participation of some of the signal molecules such as of H_2O_2 and salicylic acid is also known. It is believed that the generation of oxy-free radicals is the principal biochemical event in responses of plants to any kind of environmental stress. Plants respond through increased activities and levels of antioxidant enzymes and metabolites, so that the oxy-free radicals are removed and minimum damage is done. Attempts have been made to modify the plant's responses to pollutant gases, through alteration in the levels of enzymes and metabolites involved in free radical scavenging. While earlier attempts were largely limited to agronomic practices or in some cases to breeding programmes, attempts are now being made to produce transgenics and mutants with increased tolerance to the pollutants.

THE problem of air pollution has been a major environmental hazard of industrialization and urbanization during the 20th century, affecting adversely both physical as well as biological systems. The important pollutant gases are O_3, SO_2, NO_x and peroxyacetyl nitrate (PAN), although several other gases such as H_2S, HF, NH_3, CH_4, etc. also acquire importance in some localized areas during episodic and accidental releases. The phytotoxicity of most of these gases and their adverse effects on agricultural crops and forest trees are well documented, which depend primarily on the dose of the pollutant and on the species[1,2]. At acute dosage, which vary according to the pollutant and the plant species, severe morpho-physiological aberrations such as yellow, brown or necrotic patches or bleaching of the leaves, acceleration of senescence and reduction in growth and productivity are apparent. But at chronic dosage, only aberrations at metabolic and enzymic levels, such as inhibition of photosynthesis and overall gas exchange, of protein synthesis and of ribulose bis phosphate carboxylase, nitrogenase, etc. are recorded[2–4], which of course may lead ultimately to qualitative and sometimes quantitative losses in plant products. The plants absorb large quantities of air pollutants, acting as their natural sinks and thus 'purifying' the air for other forms of life. The suggestion of a 'green belt' or a buffer zone of vegetation between industrial establishments and the residential areas is borne out of this property of the plants. However, in order to perform the role of an air cleanser, the plants themselves must be fairly tolerant to these pollutants. Otherwise also, the agriculturally important plants have to be protected from toxic effects of air pollutants to maintain adequate quantity of food and fodder production for humans and cattles. Attempts have been made in the past to increase air pollution tolerance of plants by using a variety of chemicals such as fungicide benomyl[5], triazoles[6], cytokinins[7], polyamines[8], antioxidants[7], stomatal regulating chemical phenyl mercuric acetate[9] and so on. However, modifying plant responses through foliar or soil application of these chemicals is a cumbersome process and in most cases the protective response is temporary and species-based and effective only against low dosage of the pollutants. Recent developments in the understanding of plant responses at molecular level and in DNA technology have raised the possibilities of modifying these responses through genetic manipulations. There has been only a limited sucess in producing genetically transformed plants with increased tolerance, so far, although the future posssibilities seem to be bright. The literature pertaining to these aspects of plant responses to air pollutants has been reviewed in this article.

The biochemical mechanism of protective action of plants

The morpho-physiological responses of plants may vary according to the nature and the dose of the pollutant and the species. However, at biochemical and molecular

levels, there appears to be a similarity among different pollutants and also among most of the environmental stresses. Several lines of evidences indicate that most air pollutants enter the plant tissues and act primarily through the production of reactive oxygen species (ROS) also called oxidative-free radicals, so is the case with most other abiotic stresses[10]. Three important ROS, superoxide anion (O_2^-), hydroxy-free radical (OH^-) and H_2O_2 are highly toxic and cause changes in DNA, proteins and lipids and in membrane organizations[11]. The generation of superoxide anion in chloroplasts is believed to take place in normal unstressed photosynthetic conditions also, by photoreduction of O_2 at PS I and PS II, when the energy from the triplet excited state of chlorophyll is transferred to O_2 (ref. 12). Superoxide anion is a charged molecule and cannot cross biological membranes. Thus, it is to be removed at the site of its generation. Hydroxyl ion is also a charged molecule and is highly reactive. Hydrogen peroxide does not exhibit radical properties and is relatively a stable ROS. However, it is a strong nucleophilic oxidizing agent, and oxidation of –SH group in enzymes and other proteins is considered to be a major mode of its phytotoxic action[13]. Evidences for the production of ROS during exposure to air pollutants specially with O_3 have been obtained through several investigations. Various modes of ROS production in the O_3 polluted enviroment are: (i) production of OH^- from the dissolution of O_3 in the cell sap at physiological pH[14], (ii) production of OH^- from the reactions of O_3 with terpenes[15] or with the components of plasma membrane[16], (iii) production of ROS from interaction of ethylene (induced during ozone exposure) with O_3 (ref. 17), (iv) production of O_2^- through the activation of plasma membrane associated NADH-dependent superoxide synthase[18] and (v) production of H_2O_2 from the interaction of O_3 with unsaturated fatty acids[19]. Evidence for the formation of O_2^- has been obtained by Runeckles and Vaartnou[20], who demonstrated the appearance of an EPR signal with typical characteristics of O_2^- in O_3 exposed leaves of different plant species.

Plants try to respond suitably by adjusting their metabolism so that minimum damage is done due to air pollutants. Broadly, two types of protective responses are recorded at molecular level: (i) Increase in antioxidant enzymes and metabolites and (ii) Induction of protection-related secondary metabolite genes. If the plants are able to express these responses adequately, pollution-induced 'visible' or 'hidden' damages do not occur. However, if these protective responses are inadequate and are unable to cope with the incidence and the dose of the pollutant, the injury occurs. These responses therefore, can be compared to immune responses in animals, which of course are evoked in response to pathogens. The role of various antioxidants and metabolites and enzymes, in scavenging of ROS, is described in the following paragraphs.

Antioxidant enzymes and metabolites of Asada–Haliwell cycle

The increase in enzymes and metabolites involved in the scavenging of ROS in response to some air pollutants and other oxidative stresses has been reported in several studies[21,22]. The first reaction in detoxification of superoxide anion is its conversion (a dismutation reaction) to H_2O_2 by the enzyme superoxide dismutase (SOD, 1.15.1.1.). Initially this enzyme was considered to be an intracellular enzyme, but in the recent past its occurrence in extracellular matrices has also been reported in *Pinus sylvestris*[23,24] and in spinach[25]. In pine needles, the extracellular Cu–Zn SOD represents only about 0.1% of the total Cu–Zn SOD in these needles[23,24]. But, even at such a small concentration it may play a significant role in free radical scavenging. At least three types of SODs with several isoforms are present in plants. These are: (i) chloroplastic or cytosolic Cu–Zn SOD; the cytosolic Cu–Zn SOD is referred to as Cu–Zn SOD I and while chloroplastic one is referred to as Cu–Zn SOD II. (ii) mitochondrial Mn SOD and (iii) chloroplastic Fe SOD[26]. The amino acid sequence of Fe–SOD and Mn–SOD apoproteins are similar, whereas Cu–Zn SOD is different. The genes for different types of SODs are also identified. For example, *Arabidopsis* contains multiple SOD genes encoding at least three Cu–Zn SODs, three Fe–SODs and one Mn–SOD[27]. Hydrogen peroxide can also be formed non-enzymatically from O_2^-, specially in the peroxisomes and mitochondria[28]. Increase in one or the other type of SOD activities in response to air pollutants has been demonstrated in some investigations at both acute as well as chronic dosage of the pollutant (Table 1). Ethylene diurea, a compound which confers tolerance to O_3 susceptible plants also causes an increase in SOD activity in bean leaves[29].

Besides affecting through dismutation of O_2^-, there is evidence that the extracellular SODs might as well be directly involved in nitric oxide (NO) metabolism. Superoxide anion radicals react with nitric oxide to form a more toxic peroxynitrite[30]. Nitric oxide in the environment may be present as an air pollutant and may enter the plant cells as such. Endogenous nitric oxide has recently been detected in pea leaves[31], and is proposed to be partitioned in the apoplast[32]. The extracellular SOD in the apoplast may inhibit the formation of toxic peroxynitrite by dismutating the O_2^- in to H_2O_2.

Hydrogen peroxide is not the final product in the series of reactions involved in the scavenging of ROS. It is further reduced to H_2O by catalases in peroxisomes and by ascorbate peroxidase in the chloroplasts and cytosols.

Table 1. Effect of atmospheric pollutants on superoxide dismutase activity

Plant system	Pollutant level [nl l^{-1}]	Response	Ref.
Ozone			
Arabidopsis thaliana	330 for 8 h	Increase	27
Glycine max	200 for 4 h	No effect	123
Hypogymnia physodes (a lichen)	Varying levels (field study)	Increase	124
Nicotiana tabaccum	125 for 6 h	Increase	125
Phaseolus vulgaris	20–50 pretreatment and then 400 for 6 h	No effect	21
Picea abies	160 for 7 h per day for 30 days	Increase	126
	600 for 16 h per day for 4 weeks	Increase	127
	75–600 for 5 weeks	No effect	128
	80 for 6 months	Decrease	129
Picea rubens	*Ca* 90 for 7 months	Decrease	58
Pinus sylvestris	300 for 8 h per day for 5 days	No effect	130
Pinus taeda	*Ca* 129 for 3 months	Increase	131
Populus spp.	150 for 1.5 h	Increase	57
Spinacea oleracea	125–250 for 6 h	Increase	132
	500 for 8 h	Decrease	133
	100 for 7 days	Decrease	38
Trifolium repens	20–80 for 8 h per day for 8 days	No effect	134
	60 for 8 h per day for 33 days	No effect	135
Zea mays	500 for 8 h	No effect	136
Sulphur dioxide			
Pisum sativum	800 for 3.5 h	Increase	56

The ultimate scavenging of H_2O_2 involves at least two antioxidants, ascorbate and reduced glutathione. The involvement of these oxidants and their cyclic regeneration for further involvement in free radical scavenging[12,33] is popularly known as Asada–Halliwell cycle (Figure 1).

Ascorbate peroxidase (E.C. 1.11.1.11), a heme protein, is one of the important peroxidases, of ubiquitous occurrence in plants. It is regarded as a universal housekeeping protein in the cytosol and chloroplasts of plant cells. In the cytosol of nitrogen fixing root nodules, where ROS are produced under unstressed conditions also, it may constitute up to 1% of the total protein[34]. Ascorbate peroxidases use ascorbate as a substrate and are believed to scavenge excess of H_2O_2 formed in plant cells under both normal and stress conditions[35]. The product of ascorbate oxidation by ascorbate peroxidase is an ascorbate-free radical which is reduced back to dehydroascorbate by the enzyme monohydroascorbate reductase with NAD(P)H as the electron donor[36]. Increase in ascorbate peroxidase (E.C. 1.8.5.1) activity in response to air pollutants specially with O_3 has been demonstrated in several species such as in wheat[37], spinach[38], pumpkin[39], and *Picea abies*[40]. An increase in apoplastic ascorbate peroxidase in response to O_3 (100 nl l^{-1} for 10 days) has been reported in bean[41]. The protective action of ascorbate in the response of plants to O_3 was realized as early as in 1964 by Menser[42]. Its concentration in the chloroplasts is quite high (10–20 mM), and apart from its obvious role as a substrate for ascorbate peroxidase, it can react chemically with almost all forms of ROS[43]. Rapid changes in the concentration or redox status of ascorbate have also been reported in response to O_3 (refs 44–47) and to NO_2 (ref. 48). Bean genotypes tolerant to O_3 stress had a higher ascorbate content than the susceptible genotypes[49]. However, the exposure of Norway spruce and red spruce to 37 nl l^{-1} O_3 for 12 h a day during the summer (on sunny days) for two years had no effect on the ascorbate content[50]. It appears that the response of ascorbate is dependent upon its endogenous level in the plant and also to the dose of the pollutant. For example, ascorbate deficient mutants of *Arabidopsis* are more susceptible to O_3 injury than the wild type with normal ascorbate concentrations[51]. The peroxidases are usually intracellular in location, where they can detoxify H_2O_2 (refs 13, 52), although in many species including *Sedum album*[53], bean[41] and in Norway spruce (*Picea abies*)[46], the metabolism of H_2O_2 by extracellular or apoplastic ascorbate peroxidase has also been reported. In a study with *Cucurbita pepo* exposed to 150 nl l^{-1} O_3 for 5 h day^{-1} for 5 days, the ascorbate peroxidase activity and the ascorbic–dehydroascorbic acid system increased in the extracellular matrix of young as well as mature leaves, while at the intracellular level only small changes in the metabolites were recorded[54]. So even if the air pollutant is unable to penetrate the leaf cells and if it produces toxic ROS in the apoplastic environment itself, the peroxidases may take care of the ROS. Such a situation is

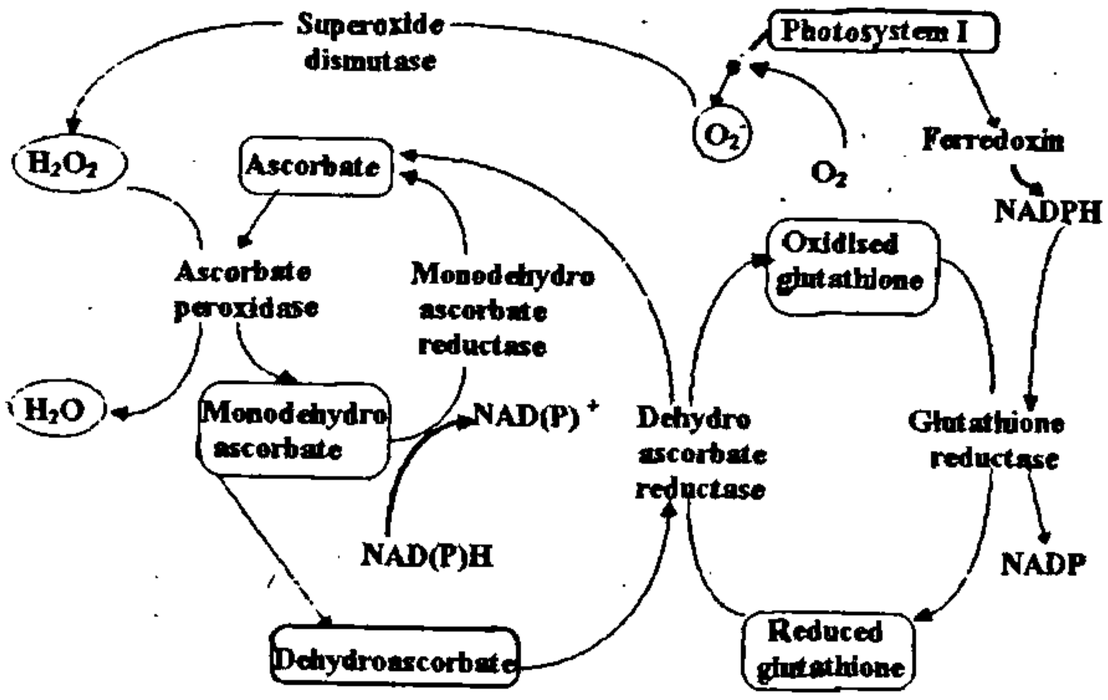

Figure 1. Ascorbate-glutathione (Asada–Haliwell) cycle showing photosynthetic generation of reactive oxygen species and their scavenging. (e^- = electrons).

often realized with O_3. As mentioned earlier, significant activity of SOD has also been detected in the apoplasts[23–25] and thus the apoplast may play an active role in neutralizing the free radical toxicity, before they enter the cellular environment. This might have been evolved as a safety device by the plants.

The tripeptide thiol glutathione is another important low molecular weight oxidant, which has many functions in plants including stress management[55]. Reduced glutathione can readily undergo oxidation and is believed to participate in the maintenance of redox balance of the cell and in the free radical scavenging cycle primarily because it is able to convert dehydroascorbate to ascorbate (Figure 1). Possible involvement of glutathione and glutathione reductase (E.C. 1.6.4.2) in tolerance towards SO_2 has been demonstrated in pea. Two pea cultivars, a sensitive 'nugget' and insensitive 'progress' exposed to 8 ml l^{-1} SO_2 up to 3.5 h showed increased glutathione biosynthesis and glutathione reductase activity, the insensitive cultivar 'progress' showing better response[56]. Increased glutathione level has also been detected in response to O_3 in *Populus*[57] and red spruce needles[58]. Further, the induction of glutathione reductase has also been demonstrated in response to ozone in spinach[59]. Elevated levels of total glutathione and enhanced activities of ascorbate peroxidase were also found in the needles of red spruce which had been exposed to acidic mists[60]. However, in the leaves of *Cucurbita pepo* exposed to 150 nl l^{-1} O_3 for 5 h day^{-1} for 5 days, there was no change in the glutathione level[54]. Another possible mode of glutathione action in stress management is through its direct participation in H_2O_2 reduction catalysed by the enzyme glutathione peroxidase. Glutathione peroxidase activity and sequences encoding glutathione peroxidase-like genes have been demonstrated in several species including *Nicotiana sylvestris, Citrus sinensis, Arabidopsis thaliana, Avena fatua,* and *Brassica compestris*, indicating that glutathione peroxidase is present in plants[61]. Recently Roeckel-Drevet *et al.*[62] have generated gluathione peroxidase encoding cDNA fragments from *Helianthus annuus* hypocotyl RNA using reverse transcription amplification strategy. Perhaps glutathione peroxidase is present in plants to back up ascorbate peroxidase and catalase systems which are primarily responsible for the oxidation of H_2O_2.

Attempts have been made to understand the molecular mechanism of the increase in ROS scavenging enzymes in response to the air pollutants. In many experiments, the increase in enzymes has been demonstrated to be at the gene level[63]. In *Arabidopsis thaliana,* RNA blot analysis has demonstrated that mRNA levels for several defence-related enzymes and of cytosolic Cu–Zn SOD and neutral peroxidase are found to be higher in plants treated with 300 nl l^{-1} O_3 than in ambient air treated controls[64]. In *Helianthus*, wounding or infection with the fungus *Plasmopara halstedii* induces glutathione per oxidase-like transcripts[62]. However, post-transcriptiona regulation of SOD expression in response to SO_2 ha: been demonstrated in pea[65].

Flavonoids

Flavonoids are one of the most common secondary metabolites in higher plants. Several *in vitro* studies have demonstrated that flavonoids can directly scavenge ROS including superoxide anion, hydrogen peroxide and hydroxy radical[66,67]. This is apparently through the participation of flavonoids in peroxidase-mediated catabolism of H_2O_2, where flavonoids may act as electron donors[68,69]. Two major flavonoids, quercetin and kaempferol and their glycosides could be oxidized by H_2O_2 in the presence of horse radish peroxidase or in a cell-free extract of the leaves[70]. The leaf extract apparently contains a peroxidase which could use flavonols as a reducing agent for the oxidation of H_2O_2. The flavonol could be reduced back by the ascorbate. The following scheme of reactions has been proposed[70,71] for the scavenging of H_2O_2 by flavonols (flav) involving ascorbic acid:

$$2\ \text{Flav OH} + H_2O_2 \rightarrow 2\ \text{Flav O}\cdot + 2\ H_2O$$

$$2\ \text{Flav O}\cdot + 2\ \text{ascorbic acid} \rightarrow 2\ \text{Flav OH} + 2\ \text{monodehydroascorbic acid radical}$$

$$2\ \text{monodehydroascorbic acid radical} \rightarrow \text{ascorbic acid} + \text{dehydroascorbic acid}$$

$$H_2O_2 + \text{ascorbic acid} \rightarrow 2\ H_2O + \text{dehydroascorbic acid}$$

where Flav OH is a flavonoid containing a free hydroxyl group and Flav O· is a flavanoid phenoxy radical. The enzymes catalysing these reactions have not been characterized.

Increase in flavonoid content of the plants in response to various types of stresses including exposure to the air pollutant ozone has been demonstrated[72]. These observations suggest that flavonoids may be involved as a defence element against abiotic stresses including air pollutants, although experiments involving air pollutants other than O_3 are lacking to support this suggestion.

Polyamines

Polyamines are present in plant cells in millimolar concentrations, and they can act as free radical scavengers either directly[73] or after interacting with other molecules such as free ferulic and caffeic acids[74]. Increase in polyamine level in response to air pollutants has been

observed in pea in response to SO_2 (ref. 75), in tobacco in response to ozone[76] and in *Azola–Anabaena* symbiont in response to NO_2 (ref. 77). In O_3 tolerant tobacco cultivar Bel B, conjugates of polyamines with hydroxycinnamic acid have been detected, which are better scavengers of O_3 and oxy-free radicals than the polyamine itself[76]. Exogenous supply of the polyamines putrescine, spermine and spermidine could protect the tomato plants against ozone-induced damage[78]. Also, in bean plants, the NO_2-induced decline in leaf growth could be prevented to some extent by the supply of polyamines spermine and spermidine[8]. However, the exact mechanism of the protection against air pollutants, by polyamines is not understood. Various possibilities as suggested by Ye *et al.*[79] for the protection by polyamines against oxidants include: (i) scavenging of ROS, (ii) increasing the permeation of antioxidant enzyme SOD through the membranes, (iii) protecting the membranes against oxidant damage, (iv) changing the redox state of the cells or (v) regulating the expression of genes.

Cell wall metabolites

The plant cell walls provide a mechanical barrier to the entry of air pollutants inside the cells. In some instances it has been found that the plants respond through the modification of the structure and the permeation of cell wall and cell membranes so that the entry of the air pollutant inside the cell is restricted. Exposure to air pollutants, specially to O_3 has been demonstrated to induce changes in wound and pathogen-related secondary metabolism such as phenylpropanoid, flavonoid and lignin biosynthesis pathways which are responsible for the synthesis of many potentially protective compounds such as phytoalexins and lignin[80]. These responses are also non-specific and common to many pathogenic stimuli as well.

Ethylene plays an important role in cell wall metabolism and accumulation of ethylene in response to O_3 has been reported in tobacco[76]. Increased ethylene production has been reported in response to NO_2 in rice[81]. Besides taking part in the cell wall metabolism, ethylene may also act as a signal molecule in O_3 response[82].

Guaiacol peroxidases, another class of peroxidases, are also involved in the protective action in plants. They participate in lignification of cell wall, degradation of IAA, biosynthesis of ethylene, wound healing and defence against pathogens[83,84].

Gene induction by air pollutants has been demonstrated in the woody species *Atriplex canescens* also[85]. In this species, two c-DNA clones O12-2 and O1 14-3 were induced by O_3, SO_2 and water deficit. These genes code for glycine-rich proteins which are associated with the cell wall. This alteration in the cell wall metabolism could be linked with the cell wall elasticity which is induced by O_3 (ref. 86) as well as by water deficit[87].

Transgenics and mutants with increased air pollution tolerance

Various approaches and strategies in the production of genetically transformed crop plants tolerant to abiotic stresses have been described in a recent publication by Grover *et al.*[88]. As far as the atmospheric pollutants are concerned, attempts have been made to genetically engineer the plants with higher SOD levels with a view to increasing tolerance to O_3. However, in a study with tobacco, variety W_{38} engineered to overproduce chloroplastic Cu–Zn SOD by 15 folds in the chloroplasts was equally sensitive to O_3 as the non-engineered one[89]. On the other hand, in PBD 6 variety of tobacco, there was a 3–4 fold reduction in visible necrotic damage when chloroplastic Mn–SOD was overproduced by genetic manipulations, although over-production of mitochondrial SOD had little effect on O_3 tolerance[90]. This may be due to difference in either the response of two varieties or due to the dosage used in the two experiments[89,90]. In another set of experiments, Pitcher and Zilinskas[91] produced transgenic Bel W_3 and Wisconsin 38 varieties of tobacco by introducing pea cytosolic Cu–Zn–SOD c-DNA. Young and recently expanded leaves of transgenic plants of both cultivars which had 2- to 6-fold higher cytosolic SOD activity, showed less foliar necrosis than non-transformed controls when exposed to 200 to 300 nl l^{-1} O_3 for 4.5 or 6 h. The authors have suggested that cytosolic Cu/Zn–SOD was important in protecting the integrity of plasma membranes and possibly other cellular constituents. However, a 10 fold over production of chloroplastic ascorbate peroxidase in transgenic tobacco (*Nicotiana tabaccum* cv. Bel W_3) did not protect the plants against O_3 injury[92]. There were no significant differences in O_3 response of transgenic and the non-transgenic plants based on visible injury and on some physiological parameters. On the other hand, Tepperman and Dunsmuir[93] showed that transgenic petunia overexpressing chloroplastic Cu/Zn–SOD by 40 times were more sensitive to O_3 than the non-transformed plants. This might be due to accumulation of H_2O_2 in these transgenics since both Cu/Zn–SOD and ascorbate peroxidase are inhibited by H_2O_2 (ref. 94).

Transgenics with elevated levels of free radical scavenging enzymes and metabolites have been produced to assess the role of these enzymes and metabolites in tolerance to other stresses also. In a recent publication, Mock *et al.*[95] have produced transgenic tobacco by using anti-sense RNA technology, which had increased levels of cytosolic Cu/Zn–SOD and mitochondrial Mn–SOD than the wild type. Most other enzymes of the Asada–Halliwell cycle also had higher levels in the transfor-

mants. Despite the elevated enzyme activities, the antioxidative action of tetrapyrroles (an oxidative stress factor) could not be overcome in the transgenics apparently due to decreased levels of ascorbate and glutathione. Apparently a coordinated increase in all the enzymes and metabolites is required for conferring tolerance to the stress.

Trangenics with altered levels of glutathione reductase have also been produced with a view to inducing air pollution tolerance[96,97]. In studies with tobacco, some of the transformants overproducing glutathione and glutathione reductase were found to be tolerant to atmopsheric O_3 but not to SO_2 (ref. 98). In another investigation, Youssefian *et al.*[99] produced transgenic tobacco overexpressing *Cys1*, a wheat cysteine synthase gene. The transgenic had up to 5-fold the cysteine synthase activity of the control plants and showed enhanced tolerance to H_2S-induced damage.

There have been only a few attempts to induce tolerance in plants through induced mutation. The group led by P. J. Lea and A. R. Wellburn at Lancaster University, Lancaster, UK, however, has produced several mutants of barley, some of which are visibly tolerant to the air pollutant NO_2. The mutants B_1 and W_5 did not develop any visible injury even when exposed to up to 5000 nl l^{-1} NO_2 for up to 5 days while the wild type barley developed brownish, reddish streaks and patches on the margin of the leaf by similar exposure[100]. The mutants, however, had smaller root mass than the wild type, indicating thereby that the roots contributed somehow in the development of visible symptoms of injury. Mutants with respect to altered response to other air pollutants are not known.

Signal transduction pathway

The signal transduction pathway in the injury either due to the pollutant itself or due to a secondary response of ROS is not clearly understood. However, experiments conducted with O_3 have demonstrated that molecules like ethylene[82], H_2O_2 and Ca^{2+} (ref. 101), and salicylic acid[102–107], might be involved in the phytotoxic responses of plants to the pollutant. A tentative model linking these possible signal molecules with the O_3 is proposed (Figure 2), which might be applicable for other air pollutants and abiotic stresses also. According to the model, the O_2^- generated in response to the air pollutant is converted rapidly to H_2O_2 which is the most stable amongst ROS and plays a key role in phytotoxic/tolerance responses of the plant. H_2O_2 in turn may affect the level of salicylic acid. Application of high concentration of H_2O_2 is known to increase the biosynthesis of salicylic acid[106], which acts as a signal molecule in acquired resistance to pathogens and/or tolerance to other abiotic stresses[102,108,109]. Accumulation of salicylic acid has been reported during O_3 exposure also, in *Arabidopis thaliana*[103] and in tobacco[104]. The acid might also have a kind of feedback effect on the generation of H_2O_2 (refs 109, 110). Salicylic acid either on its own or through some other signal molecules such as Ca^{2+}, calmodulin, phosphoinositides, cyclic GMP, jasmonic acid, etc. may influence the level of critical enzyme activity/protein leading to the altered metabolism involved in phytotoxic or tolerance responses of the plant. Among other signal molecules, abscisic acid, or a hypothetical molecule 'noxine' have also been proposed to act as secondary messenger molecules in phytotoxic responses of plants to NO_2 (ref. 111). Salicylic acid may act directly through the critical enzyme as well. One of the possible ways of enzyme modulation by salicylic acid may be through the phosphorylation of enzymic proteins[112]. The role of Ca^{2+}, calmodulin and cGMP[113,114] and of phosphoinositide[115,116] in signal tranduction has been demonstrated for several physical and chemical stimuli, in plants and because of their multifunctional roles it is assumed here that they participate in air pollution responses also. However, this has to be demonstrated experimentally. Jasmonic acid is a part of signal transduction system in plants regulating expression of defence-related genes[117,118]. Recently, the possibility of its signalling role in cadmium-induced glutathione biosynthesis has also been explored, although the findings were not supportive of its role[119]. No such studies have been conducted with air pollutants.

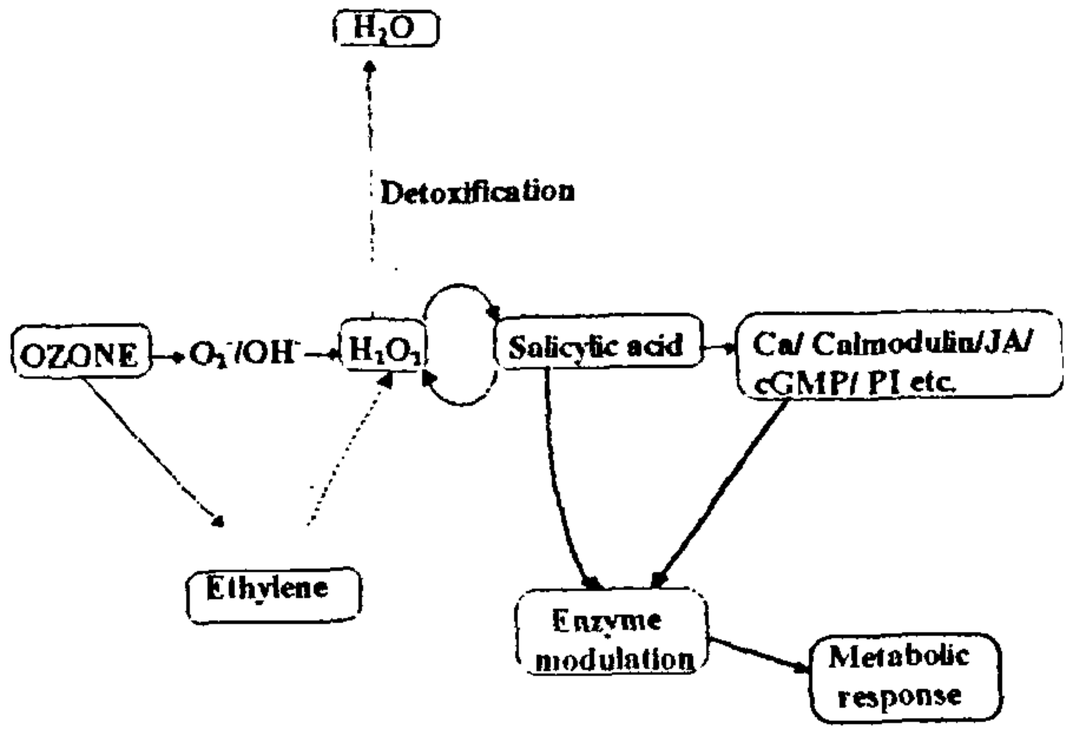

Figure 2. Signal transduction pathway in ozone-induced phytotoxic responses. (cGMP = cyclic GMP; Pi = phosphoinositides).

Alternatively or additionally, the pollutant may act through the generation of ethylene[82] (Figure 2), which may cause the generation of ROS either on its own or through the reaction with the air pollutant. Ethylene is also known to affect a variety of metabolic processes leading towards the toxic/tolerance response of the plant. The messenger role of ethylene in O_3 action has been demonstrated in quite a few investigations. For

example, O_3 exposure did not cause stomatal closure and reduction in carbon assimilation in soybean in the presence of ethylene synthesis inhibitor, 2-aminoethoxy vinyl glycine (AVG), which was otherwise seen in the absence of AVG[120]. Similarily, *N*-2-(2-oxo-1-imidazolidinyl) ethyl-*N*-phenylurea (EDU), which prevents the development of symptoms of O_3 toxicity also prevents O_3-associated ethylene burst[121]. Further, there is a temporal sequence of events in O_3-induced visible damage in sensitive tobacco variety Bel W_3 (ref. 122). In this system, ethylene burst takes place within one h of O_3 exposure, the formation of β-1,4-glucanase takes place 5 h after the O_3 exposure and the necrotic lesions are observed 15–72 h after the O_3 exposure. This simple model, as proposed in Figure 2, however, has to be elaborated as the possibility of the involvement of other regulator/signal molecules is realized in due course of time. The temporal sequence of the inducible expression of various types of signal molecules is also to be determined.

Conclusions and perspectives

It is apparent that at the biochemical level, the generation of ROS is the principal phytotoxic response of plants to air pollutants. As an initial response, the plants potentiate their inherent ROS scavenging mechanism by the activation/induction of enzymes and by the increase in the levels of antioxidant metabolites. If the ROS scavenging metabolism is adequate enough to prevent their accumulation, there is little 'visible' or 'hidden' injury to the plant. The scavenging metabolism seems to be operational in both the apoplast as well as the symplast of the cell. The cellular organelles, chloroplasts and mitochondria also possess the relevant enzymes and metabolites. Only in a few investigations, it has been possible to increase the tolerance of the plant to the air pollutants through genetic manipulation leading to the increased activity of SOD, the first enzyme in ROS metabolism. Perhaps it will be necessary to manipulate genes in such a way that not only the SOD, but all the enzymes and metabolites involved in ROS metabolism are increased.

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