Crop adaptation to air pollution II. Tolerance to SO$_2$ stress is regulated by oxidative and antioxidative characteristics and sulphur assimilation

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ABSTRACT

Sulphur dioxide (SO$_2$) and particulate matter (PM) are one of the major air pollutants emerging out of the industrial development and human activities. Plants exhibit differential sensitivity to SO$_2$ pollution and its effects on plant growth can be both direct and/or indirect. We have earlier reported that a high SO$_2$ stress contributes toward the S-nutrition of crops. The SO$_2$ enriched environment significantly improved the activity of serine transacetylase (SAT) in all the experimental crops, however, the activity of O-acetylserine (thiol) lyase (OAS-TL) was enhanced chiefly in wheat but not in chickpea and barley. Further, the relative tolerance of crops to the particulate and gaseous pollutants was related to a lower level of superoxide and H$_2$O$_2$ radicals and lipid peroxidation and a higher level of antioxidants such as ascorbic acid and peroxidase activity. Relative tolerance of crops to the particulate and gaseous pollutants was related to a lower oxidative stress and a higher anti-oxidative defence that elevated SO$_2$ contributes to S-nutrition of crops however, the threshold value of phytotoxicity need to be determined across the crops.

Key words: Antioxidant cascade, Oxidative stress, Particulate pollution, S-metabolism, SO$_2$ stress.

INTRODUCTION

Plant growth and development are adversely affected under abiotic stress such as nutrient toxicity and deficiency, high and low temperatures, salinity, water scarcity and flooding etc (Gupta and Singh 2017). However, plant growth is affected not only by the soil conditions but also by the air quality (Emerson et al. 2003). Built up of sulfur dioxide in the atmosphere can also be attributed to burning of fossil fuels for power generation besides to the industrial processing such as in steel and other ores. National air quality data for SO$_2$ in the environment normally ranges between 2-23 μg m$^{-3}$, with 60-125 μg m$^{-3}$ in Industrial regions (SO$_2$ level >50 are considered high and >75 μg m$^{-3}$ are considered critical) while particulate pollutants average at 80 μg m$^{-3}$. Sulfur dioxide (SO$_2$) induced damage in plants is chiefly observed around the industrial polluting units but the effect appears to be transit or temporary as at other places where such polluting sources have been closed the proceeding plant damage is overshadowed by revegetation and no drastic morphological or phonological injuries are observed. No acute plant damage was observed even when these polluting units used tall stacks since it yielded a height distribution and concentration dilution advantage (Rabl et al, 2008). Quality of air can also alter the plant communities at the ecosystem level since species sensitive to SO$_2$ or other pollutants are likely to perish leaving way for the dominance of the tolerant species. This may also however, alter the animal and microbiological populations in the ecosystem owing to the SO$_2$ mediated disturbance in the native plant community structure. However, terrestrial plants vary widely in their tolerance to sulfur dioxide with lichens and bryophytes being the most sensitive plantae. In fact, these plants have also been successfully used as bioindicators of SO$_2$ pollution (Democker, 2010) (Fig 1).

Plants absorb SO$_2$ via stomata and its flux is proportional to the concentration of SO$_2$ in air with mesophyll chloroplasts having highest trapping potential for SO$_2$. Further, the SO$_2$ in leaf is reduced to bisulphite and sulphite ions which are phytotoxic and enhance generation of ROS, if not oxidized to sulphates, which are stored in vacuoles. Damage occurs when detoxification of SO$_2$ is insufficient and/or when storage capacity of vacuoles for sulphates is exhausted. Sulfur dioxide sensitive plants exhibited bleaching of leaves depending upon the plant sensitivity, plant age and the pollutant concentration. The youngest and fully opened leaves exhibit a greater sensitivity to SO$_2$ pollution than fully matured or relatively younger groups. The foliage exposed to SO$_2$ may reveal tan to reddish brown or dark brown necrotic patches, depending on species. Relative phytotoxicity of SO$_2$ may be guided by relative induction of the free radicals and the reactive oxygen species which are deleterious to cell integrity and consequently inhibit growth and their removal or counter-balancing by the induced enzymic and non-enzymic antioxidative defense.

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components (Fig 1). A relatively lower generation of free radicals and a higher level of antioxidant may impart crop tolerance to SO$_2$ stress. Further, the contribution of an elevated SO$_2$ stress towards improving the S-nutrition of crops has been examined. However, utilizing gaseous S as a nutrient would essentially be mediated via the S assimilation pathway which may involve a regulated activity of the key S assimilating enzymes viz., OAS-TL and SAT (Prasad and Rao, 1982; Hongfa et al., 1999). No clear information is available on mechanisms that regulate the relative sensitivity of crops to SO$_2$ and particle pollution (Sha et al., 2010). The present study attempts to decipher the mechanism of SO$_2$ tolerance at the cellular level by measuring the induction of oxidative stress and the activity of key antioxidants and S assimilating enzymes in cereal and legume species.

**MATERIALS AND METHODS**

**Experimental setup and plant material:** Experiment was conducted in enclosed tunnels (size 10 x 2.5 x 2.5 m; PAR 800-1200 µmol m$^{-2}$s$^{-1}$; day temperature ~25±5 °C, RH ~60-70 %) to assess the effect of particulate and gaseous air pollutants on growth and sulfur nutrition of bread wheat i.e, (Triticum aestivum var. HD-2967), durum wheat (Triticitum durum var. HI-8663), chickpea (Cicer arietinum var. Pusa-391 and barley (Hordeum vulgare var. BHS 380), procured from the Division of Genetics and Plant Breeding, ICAR-IARI, New Delhi. Four different growth environments, under field condition were created, in the experimental tunnels, by using gas and PM filters and by SO$_2$ enrichment. Tunnel with charcoal filter was used to achieve gas and particle free environment (T1); tunnel with particulate filter removed PM (T2); tunnel without any filter served as the ambient control (T3) and; tunnel with elevated level of SO$_2$ (T4) achieved by releasing 25 µgm$^{-3}$ into the tunnel for 3 hours each day for 30 days continuously starting at 30 days of the crop growth stage from the SO$_2$ cylinder (~1200 ppm SO$_2$ supplied by M/s Amit Gas Limited, New Delhi). Different physiological and biochemical attributes were measured at 60 days after sowing in triplicates (n=3).

**Activity of key sulphur assimilating enzymes Serine transacetylase /acetyltransferase (SAT) and O-acetylsereine (thiol) lyase (OAS-TL) activity:** SAT and OAS-TL activities were measured in crude leaf extract following the method of Kredich and Tomkins (1966). 200 mg of the freshly harvested leaf samples were ground in a chilled mortar and pestle with a fixed volume of the chilled extraction buffer containing 100 mM Tris–HCl (pH 8.0), 100 mM KCl, 20 mM MgCl$_2$, 1% Tween 80 and 10 mM DTT. Supernatants were collected and used for SAT and OAS-TL activity assays.

**Induction of oxidation stress and antioxidant activity in response to particulate and SO$_2$ pollution**

**Superoxide radical (O$_2^-$) production:** Superoxide radical (O$_2^-$) production under different experimental environments
was measured following the method of (Chaitanya and Naithani, 1994) and expressed as $\Delta A_{415} \text{min}^{-1} \text{g}^{-1} \text{dry weight}$.

**Hydrogen peroxide content:** $\text{H}_2\text{O}_2$ production was measured as per the method of (Teranishi et al, 1974) at 415 nm and was computed by referring to a standard curve prepared following (Rao et al, 1996).

**Lipid peroxidation (thiobarbituric acid reactive substance, TBARS) assay:** The level of MDA (malondialdehyde), the major lipid oxidation product and a reliable indicator of the level of oxidative stress in a biological sample, was measured (Heath and Packer, 1968). In the assay, thiobarbituric acid reacts with MDA to form a red product which was measured colorimetrically at 532 nm. The molar extinction coefficient used to calculate TBARS concentrations is $1.56 \times 10^5 \text{ M}^{-1} \text{cm}^{-1}$.

**Ascorbic acid:** Ascorbic acid content was measured as per the method of (Mukherjee and Choudhuri, 1983) by measuring the absorbance at 530 nm.

**Peroxidase activity:** Peroxidase activity was determined in terms of formation of tetra-guaiacol which was measured at 470 nm (Castillo et al. 1984). Peroxidase activity was calculated as per extinction coefficient of its oxidation product, tetra-guaiacol $e = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed as $\mu$mol tetra-guaiacol formed per min per gram fresh weight (fw).

**Statistical analysis:** Data was statistically analyzed by using SPSS version 23 statistical program and significant difference between the treatments within the selected crop were determined using one-way ANOVA at $n=3$, as described by (Snedecor and Cochran, 1980) in combination with Duncan’s Multiple Range Test (DMRT) at $P<0.05$.

**RESULTS AND DISCUSSION**

Effect of gaseous and particulate pollutants on the activity of key sulphur assimilating enzymes in cereal and legume crops

**O-acetylsersine (thiol) lyase (OAS-TL) and Serine transacetylase (SAT) activity:** Variation in activity of key sulphur assimilating enzymes viz. O-acetylserine (thiol) lyase (OAS-TL) and Serine transacetylase (SAT) in response to gaseous and particulate pollutants and SO$_2$ enrichment were measured at 60 days into the vegetative growth stage and are presented in (Fig 2). O-acetylserine (thiol) lyase (OAS-TL) activity, in general, was significantly improved in response to SO$_2$ enrichment (T4) then the control treatment (T3) in bread wheat, durum wheat, and barley (Fig 2). Removal of particulate pollutant alone (T2) did not alter significantly the O-acetylserine (thiol) lyase (OAS-TL) activity with respect to the ambient control across the experimental crop and in general, a relatively higher mean OAS-TL were measured in bread wheat and barley then durum wheat and chickpea.

Serine transacetylase (SAT) activity, on the others hand, was significantly induced across the experimental crops under the SO$_2$ enrichment treatment (T4) when compared with the ambient control (T3) and other growth environments (T1 and T2). Highest SAT activity in the T4 treatment, over the ambient control, was measured in bread wheat (~3 fold increase) followed by chickpea, durum wheat and barley in the same order (Fig 2). Barley measured highest SAT activity under the ambient control treatment followed by chickpea. Removal of gaseous and particulate pollutant (T1) or to some extent even particulate pollutants (T2) from the growing environment of durum wheat, chickpea and barely inhibited the SAT activity significantly over the ambient condition control treatment. Biochemical effects of sulphur dioxide arises from its unique ability to act as an oxidizing or a reducing agent. SO$_2$ can directly interfere with the photosynthetic machinery and the energy metabolism, while indirect effects arise from the formation of sulphites and sulphonates to inhibit activity of several enzyme system (Malhotra and Hocking, 1976). Randewig et al (2012) investigated the effect of SO$_2$ fumigation on activities of different S assimilating enzymes and reported a positive effect on the OAS-TL but not the SAT activity in Arabidopsis. They further mooted that the activity of sulfate oxidase...
controls the sulfur metabolism under SO\(_2\) exposure in Arabidopsis. Further, an optimized N nutrition of crops was shown to help plant’s adapt under the SO\(_2\) enriched environment (Milchunas et al., 1981; Borland and Lea 1991).

**Effect of gaseous and particulate pollutants on oxidative stress induction and activity of key antioxidants:** Gaseous pollutants lead to oxidative stress by producing damaging reactive oxygen species (ROS). However, the oxidative damage can be minimized by antioxidant defenses for example superoxide dismutase (SOD), catalase, peroxidase, ascorbic acid, glutathione etc (Allgrove and Davison, 2014). Air pollutants like SO\(_2\) and NO\(_x\) do cause oxidative stress in plants, however, their damage causing properties will depend upon the regulatory balance between the oxidative and the antioxidative processes with a higher induction of antioxidative defenses favoring SO\(_2\) resistance.

**Superoxide radical, hydrogen peroxide production and lipid peroxidation:** Relatively higher superoxide radical activity were measured under control treatment in barley and durum wheat over the other treatments. Superoxide radical activity was least across crops under the SO\(_2\) enrichment treatment (T4) when compared with the ambient control (T3). Removal of gaseous and / or particulate pollutant from the crop growing environment (T1) did not alter the superoxide radical production significantly over the control, for all crops except chickpea (Fig 3). Variation in hydrogen peroxide accumulation in leaves of different cultivar at 60 day growth stage showed least H\(_2\)O\(_2\) production in durum wheat while the highest level was measured in chickpea across treatments. SO\(_2\) fumigation caused a decline in H\(_2\)O\(_2\) production across the monocot and dicot species when compared to respective H\(_2\)O\(_2\) production level under the ambient control treatment (T3). Removal of gases and/or particulate pollutant (T1 and T2) from the growing environment caused significant reduction in hydrogen peroxide accumulation over the ambient control (T3). H\(_2\)O\(_2\) production values were, however, still higher in ambient control than those measured under the SO\(_2\) enrichment treatment (T4; Fig 3). Lipid peroxidation which is a measure of oxidative degradation of lipids, was highest under the treatment where charcoal and particulate filters (T1) were used in barley. Relatively lower lipid peroxidation activity was measured under SO\(_2\) enrichment treatment (T4) across the experimental crops when compared to the ambient control. Further, lipid peroxidation, across the growth environment, did not vary significantly for the bread wheat and chickpea (Fig 3). (Zhu et al, 2015) while studying the role of SO\(_2\) in alleviating aluminum toxicity stress showed that pretreatment of wheat seeds with SO\(_2\) donar such as NaHSO\(_3\)/Na\(_2\)SO\(_3\) under Al stress reduced reactive oxygen species (ROS) activity and that SO\(_2\) exposure at low doses may not induce ROS related deleterious effects at the cellular level. In fact a low dose of SO\(_2\) fumigation may be acting as source of nutrient S fertilizer to promote vegetative growth. However, the above effect seems to take toll on the reproductive potential or yield performance of crops. In contrast, (Muneer and Co workers, 2014) investigated the effect of CO, NOx and SO\(_2\) on ROS activity, antioxidants and photosynthesis in strawberry plants and observed a dose concentration dependant increase in activity of singlet oxygen (O\(_2\)^1) and H\(_2\)O\(_2\) in the exposed plants. However, the negative effects of these deleterious species can be curtailed by simultaneous and at least matching induction of the antioxidative defense cascade of metabolites and enzymes (Kumar et al., 2017).

**Peroxidase and ascorbic acid activity:** Variation in peroxidase activity, responsible for removal of deleterious hydrogen peroxide radical is presented in Fig 4. In general, an increase in peroxidase activity was measured under the SO\(_2\)-enrichment condition (T4) over the ambient control (T3) in cereal species but not in chickpea. A relatively higher peroxidase activity was measured in durum wheat than the other experimental crop species. Removal of obnoxious gases and/or pollutant in growing environment (T1), in general, did not induce the peroxidase activity significantly over the untreated ambient control, except for chickpea. Further, a

**Fig 3:** Effect of gaseous and particulate pollutants on leaf (A) superoxide radical production (B) hydrogen peroxide accumulation and (C) lipid peroxidation in cereal growing environments i.e., T1 (Treatment tunnel 1): Charcoal and particulate filter (Removes noxious gases and particle pollutants); T2 (Treatment tunnel 2): Particulate filter (Removes particle pollutants); T3 (Treatment tunnel 3): No Filter (Ambient control); T4 (Treatment tunnel 4): SO\(_x\) enrichment (25 μg m\(^{-2}\) over ambient). Data bars for the treatment, Within a crop, depicting different letters are significantly different as per DMRT at p≤0.05 n=3).
significantly higher accumulation of ascorbic acid level was measured under the SO\(_2\) enrichment treatment (T4) when compared with the ambient control (T3) across the investigated crops. In general, irrespective of the treatment, highest ascorbic acid levels were recorded in bread and durum wheat while chickpea recorded a minimal activity (Fig 4). Removal of gaseous/particulate pollutants (T1 and T2) did not induce ascorbic acid accumulation over the control treatment (T3) as in case of bread and durum wheat than that observed in chickpea and barley. Varshney and Varshney (1984) measured the effect of low levels of SO\(_2\) (78.6, 131 and 262 \(\mu g\) m\(^{-3}\)) on ascorbic acid in *Brassica nigra* L., *Phaseolus radiatus* L. and *Zea mays* L and suggested that a relatively higher SO\(_2\)-resistance of *Z. mays* was related to its comparatively higher ascorbic acid accumulation but higher level of SO\(_2\) exposure caused a reduction in the ascorbic acid level. They further observed a decline in ascorbic acid level of the SO\(_2\) sensitive species within a week of SO\(_2\) fumigation. It is likely that the ascorbic acid, a major antioxidant and a powerful reductant, facilitates the reduction of sulphite to hydrogen sulphide, thus contributes significantly towards the plants’ strategy of detoxifying SO\(_2\).

**CONCLUSION**

Our results reveal that SO\(_2\) stress, in general, helps in improving S-nutrition of crops and suggests that SO\(_2\) stress at low concentration can benefit the crops, particularly on sulfur-deficient soil. We further showed that SO\(_2\) enrichment significantly improved SAT activity, but OAS-TL activity was enhanced only in wheat. Further, we observed a reduced ROS activity and an increased antioxidant activity at low dose of SO\(_2\) stress, which indicates that antioxidant activity may be a significant player in determining plant protection against the air pollutants. Further, an induced antioxidant activity under SO\(_2\) stress can be used as a physiochemical marker to differentiate between sensitive and resistant plant types. However, to achieve SO\(_2\) tolerance, the defense capacity must be induced to level sufficiently high enough to alleviate oxidative damage induced under the SO\(_2\) stress.

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