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Effect of Sulphur Dioxide Exposure on Chlorophyll Content and Nitrogenase Activity of *Vicia faba* L. Plants

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Sulphur dioxide occupies leading position as an air pollutant due to its potential hazard for vegetation as well as due to its wide distribution over the world. Sulphur dioxide has been reported to induce visible injury to leaves and leads to reduction in photosynthetic pigments (Malhotra, 1977; Nandi *et al.*, 1990), inhibition of metabolic processes (Darrell, 1989) and suppression of growth and yield of plants of natural and agricultural ecosystems (Nandi *et al.*, 1990). The studies related to the effects of SO_2 on nitrogen fixation are limited. Hallgren and Huss (1975) reported greater inhibition of nitrogen fixation than that of photosynthesis in lichens and algae treated with aqueous solutions of $NaHSO_3$ and $NaHSO_4$. Stimulation of N_2-fixation at lower SO_2 concentrations and inhibition at higher concentrations were reported for soybean plants (Scheridan, 1979). Other workers (Varshney and Varshney, 1979; Agrawal *et al.*, 1985; Griffith and Campbell, 1987) have also reported the adverse effects of SO_2 on nitrogen fixation, photosynthetic pigments, growth and yield of certain leguminous plants.

The annual average concentrations of SO_2 around Obra thermal power plant and nonpolluted sites in India were reported as 0.06, and 0.007 ppm, respectively (Rao *et al.*, 1990). However, daily average concentrations in areas close to the emission source may be as large as 0.34 ppm (Dubey *et al.*, 1982). Therefore, in the present investigation an attempt has been made to determine the potential effects of such episodic and exceptionally high intermittant concentrations of SO_2 on total chlorophyll content and nitrogenase activity of *Vicia faba* (broad bean) plants.

MATERIALS AND METHODS

Vicia faba L. plants were raised in plastic pots (25 cm diameter) containing sandy loam soil. When 45 days old, plants with uniform growth were separated into three batches of 15 pots each

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designated as C for control, S_1 for plants exposed to 0.25 ppm for 1.5 hr and S_2 for plants exposed to 0.5 ppm for 1.5 hr. Plants were exposed at the interval of 10 days until they became 84 days old. The SO_2 concentration was monitored by using Kimoto-319 SO_2 analyser (Japan). For analysis of plant samples i.e. leaves and active root nodules from control, 0.25 ppm SO_2 and 0.5 ppm SO_2 exposed plants were collected at 45, 55, 65, 75, and 85 day ages.

The plants were visually observed for the presence of injury symptoms. The amount of total chlorophyll as expressed in mg g^{-1} dry leaf weight was measured by using the method of Maclachlan and Zalik (1963). For measuring the nitrogenase activity the acetylene reduction method was used (Stewart *et al.*, 1967). The nitrogenase activity was expressed as n mol C_2H_2 g^{-1} nodule fresh wt min^{-1}.

RESULTS AND DISCUSSION

Vicia faba plants fumigated at 10 days interval with 0.25 and 0.5 ppm SO_2, for 1.5 hr between 45 and 84 days plant ages, showed interveinal chlorosis on both leaf surfaces, after 3 days of initial exposure (Table 1). Similar symptoms were reported for *Vicia faba* plants treated with SO_2 (Nandi *et al.*, 1990).

Table 1. Percent leaf area injury in plants exposed to SO_2 at different ages of their growth

Plant age (days)	Control (C)	0.25 ppm SO_2 (S_1)	0.5 ppm SO_2 (S_2)
45	0.0	0.0	0.0
55	0.0	4.0	7.5
65	0.0	8.5	14.9
75	0.0	12.3	21.8
85	0.0	19.2	31.5

The amount of total chlorophyll in SO_2 exposed leaves decreased significantly with the increase in SO_2 concentration and exposure time (Table 2). The reductions were of higher magnitude in S_2 plants exposed to 0.5 ppm SO_2 in comparison to S_1 plants fumigated with 0.25 ppm SO_2. The maximum reduction of 53.48% in total chlorophyll was observed at 75 day age in S_1 plants. Reduction in chlorophyll content was found positively correlated ($P > 0.01$) with SO_2 concentration as well as duration of exposure. Decrease in chlorophyll content of higher plants due to SO_2 treatment was also reported earlier (Malhotra, 1977). Such decrease were ascribed either to inhibition of chlorophyll synthesis (Spedding and Thomas, 1973) or its destruction (Malhotra, 1977; Shimazaki *et al.*, 1980). Shimazaki *et al.*(1980) suggested that SO_2 induced increase in oxygen free radicals (O_2-)

in chloroplasts leads to destruction of chlorophyll molecules. Nandi (1984) suggested H_2O_2 dependent and peroxidase mediated oxidation of chlorophyll in SO_2 exposed rice plants.

Table 2. Total chlorophyll content (mg g^{-1} dry wt) of control (C), 0.25 ppm SO_2 exposed (S_1) and 0.5 ppm SO_2 exposed (S_2) plants at different ages of their growth (mean ± SE)

Plant age (days)	C	S_1	S_2
45	15.65±0.17[a]	14.20±0.32[a]	13.85±0.34[a]
55	16.28±0.34[a]	13.84±0.60[b]	12.19±0.32[b]
65	16.88±0.19[a]	13.18±0.52[b]	10.54±0.17[b]
75	17.20±0.32[a]	12.62±0.26[b]	8.02±0.19[b]
85	14.20±0.34[a]	10.06±0.17[b]	7.28±0.17[b]

Mean in rows followed by different letters are significantly different according to LSD ($P < 0.05$).

The nitrogenase activity in the root nodules of control plants remained always higher in comparison to that of S_1 and S_2 plants (Table 3). The N_2-ase activity in root nodules decreased gradually with the increasing age of control and SO_2 exposed plants. The maximum reduction of nitrogenase activity was measured in S_2 plants at the age of 85 days; the reduction being 77.5%, as compared to the control. Inhibition of N_2 fixation in lichens treated with different sulphite solution was mainly

Table 3. Effects of SO_2 on nitrogenase activity (n mol C_2H_4 g^{-1} fresh wt min^{-1}) in *Vicia faba* plants (mean ± SE)

Plant age (days)	Control (C)	0.25 ppm SO_2 exposed (S_1)	0.5 ppm SO_2 exposed (S_2)
45	2.806±0.03[a]	2.424±0.17[a]	2.136±0.07[a]
55	2.822±0.16[a]	2.106±0.02[a]	1.824±0.05[b]
65	1.824±0.07[a]	1.655±0.05[a]	1.368±0.07[b]
75	1.604±0.12[a]	1.367±0.01[b]	1.092±0.02[b]
85	0.818±0.01[a]	0.418±0.02[b]	0.184±0.02[b]

Means in rows followed by different letters are significantly different according to LSD test ($P < 0.05$).

attributed to SO_3^{2-} induced inhibition of nitrogenase activity (Hallgren and Huss, 1975). Griffith and Campbell (1987) also reported a reduction of 59% in specific nodule activity of SO_2 exposed *Phaseolus vulgaris* plants in comparison to the control. The results of present study clearly show that SO_2 had a direct unfavourable effect on nitrogenase activity as other environmental factors remained same throughout the experiment.

This enzyme plays a key role in biological nitrogen fixation, hence any change in its activity would reduce the ability of *Rhizobium* bacteria to fix nitrogen. The reduction in N_2-ase activity of *Vicia faba* plants could be either due to toxic effects of SO_2 on bacteroids and/or due to changes in the permeability of the nodules and hence their normal functioning may be unfavourably affected. But the chances of direct effect of SO_2 on root nodules seem not possible as the intermittent SO_2 exposure did not change soil pH appreciably. Chlorophyll reductions due to SO_2 exposure have a direct effect on the photosynthate production (Nandi *et al.*, 1990). The significant positive correlation ($P > 0.01$) between chlorophyll content and nitrogenase activity suggests a possible explanation of SO_2 induced changes in nitrogenase activity. Reduced translocation of photoassimilate to other growth sites due to SO_2 exposure was reported (Noyes, 1980). Nodule development depends upon the photosynthate supply. Hence, the reduction in photosynthetic potential of plant may restrict the photoassimilate supply to root and developing nodules and thus limits N_2-fixation.

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