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Exogenous Sulphur Dioxide Induced Changes in Superoxide Dismutase, Peroxidation and Non-Protein SH Content in Mung Bean (Vigna Radiata) Seedlings

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Abstract: This study investigated the effect of exogenous sulphur dioxide gas for different durations the superoxide dismutase, peroxidase, lipid peroxidation and non-protein SH content in mung bean *(Vigna radiata)* seedlings. It has been found that the activity of superoxide dismutase is enhanced at low concentration but decreased when the seedlings are exposed to higher concentration of SO_2; the peroxidase activity and lipid peroxidation decreased and the non-protein SH content increased significantly. The results indicate that superoxide dismutase, peroxidase, lipid peroxidation and non-protein SI-I content metabolisms are associated with the growth of the organism and are susceptible to alterations by exogenous sulphur dioxide stress.

Keywords: Sulphur dioxide, superoxide dismutase, peroxidase, lipid peroxidation, non-protein SH content, mung bean *(Vigna radiate)*.

1. INTRODUCTION

Sulhpur dioxide(SO_2) is a major atmospheric contaminant resulting from volcanoes and combustion of sulfur containing fossil fuels in industries like brick kilns, thermal power plants, etc. (Dwivedi and Tripathi 2007). Further, oxidation of SO_2 usually in the presence of catalysts like finely divided metal particulate matter, NO_2 etc. forms H_2SO_4 and thus causes acid rain, and is major cause of environmental concern.

SO_2, a colorless, corrosive, non-inflammable gas with irritating and pungent odour may cause temporary and permanent injury to vegetation

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(Tripathi and Dwivedi, et. al. 2002), Health (1980) and (Renuga and Paliwal 2004). The phytotoxic behaviour of SO_2 has been reported in many. Studies (Daines 1968); Jacobson and Hill 1971 and Thomas et. al. 1956) Changes in the physical appearance of vegetation is an indication that the plant metabolism is impaired (Puckett et. al. 1973 and Zieoles 1972) SO_2 directly alter the ability of mesophyll cells to fix CO_2 (Winner et. al. 1985). Thus influence the photosynthetic capacity of the plant (Silvius et. al. 1978). Much of the SO_2 absorbed by leaves enters through stomata and dissolves in the moist surfaces of mesophyll cells (Thomas et. al. 1950). The resulting sulfrous acid dissociates into H^+, HSO_3- SO_3-^{2} and SO_4-^{2} and there ions get accumulated these. SO_2 toxicity is due to the reducing property of the gas. SO_2 also interfere with the structure and permeability of cellular membranes and with enzymatic activity which affects many biochemical processes in the cell (Irshad et. al. 2011) by causing oxidative stress. (Khan and Khan 2011) have critically analysed the plant response to diseases in sulphurdioxide stressed environment.

Superoxide radicals (O_2^-) and other activated oxygen species (singlet excited oxygen hydroxy radicals and hydrogen peroxide) are generated in probably all the aerobically living cells (Fridovich, 1974). These forms of capable of damaging cellular components, e.g. they may initiate lipid peroxidation or cause inactivation of enzymes. Cells are protected against the deleterious superoxide radicals by various kinds of superoxide dismutase which specifically scavenge these radicals. This enzymes plays a vital role in protecting cells against the activated oxygen species, however, their precise significance is not clear. The hydrogen peroxide produced directly or indirectly, being toxic to the biological system, is metabolized by peroxidases in plants.

Since superoxide dismutase is a sulphydral enzyme and also SH groups help in the maintenance of oxidation-reduction potential of the cell it is felt to determine the non-protein SH content during the course of SO_2 stress.

The present investigation is aimed to look into the oxidative metabolism in terms of radical scavenging enzymes, viz. superoxide dismutase and peroxidase, and cellular damage in terms of lipid peroxidation and non-protein SH content on exposure of seeds to sulfur dioxide and to determine the effect of exogenous SO_2 in germinating mung beans. This will help in better understanding the role of antioxidative enzymes in physiological processes.

2. MATERIALS AND METHODS

Healthy seeds of mung bean were purchased locally and sterilized with 0.2% $HgCl_2$ solution. The seeds were then thoroughly washed with 0.2% distilled water solution and soaked in doubly distilled water in petridishes for 5h for imbibitions and then were exposed to 0.025 mole of SO_2 for different intervals.

of time i.e. from half hour to two hours. The seeds taken out after different intervals of time were washed thoroughly with distilled water and germinated for 72h. The seedlings were taken after 24, 48 and 72 h of germination and ground in a prechilled mortar and pestile with distilled water. The slurry was then centrifuged at 1000rpm for 10 min. The clear supernatant was used for the determination of enzyme activities.

Superoxide dismutase was estimated by the method of (Fridovich 1974). Peroxidase was assayed using p-phenyldiamine as the substrate by the method of (Briton and Mehley et.al 1955). Lipid peroxidation and non protein SH content were estimated by the methods of (Krishnamurty and Bieri 1962) and (Sedlak and Lindsay 1968) respectively.

Protein concentration in the seedling extracts were determined by the method of (Lowry et. al. 1951) using bovine serum albumin as the standards.

3. RESULT AND DISCUSSION

3.1 Superoxide dismutase activity

The results (Table 1) reveal that the enzyme activity is increased significantly ($P<0.05$) in the seedlings germinated in water, as the germination is advanced.

Table 1: Effect of Sulphur dioxide gas on the superoxide dismutase activity, peroxi dase activity, lipid peroxidation and non-protein SH content in mung bean seedling germinated for 72 hours at 30° C.

Germination time (hr.)	Controlled seedlings (germinated in water)	Exposed seedlings (germinated in water exposure to Sulphur dioxide gas for different periods)			
		1/2 hour	1 hour	1/2 hour	2 hours
Superoxide dismutase activity (E.U/mg protein)					
24	2.86 ± 0.01	2.97 ± 0.04	3.74 ± 0.11	2.56 ± .07	1.71 ± 0.04
48	3.96 ± 0.13	3.84 ± 0.01	4.98 ± 0.27	1.56 ± 0.05	1.15 ± 0.03
72	4.72 ± 0.34	5.50 ± 0.29	3.86 ± 0.13	1.03 ± 0.04	0.94 ± 0.02
Peroxidase activity (E.U/mg protein)					
24	300.72 ± 4.52	301.86 ± 1.39	299.29 ± 1.52	268.34 ± 0.38	187.27 ± 11.12
48	512.42 ± 33.54	511.81 ± 10.33	490.89 ± 26.83	300.05 ± 2.27	272.53 ± 5.46
72	754.18 ± 61.89	665.37 ± 6.48	662.79 ± 40.92	390.94 ± 3.48	363.87 ± 21.02
Lipid peroxidation (O.D/mg protein)					
24	0.0076 ± 0.0004	0.0083 ± 0.0007	0.0101 ± 0.001	0.0068 ± 0.0005	0.0059 ± 0.0003
48	0.0112 ± 0.0013	0.0104 ± 0.0009	0.0173 ± 0.0014	0.0092 ± 0.0003	0.0063 ± 0.0001
72	0.0201 ± 0.0018	0.0203 ± 0.0001	0.0220 ± 0.0017	0.0108 ± 0.0001	0.0069 ± 0.0002
Non-protein SH content (uM/mg protein)					
24	3.76 ± 1.05	3.77 ± 0.14	3.56 ± 0.17	7.56 ± 1.38	13.56 ± 1.45
48	3.73 ± 0.34	3.45 ± 0.98	5.08 ± 0.39	10.45 ± 0.21	13.86 ± 0.47
72	5.32 ± 0.58	6.99 ± 1.11	8.03 ± 0.12	11.37 ± 0.90	14.49 ± 0.13

The values reported here the mean ± SD of six determinations of two independent experiments.

The enzyme activity did not change significantly in half an hour exposed seedlings germinated for 24 and 48 hours. But on third day of germination, the enzyme activity is induced and the induction is 17 per cent as compared to the control values. Similarly, increase in the enzyme activity is observed in one hour exposed seedlings germinated for 24 and 48 hours although the induction is low in the 48 hours germinated seedlings. In 72 hours germinated seedlings, significant decrease in the enzyme activity is observed. Similarly, the enzyme activity is found to be inhibited in one and a half and two hours, exposed seedlings. On first day f germination, inhibition is less than that on the second day of germination period. Similarly, on third day of germination, the inhibitory effect is more pronounced showing the inhibition to be germination time dependent.

3.2 Peroxidase activity

It is evident from the data (Table 1) that significant ($P<0.05$) increase in the enzyme activity is there in the control as well as in the treated seedlings. Sulphur dioxide gas is found to have no effect on the enzyme activity in the half and one hour exposed seedlings germinated for 24 and 48 hours, however, on third day of germination the enzyme activity is decreased significantly ($P<0.05$) as compared to the control values. In the one and a half and two hours exposed seeds germinated for 24 hours, inhibition in the enzyme activity is observed and the inhibition is more in two hours exposed seedlings. The inhibitory effect is more pronounced on second and third day of germination.

3.3 Lipid peroxidation

It is clear from the data (Table 1) that increase in lipid peroxidation is observed in the control as well as in the treated seedlings as the germination advanced. In two hour exposed seedlings, the increase in not much as the germination time increases. No significant change in lipid peroxidation is observed in half an hour exposed seedlings germinated for 24, 48 and 72 hours as compared to the control seedlings. Increase in lipid peroxidation is observed in one hour exposed seedlings (33, 54,s and 9 per cent as compared to control values on first, second and third day of germination, respectively). Sulphur dioxide gas has inhibitory effect on lipid peroxidation in mung bean seedlings exposed for one and a half and hour and two hours and inhibition is found to be germination time dependent, i.e. the lipid peroxidation decrease being 22, 44 and 66 percent as 24, 48 and 72 hours of germination, respectively in the seedlings exposed for two hours with SO_2 gas.

3.4 Non-protein SH content

From the data (Table 1) it is clear that sulphur dioxide gas has no effect on non-protein SH content in 24 hour mung bean seedlings exposed for half an hour and one hour, with gas. Non-protein SH content is increased significantly ($P<0.05$) in 72 hours seedlings exposed for different periods under study and enhancement is found to be exposure time dependent as non-protein SH content increases with the increase in the exposure time. Similar results are obtained in 24 and 48 hours germinated seedlings but the effect is more pronounced in 48 hours germinated seedlings.

CONCLUSION

A variety of biochemical and physiological changes take place in plants subjected to SO_2 stress (Rao and Blanck 1996; Katzel and Moller 1993 and Irshad et. al. 2011). Sulphurdioxide gas enhanced the superoxide dismutase activity when the seedlings were exposed to SO_2 for short duration, but the activity was decreased when the seedlings were exposed to SO_2 for a longer time and SO_2 became toxic at higher concentration. The peroxidase activity and lipid peroxidation significantly in seedlings exposed to SO_2 for different duration and the results are in agreement with the results obtained by (Ketzel and Moller 1993) who have observed that apples exposed to 0.5 percent sulphurdioxide have substantially low activity of polyphenol oxidase and peroxidase. The non-protein SH content was found to be increased significantly and the results are in agreement with those of (Irshad et. al. 2011). The increase in protein content could be due to the utilization of sulphur for synthesis of more amino acids (Mlodzinowski and Bialobok 1977).

The results clearly show that gases affect the oxidative metabolism when the seedlings are exposed to sulphurdioxide gas.

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