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Peroxidase Activity as an Indicator of SO_2- Tolerance in Crop Plants

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Abstract:
Effect of different SO_2 concentrations on the peroxidase activity in the leaves of three economically important plant species, viz., Vigna radiata (Mung bean), Solanum esculentum (= Lycopersicon esculentum) [Tomato] and Zea mays (Maize) was studied. Controlled fumigation experiments were carried out using three different treatments of SO_2 : ***T-1*** *= 0.05 ppm(134.0µg m^{-3} SO_2) [x 4h],* ***T-2*** *= 0.1 ppm(268.0 µg m^{-3} SO_2) [x 2h] and* ***T-3*** *= 0.2 ppm(536.0 µg m^{-3} SO_2) [x 1h] for 60 days. Although the peroxidase levels in controls were highest in Z.mays, followed by V.radiata and S.esculentum, it was S.esculentum which recorded maximum increase in peroxidase activity after SO_2fumigation, followed by V.radiata and Z.mays. Enhanced peroxidase activity is indicative of greater tolerance toSO_2 stress.*

Keywords*: Free-radical scavengers, Maize, Mung bean, Peroxidases, SO_2-tolerance, Tomato*

1. Introduction

Detrimental effects of SO_2 on plantsas a potent phytotoxicant have been well documented. A series of physiological and biochemical changes precede the visible SO_2 -injury symptoms. These include alterations in parameters like contents of chlorophyll, proteins, reducing sugars ascorbic acid, activities of free-radical scavenger enzymes like superoxide dismutase and peroxidases(POD) along with the pattern of volatile emissions(Chauhan 1989a,1989b, 1990, 2015a,2015b, 2015c, 2015d, Varshney and Varshney, 1985, Rai et al. 2011, Singh et al. 2012).Among these, the peroxidases have been ascribed importance in order to understand the early effects of chronic injury to plants by air pollutants. An increase in the level of peroxidases due to SO_2 exposure is thought to be indicative of a plant's potential response to this stress (Fridovich and Handler, 1961, Asada, 1080, Tanaka and Sugahara, 1980), POD, thus constitutes a very sensitive indicator of plant response to air pollution stress, reflecting the so-called "hidden injury" (Keller and Schwager, 1971). Present investigations on three crop plants also substantiate the notion that an increase in POD activity helps the plant to resist SO_2 exposure stress by quantitative readjustment of intermediary metabolism. Among many others, increase in metabolic potentiality for SO_2 resistance is achieved by faster SO_2 mobilization, stabilization of internal pH, and scavenging of toxic H_2O_2.

2. Materials and Methods

Three economically important cultivated plant species viz., *Vigna radiata* (L.) Wilczek [Mung bean], *Solanum esculentum* [Tomato], and *Zea mays* L. [Maize] were grown from seeds in the nursery. Fifteen-day-old seedlings of these plants were subjected to different SO_2 treatments through an artificial fumigation system. Sulfur dioxide was generated by bubbling $Na_2S_2O_5$ in water and circulated in closed-top fumigation chambers (1 x 1 x1m=1^{m3}) at temperatures ranging between 25-29^{0}C ± 1^{0}C and at a RH of 60 ± 5%. Two 200W metal halide lamps were used for illumination with a light/dark cycle of 12/12 hours.

2.1. Treatment protocols of SO_2

T-1=0.05 ppm (134.0µg $^{m-3}$ SO_2) [x 4h], **T-2**=0.1 ppm (268.0 µg $^{m-3}$ SO_2) [x 2h] and **T-3=**0.2 ppm (536.0 µg $^{m-3}$ SO_2) [x 1h] for 60 days, thus keeping the SO_2 dose constant. *V. radiata* was fumigated for only 45 days. Controls (C) were maintained simultaneously by exposing the plants to air alone.

2.2. Determination of Peroxidase Activity

The activity of peroxidases (POD: EC 1. 11. 1. 7) was determined according to the method given by Gasper et al., 1982. Peroxidases from the three plants were extracted in different buffers, viz., 0.2 M potassium phosphate buffer (pH 7.8) for *S.* esculentum, 0.2M potassium phosphate buffer (pH 6.0) for *V. radiata*, and 0.1M Tris-HCl buffer (pH 6.3) for *Z. mays*. Fresh leaves (0.2g) were

homogenized in ice-chilled 5ml of the specific extracting buffer and later centrifuged at 16,ooo x g for 20 min at 4^0C in a K-24 refrigerated centrifuge. The clear supernatant so obtained was used as a crude enzyme extract.
200µl of the crude enzyme extract was added to 7.8ml of 0.2 M potassium phosphate buffer (pH 6.3). After adding 1.0ml of 1% guaiacol solution, the contents were mixed thouroughly. 1% H_2O_2 (1.0 ml) was added to this mixture just prior to recording of the extinction E, measured at 420 nm at 10 sec intervals. The blank was set with the mixture without H_2O_2. Peroxidase activity was expressed in terms of conversion of guaiacol or H_2O_2 per minute from the amount of tetraguaiacol produced under the assay conditions. One molecule of tetraguaiacol developed corresponds to the conversion of four molecules of guaiacol and four molecules of H_2O_2. The enzyme activity was represented as unit's mg^{-1} protein.

2.3. Statistical Analysis
Analysis of variance (ANOVA) and multiple regression analysis were employed to test the significance of individual as well as interactive effects of SO_2 concentration (ppm) and the exposure time (h) upon the activity of peroxidases. The relationship between these variables was calculated with the help of an empirical (statistical regression) model and correlation coefficient I. This model explains the relationship between chronic exposure to different SO2 regimes and plant response, as manifested by alterations in their peroxidases. The empirical model is based on multiple regression. To facilitate the visualization of the regression fitting process, the observations have been plotted in the form of a three –dimensional scatter diagram.

3. Observations
The activity of peroxidases (oxidoreductase EC 1. 11. 1. 7) increased gradually during development in the control plants. Enzyme levels were highest in *Z. mays*, followed by those in *V. radiata* and *S. esculentum*. There was a significant increase in peroxidase activity in response to SO_2 exposure in all the three plant species investigated. Following SO_2-fumigation, *S. esculentum* showed maximum increase in peroxidase activity, followed by *V. radiata* and *Z. mays*.
In *S. esculentum* highest increase in POD levels was recorded after 75 days of SO_2- treatment T-3(92.04%), whereas the increment in enzyme activity with T-2 in the same period was slightly lower (90.54%). Treatment T-1 resulted in an increase in POD levels by only 61.11% at the end of 60 days of SO_2-fumigation (Table 1, Fig. 1).
Fumigated plants of V. radiata with treatment T-1 exhibited an increase of 39.42% after 45 days of SO_2-exposure. The quantum of increase in POD levels in T-2 and T-3 treatments on the same time period was 66.10 and 77.03% respectively (Table 2, Fig. 2). Although the intrinsic levels of peroxidases in control plants of *Zea mays* were the highest (e.g., 182.40 unit's mg^{-1} protein), there was least increase in enzyme activity in response to SO_2-fumigation with respect to the other two species investigated. Highest increment in POD activity after 60 days of SO_2- treatment for and T-3 was recorded at42.78% (Table 3, Fig.3).
Data on peroxidase activity subjected to ANOVA indicates that the effect of fumigation period was significant in plants of all ages (P= 0.05).SO_2-treatments in combination with fumigation period also exercised a significant effect (P =0.05-0.001). However, SO_2 treatment alone was not significant (Tables 1-3).

4. Discussion
Peroxidase activity increased in all the three plant species fumigated with SO_2. Similar increase in leaves of plants growing in areas heavily polluted with SO_2, or under conditions of controlled SO_2-fumigation have also been observed by Horsman and Wellburn (1976), Pierre and Queiroz (1982), Khan and Malhotra (1982), Varshney and Varshney (1985) and Sarkar et al. (1986). High concentrations of SO_2 have been shown to result in upto six-fold increase in peroxidase levels in many forest species.
Since peroxidase activity is known to increase under various stress conditions such as the influence of toxic gases, mechanical injury, or attack by parasitic organisms (see Treshow,1984), and high enzyme levels are believed to be connected with manifold physiological functions of these enzymes. This makes peroxidases good indicator of chronic injury in plants in the vicinity of large cities, this is because SO_2 causes an increase in peroxidase activity much before the appearance of any visible symptoms of leaf injury. In the present investigation, SO_2-induced higher peroxidase levels are suggestive of their participation in protective mechanisms of plants for the removal of H_2O_2. An increase in the peroxidase activity following SO_2 –exposure protects the plant on one hand by oxidizing sulphite to sulphate, and on the other hand byscavenging the superoxide radical, formed due to initiation of sulphite oxidation in the illuminated chloroplasts (see Asadaand Kiso, 1973). Higher peroxidase and superoxide dismutase levels are also thought to increase with age of the plant, thereby increasing the cellular detoxification capacity (Rao, 1992). Present observations are amply corroborated by a parallel increase in the superoxide dismutase (SOD) activities (Chauhan, 1989b) and reduction in ascorbate contents (Chauhan, 2015d) in these crop plants and alteration in the pattern of volatile emissions (Chauhan, 1990) which indicate a strong participation of free-radical scavenger enzymes. Thus differential increase in peroxidase activity following exposure to SO_2 in the crop plants investigated is considered to be a function of the resistance to the pollutant (see Rao and Dubey, 1990).

4.1. Acknowledgements
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5. References

i. Asada, K. 1980. Research Report, Natl.Inst.for Environ.Studies, Japan. 11: 165-179.
ii. Asada, K. and Kiso, K. 1973. Initiation of aerobic oxidation of sulphite by illuminated spinach chloroplasts. Eur.J.Biochem. 33: 253-257.
iii. Chauhan,A. 1989a. Effect of sulphur dioxide on plants at biochemical and physiological levels. PhD. Thesis, University of Garhwal, India.
iv. Chauhan,A. 1989b. Superoxide dismutase—a bioindicator of plant response to SO_2-stress. In M.A. Öztürk (Ed.), Plants and pollutants in developed and developing countries (pp 549-568). Izmir, Turkey:Ege University.
v. Chauhan, A. 1990. Early diagnosis of SO_2-stress by volatile emissions in some crop plants. Oecologia84: 289-294.
vi. Chauhan, A. 2015a. Phytotoxic effects of SO_2 on crop plants: total chlorophyll content. Int. J. Chem.and App. 7: 51-61.
vii. Chauhan, A. 2015b. Phytotoxic effects of SO_2 on crop plants—total leaf protein content. Int.Adv. Jour.Sci.9:5-16.
viii. Chauhan, A. 2015c. Phytotoxic effects of SO_2 on crop plants—abiotic stress and reducing sugars.Int.J.Sci.& Technol. 3: 25-29.
ix. Chauhan, A. 2015d. Effect of SO_2 on ascorbic acid content in crop plants—First line of defense against oxidative stress. Int. J. Innov.Res.& Dev. 4(11): 8-13.
x. Fridovich, I. and Handler, P. 1958. Xanthin oxidase III. Sulphite oxidation as an ultrasensitive assay. J.Biol. Chem. 233: 1578-1580.
xi. Gaspar, T., Penel, C., Thorpe, T. and Greppin, H. 1982. Peroxidases 1970-1980. A survey of their biochemical and physiological roles in higher plants. University of Genève, Switzerland.
xii. Horsman, D.C. and Wellburn, A.R. (1976). Guide to the metabolic and biochemical effects of air pollutants on higher plants. In T.A. Mansfield (Ed.), Effects of air pollutants on plants (pp. 185-199). Cambridge: Cambridge University Press.
xiii. Keller, T. and Schwager, H. 1971. Der. Nachweis unsichtbarer ("physiologischer") Fluor-Immission schädingungen an Waldbäumen durch eine einfache kolorimetrische Bestimmung der Peroxidase-Aktivität. Eur.J. For.Pathol. 1: 6-18.
xiv. Khan, A.A. and Malhotra, S.S. 1982. Peroxidase activity as an indicator of SO_2 injury in jack pine and white birch. Biochem. Physiol. Pflanzen, 177: 643-650.
xv. Pierre, M. and Queiroz, O. 1982. Modulation of leaf age and SO_2 pollution. Environ. Pollut. 28: 209-217.
xvi. Rai, P.K. and Panda, Lalita,L.S. 2015. Roadside plants as bioindicators of air pollution in an industrial region, Rourkela,India. Int. J. Adv. Res. & Technol. 4(1):14-36.
xvii. Rao, M.V. 1992. Cellular detoxifying mechanisms determine the age dependent injury in tropical trees exposed to SO_2. J.Plant Physiol. 140(6): 733-740.
xviii. Rao, M.V. and Dubey, P.S. 1990. Explanations for the differential response of certain tree species to SO_2 under field conditions. Water,Air and Soil Pollut. 51(3): 297-305.
xix. Sarkar, R.K., Banerjee, A. and Mukherji, S. 1986. Acceleration of peroxidase and catalase activities in leaves of wild dicotyledenous plants as an indication of automobile exhaust pollution. Environ. Pollut.(Series A) 42: 289-295.
xx. Singh,L.P.,Gill,S.S., Gill,R. and Tuteja,N. (2012). Mechanism of sulphur dioxide toxicity and tolerance in crop plants In N. Tuteja, A.F.Tiburcio, S.S. Gill and R. Tuteja (Eds.), Improving crop resistance to abiotic stress (pp. 133-163). Berlin: Wiley-VCH Verlag GmBh & KgaA.
xxi. Tanaka, K. and Sugahara, K. 1980. Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity. Research Report from the Natl. Inst. Of Environ. Stud. Japan. 11: 155-164.
xxii. Treshow, M. (ED.) 1984. Air Pollution and Plant Life. Chichester, U.K.: Wiley.
xxiii. Varshney, S.R.K. and Varshney, C.K. 1985. Response of peroxidase to low levels of SO2. Environ.Exp.Bot. 25: 107-114.

Annexure

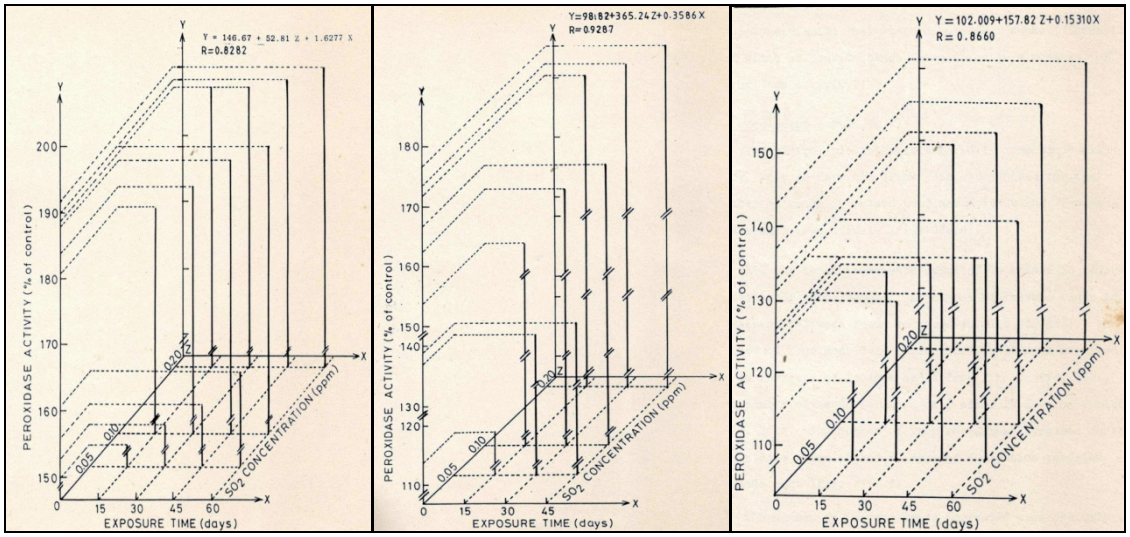

Figure 1: Solanum esculentum *Figure 2: Vigna radiata* *Figure 3: Zea mays*

Figures 1-3: Significance of Factorial Effects

Period of fumigation (days)	**15**		**30**		**45**		**60**	
Treatment conc. (ppm) X time (h)	Peroxidase Activity (µ/mg) protein	Percent increase	Peroxidase Activity (µ/mg) protein	Percent increase	Peroxidase Activity (µ/mg) protein	Percent increase	Peroxidase Activity (µ/mg) protein	Percent increase
C-1 (0 × 4)	12.50 ± 0.270		17.52 ± 0.1725		20.0±1.581		17.5±1.06	
T–1 (0.05 × 4)	25.10±0.0707	49.80	37.5±0.223	53.28	45±1.414	55.1	45±1.58	61.11
C-2 (0 × 2)								
T–2 (0.10 × 2)	67.48±0.2167	81.47	112.30±7.07	84.42	171.2±1.09	83.31	18.5±3.53	90.54
C-3 (0 × 1)								
T–3 (0.20 × 1)	112.5±0.707	88.88	116.25±14.14	89.46	208.0±14.83	90.38	220±12.24	92.04

Table 1: Effect of So_2 Treatments on Peroxidase Activity in S. esculentum
Mean (±SD) of 5 replicates C – 1, C – 2, C – 3 : Controls [air ×time (h)] ;
T – 1, T – 2, T – 3; Treatments [Conc. Of SO_2 (ppm) ×Exposure time (h)]

Period of fumigation (days)	**15**				**30**				**45**				**60**			
Source of variation	df	Sume of squares	Mean source	F	df	Sume of squares	Mean source	F	df	Sume of squares	Mean source	F	df	Sume of squares	Mean source	F
SO_2 Conc. (ppm) Exposure	9	58588.9	9502.8	0.90 **	9	134259.53	20908.854	0.95 **	9	231916.1	36541.7	0.97 **	9	253296.8	42874.92	0.98 **
Time (h)	14	72400.44	23314.36	2.41 **	14	171.294.00	57943.32	2.60 **	14	305909.4	110534.7	2.95 **	14	342093.75	131671.87	3.02 **
SO_2 Conc. × Exposure time	29	94560.94	42474.36	4.39	29	214112.65	100761.97	4.59	29	379915.00	184540.3	4.92	29	428523.25	218101.37	5.01
Error	6		9657.705		6		21909.996		6		37464.6		6		43554.58	

Significance of Factorial Effects:
*Levels of significance : ** P = 0.001 ; * P < 0.25*

Period of fumigation (days)	15		30		45	
Treatment conc. (ppm) X time (h)	Peroxidase Activity (μ/mg) protein	Percent increase	Peroxidase Activity (μ/mg) protein	Percent increase	Peroxidase Activity (μ/mg) protein	Percent increase
C-1 (0 × 4)	10.4 ± 0.961		30.0±1.414		50.2±0.9071	
T–1 (0.05 × 4)	12.6±0.418	17.46	47.3±1.303	36.57	82.54±0.1516	39.42
C-2 (0 × 2)						
T–2 (0.10 × 2)	21.71±0.074	52.09	80.0±1.581	62.5	147.5±0.7071	66.10
C-3 (0 × 1)						
T–3 (0.20 × 1)	35.72±0.083	70.88	115.36±1.937	74.02	217.7±0.8366	77.03

Table 2: Effect of So_2 Treatments on Peroxidase Activity in V. radiata
Mean (±SD) of 5 replicates C – 1, C – 2, C – 3 : Controls [air ×time (h)] ;
T – 1, T – 2, T – 3; Treatments [Conc. Of SO_2 (ppm) ×Exposure time (h)]

Period of fumigation (days)	15				30				45			
Source of variation	df	Sume of squares	Mean source	F	df	Sume of squares	Mean source	F	df	Sume of squares	Mean source	F
SO_2 Conc. (ppm) Exposure	9	9217.776	678.186	0.98	9	980165.1	5794.186	0.99 **	9	320590.98	22846.71	0.99 **
Time (h)	14	9796.068	1256.478	1.82 **	14	111644.65	19422.42	3.32	14	371618.51	73874.25	3.23 **
SO_2 Conc. × Exposure time	29	11165.26	2624.67	3.80	29	123288.84	31066.617		29	417322.85	119578.595	5.23
Error	6		690.006		6		5850.011		6		22857.62	

Significance of Factorial Effects:
*Levels of significance : ** P < 0.001 ; * P < 0.25*

Period of fumigation (days)	15		30		45		60	
Treatment conc. (ppm) X time (h)	Peroxidase Activity (μ/mg) protein	Percent increase	Peroxidase Activity (μ/mg) protein	Percent increase	Peroxidase Activity (μ/mg) protein	Percent increase	Peroxidase Activity (μ/mg) protein	Percent increase
C-1 (0 × 4)	60.0±1.581		120±1.0		182.4±4.56		107.48±0.083	
T–1 (0.05 × 4)	70.0±0.707	14.28	156.6±0.707	24.81	247.5±5.85	26.3	155.27±0.2190	30.96
C-2 (0 × 2)								
T–2 (0.10 × 2)	78.4±0.8366	23.46	159.8±1.303	24.90	247.5±1.118	26.3	155.25±0.270	30.96
C-3 (0 × 1)								
T–3 (0.20 × 1)	95.0±0.7071	36.8	175.0±0.707	31.42	270.2±2.86	32.59	187.51±0.041	42.78

Table 3: Effect of So_2 Treatments on Peroxidase Activity in Z. mays
Mean (±SD) of 5 replicates C – 1, C – 2, C – 3 : Controls [air ×time (h)] ;
T – 1, T – 2, T – 3; Treatments [Conc. Of SO_2 (ppm) ×Exposure time (h)]

Period of fumigation (days)	15				30				45				60			
Source of variation	df	Sume of squares	Mean source	F	df	Sume of squares	Mean source	F	df	Sume of squares	Mean source	F	df	Sume of squares	Mean source	F
SO_2 Conc. (ppm) Exposure	9	150198.9	809.27	0.956 **	9	60872.3	1390.20	0.944 **	9	1435972	862.65	0.87 **	9	562974.77	1996.22	1.357 **
Time (h)	14	152739.27	3349.64	3.95 **	14	623385.6	15052.8	36.44 **	14	1474676.4	39567	39.87 **	14	586672.38	25693.80	17.47 **
SO_2 Conc. × Exposure time	29	15394.6	5004.9737	5.91	29	624188.8	158560	38.39	29	1476531.3	41421.85	41.74	29	590139.27	29160.69	19.82
Error	6				6		413.0		6		992.25		6		1470.67	

Table 4: Significance of Factorial Effects
*Levels of significance : ** P < 0.001 ; * P < 0.25*