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Peroxidase Isoenzyme Profiles as Indicators of Sensitivity in SO_2-fumigated Crop Plants

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ABSTRACT

A study was made on the isoenzyme patterns of the anti-oxidative enzyme peroxidase in three crop species viz., *Solanum esculentum* (tomato), *Vigna radiata* (mung bean) and *Zea mays* (maize) exposed to low levels of SO_2. Exposure to the phytotoxicant gas resulted in alteration in isozyme profiles. The intensity and number of Peroxidase (POD) bands increased in *S. esculentum* and *V. radiata*. Changes in isoperoxide zymograms are indicative of enhanced peroxidation processs in response to pollutant-stress. This method also underlines the value of Polyacrylamide Gel Electrophoresis (PAGE) in sensitivity screening of degrees of SO_2- tolerance, prior to the manifestation of visible injury symptoms.

Keywords: Mung bean, tomato, maize, electrophoresis, peroxidases, isozyme profiles, SO_2 -tolerance

I. INTRODUCTION

SO_2 is a major phytotoxicant and is known to induce a variety of detrimental physiological and biochemical changes inside plants, long before the appearance of visible-injury symptoms (1-4). One of the most notable effects of SO_2, along with other pollutants like NO_x and O_3, is the excessive generation of free radicals, which in turn induces acute oxidative stress. The ROS (Reactive Oxygen Species) like H_2O_2, $OH^{\cdot}$ and $^{\cdot}O_2^-$ cause serious damage to the cell constituents (see 5). Plants possess various enzymatic as well as non-enzymatic detoxification mechanisms to counter SO_2 –induced biochemical alterations. Among the enzymatic protectors, superoxide dismutases (SOD) and peroxidases (POD) are the most notable ones functioning against oxidative stress (see 6-8). Ascorbic acid also constitutes the first line of defense against SO_2 induced stress (9). In our earlier communication, activity of peroxidases as an indicator of SO_2 –tolerance has been reported (8). Higher peroxidase activities in all the three plant species investigated may be interpreted as an indication of the participation of these enzymes as effective scavengers of toxic ROS (7-11). Studies on a variety of higher and lower plants suggest that peroxidases may serve as a good indicator of the distribution of gaseous pollutants in the vicinity of large metropolitan cities with a higher level of SO_2, HF and O_3 (7,10-19).

Peroxidases are known to exist in multiple molecular forms (20).The multiple gene loci and their dependent isoforms may well provide means for adaptation of metabolic patterns to the changing needs of different tissues and organs in the course of normal development,in response to environmental alterations(see 21-23).In the present study , various isozymes of peroxidases were investigated . The electrophoretic pattern of peroxidase isoenzymes in SO_2 –fumigated plants provides additional useful information to detect biochemical alterations caused by this phytotoxicant, thereby helping in screening of SO_2 sensitivity.

II. METHODS AND MATERIAL

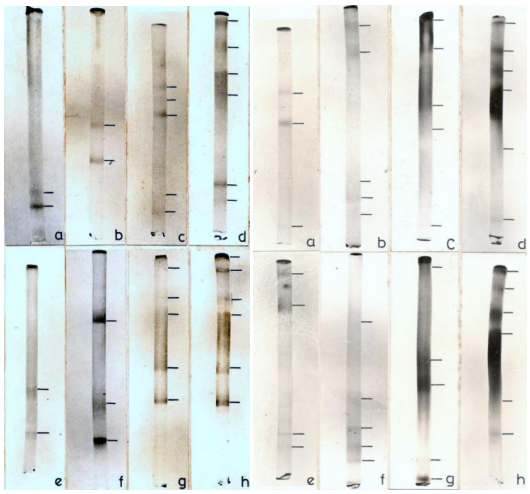
Three economically important cultivated plant species viz., *Vigna radiata* (L.) Wilczek [Mung bean],*Solanum esculentum*(= *Lycopersicon esculentum* Mill.) [Tomato], and *Zea mays* L. [Maize] were grown from seeds in the nursery. Fifteen-day-old seedlings of these plants were subjected to different SO_2 treatments through an artificial fumigation system. Sulfur dioxide was generated from an aqueous solution of $Na_2S_2O_5$ and

circulated over the plants kept in specially designed closed-top fumigation chambers (1 x 1 x1m= $1m^3$) 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 h.

A. Treatment Protocols of SO_2

Artificial fumigations were carried out according to the following protocols: **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.

B. Extraction and Determination of Peroxidase Activity



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Figures 1-2. Peroxidase Isozyme Profiles in SO_2-fumigated *S.esculentum*

Peroxidases (POD: EC 1.11. 1. 7) were extracted and their activity was determined by the method outlined by Gasper et al.(22). The entire methodology is detailed in our earlier communication (7).

C. Separation of POD Isozymes

Isoforms of peroxidases were separated using polyacrylamide gel electrophoresis (PAGE) technique according to the procedure outlined by Davis (24). Tris-glycine buffer (pH 8.3) was used as a running buffer. After electrophoresis the isozymes were visualized by the method suggested by Mitra et al.(25). The gels were first immersed in 2.4x 10^{-4}M benzidine containing 7x10^{-3}M (v/v) acetic acid solution for 15 minutes in dark. Thereafter, the gels were transferred to 3x10^{-2}M H_2O_2 solution maintained in dark for another 5 min. Intense blue isozyme bands appeared which later turned brown. Gels were immediately washed and stored in 7% acetic acid at 25^{0}C. The gels were later photographed.

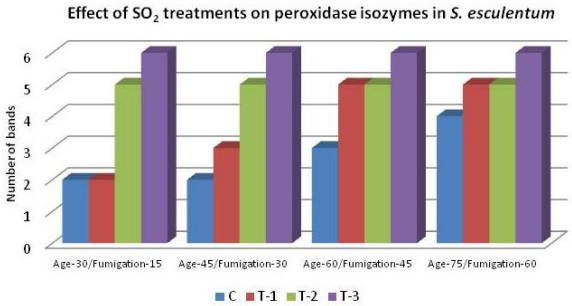
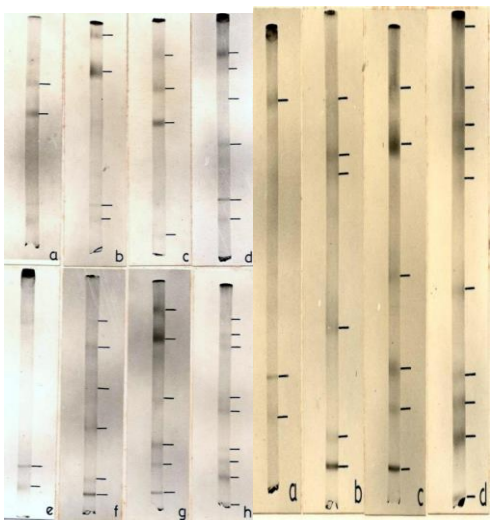


Table 1. Effect of SO_2 treatments on peroxidase isozymes



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Figures 3-4. Peroxidase Isozyme Profiles in SO_2-fumigated *V.radiata*

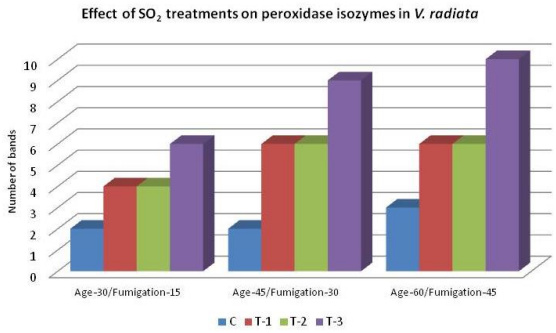
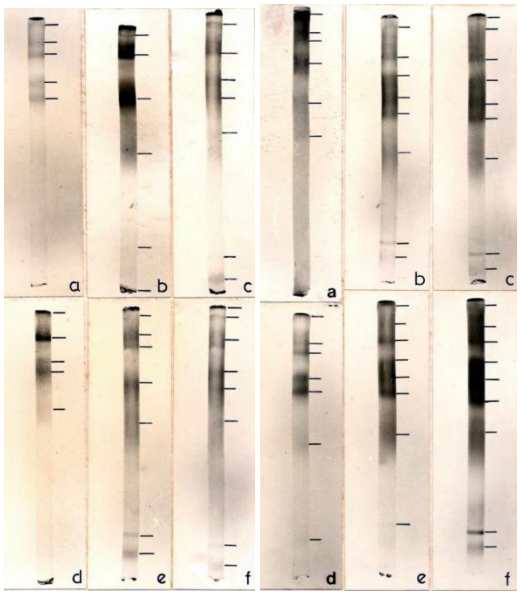


Table 2. Effect of SO_2 treatments on peroxidase isozymes



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Figures 5-6. Peroxidase Isozyme Profiles in SO_2-fumigated *Z.mays*

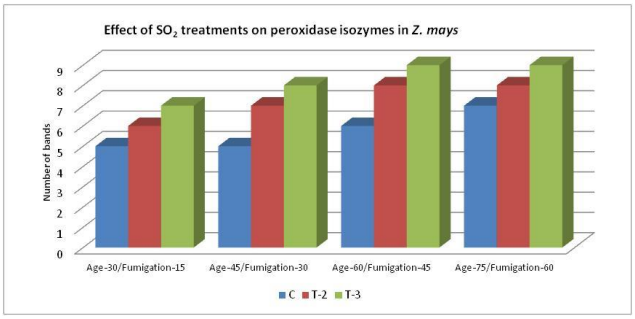

Table 3. Effect of SO_2 treatments on peroxidase isozymes

III. OBSERVATIONS

Number, intensity and width of isozyme bands were taken as a measure of peroxidase activity. Control plants of *S.esculentum* exhibited two isozyme bands till they were 45-day old (Figs 1a, 1e). Thereafter, the number of isobands increased to 3 and 4 in 60 and 75-day old plants respectively(Table 1;Figs 2 a, 2e).All the three SO_2 treatments resulted in change in isozyme profiles of peroxidases. In T-1 the number of isozyme bands increased to three only after 30 days of SO_2 fumigation (Fig 1f),and increased further to 5 bands each after 45 and 60 days of fumigation respectively (Figs 2b, 2f). In treatment T-2, there was a progressive change in isoperoxide zymogram showing 5 bands in all plants following 15, 30, 45and 60 days of SO_2 - fumigation respectively (Table 1; Figs 1c,1g,2c , 2g). The number of isozyme bands increased to 6 progressively increasing periods of SO_2 - fumigation (Figs 1d,1h ,2d, 2h).In addition to increase in number, there was also an increment in width and relative intensity of bands.

In *V.radiata*, two multiple forms of peroxidases were observed in 30 – 45 day control plants (Table 2; Figs 3a, 3e). There was an additional band in 60-day old plants (Fig 4a). All three SO_2 – treatments in T-1 showed an increase in the number of isoperoxidase forms. The number of multiple forms after 15 days of SO_2 – fumigation was 4 (Fig 3b) and this increased to 6 after 30-45 days of SO_2 - treatment respectively (Figs 3f , 4b).A similar pattern of the number of bands(6) was seen after 30 and 45 days of SO_2 – fumigation in T-2 (Figs 3g , 4c).There was, however, a progressive increase in the number of multiple forms of peroxidases in T-3. Peroxidase isoenzyme profile showed 6, 9 and 10 isobands after 15,30 and 45 days of SO_2 - fumigation respectively (Figs 3d,3h, 4d).

Thirty-day old control plants of *Z.mays* had 5 isoperoxide bands(Table 3; Fig 5a), which also remained the same in 45-day old plants (Fig 5d) and increased to 6 and 7 in 60 and 75-day plants respectively (Figs 6a and 6d).Not much significant change in isoperoxide zymogram occurred in plants after treatments T-2 and T-3. Whereas the number of bands increased from an initial 5 to 6, 7 and 8 after 15, 30 and 45 days of fumigation respectively (Figs 5b,5e and 6b).No new isozymic form was induced after 60 days as the number of bands remained at 8 (Fig. 6e).Number of isoforms increased to 7 after 15 days of SO_2 – fumigation in T-3 (Fig. 5c). Eight anionic bands were observed after 30 days (Fig.5f) and the number of bands after 45 and 60 days of SO_2 – fumigation was 9 (Table 3; Figs 6c , 6f).

IV. DISCUSSION

Peroxidases are known to catalyse the H_2O_2-dependent oxidation of a number of substrates, and exist in a number of multiple molecular forms (20, 26). SO_2-induced changes in POD may also be reflected as alterations in the activities of specific isozymes. The distribution and characteristics of these isoforms, which are not under genetic control, may be indicative of the needs of the developing tissue under SO_2 –stress. Since genetic variability increases with age, so does the pattern of scavenger enzymes like SOD and POD.

In the present investigations, those plants having few isozymic forms developed entirely different band patterns which could be well designated as induced isozymes. In *S. esculentum*, the control plants of all ages (viz., 40, 45, 60 and 75 days) exhibited only 2,3 and 4 isozyme bands respectively as compared to the SO_2-fumigated plants, which showed additional isoforms for the same respective ages. This alteration in isozyme profile can be ascribed to the *de novo* synthesis of POD as anionic bands 5 and 6, which never appeared in the control plants during their entire life span. In the relatively SO_2-tolerant species *V. radiata* and *Z. mays*, SO_2 perhaps does not act as an inducer of new isozymes as similar Rf values were located in comparatively older control plants. It is therefore, apparent that SO_2-stress simply advanced the appearance of those isozymes which were yet to appear in the control plants. Resistance to SO_2 is thus a function of the number of isoenzymes of POD *ab initio.* A similar trend is also observed for SOD (see 6). More isozymic forms would mean greater resistance, as is clearly documented in *Z.mays*. Number of POD isozymes is 5-6 and 7 respectively in control plants of progressively increasing ages. This number remained fairly constant throughout the life span. Consequently maize is able to resist SO_2-stress more successfully. Biochemical studies on peroxidases corroborate this viewpoint (7).Similar pattern of SO_2-induced POD isozyme profile has been reported in *Vigna sinensis* and *Capsicum* (27), in pollen of *Argemone mexicana* (16), and also in sunflower cotyledons exposed to UV-B radiations (20), as well as in low level O_3- exposed bean leaves (28).

V. CONCLUSION

Electrophoretic patterns of POD isozymograms are a useful diagnostic tool for detecting certain biochemical alterations occurring in a plant in response to air pollutants, thereby assisting in their usage as reliable biochemical markers of stress tolerance.

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