

Peroxidase and Polyphenol Oxidase Activity in Malformed Mango Inflorescence Caused by *Fusarium moniliforme* var. *subglutinans*

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Abstract. Peroxidase and polyphenol oxidase activities in malformed mango inflorescences of 'Himsagar' and 'Bombay green' cultivars were found to be increased considerably following infection by *Fusarium moniliforme* var. *subglutinans*. Whether such increased activities were due to their synthesis by the pathogen or the host, or both, was not studied although it was found that the pathogen was incapable of producing the enzymes *in vitro*. The activities of both the enzymes in infected tissues were found to increase considerably during the experimental period. It was found that activities of polyphenol oxidase were inhibited in the presence of sodium diethyldithiocarbamate and phenylthiourea; the former acted as chelating agent of Cu of the enzymes and the latter as a competitive inhibitor. Similarly, peroxidase activity was found to be inhibited by cycloheximide which acted as inhibitor of enzyme protein synthesis. The fact that the 'Himsagar' cultivar showed greater enzyme activity than the 'Bombay green' cultivar possibly suggests its higher resistance to the pathogen.

In many plant diseases, the activation of peroxidase and polyphenol oxidase in the host tissues following infection by pathogens has been reported by a number of workers (FARKAS and STAHMANN 1966, FEHRMANN and DIMOND 1967, HARE 1966, KUĆ 1964, MEAGHER *et al.* 1967, WEBER *et al.* 1967). Although no definite biochemical role has yet been proposed, these enzymes are considered to play an active role in contributing to disease resistance in certain plant host-pathogen interaction. The present investigation has been undertaken to study the activities of these enzymes in malformed mango inflorescence caused by *Fusarium moniliforme* var. *subglutinans* WR. et RG. This disease of one of the principal fruit crops in the Indian sub-continent and other adjacent countries of the East, produces abnormal vegetative shoots and inflorescences which fail to fruit, thus resulting in tremendous economic losses. The identification of the causal organism, which was isolated from a large number of inflorescences collected from different areas of Burdwan, was confirmed by the Indian Agricultural Research Institute, New Delhi.

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Material and Methods

Healthy mango plants of the popular 'Himsagar' and 'Bombay green' cultivars in flowering condition were inoculated with the spores of the test-fungus keeping adequate controls. Spore suspension of the fungus was prepared in distilled water with spores collected from mycelia grown on synthetic medium for 12 days. The density of the spores was adjusted to an absorbance of 0.40 at 360 nm by a Hilger pattern biochemical absorptiometer. The suspension (10 ml) was then sprayed in the form of fine mist on the inflorescences. The control plants were sprayed with sterile distilled water. The plants were then incubated in a water saturated atmosphere for different periods of time.

Extraction and Assay of Peroxidase

To extract the enzyme, 4 g of each of the diseased and healthy tissues were ground separately in 20 ml cold distilled water in a mortar at 0°C. A pinch of sand was added to facilitate crushing. The extract was obtained by filtering off the debris with a clean cloth and centrifuging at 3000 *g* for 15 min in a refrigerated centrifuge. The supernatants were recovered and kept in a tube in an ice-bath until assayed. 5 ml of the freshly prepared pyrogallol reagent (prepared by mixing 10 ml of 0.5 M pyrogallol solution and 12.5 ml of 0.66 M phosphate buffer and the volume made up to 100 ml with distilled water) and 1.5 ml of the enzyme extract were mixed in a colorimeter tube and the mixture was immediately adjusted to zero absorbance of a colorimeter. 0.5 ml of 1 % hydrogen peroxide was added to it and the content was mixed by inverting the tube. The reaction was initiated by the addition of H₂O₂. Enzyme activity was recorded as the change in absorbance per minute ($\Delta A \text{ min}^{-1}$) at 430 nm immediately after the addition of substrate. Similarly, control on non-enzymatic oxidation was maintained at different times by heating the extract at 100 °C for 10 min. The activity was always measured zero indicating its complete inactivation by the heat treatment.

Extraction and Assay of Polyphenol Oxidase

2 g of healthy and diseased tissues were homogenised separately with a pinch of sand in 6 ml phosphate buffer of 0.1 M at pH 7.0 at 0°C. The extract was filtered, centrifuged and stored in an ice-bath until used.

2 ml of the enzyme extract and 3 ml of distilled water were mixed together in a colorimeter tube and adjusted to zero absorbance of a colorimeter. 1 ml of catechol solution (0.4 mg ml⁻¹) was added to the above mixture and the reactants were quickly mixed. The enzyme activity was measured as the change in absorbance per minute ($\Delta A/\text{min}$) at 490 nm immediately after the addition of catechol, which initiated the reaction. Control in a similar manner was maintained at different times by heating the extract to 100 °C for 10 min. The activity was measured zero indicating complete inactivation of the enzyme by heat treatment.

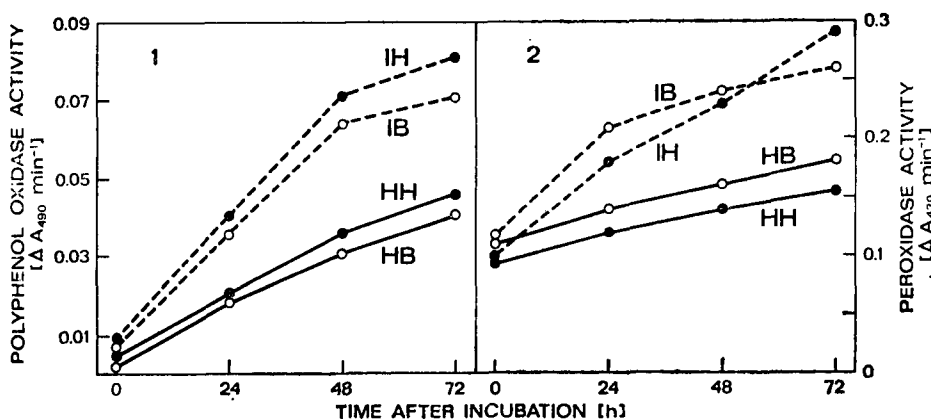
Results

Following infection after 24 h, polyphenol oxidase activity showed a considerable difference, being much higher in both the cultivars of infected hosts than in the healthy control tissues (Fig. 1). With increasing time of incu-

bation, the differences in the activity became much higher in both the cultivars of the host than in the control. After an initial increase, there was a slight depression in the activity of the enzyme. Of the two, the 'Bombay green' cultivar shows a slightly lower activity than the 'Himsagar' cultivar.

Similarly, the peroxidase activity also showed a marked difference after 24 h (Fig. 2), then increasing considerably more with the increase in the period of incubation in both the cultivars than in the control. Peroxidase activity of 'Bombay green' cultivar was also found to be much lower than that of the 'Himsagar' cultivar.

Since differences were noted in the two enzyme systems from the early stages of host infection, the increased activity might well be due to increased synthesis of the enzymes.



Figs. 1 and 2. Polyphenol oxidase (1) and peroxidase (2) activities in healthy and infected tissues of two cultivars of mango inflorescence infected with *Fusarium moniliforme* var. *subglutinans* at different incubation periods. HH — Healthy 'Himsagar'; IH — Infected 'Himsagar'; HB — Healthy 'Bombay green'; IB — Infected 'Bombay green'.

Extraction and Assay of Enzymes from the Pathogen in Culture

For the assay of the enzymes present in the mycelium, the fungus was grown in a liquid synthetic medium for 6 days in complete darkness. The mycelium was gathered, washed thoroughly with distilled water and blotted dry. 1 g in 5 ml cold distilled water for peroxidase and 1 g in 3 ml phosphate buffer of 0.1 M at pH 7.0 for polyphenol oxidase were used for extraction of the enzymes.

The activities of the enzymes were assayed following the same method used in infected tissues. The peroxidase activity was found to range between 0.005 and 0.012 $\Delta A \text{ min}^{-1}$, but no activity of polyphenol oxidase was recorded.

Inhibition of Peroxidase Activity by Cycloheximide

The previous experiment showed that the peroxidase activity increased as a result of its synthesis following infection. Therefore, it was expected that in the presence of cycloheximide, an inhibitor of protein synthesis, interruption or inhibition of further increase in the activity of the enzyme

in the infected host should result. To study this, another set of experiments was undertaken by placing infected cut tissues in either water or a solution of cycloheximide ($10 \mu\text{g ml}^{-1}$). The tissues were kept in a humid condition to check desiccation and under regular growth chamber conditions of light and temperature. The tissues were then assayed for peroxidase at different incubation periods.

TABLE 1

Inhibition of increased peroxidase activity by cycloheximide in mango tissues infected with *F. moniliforme* var. *subglutinans* at different incubation periods expressed in $\Delta A \text{ min}^{-1}$ at 430 nm

Material	Treatment	Incubation periods [h]					
		0		24		48	
		Himsagar	B. green	Himsagar	B. green	Himsagar	B. green
Healthy	Water	0.096	0.114	0.110	0.131	0.132	0.144
	Cycloheximide			0.073	0.081	0.095	0.100
Infected	Water	0.104	0.120	0.186	0.204	0.235	0.245
	Cycloheximide			0.112	0.105	0.125	0.132

It became evident from Table 1 that the cycloheximide treatment resulted in the reduction of peroxidase activity in healthy tissue as compared to the water treated control. As the level of the enzyme increased only to a small extent in healthy tissues, it seemed that an apparent low level of turnover of peroxidase occurred in healthy plants. Peroxidase activity which increased considerably in response to infection, was also inhibited by the compound.

Inhibition of Polyphenol Oxidase Activity by Phenylthiourea and Sodium Diethyldithiocarbamate

Another set of experiments was made following BATEMAN (1962) to study the effect of the compounds on the polyphenol oxidase. For this, cut infected tissues were dipped in either water or solutions of phenylthiourea and diethyldithiocarbamate (6×10^{-3} M) separately for different incubation periods under humid conditions. At the end of the periods the tissues were analysed for the enzyme.

The results from Table 2 showed that the enzyme activity, which was comparatively low in the healthy host, was completely stopped in both the treatments. Infected tissues of both the cultivars, on the other hand, showed significant reduction when treated with phenylthiourea. Treatment with sodium diethyldithiocarbamate showed only slight activity after 24 h but complete inhibition in both the cultivars of hosts in later observations.

Discussion

The results clearly indicate a considerable increase, in both peroxidase and polyphenol oxidase activities following infection, which is in full accordance with similar observations made by previous workers in other cases (FEHR-

TABLE 2

Inhibition of increased polyphenol oxidase activity by phenylthiourea (PT) and sodium diethyl-dithiocarbamate (SD) in mango tissues infected with *F. moniliforme* var. *subglutinans* at different incubation periods expressed in $\Delta A \text{ min}^{-1}$ at 490 nm

Material	Treatment	Incubation periods [h]					
		0		24		48	
		Himsagar	B. green	Himsagar	B. green	Himsagar	B. green
Healthy	Water	0.004	0.002	0.021	0.020	0.038	0.034
	PT			0	0	0	0
	SD			0	0	0	0
Infected	Water	0.009	0.011	0.042	0.037	0.073	0.065
	PT			0.001	0.001	0.002	0.003
	SD			0.002	0.001	0	0

MANN and DIMOND 1967, JENNINGS *et al.* 1969). The increased activity, which has been recorded from the very early stage of infection, might well be due to activation in the synthesis of the enzyme proteins in the host. The complete absence of polyphenol oxidase and the presence of only a small amount of peroxidase in the culture of the pathogen *in vitro*, however, does not preclude their pathogenic origin.

It is known that in the course of molecular interaction between the pathogen and the host tissues, the latter is metabolically activated to produce abnormal metabolites. These metabolites are finally oxidised by polyphenol oxidase to quinones, which by their toxicity against the pathogen account for increased host resistance. Thus, increased polyphenol oxidase activity can be correlated with the host resistance since the degree of defense reaction is sometimes parallel to the amount of the enzyme.

Peroxidases are also known to be strikingly correlated to host resistance but the mechanism of their action is still unknown. Possibly the increased activity is responsible for contributing to a physiological status in the host tissues that become inhospitable to the pathogen.

The higher activity of the enzymes in the 'Himsagar' cultivar probably suggests the comparatively greater resistance of this cultivar than 'Bombay green' and is in conformity with the slightly lower disease frequency in nature as observed by the authors.

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BOOK REVIEW

POLJAKOFF - MAYBER, A., GALE, J. (ed.): *Plants in Saline Environments*. — *Ecological Studies*, Volume 15, Springer-Verlag, Berlin—Heidelberg—New York 1975. 213 pp., in English.

The problem of salinity becomes more relevant every year because not only further expansion of agriculture must consider the cultivation of saline soils and use of water with relatively high content of soluble salts but also water pollution due to industrial development often induces secondary salinization of soils and sources of irrigation water. Therefore this very readable monograph of high scientific level will be certainly welcomed not only in basic and agricultural research but also in practice.

The first chapter gives a general survey of the salinity problem with special reference to the distribution of saline areas and halophytic vegetation throughout the world. Chapter II emphasizes the severity of the salinity problem in modern agriculture and points to some possible practices which enable to cope with damage caused by salinity. The main purpose of chapter III is to describe the effect of the concentration and composition of salts on the physical and chemical properties of the soil. Chapter IV reviews the criteria for water quality for irrigated agriculture and chapter V examines factors influencing the distribution of soluble salts in the natural environments, and the redistribution of salts which results from environmental disturbances. Various responses of plant to salinity and the nature of damage of agricultural crops caused by salinity (both the osmotic effect and the specific ionic effects) is a common feature of the following chapters. Chapter VI deals primarily with the morphological and anatomical changes in plants as a response to salinity stress. In chapter VII special attention is given to the structure and function of salt glands common in the families *Plumbaginaceae* and *Frankeniaceae*. The further chapter VIII is focused to the metabolic and biochemical aspects of salt tolerance. Water balance and gas exchange of plants under saline conditions is the subject of chapter IX and in the last chapter X, the combined effect of environmental factors and salinity on the plant growth is examined. The parts Introduction, Intermediary Remarks and General Discussion aptly complement the book.

It is well produced and the text is illustrated with many graphic figures, excellent microphotographs, maps and tables. The plant and subject indexes are also included.

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