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Short Communication

Studies on Certain Oxidases, Their Isozymes and Phenols as Related to Gall Formation in *Leea indica*

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Summary

Leea leaf gall tissue showed increased PPO activity and protein content followed by normal from diseased leaf and normal tissues. A reverse picture was obtained for IAA-oxidase activity. The gall tissue showed decrease in PRO activity as compared to normal, whereas a very high activity of this enzyme was observed in normal from diseased tissue. The *O*-dihydroxy and total phenol contents were high in the gall tissue in contrast to normal. The normal from diseased tissue showed the highest accumulation of phenols. A correlation existed between quantitative and qualitative assays of the above enzymes. Specific isozyme bands, of PPO & PRO for normal from diseased, and of IAA-oxidase and PPO for gall, characteristic of these tissues were recorded.

A large number of plant insects and mites incite the tissue of the host plants to initiate galls showing hypertrophy and hyperplasia. Some interesting reviews on the problem have appeared (Arya et al. 1975; Rohfritsch and Shorthouse 1982). The plant galls showing neoplastic growth reflect an abnormal auxin and phenol metabolisms. The hyperauxinity exhibited by gall tissue could be due to increased synthesis and or retention of this compound (Purohit et al. 1980; Tandon and Arya 1980a; 1982). The role of oxidative enzymes and phenols in auxin catabolism in plant tumors have been reported (ref. Tandon and Arya 1982). However, the isozyme forms of these enzymes and their functional requirements in gall formation has not been worked out in detail. The present investigation was undertaken to study the effect of mite infection on PRO, PPO and IAA-oxidase activities, their isozymes and phenols in gall development on leaves of *Leea indica* (Burm.) Merr.

Leea indica showed severe leaf gall formation in the months from November to May, thereafter decrease in disease incidence was observed. Leaf galls are bluntly conical or subglobose, sessile, visible on both sides of the leaf. Their size ranges from 2—15 mm in length and 2—5 mm in diameter. Normal leaf, normal looking portions from diseased leaf and galled materials were collected in the months from January to March from a forest in Upper Shillong.

For the extraction of IAA-oxidase, phosphate buffer (0.2 mol, *p*H 6.0) and for PRO and PPO the same buffer having 5% PVP was used. One g each of fresh tissues were homogenised in 10 ml of

Abbreviations: PPO, polyphenol oxidase; IAA, indole-3-acetic acid; PRO, peroxidase; PVP, polyvinyl pyrrolidone

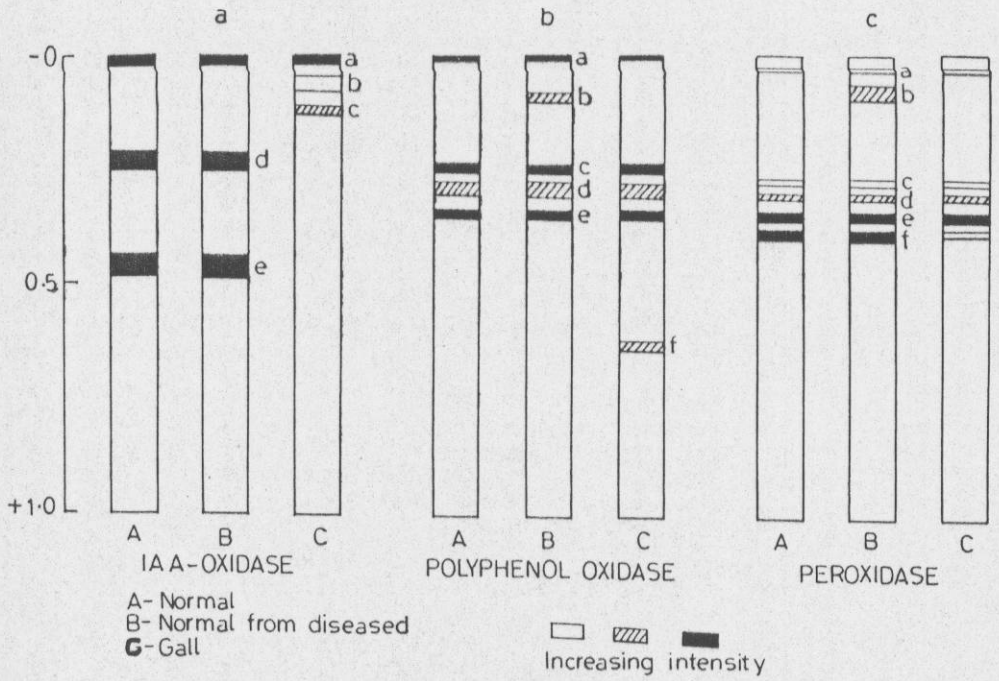

Fig. 1. *Isoenzyme patterns of IAA-oxidase, polyphenol oxidase and peroxidase of normal leaf, normal from diseased leaf and gall tissues.*

chilled buffer and the homogenates were centrifuged twice at 15,000 rpm for 30 min at 2 °C. The supernatants were used for enzyme assays.

Assay of 1.11.1.7 PRO, 1.10.3.1 PPO and IAA-oxidase and estimations of protein, *O*-dihydroxy and total phenol contents were done by the procedures described earlier (Tandon and Arya 1982). The activities of PRO and PPO are expressed as change in absorbance min^{-1} g^{-1} fresh weight at 460 and 420 nm, respectively. The IAA-oxidase activity is expressed as mg IAA destroyed g^{-1} fresh mass of tissue h^{-1} at 37 °C. Isozymes were separated on polyacrylamide gels (7.5 %) at 4 °C following the method of Davis (1964). The isozymes of PRO, PPO and IAA-oxidase were located using the methods of Mitra et al. (1970); Vanloon (1971); and Endo (1968), respectively.

The PRO, PPO and IAA-oxidase activities, *O*-dihydroxy and total phenols and protein contents in normal leaf, normal from diseased leaf and gall tissues is shown in Table 1. In contrast to normal and normal from diseased tissues, the gall tissue showed low IAA-oxidase activity. Similar decrease in the activity of IAA-oxidase associated with mite-incited galls on *Ficus mysorensis*, *Achyranthes aspera* and *Zizyphus mauritiana* has been reported (Purohit et al. 1980). The IAA-oxidase isozyme pattern was similar in both normal and normal from diseased tissues (Fig. 1a). On the other hand, two intense bands (d & e) were absent in the gall tissue which is also consistent with its low IAA-oxidase activity. However, two new low intensity isozyme bands (b & c) appeared which were characteristic of the gall tissue. The IAA-catabolism in plant tumors has been a subject of extensive investigations and there are a number of conflicting reports (Bouillenne and Gaspar 1970; Stonier 1972; Butcher 1973). A perusal of literature shows that in contrast to gall tissue, the normal tissue was reported to possess IAA-inactivating enzymes or during gall induction a decrease in IAA destruction was reported. A low IAA-oxidase activity in crown-gall tissue as compared to normal was also reported resulting in its hyperauxinity. On the other hand, in plant tumor tissues, the presence of

Table 1. *Activities of IAA-oxidase, polyphenol oxidase, peroxidase and contents of total and O-dihydroxy phenols and protein in normal, normal from diseased and gall tissues*

Analysis	Normal	Normal from diseased	Gall
IAA-oxidase mg IAA destroyed g^{-1} fresh mass h^{-1}	5.60 ± 0.05	5.40 ± 0.02	4.65 ± 0.03
Polyphenol oxidase ΔA min^{-1} g^{-1} fresh mass	0.21 ± 0.07	0.69 ± 0.06	1.00 ± 0.02
Peroxidase ΔA min^{-1} g^{-1} fresh mass	4.60 ± 0.10	8.60 ± 0.09	2.14 ± 0.07
Total phenols mg g^{-1} fresh mass	1.35 ± 0.03	3.95 ± 0.05	2.75 ± 0.07
O-dihydroxyphenol mg g^{-1} fresh mass	1.10 ± 0.03	2.85 ± 0.07	2.40 ± 0.02
Protein mg g^{-1} fresh mass	25.00 ± 0.04	35.00 ± 0.02	56.50 ± 0.04

± Standard error.

IAA-oxidase inhibitors has been reported by several workers. The naturally occurring IAA-oxidase inhibitors of phenolic nature range from free low molecular weight compounds such as chlorogenic, ferulic and protocatechuic acids to high molecular weight auxin protectors (Schneider and Wightman 1974).

Increased accumulation of total and *O*-dihydroxy phenols in *Leea* gall tissue as compared to normal could be due to higher activity of PPO in the former. The normal from diseased tissue showed one PPO band (b) in excess as compared to normal tissue (Fig. 1b) which is also consistent with its higher PPO activity and phenolic contents. The gall tissue showed PPO bands similar to normal but for band (f) which is characteristic of gall. The higher amounts of *O*-dihydroxyphenols in normal from diseased and gall tissues might be responsible for their low IAA-oxidase activities (Table 1). The *O*-dihydroxyphenols are considered to be auxin protectors which prevent peroxidase-catalysed oxidation by inducing a lag period in juvenile tissue, in wounded tissue and also in plant tumor tissue (ref. Tandon and Arya 1980b).

The results presented in Table 1 showed highest *O*-dihydroxy and total phenols and also PRO activity in the normal from diseased tissue probably to check further attack of mites. This is consistent with the general belief that PRO and PPO activities and phenolic compounds play an important role in disease resistance (Fric 1976). The normal from diseased tissue of *Leea* showed one PRO isozyme (b) in excess to the normal tissue (Fig. 1c) which is in line with increased PRO activity in the former and is probably associated with resistance against further invasion by mites. Increase in PRO isozymes as a result of infection has been reported by a number of workers (ref. Curtis 1971; van Lilyveld and Brodrick 1975). On the other hand, gall tissue showed PRO isozyme pattern similar to normal tissue. However, the intensity of band (f) decreased considerably in the gall tissue. The enzyme multiplicity could be either genetic or due to liberation of proteolytic enzymes upon disruption of cells that may result in partial degradation of enzymes. The changing patterns of isozymes from one tissue to another and one developmental stage to another are helpful in understanding the basic mechanisms of cellular differentiation. The changes in isozyme patterns in leaves of *Leea* following mite attack resulting in gall formation should be observed in the light of transformation of normal to abnormal growth behaviour. The gall tissue of *Leea* showed

highest protein content as compared to normal and normal from diseased tissues. The higher rate of protein synthesis in gall tissue has been correlated with its uncontrolled rapid growth in a number of plant tumors (Tandon and Arya 1982).

Conclusion can be drawn that both quantitative and qualitative assays of PPO, PRO, IAA-oxidase and phenolics play an important role in gall induction in *Leea*. The accumulation of *O*-dihydroxyphenols due to PPO activity may cause hyperauxinity in the gall tissue thus resulting in its abnormal growth.

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