Changes in certain oxidative enzymes and phenolics in *Camellia sinensis* and *Elaeocarpus lancifolius* leaf roll-galls

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ABSTRACT

The polyphenol oxidase activity could not be observed in extracts of fresh material and acetone powders of the normal leaf and leaf roll-gall tissues of *Camellia sinensis* and *Elaeocarpus lancifolius*. However, it was recorded in tissue extracts of *C. sinensis* and of *E. lancifolius* in a buffer containing sodium dodecyl sulfate and polyvinyl pyrrolidone, respectively. In both the species, the gall tissue showed higher polyphenol oxidase activity as compared to the normal. Lower levels of IAA-oxidase activity and phenolic contents were recorded in the gall tissue. In the two species, a different pattern of peroxidase was observed. The gall tissue showed more protein in extracts of acetone powders and fresh tissues in antioxidant-containing buffer.

Introduction

Various agents or conditions have been reported to act as incitants of abnormal growths in plants. Insect or mite-incited abnormal growths are commonly known as galls or cecidia. The size, shape and structure of the gall is characteristic of its causal agent.

It is now generally accepted that visible symptoms induced in plant tissues infected by pathogens are manifestations of disturbances in the host metabolism. The qualitative and quantitative changes in host proteins, particularly in enzymes, have been demonstrated during host-parasite combinations (Burnett, 1973; Fric, 1976 ; Lazarovits and Ward, 1982 ; Vaughan and Duke, 1984). Increased hormonal

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levels and oxidative enzymes are associated with mite-incited galls (Purohit et al., 1980 ; Tandon and Arya, 1980, 82 ; Malan et. al., 1982).

Phenolics are widely distributed in plant kingdom. Besides their role in defense reactions of the host (Mayer and Harel, 1979 ; Friend, 1981 ; Zucker, 1982), a number of other functions in plant metabolism have been attributed to them (Gordon and Paleg, 1961 ; Vaughan and Duke, 1984). Inactivation of enzymes by phenolics or by their oxidation products is also well documented (Sermani et. al., 1982). Therefore, a careful formulation of the medium for extracting enzymes in such cases is required. A large number of reducing agents or phenolic scavengers have been used for enzyme extraction by various workers (Loomis and Battaile, 1966 ; Baijal et. al., 1972 ; Werth et. al., 1982).

This paper deals with the extraction of certain oxidative enzymes and changes in their activities along with phenolics in the leaf roll-gall formation in *Camellia sinensis* and *Elaeocarpus lancifolius* by some apids (Homoptera).

MATERIALS AND METHODS

Plant Tissue

In both *Camellia sinensis* (L.) O. Ktze (Fig. 1) and *Elaeocarpus lancifolius* Roxb. (Fig. 2) young leaf blades, upon mite-attack, roll upward or downward from the leaf margins towards the midrib followed by swelling of the rolled up part of the leaf blade. Young leaf roll-galls are green in color and they become purple in *C. sinensis* and reddish in *E. lancifolius* in the latter stages of gall development.

Normal leaf and leaf roll-galls of these plants were collected from a forest in Upper Shillong, Meghalaya for the present studies.

Tissue extraction

Fresh tissues or acetone powders were homogenized in phosphate buffer (0.2M, pH 6.0) with or without antioxidants for the extraction of peroxidase, polyphenol oxidase, indole-3-acetic acid (IAA)-oxidase and protein contents in both the normal and diseased tissues. Polyvinyl pyrrolidone (PVP : 0.1, 0.2, 0.5 g/g fresh weight of tissue) and sodium dodecyl sulfate (SDS : 0.1 and 1.0 g/g fresh weight of tissue) were used as antioxidants.

In all cases the homogenized mixture was squeezed by two-layered cheese cloth and centrifuged at 15,000 rpm for 20 min. at 0 C. The supernatants were used for enzyme assays.

An alcohol extract was prepared for phenol estimations following the method of Mahadevan and Sridhar (1982).

Assay of Enzymes

1.11.1.7 peroxidase activity was determined by using 0.05 M pyrogallol as hydrogen donor. The rate of formation of purpurogallin was measured at 420 nm (Mahadevan and Sridhar, 1982). 1.10.3.1 polyphenol oxidase activity was measured following Ponting and Joslyn (1948). Method of Tandon and Arya (1982), with slight modification, was used for assay of IAA-oxidase activity. The reaction mixture for IAA-oxidase activity was buffered by 0.2M phosphate buffer (6.0). The activities of enzymes are expressed as change in absorbance/min/g fresh weight of tissue for peroxidase and polyphenol oxidase ; and mg IAA destroyed/g fresh weight of tissue/h at 37 C for IAA-oxidase.

Estimation of Protein

Method of Lowry et al (1951) was followed for protein estimation. The amount of pro

tein is expressed as mg/g fresh weight of tissue.

Estimation of Phenols

An alcohol extract was used to estimate the O-dihydroxy and total phenols using Folin-phenol and Arnow's reagent, respectively (Mahadevan and Sridhar, 1982). Phenols are expressed as mg/g fresh weight of tissue.

Results

In both normal and gall tissues of *C. sinensis* and *E. lancifolius*, polyphenol oxidase activity could not be observed in extracts made in phosphate buffer from fresh tissues as well as acetone powders of the same (Table 1 and 2). While the activity of polyphenol oxidase was recorded in *C. sinensis* extracts prepared in phosphate buffer containing SDS (0.1 g/g fresh weight of tissue), in *E. lancifolius* it was recorded in extract prepared in phosphate buffer containing PVP (0.2 g/g fresh weight of tissue) (Table 3). In both the species the gall tissues showed more polyphenol oxidase activities as compared to the normal. In *C. sinensis*, the gall tissue showed a higher peroxidase activity in contrast to the normal (Table 1, 2 and 3). However, a reverse picture was obtained for *E. lancifolius*. In both the species the IAA-oxidase activities (Table 1, 2 and 3), and O-dihydroxy and total phenol contents (Table 4) were less in gall tissues as compared to the normal. The protein contents were also less in gall tissues of both the species in extracts prepared in phosphate buffer (Table 1). However, the gall tissue extracts prepared from acetone powders and with antioxidants in buffer, revealed a higher protein content (Table 2 and 3).

Discussion

Changes in oxidative enzymes and phenolics are associated with gall formation. An altered host metabolism favours the growth of the pathogen in many-pathogen interaction (Sequeira, 1963). The leaf roll-galls of *C. sinensis* and *E. lancifolius* showed more polyphenol oxidase in comparison to the normal leaf—an observation consistent with the work of Burnett (1973) and Bomhoff (1974) on crown-gall, and that of Tandon and Arya (1982) on mite—incited gall. This increase in the enzyme activity could either be due to the increased solubilization or activation of the enzyme present in the latent form by reducing agents (Penefsky and Tzadgoloff, 1971 ; Mayer and Harel, 1979). It is quite clear that polyphenol oxidase is the key enzyme which regulates the levels of phenolic compounds by converting monophenols (electron acceptors) to O-diphenols (electron donors) to quinones (strong electron acceptors) which further polymerise to form high molecular weight compounds. These phenolic compounds influence both functional as well as structural proteins. The low protein contents in gall tissues extracted with buffer without antioxidants might be accounted for by the protein-phenol/quinone complex formation and their precipitation during homogenization (Leatham et al., 1980). This precipitation is prevented by using antioxidants in the extracting buffer (Loomis and Battaile, 1966).

In response to the various external factors changes in phenols occur in plants. (Purohit et al., 1980 ; Woodhead, 1981). In the present studies, a low level of phenolic compounds was observed in leaf roll-galls. This is in line with the findings on stem galls in *Prosopis* (Purohit et al., 1979). However, in contrast to normal tissue, a higher phenolic content has been reported in many gall tissues (Wegen and Glase, 1981 ; Tandon and Arya, 1982). A decrease in phenolics in the gall tissues

TABLE 1

Activities of peroxidase, polyphenol oxidase, IAA-oxidase and protein contents in normal and gall tissues of *C. sinensis* and *E. lancifolius* in extracts prepared in phosphate buffer.

Analysis	*C. sinensis*		*E. lancifolius*	
	Normal	Gall	Normal	Gall
Peroxidase : △A/min/g fresh weight	0.8±0.01	1.8±0.04	3.3±0.07	0.8±0.01
Polyphenol oxidase : △A/min/g fresh weight	—	—	—	—
IAA-oxidase : mg IAA destroyed/g fresh weight/h	1.4±0.07	0.4±0.02	5.5±0.10	5.0±0.20
Protein mg/g fresh weight	1.02±0.03	0.84±0.02	2.9±0.20	2.58±0.17

±S. E.
—no activity.

TABLE 2

Activities of peroxidase, polyphenol oxidase, IAA-oxidase and protein contents in extracts prepared from acetone powders of normal and gall tissues of *C. sinensis* and *E. lancifolius*.

Analysis	*C. sinensis*		*E. lancifolius*	
	Normal	Gall	Normal	Gall
Peroxidase : △ A/min/g acetone powder	1.80±0.02	4.5±0.06	13.5±0.20	11.8±0.3
Polyphenol oxidase : △A/min/g acetone powder	—	—	—	—
IAA-oxidase : mg IAA destroyed/g acetone powder/h	2.9±0.04	1.48±0.08	1.04±0.07	0.80±0.03
Protein mg/g acetone powder	0.55±0.03	0.61±0.02	0.24±0.01	0.31±0.02

±S. E.
— no activity.

TABLE 3

Activities of peroxidase, polyphenol oxidase, IAA-oxidase and protein contents in extracts of normal and gall tissues of *C. sinensis* and *E. lancifolius* prepared in buffer containing antioxidants.

Analysis	*C. sinensis* Normall	*C. sinensis* Gall	**E. lancifolius* Normal	**E. lancifolius* Gall
Peroxidase : △A/min/g fresh weight	0.45±0.01	2.60±0.03	7.30±0.06	3.50±0.02
Polyphenol oxidase : △A/min/g fresh weight	0.13±0.00	1.5±0.01	0.46±0.00	2.77±0.01
IAA-oxidase : mg IAA destroyed/g fresh weight/h	1.60±0.01	0.50±0.00	3.50±0.04	2.50±0.03
Protein mg/g fresh weight	0.50±0.01	2.75±0.02	3.38±0.20	6.00±0.30

Extract prepared in phosphate buffer containing *SDS (0.1 g/g fresh weight) and **PVP (0.2g/g fresh weight).
±S.E.

TABLE 4

Contents of O-dihydroxy and total phenols in normal and gall tissues of *C. sinensis* and *E. lancifolius*.

Analysis	*C. sinensis* Normal	*C. sinensis* Gall	*E. lancifolius* Normal	*E. lancifolius* Gall
O dihydroxyphenols mg/g fresh weight	0.15±0.01	0.03±0.01	1.31±0.05	0.45±0.01
Total phenols mg/g fresh weight	0.65±0.01	0.23±0.01	13.5±0.40	5.50±0.20

±S. E.

could either be due to their utilization by insects (Bernays et al., 1983) or their conversion into higher molecular weight substances which were not extracted by the method employed in the present investigation. The phenolics also act as substrates of some enzymes, such as peroxidase. Many investigators have shown a correlation between disease resistance and peroxidase activity (ref. Frie, 1976). The differences in peroxidase activities between the two species studied might be due to the differences in their metabolism. Decreased levels of peroxidase and polyphenol oxidase have also been reported in sunflower crown-

gall tumors (Kado, 1975). A low IAA-oxidase activity in gall tissue of *C. sinensis* and *E. lancifolius* might be involved in its hyperauxinity (unpublished data) and abnormal growth behaviour.

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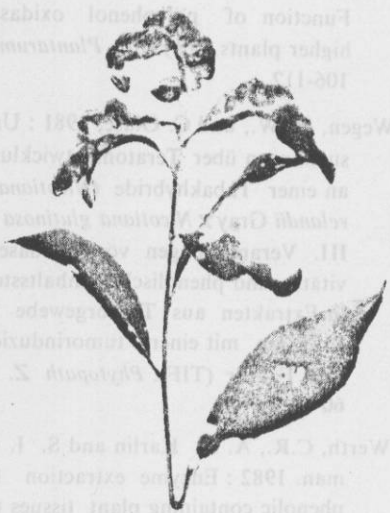

Fig. 2

Fig. 1

Fig. 1 : *Camellia sinensis* and Fig. 2 : *Elaeocarpus lancifolius* leaf blades showing rolling towards the mid rib forming leaf roll-galls.