

Phenolics and Enzymes Involved in Phenol Metabolism of Gall and Normal Tissues of *Prosopis cineraria* (Linn.) Druce *in vitro* and *in vivo*

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Phenolic (Total phenols and *O*-dihydroxyphenols) contents of *Lobopteromyia*-induced gall and normal tissues of *Prosopis cineraria* as also changes in the activities of enzymes peroxidase, polyphenoloxidase, phenylalanine ammonia lyase and tyrosine ammonia lyase were studied. Total phenolic contents were higher in the normal compared to gall tissues both *in vitro* and *in vivo*. Hypophenolicity in the gall tissues is correlated to high activities of peroxidase and polyphenol oxidase. High activity of phenylalanine ammonia lyase (PAL) and low activity of tyrosine ammonia lyase (TAL) was recorded in the gall tissues both *in vitro* and *in vivo* conditions.

Key Words: Phenolics, Gall tissues, *Prosopis cineraria*, *Lobopteromyia prosopidis*, Enzymes, *In vitro*, *In vivo*

Introduction

Four types of galls may occur on *Prosopis cineraria*, the stem gall caused by an unknown chalcid, the leaflet and flower galls caused by *Eriophyes prosopidis* Saksena and the leaf-rachis gall caused by *Lobopteromyia prosopidis* Mani. Biochemical studies and histopathology of stem gall have been carried out (Kant & Ramani 1988). The leaf-rachis gall of *Prosopis cineraria* induced by *Lobopteromyia prosopidis* Mani from infected plants and developed *in vitro* have been studied here. Kant and Ramani (1987) have studied auxin-kinetin interaction on the growth of rachis gall tissues of *Prosopis cineraria*. In the present study changes in phenolic levels and activities of enzymes involved in phenol metabolism have been determined.

Materials and Methods

Normal and gall tissues of *Prosopis cineraria* were isolated and maintained on Murashige and Skoog's (1962) medium supplemented with 8.0 mg/l NAA, 0.2 mg/l kinetin and 2.0 mg/l 2, 4-D. Thirty days old gall

callus, normal callus, galled rachis and normal rachis were used for estimation of phenolics and enzymes *in vitro* and *in vivo* conditions. The procedures followed were as follows: Total phenols (Bray & Thorpe 1954) Ortho dihydroxy phenols (Johnson & Schall 1952). Phenylalanine ammonia lyase (EC 4.3.1.5) and Tyrosine ammonia lyase (Higuchi 1966), Polyphenol oxidase (EC 1.10.3.1; Palmer 1963), Peroxidase (EC 1.11.1.7; Worthington Enzyme Manual 1972).

Results

Phenolic (total phenols and orthodihydroxy phenols) contents were higher in the normal tissue compared to the galled counterpart, both *in vitro* and *in vivo* conditions (figures 1,2,3). Total phenolic contents were higher *in vivo* conditions. The difference in the orthodihydroxy phenolic contents between the two tissues was not appreciable. The gall tissue showed higher PAL activity and lower TAL activity. The activities of these enzymes was higher *in vitro* than *in vivo* conditions. High Polyphenol oxidase (PPO) and peroxidase

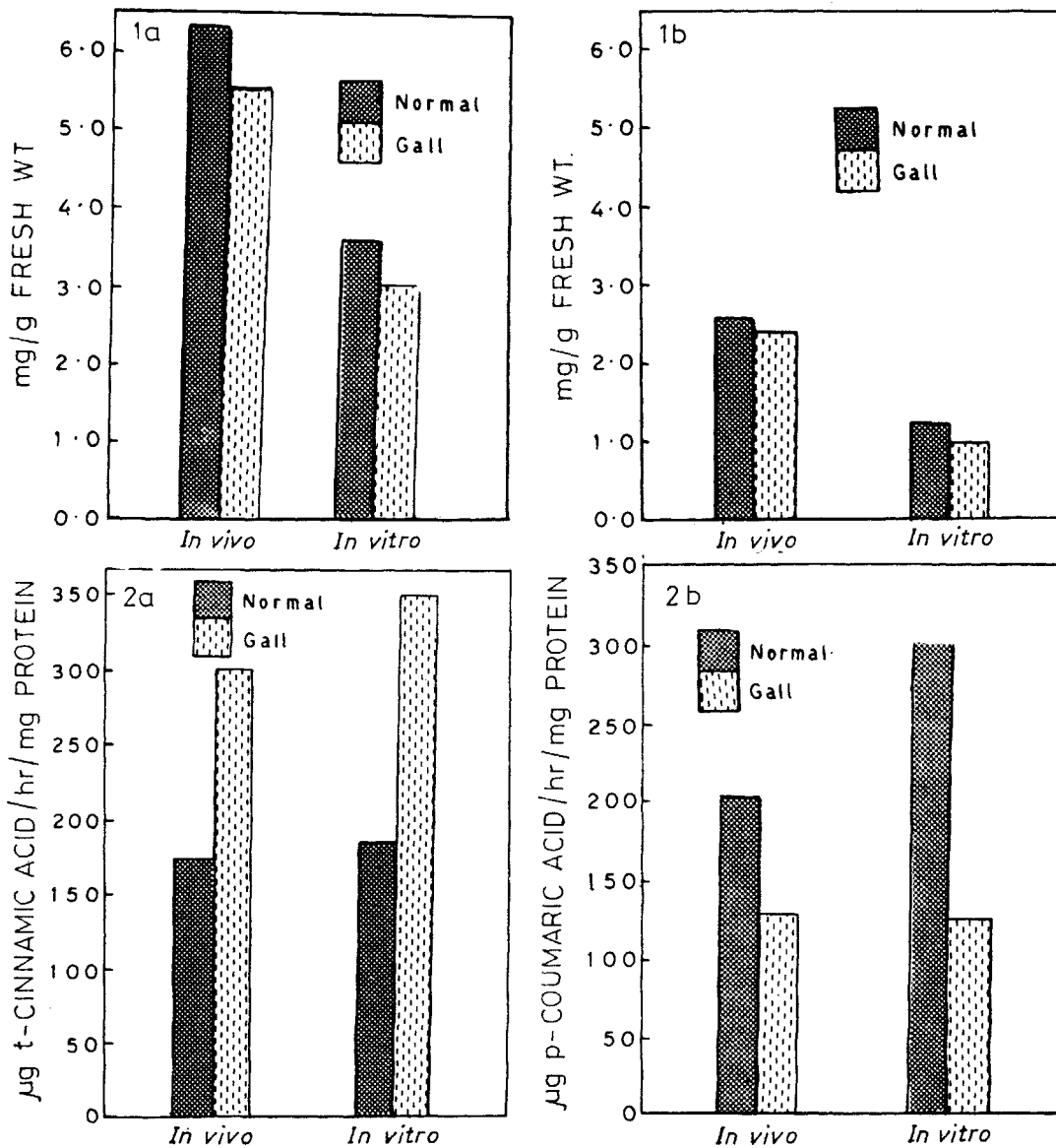


Figure 1a Total phenolic contents; .

Figure 1b Orthodihydroxy phenolic contents;

Figure 2a Phenylalanine ammonia lyase activity;

Figure 2b Tyrosine ammonia lyase activity;

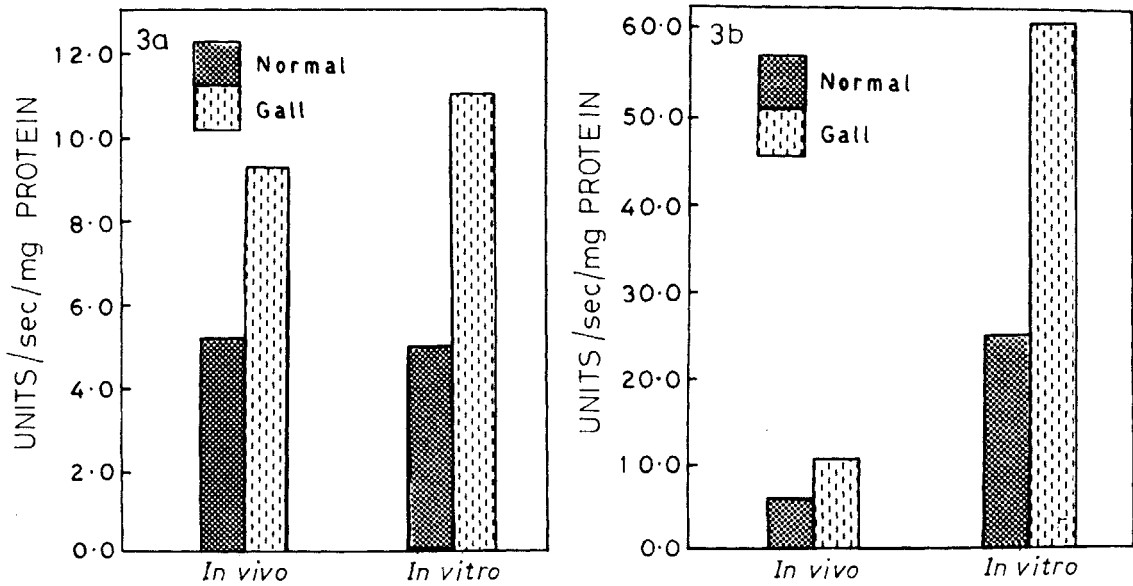


Figure 3a Polyphenoloxidase activity; and
Figure 3b Peroxidase activity in normal and
gall tissues *in vivo* and *in vitro*

(PO) activities were recorded in the gall tissues compared to the normal counterpart, both *in vitro* and *in vivo* condition. Polyphenol oxidase activity in the normal tissue *in vitro* and *in vivo* conditions did not differ appreciably. However, increase in the activity of peroxidase was recorded *in vitro* conditions in both gall and normal tissues, compared to *in vivo* conditions.

Discussion

Decrease in phenolics with increased peroxidase and polyphenol oxidase activity were recorded in the rachis galls of *Prosopis cineraria*. Similar results have been reported by Purohit et al. (1979) in the stem gall of *Prosopis cineraria*. Low phenolics have also been reported in the leaf-roll galls of *Camellia sinensis* and *Elaeocarpus lancifolius* (Joshi et al. 1985). Decrease in phenolic contents have been attributed to their utilization by insects (Bernays et al. 1983) or their conversion into high molecular weight substances not extracted by the method employed in the present study. High level of PAL has been recorded in several diseased tissues (Pascholati et al. 1986, Saini et al. 1986). Low activity of TAL in gall tissues could be correlated with high level of tyrosine in gall tissue (Ramani 1987). Phenolics also

act as substrates for some enzymes such as peroxidase. Gopinathan and Ananthakrishnan (1985) have reported increased polyphenol oxidase and peroxidase activities in galls of *Mimusops elengi* and *Calycopteris floribundus*.

Increase in severity of gall disease with increased peroxidase and polyphenol oxidase activity has been recorded (Ramani 1987). Hence, in the present study, increased activity of polyphenol oxidase and peroxidase indicates that the cecidozoan has the capacity of detoxifying the effect of oxidized phenols. Peroxidases are responsible for oxidation of phenolics (Kosuge 1969). Decrease in the level of phenolics is attributed mainly to the increased activity of peroxidase and polyphenoloxidase. Monophenols are considered to be activators of enzymes to help accelerate the activity of peroxidase and IAA oxidase, whereas *o*-dihydroxyphenols are known inhibitors of these two enzymes (Kosuge 1969). Increased peroxidase and polyphenol oxidase converts phenolics into Quinones (Farkas & Kiraly 1962). Increased activity of these oxidative enzymes indicates a state of high catabolism induced by pathogenesis.

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References

- Bernays E A, Chamberlain D J and Woodhead S 1983 Phenols as nutrients for a phytophagous insect, *Anacridium melanorhodon*; *J Insect Physiol* **29** 535-539
- Bray H C and Thorpe W V 1954 Analysis of phenolic compounds of interest in metabolism; *Meth Biochem Analysis* **1** 27-52
- Farkas G L Kiraly Z 1962 Role of phenolic compounds in the physiology of plant disease and disease resistance; *Phytopathology* **44** 105-150
- Gopinathan K and Ananthakrishnan T N 1985 Morphogenesis and histochemistry of some thrips (Thysanoptera: Insecta) induced galls; *Proc Indian natn Sci Acad* **B51** 413-456
- Higuchi 1966 Role of phenylalanine deaminase and Tyrase in the lignification of bamboo; *Agr Biol Chem* **30** 667-673
- Johnson G and Schall L A 1952 Relation of chlorogenic acid to scab resistance in potatoes; *Science* **115** 627-629
- Joshi S C, Tandon P and Rajee A L S 1985 Changes in certain oxidative enzymes and phenolics in *Camellia sinensis* and *Elaeocarpus lancifolius* leaf roll-galls; *Cecid Int.* VI 1-3, 51-57
- Kant U and Vidya Ramani 1987 Auxin-kinetin interaction on the growth of tumor tissues of *Prosopis cineraria* in tissue culture; in *Plant cell and Tissue Culture of Economically Important Plants* pp 331-335 ed. G M Reddy
- and — 1988 Insect induced galls of certain economically important plants of arid and semi-arid regions; in *Dynamics of Insect—Plant Interaction, Recent Advances and Future Trends* pp 165-176 ed T N Ananthakrishnan and A Raman (New Delhi: Oxford and IBH Publ. Co.)
- Kosuge T 1969 The role of phenolics in host response to infection; *Ann Rev Phytopath* **7** 195-222
- Murashige T and Skoog F 1962 A revised medium for rapid growth and bio-assays with tobacco tissue cultures; *Physiol Plant* **15** 473-497
- Palmer J K 1963. Banana polyphenol oxidase: Preparation and properties; *Plant Physiol Lancaster* **38** 508-513
- Pascholati S F Nicholson R L and Buttler L G 1986 Phenylalanine ammonia lyase activity and anthocyanin accumulation in wounded maize mesocotyls; *J Phytopath* **115** 165-170
- Purohit S D Ramawat K G and Arya H C 1979 Phenolics, peroxidase and phenolase as related to gall formation in some arid zone plants; *Curr Sci* **48** 714-716
- Ramani Vidya 1987 *In vitro* and *in vivo* Studies on the Leaf-Rachis Gall of *Prosopis Cineraria* (Linn.) Druce induced by *Lobopteromyia prosopidis* Mani; Ph.D. Thesis, University of Rajasthan, Jaipur
- Saini R S Arora Y K and Wagle D S 1986 Phenylalanine ammonia lyase and aromatic amino acids in wheat cultivars resistant and susceptible to *Ustilago tritici* (Per.) *Biochemie und Physiologie der Pflanzen* **181** 53-56
- Worthington Enzyme Manual 1972 *Enzymes, Enzyme Reagents, Related Biochemicals* (New Jersey: Worthington Corporation) 216 pp