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LOCALISATION OF METABOLITES AND ENZYMES IN INSECT INDUCED RACHIS GALL AND NORMAL TISSUES OF *PROSOPIS CINERARIA* (LINN.) DRUCE

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Histochemical localisation of metabolites in the rachis galls of *Prosopis cineraria* (Linn.) Druce induced by a dipteran insect, *Lobopteromyia prosopidis* Mani (Diptera : Cecidomyiidae) have been made. Results indicated a greater localisation of proteins, tannin, lignin, polyphenol oxidase, peroxidase and acid phosphatase in gall tissues especially in the nutritive zone as compared to the normal tissues. Hence, a functional elaboration in the cells closer to the feeding sites of the cecidozoan has been established.

Keywords : Galls; Histochemistry; *Lobopteromyia prosopidis*; *Prosopis cineraria*.

Introduction

Plant galls exhibit the most elaborately evolved insect-plant relationships. Investigations of developmental morphology, anatomy and biochemistry of *Prosopis cineraria* (Linn.) Druce galls have been carried out[1]. Four types of galls may occur on *Prosopis cineraria*, the stem gall caused by an unknown chalcid, rachis gall caused by *Lobopteromyia prosopidis* Mani and leaflet and flower galls induced by a mite, *Eriophyes prosopidis* Saksena. An attempt has been made to study the histochemistry of the rachis galls of *P. cineraria* induced by *L. prosopidis*.

Materials and Methods

Rachis galls at various stages of development were collected from their natural habitat. Depending on their approximate age, they were graded into various developmental stages as young,mature and old.Normal rachis of comparable age was also obtained for the investigation. Fresh hand-cut sections were used.

Histochemical localisation of proteins, lipid, starch, cellulose, tannin, lignin and enzymes such as acid phosphatase, peroxidase and polyphenol oxidase was carried out in normal and gall tissues. The qualitative increase or decrease in enzymes and other compounds studied, was assessed in terms of intensity of staining and the degree of distribution in tissues. Proteins were localised using filtered Amido Back stain[2], lipids by Sudan III dye[3], starch by IKI reaction[4], celluose by IKI-H_2SO_4 method[4], tannin by Lugol's iodine[5] and lignin by Phloroglucinol test[4].

The enzyme activity was assayed by the following methods; peroxidase[6], acid phosphatase[7] and polyphenol oxidase[8].

Results and Discussion

Histochemical localization of metabolites in normal and gall tissues of *P.cineraria* rachis has been shown in table 1.

Proteins stained blue in colour and were very high in concentration in the cells around the gall cavity. Proteins were also observed throughout the young gall parenchyma in contrast to the normal where these were localised in the cortex, phloem and pith. Starch grains (black deposits) were present in the pith and phloem cells in normal tissues, whereas in gall tissues they appeared in the outer region of the pith and cortex particularly away from the nutritive zone. Lipids stained orange and were sparse in normal rachis. In gall tissues, lipid droplets accumulated in the epidermal cells and nutritive tissues.Tannins which gave bright red colour occurred in the pith cells in the normal tissues and in gall tissues they were predominant and confined to nutritive tissues and epidermal cells. Lignin appeared yellow orange. In galls it was seen surrounding the nutritive region and distributed around the gall cavity. Cellulose was observed in the outer surface of the normal and galled rachis.

Table 1. Histochemical localization of metabolites in normal and gall tissues of *Prosopis cineraria* rachis.

S. No.	Metabolites	Type of Tissues	Region localized	Intensity
1.	Protein	Normal	Cortex, phloem and	++
			pith	+
		Gall	Nutritive zone and	++++
			gall parenchyma	+++
2.	Starch	Normal	Phloem and pith	++
		Gall	Pith, and	+
			cortex	+++
3.	Lipid	Normal	Cortex, phloem and xylem	+
		Gall	Epidermis, cortex and	++
			nutritive tissues	+++
4.	Tannin	Normal	Pith	++
		Gall	Epidermis and	++
			nutritive tissues	+++
5.	Lignin	Normal	Epidermis and	+
			cortex	++
		Gall	Epidermis, cortex	++
			gall parenchyma and	+
			nutritive zone	+++
6.	Cellulose	Normal	Cork cells	+
		Gall	Cork cells and	+
			cortex	++
7.	Peroxidase	Normal	Cortex and pith	+
		Gall	Cortex and	+
			nutritive tissues	+++
8.	Acid phosphatase	Normal	Cortex and pith	+
		Gall	Epidermis and	+++
			cortex	++++
9.	Polyphenol oxidase	Normal	Cortex, phloem,	++
			xylem and pith	++
		Gall	Cortex	++
			gall parenchyma	+
			xylem and	+++
			Nutritive zone	++++

+ very low intensity; ++ low intensity; +++ high intensity; ++++ very high intensity.

The inner lining of the gall cavity also exhibited a positive reaction for cellulose.

Wall bound activity of the enzyme peroxidase indicated by brown colour was seen in the gall cells of cortical region and isolated cells of nutritive tissue. Few cells of nutritive zone abounding in peroxidase were considerably larger and high activity of the enzyme was evident in cells near the larval locations. Significant activity of the enzyme acid phosphatase was detected in mature and old galls, compared to normal tissues. Mature gall exhibited high activity of the enzyme in nutritive tissues and cells away from nutritive zone with scattered activity in cortical parenchyma. In old galls however, intense enzyme activity in the cell surrounding gall cavity was evident.

The nutritive zone and associated vascular tissues of *P. cineraria* young and mature galls showed increase in proteins. Wounding as a result of feeding in known to accelerate protein synthesis[9]. Starch deposits found in the later stages of gall development appear significant and as suggested[10] there are possibilities of diffusion of soluble saccharides produced by starch hydrolysis towards nutritive tissues which could be utilized by the cecidozoan. Higher incidence of lipids in the nutritive zone could be related to continuous wounding as a result of feeding activity of the cecidozoan, altering the metabolic pathway to synthesize more lipids near the feeding zone. Increase in tannin and lignin in gall tissues is attributed to hyperactivity of polyphenol oxidase and peroxidase. Phenolics such as tannin and lignin are being utilized by acridids implying adaptive strategy for the cecidozoan[11]. Hyperactivity of the enzymes peroxidase, polyphenol oxidase and acid phosphatase in the nutritive tissues of gall suggests a triggering of these enzymes as a result of feeding stimulus of *Lobopteromyia*. This has also been reported in some thrips galls[12]. Hence, a functional elaboration in the cells closer to the feeding sites of the cecidozoan has been established.

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