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Auxin profile of gall and normal tissues of *Prosopis cineraria* (Linn.) Druce induced by *Lobopteromyia prosopidis* Mani, *in vitro* and *in vivo*

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Abstract. Effect of various auxins on the growth of gall and normal tissues of *Prosopis cineraria* (Linn.) Druce induced by *Lobopteromyia prosopidia* Mani has been discussed. Differential response of gall and normal tissues to various auxins was observed. α-Naphthalene acetic acid (8 mg/l) and indole-3-acetic acid (4 mg/l) sustained excellent growth of gall and normal tissues respectively. Biochemical studies revealed hypoauxiny and high indole-3-acetic-acid oxidase activity in the gall tissues which have been discussed *in vitro* and *in vivo* conditions.

Keywords. Auxin; *Prosopis cineraria*; hypoauxiny.

1. Introduction

Prosopis cineraria (Linn.) Druce presents a unique example of insect plant interaction as 4 types of galls—on the stem, leaflet, flower and rachis have been recorded on this tree. The tree locally known as 'Khejari' in the arid and semi-arid zones of India is a perennial woody legume of family Leguminosae, sub-family Mimosoideae. It offers shade, firewood timber and food to man and forage for wildlife and domestic herbivores. The leaf-rachis gall of *P. cineraria* induced by *Lobopteromyia prosopidis* Mani has been studied *in vitro* (Kant and Ramani 1987). An attempt has been made to study the auxin-profile of the normal and gall tissues *in vivo* and *in vitro*.

2. Materials and methods

Young galls obtained from Jaipur and adjoining areas were longitudinally split and the insect was removed. Split gall pieces were sterilized with mercuric chloride solution (0·1%), rinsed with sterile distilled water and transferred aseptically to flasks containing 40 ml of Murashige and Skoog's (1962) medium for gall callus initiation. The tissue was maintained through successive subcultures on MS medium supplemented with 8 mg/l α-naphthalene acetic acid (NAA), 0·2 mg/l kinetin and 2 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D). Normal callus which appeared from the hypocotyl of seedlings after similar treatment as above was also maintained on MS medium. The cultures were incubated in a dark culture chamber maintained at 26 ± 2°C and 55% relative humidity.

Effect of various auxins on the growth of gall and normal tissues was observed. NAA and 2,4-D were omitted from the medium and varying concentrations (0–16 mg/l) of various auxins were added singly before autoclaving. The auxins tried were indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), indole-3-propionic acid (IPA), NAA and 2,4-D.

Extraction of auxin was made following the method of Avery *et al* (1945). Auxin contents in the extract were determined by the method of Gordon and Weber (1951). IAA oxidase activity was determined by the method of Sequeira and Mineo (1966).

Thirty-day old gall callus tissue, normal callus galled rachis and normal rachis were used for biochemical estimation of auxins and IAA oxidase, *in vitro* and *in vivo*.

Transplant pieces weighing about 300 mg fresh weight were used for subculturing and setting experiments. Every treatment consisted of 12 culture flasks each with one tissue piece. The experiments were repeated 3 times. Growth was recorded in terms of fresh weight after 40 days of tissue growth.

3. Results

Results are presented in figures 1–3. Gall tissue showed poor growth in the absence of auxin. All auxins added were beneficial for the growth of the gall tissue. Poor growth was obtained on IPA. IAA (12 mg/l) and IBA (8 mg/l) supported good growth of gall tissue. 2,4-D sustained moderate growth of tissue at 4 mg/l. NAA (8 mg/l) supported excellent growth of tissue.

The normal tissue showed moderate growth on 2,4-D (0·1 mg/l) or NAA (8 mg/l) supplemented media. IPA and IBA did not support good tissue growth. However the optimal growth of normal tissues was recorded at 1 mg/l of IBA and 12 mg/l of IPA. IAA at 4 mg/l supported excellent growth of normal tissue of *P. cineraria.*

Hence, IAA (4 mg/l) and NAA (8 mg/l) were found to be the best auxins for growth of normal and gall tissues of *Prosopis.* Both tissues showed very poor growth on auxin-free medium.

Free auxin and total auxin contents of gall tissue were lower compared to their normal counterparts, both *in vitro* and *in vivo* conditions. High IAA oxidase activity was recorded in the gall callus tissue and rachis gall compared to their normal counterparts.

4. Discussion

The capacity of plant tissue to utilize growth regulators is directly correlated with the endogenous auxin synthesis in the tissue and external supply of auxin. Studies with crown-gall, genetic and virus wound tumor have revealed that tumor cultures grow on simple media without auxin or cytokinins (Butcher 1973). Partial auxin autotrophy has been reported for some insect-induced gall tissue cultures (Arya 1963; Vyas 1971). However in the present study gall tissue failed to grow in the absence of added auxin to the medium. Normal tissue showed poor growth in the absence of auxin. Similar observations have been reported for insect induced gall tissue of *Salvia pomifera* (Démétriadès 1953). In the present study the response to auxins of various types of tissues, varied with its kind and concentration in the medium. The differential response of gall and normal tissues to various auxins has also been elucidated by other workers (Vyas 1971; Arora 1976).

Generally, tumors are hyperauxinic and produce auxin in more than regulatory amounts. High level of auxin in tumors resulted from reduced auxin destruction. Hyperauxiny has been reported for several insect and mite induced gall tissues

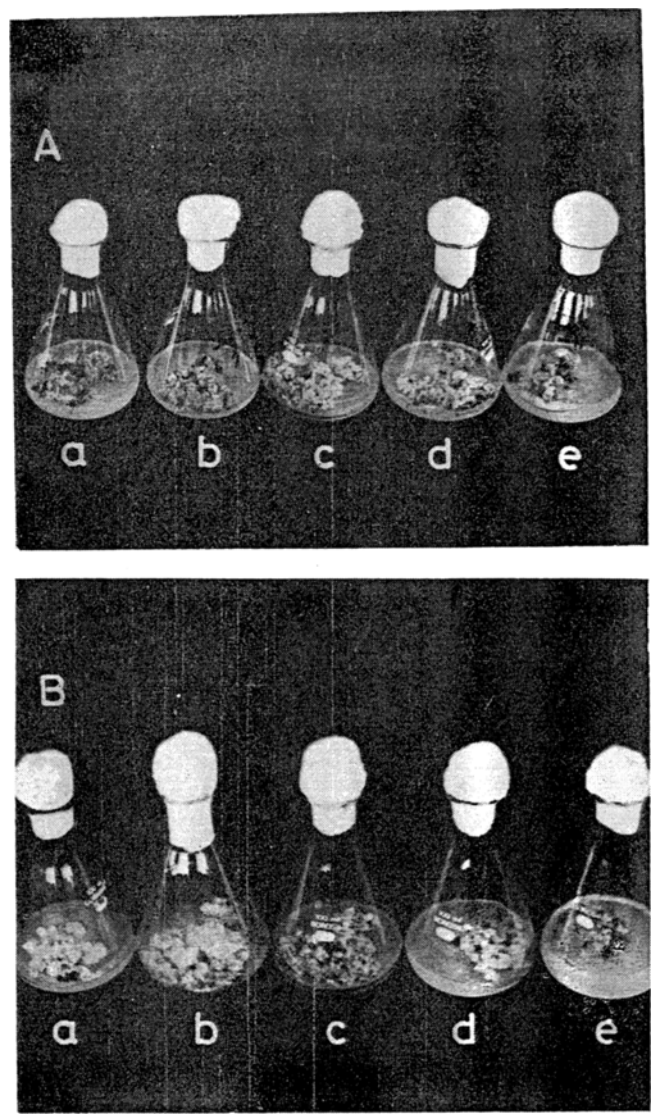

Figure 1. **A.** Optimum growth of normal tissues on various auxins. (a), 1 mg l IBA; (b), 8 mg/l NAA; (c), 4 mg/l IAA; (d), 0·1 mg/l 2,4-D; (e), 12 mg/l IPA. **B.** Optimum growth of gall tissues on various auxins. (a), 12 mg/l IAA; (b), 8 mg/l IBA; (c), 4 mg/l IPA; (d), 4 mg/l 2,4-D; (e), 8 mg/l NAA.

(Kant 1975; Kant and Ramani 1986). Nakajima *et al* (1979) who analysed the crown gall cells of tobacco concluded that enhanced hormonal content is not the only factor associated with autonomous growth. It is not clear how the tumor inducing principle causes the tumor cells to become autonomous for auxin. Nor is it known whether there is any increased endogenous synthesis of auxin by the tumor cell. Is some subtle and as yet uncharacterised auxin-inactivating system, normally concerned with regulation for growth, destroyed as a result of the action of the tumor inducing principle? The imbalance of growth substances found in crown gall tumor

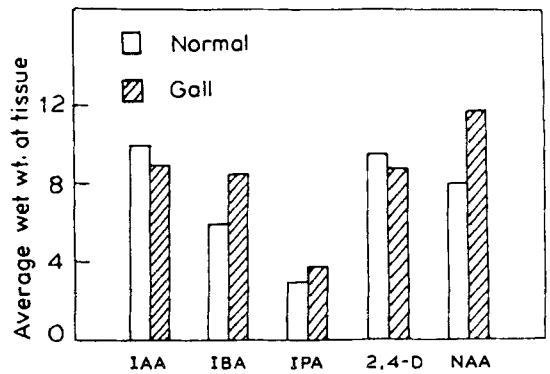

Figure 2. Histogram showing optimum growth of normal and gall tissues on various auxins.

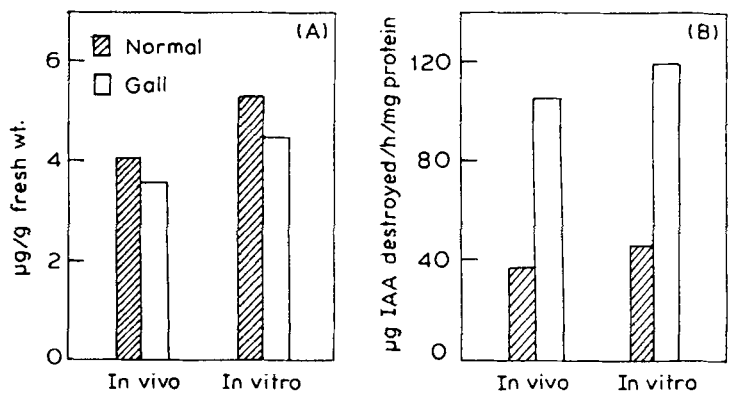

Figure 3. Total auxin content (A) and IAA oxidase activity (B) of normal and gall tissues *in vitro* and *in vivo* conditions.

cells appear not only to account for the continued abnormal growth of such cells but also plays an essential role in determining morphological, histological and cytological characteristics of the tumor.

Although most investigations report hyperauxiny in tumors, in a few cases reduced auxin levels have been reported. The mechanisms that culminate in reduced auxin level have been attributed to (i) auxin destruction by enzymes secreted by the parasite/insect (Krupasagar and Sequeira 1969), (ii) degradation of auxin by enzymes of the infected plant (Daly and Devarall 1963) and (iii) reduced concentration and conversion of auxin precursors (Schuphan 1950).

A reduction in free and total auxin content in gall tissues (*in vitro* and *in vivo*) with increased activity of IAA oxidase was recorded. The bulk of the enzyme liberated, rapidly oxidized IAA, causing reduction in auxin. Tayal *et al* (1981) found a substantial decrease in auxin contents in the galls of *Coriandrum sativum* caused by *Protomyces macrosporus* but IAA oxidase also decreased. However peroxidase increased in these tissues, which oxidized these auxins, thereby causing a marked reduction. Predominant IAA oxidase activity in the gall tissues may be elucidated

by the fact that, infection decreased IAA oxidase inhibitors in the plant which naturally raised the concentration of the enzyme consequently favouring auxin degradation (Hare 1972).

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