J. Phytol. Res. 2 (2). 1989

IN VITRO EFFECT OF VARIOUS GROWTH REGULATORS ON THE GROWTH OF NORMAL AND NEMATODE INDUCED ROOT GALL TISSUES OF *LYCOPERSICON ESCULENTUM* MILL.

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Effect of various growth regulators viz, auxins, gibberellic acid and cytokinins, was studied *in vitro* on the growth of normal and gall tissues of *Lycopersicon esculentum* Mill. IAA and 2, 4-D supported good growth of normal and gall tissues as compared to IPA and IBA. While NAA was the best among all the auxins tested, enhancing maximum growth of normal and gall tissues at 10.0 mg/l and 7.5 mg/l, respectively. Gibberellic acid was found to be inhibitory for the growth of both the tissues Cytokinins (Kinetin, benzyladenine and N6-isopentenyladenine) in combination with 10.0 mg/l of NAA, enhanced the growth of both normal and gall tissues. Kinetin was proved to be most suitable cytokinin, favouring good growth of normal and gall tissues at 0.10 mg/l and 9.08 mg/l, respectively.

Keywords : Growth regulators, *Lycopersicon esculentum*, Nematode, Gall tissue, Normal tissue, Root galls.

Introduction

The role of various growth regulating substances in *in vitro* cultivation of tissues and cells derived from various plant parts has been well established (Minocha, 1981; Firn, 1982; and Ahmed *et al.*, 1986). Infection by many plant parasites, particularly biotrophs, leads to morphological disturbance of the host tissues, strongly suggesting derangement of normal phytohormone metabolism. Increasingly, attention has been focused on the role of growth hormone in the physiology of the diseased plants. Plant galls induced by virus, bacteria and insects have been studied in tissue culture (Hildebrandt, 1958; Hildebrandt *et al.*, (1946); Kant and Arya, 1969) but nematode induced galls are almost untouched. Previously we have reported the vitamin requirements of nematode induced root gall and normal tissues of *Lycopersicon esculentum* Mill. (Mathur *et al.* 1984).

During the present investigation the effect of varying concentrations of auxins, gibberellic acid and cyto-

kinins were studied on the growth of normal and nematode induced root gall tissues of *Lycopersicon esculentum* Mill. in tissue culture.

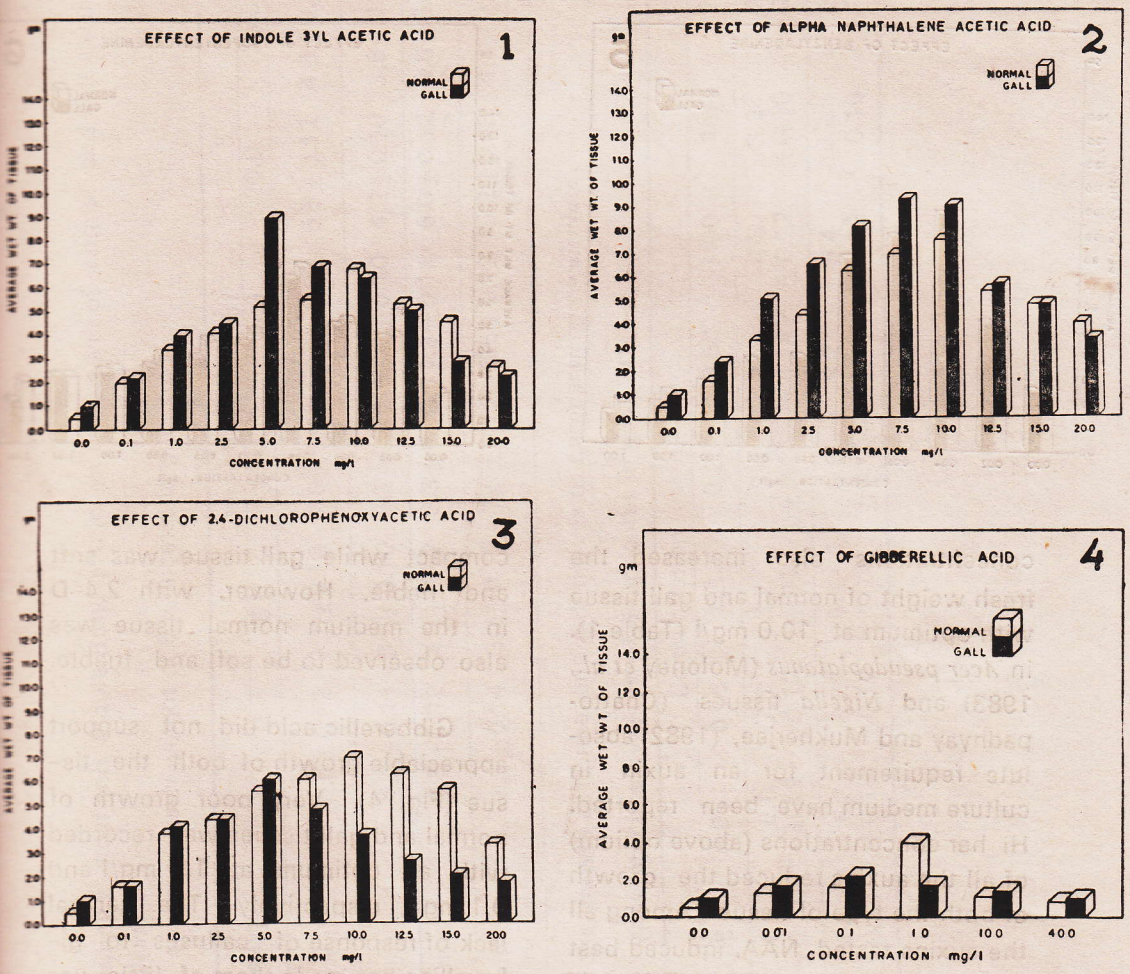
Material and Methods

Normal and gall tissues of *Lycopersicon esculentum* Mill. were isolated from the normal root (hypocotyl) and nematode (*Meloidogyne incognita*) induced root galls, respectively. The tissues were maintained on MS-medium (Murashige and Skoog, 1962) supplemented with 10.0 mg/l NAA, 0.08 mg/l kinetin, 8.0 g/l agar and 30.0 g/l sucrose. For this particular experimentation one tissue piece (about 200 mg) was grown on 40 ml of solidified MS-medium. The cultures were incubated in dark at 26±2°C and around 55% relative humidity.

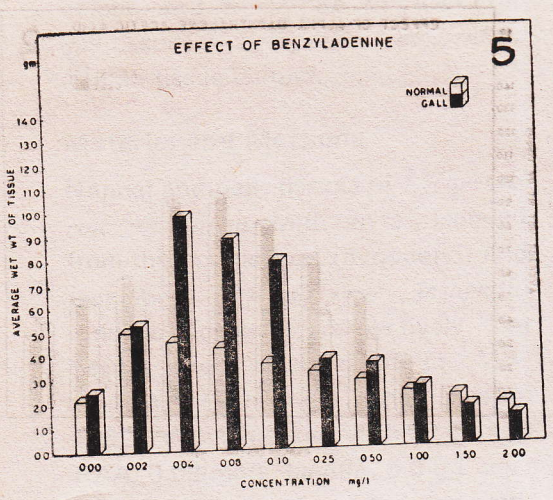
Different concentrations of auxins viz. indole-3yl-acetic acid (IAA), alpha-naphthalene acetic acid (NAA) and 2, 4-dichlorophenoxy acetic acid (2, 4-D) ranging from 1.0 to 20.0 mg/l were used separately in auxin omitted MS-medium. Indole butyric acid (IBA) and indole propionic acid (IPA) were used from 5.0 to 25.0 mg/l in MS-medium. Gibberellic acid (GA_3) was used from 0.01 to 40.0 mg/l in MS-medium (auxin omitted). Cytokinins viz. kinetin, benzyladenine and N^6—isopentenyladenine were tested from 0.02 to 2.0 mg/l in the kinetin omitted medium supplemented wtih 10 0 mg/l of NAA. The media were adjusted to pH 5.8 before autoclaving at 1.06 Kg/cm^2 and for 15 minutes. In control experiments the auxin, gibberellic acid or cytokinin, in question, was eliminated from the medium. Each combination was replicated six times and each experiment was repeated thrice. After 30 days of growth, the tissues were harvested and the fresh weight was determined.

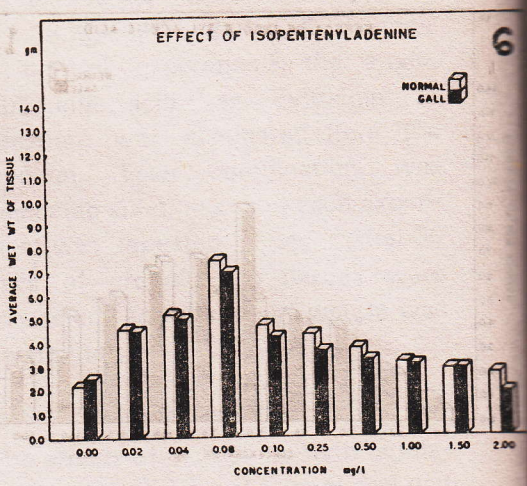
Results and Discussion

The effects of various growth regulators on the growth of normal and gall tissues of *L. esculentum* are presented in Figs. 1 to 6 and Tables and 1 & 2. Responses of the normal and gall tissues varied with the type of auxin and its concentration used in the medium. Normal tissue almost failed to grow on auxin omitted medium, while the gall tissue could grow on auxin omitted medium, though poorly. These results are in confirmity with the previous findings on *Phylloxera* (Arya, 1963) and *Salvia pomifera* (Demetriades, 1953). Addition of any of the five auxins in the medium increased the fresh weight yield of both the normal and gall tissues. The optimum concentrations of IAA and 2, 4-D in the test media were 10.0 mg/l and 5.0 mg/l for normal and gall tissue, respectively. Normal tissue grew best at 10.0 mg/l of NAA while 7.5 mg/l was optimum for gall tissue. Addition of IPA and IBA at various



Figs. 1–6 Comparative growth of normal and gall tissues of *Lycopersicon esculentum* Mill, on MS-medium supplemented with different concentrations of IAA, NAA, 2,4-D, GA, Benzyladenine and Isopentenyladenine.





concentrations also increased the fresh weight of normal and gall tissue with optimum at 10.0 mg/l (Table 1). in *Acer pseudoplatanus* (Moloney *et al.*, 1983) and *Nigella* tissues (Chattopadhyay and Mukherjee, (1982) absolute requirement for an auxin in culture medium have been reported. Higher concentrations (above optium) of all the auxins reduced the growth of both the type of tissue. Among all the auxins tested, NAA, induced best growth of the normal tissue (7.6 $\pm$ 0.12 gm/flask) and gall tissues (9.5 $\pm$ 0.15 gm/flask). Other auxins showed comparatively lower growth values. Analysis of auxin/growth relationship in auxin requiring system indicates that possibly the exogenous auxin is interacting with endogenous hormone (Moloney et al., 1983). Normal tissue growth on the test media with various auxins at different concentrations was comparatively hard and compact while gall tissue was soft and friable. However, with 2,4-D in the medium normal tissue was also observed to be soft and friable.

Gibberellic acid did not support appreciable growth of both the tissue (Fig. 4). Very poor growth of normal and gall tissues was recorded with an optimum at 1.0 mg/l and 0.1 mg/l, respectively The general lack of response of calluses to giberellins has made them of little use in tissue culture (Chattopadhyay and Mukherjee, 1982). Browning and hardening of the normal and gall tissues was observed at almost all the concentrations.

The results obtained with various cytokinins are presented in Figs. 5,6 and Table 2. Addition of benzyladenine and Kinetin in the medium increased appreciably the fresh

Table 1. Effect of Indole Propionic Acid and Indole Butyric Acid on the growth of normal and gall tissues of *Lycopersicon esculentum.*

(Average fresh weight of 6 replicates in gm±SD)

Auxin used	Tissue type	Concentration (mg/)					
		0 0	5.0	10.0	15.0	20.0	25.0
Indole Propionic Acid	Normal	0.5±0.12	3.6±0.10	7.8±0.14	7.6±0.05	5.5±0.12	4.6±0.12
	Gall	1.2±0.11	3.9±0.10	8.2±0.13	6.5±0.10	4.2±0.10	3.5±0.14
Indole Butyric Acid	Normal	0.5±0.12	4.6±0.12	8.3±0.10	7.3±0.11	5.0±0.12	3.8±0.13
	Gall	1.2±0.14	3.2±0.10	7.0±0.12	5.5±0.12	5.2±0.13	4.4±0.10

Table 2. Effect of Kinetin of the growth of normal and gall tissues of *Lycopersicon esculentum.*

(Average fresh weight of 6 replicates in gm±SD)

Tissue type	Concentration (mg/l)									
	0.00	0.02	0.04	0.08	0.10	0.25	0.50	1:00	1.50	2.00
Normal	2.4 ±0.10	4.2 ±0.11	6.0 ±0.12	7.6 ±0.12	9.7 ±0.13	8.8 ±0.14	6.2 ±0.14	4.3 ±0.12	3.2 ±0.10	2.0 ±0.10
Gall	2.6 ±0.12	4.8 ±0.13	6.8 ±0.10	9.2 ±0.10	9.2 ±0.12	8.6 ±0.15	8.2 ±0.14	5.4 ±0.12	4.4 ±0.10	3.0 ±0.12

weight of normal and gall tissues. The dependence of cytokinin action on other growth regulators is a well known phenomenon. Kinetin is particularly active as an interactant with IAA or 2,4-D in culture of tobacco pith (Yeoman, 1973). Comparatively good growth of gall tissue was recorded on benzyladenine (0.04–0 1.0 mg/l) and Kinetin (0.04–0.50 mg/l) with optimum at 0.04 mg/l and 0.08 mg/l, respectively. The normal tissue grew well with 0.10 mg/l of Kinetin as compared to 0.02 mg/l of benzyladenine. Importance of benzyladenine was also emphasized by Bergman et al., (1985) in *Salix* spp. cultured *in vitro*. The responses of both the tissues to isopentenyladenine was different. Normal and gall tissues showed optimum growth at 0.08 mg/l of isopentenyladenine in the medium. Rogozinska et al., (1964) reported isopentenyladenine about ten times as active as Kinetin while in present studies, Kinetin supported better growth of both the tissue as compared to the isopentenyladenine. Interestingly, it was observed that at all the concentrations of isopentenyladenine, the normal tissue showed more growth as compared to the gall tissues. Higher concentrations (above optimum) of all the cytokinin tested reduced the growth of both the tissues. These observations reflected variation in the metabolic capabilities of the normal and gall tissues in culture.

Acknowledgement

Thanks are due to Council of Scientific and Industrial Research, New Delhi, for financial assistance.

Accepted December, 1989

References

Ahmed, Gupta, Rina, Dutta S and Ghosh P D 1986 *Indian J. Exp. Biol.*, **24** 384

Arya H C 1963, *Indian J, Exp. Biol.* **1** 148

Bergman L, Arnold S V and Eriksson T 1985, *Plant Cell Tissue Organ Culture* **4** 135

Chattopadhyay S and Mukherjee B B 1982, *Indian J. Exp. Biol*, **20** 294.

Demetriades S D 1953, *Ann. Inst. phytopath. Benaki* **7** 21

Firn R D 1982. *Plant Physiol.* **70** 1568

Hildebrandt A C 1958, *Proc. Natl. Acad. Sei.* (U.S.) **44** 354

Hildebrandt A C, RikerA J and Duggar A M 1946, *Cancer Res.* **6** 368

Kant U and Arya H C 1969, *Indian J. Exp. Biol.* **7** 37

Mathur Anil, Singh S and Kant U 1984, *Curr. Sci.* **53** 1153

Minocha S C 1981, *Plant Physiol.* **67** S-98

Murashige T. and Skoog F. 1962. *Physiol Plant.* **15** 473.

Regozinska J, Helgeson J and Skoog F 1964. *Physiol. Plant*, **17** 165

Yeoman M M 1973, *In : Plant tissue and cell cultures* (Ed) H E. Street. Blackwell Sci. Publ. pp. 3i