

Auxin Autotrophy and Hyperauxinity of *Eriophyes* Induced *Zizyphus* Stem Galls in Culture

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Summary

In contrast to *Zizyphus* normal stem tissue, the gall tissue was able to grow on MS medium free of auxin and kinetin. With DL-tryptophan, both the tissues showed optimum growth at 5 mg/l, but the growth response of gall tissue was much higher. The optimal concentration of NAA and IAA was 10 mg/l for growth of both normal and gall tissues. *In vivo*, and *in vitro* grown gall tissues showed higher auxin contents as compared to normal tissues. The endogenous auxin level in both gall and normal tissues was highest in *in vivo* tissues, followed by DL-tryptophan, NAA, and IAA treatments in decreasing order.

Introduction

The physiological autonomy shown by crown-gall tumor cell reflects an abnormal hormone metabolism (GAUTHERET 1947; BRAUN and STONIER 1958; STONIER 1969; MEINS 1974; ARYA et al. 1975). It is now generally accepted that crown-gall tumor cells show auxin autotrophy and hyperauxinity (STONIER 1972; BUTCHER 1973; MEINS 1974). The nutritional autonomy of the crown-gall tissue could be due to either change in requirements of the gall tissue growth, or increased synthesis and retention of growth regulators. The literature is full of conflicting reports on this point (STONIER 1972). In contrast to non-self-limiting tumor tissues in plants with etiological factors like bacteria, viruses, and genetic constitution, insect- and mite-induced galls are reported to be self-limiting and continued presence of insects or mites is considered essential for gall tissue growth (BRAUN 1969). However, we reported earlier (TANDON et al. 1976) that galls induced by a plant mite, *Eriophyes cernuus*, on *Zizyphus jujuba* is of non-self-limiting type and the tissues possess tumefacient properties. In an attempt to understand the abnormal growth hormone metabolism of *Zizyphus* gall tissue, the present studies on auxin contents in *in vivo* gall and normal tissues, growth responses and auxin levels in both normal and gall tissues, subjected to three growth regulators, were carried out.

Material and Methods

Zizyphus jujuba LAMK. normal stem, stem galls incited by *Eriophyes cernuus* MASSEE and tissue cultures of the same were used as experimental material. Both normal and gall tissues were isolated and maintained on MURASHIGE and SKOOG (1962) medium as described earlier (TANDON 1976).

Abbreviations: NAA, α -naphthaleneacetic acid; IAA, indole-3-acetic acid; MS, Murashige-Skoog medium.

The pH of the medium was adjusted to 5.8 before autoclaving it at 1.06 kg/sq.cm pressure for 20 min. The cultures were maintained through regular subculturing on MS medium, and grown in dark growth chamber maintained at $26 \pm 2^\circ\text{C}$ and 50 to 60% relative humidity. Growth of the tissues on MS medium supplemented with 10 mg/l of NAA was used as control. Growth values (Final weight/initial weight) in MS medium with different growth regulators are shown as percentage of control. The growth responses of normal and gall tissues to NAA, IAA, and DL-tryptophan were studied by incorporating different concentrations (0.1 to 20 mg/l) separately in auxin free MS medium. Average growth responses of three replicate cultures for each treatment after thirty days are presented as histograms (Figs. 1–3). Studies on tissue growth and auxin contents (5th to 30th day at 5-day interval) were carried out in their third and fourth passages of growth subjected to the above mentioned growth regulators.

IAA extraction and quantitative measurement in both *in vivo* and *in vitro* gall and normal tissues was done according to the method of MAHADEVAN (1975) which is basically as follows: fresh tissues were ground to a fine paste in a mortar with pestle, extracted repeatedly with peroxide free ethyl ether, and then with 5% sodium bicarbonate solution. The extract was acidified to pH 3, and partitioned in a separatory funnel with ethyl ether. The ether extract was evaporated, dissolved in methyl alcohol, and separated by paper chromatography using isopropanol-ammonia-water (80:10:10; v/v). A reference sample of IAA was run simultaneously. The spot with the same Rf as that of known IAA was removed, eluted in 1 ml methyl alcohol. IAA content was determined by SALPER reagent using a standard curve, and expressed as μg IAA equivalent per kg fresh weight of the tissue.

Results and Discussion

During the present studies, it was found that normal and gall tissues showed poor growth on auxin-kinetin free MS medium in the first passage of growth. In subsequent passages, however, normal tissues failed to grow, while the growth of gall tissue increased considerably (Fig. 1). WHITE and BRAUN (1942) showed that bacteria-free crown-gall tissue cultures of sunflower grew vigorously on a simple medium without growth substances. These basic observations were subsequently confirmed and extended to crown-gall tissues from other plant species (BUTCHER 1973). The hybrid tumor tissue cultures derived from the tumor forming *Nicotiana suaveolus* \times *N. lundsdorffii* and *N. debney-tabacum* \times *N. longiflora* did not require auxin and kinetin for vigorous growth, whereas their non tumorous parents did (SCHAEFFER and SMITH 1963; AHUJA and HAGEN 1966).

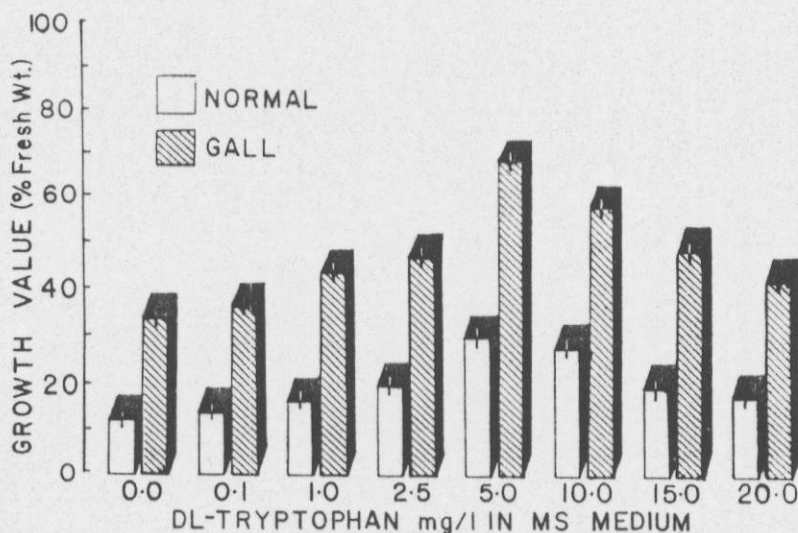


Fig. 1

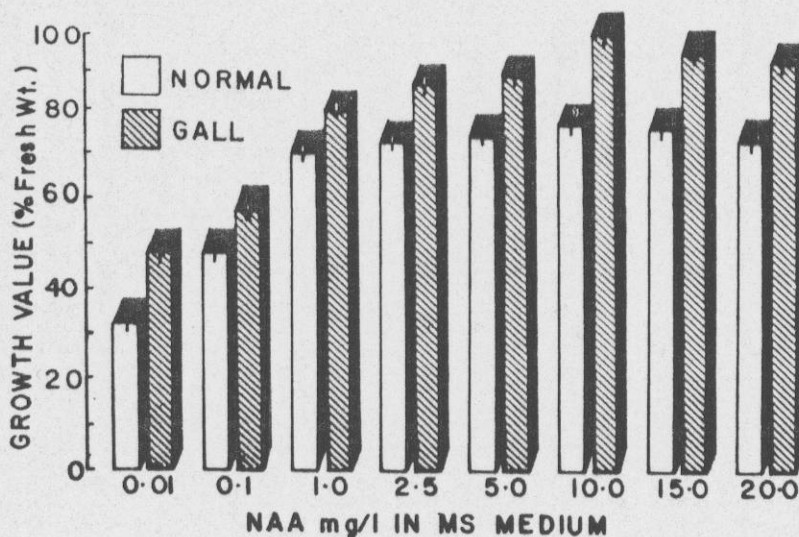


Fig. 2

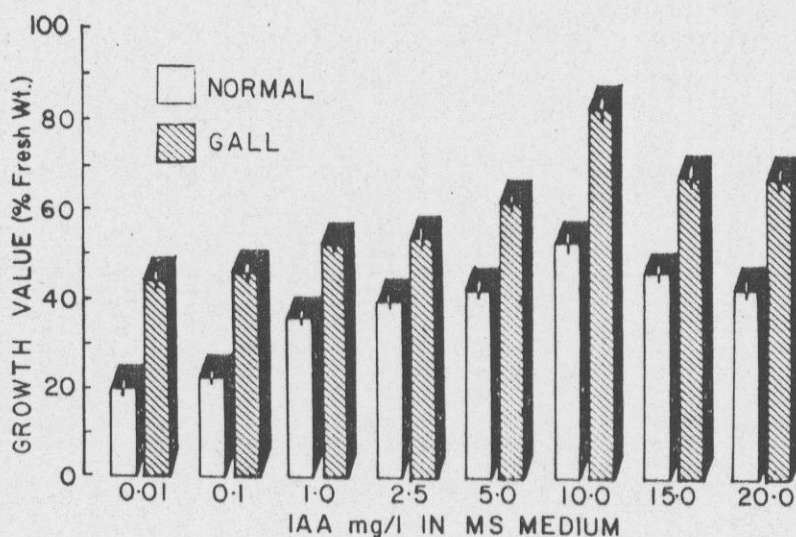


Fig. 3

The optimal concentration of NAA and IAA was 10 mg/l for growth of both the tissues. Above-optimal concentrations of NAA and IAA up to 20 mg/l in the medium, as shown in Figs. 2 and 3 respectively, showed steady growth of both normal and gall tissues. For growth of both the tissues, NAA was found a superior source (Fig. 2), therefore, was considered control. This is consistent with the findings of PELET et al. (1960) who found that NAA was a better source for the growth of *Phylloxera* gall and normal stem tissues. During the present studies, effect of DL-tryptophan on growth of *Zizyphus* gall and normal tissues, grown on auxin-free medium showed that both the tissues utilized this compound with an optimal of 5 mg/l (Fig. 1). It was interesting to note that growth response of gall tissue to DL-tryptophan was much higher as compared to normal tissue, and the gall tissue showed more auxin content in this treatment (Table 1). BOUILLENE and GASPAR (1970) reported that the capacity of the crown-gall tissues of *Impatiens* to synthesize IAA like substances from tryptophan was particularly high.

As shown in the table, from 5th to 30th day of growth, at 5-day interval, both *in vivo* and *in vitro* gall tissues showed more auxin contents than the corresponding normal

Table 1. Auxin contents ($\mu\text{g IAA equivalent/kg fresh weight}$) in *in vivo* and *in vitro* gall and normal stem tissues.

<i>in vivo</i> tissues/ treatment in culture	Days					
	5	10	15	20	25	30
<i>In vivo</i> tissues						
Normal	23.6 \pm 0.3	41.2 \pm 0.3	32.4 \pm 0.3	26.1 \pm 0.2	21.5 \pm 0.3	17.2 \pm 0.2
Gall	42.0 \pm 0.3	56.1 \pm 0.3	51.1 \pm 0.3	45.5 \pm 0.2	38.8 \pm 0.2	31.4 \pm 0.2
DL-tryptophan						
Normal	3.5 \pm 0.1	13.4 \pm 0.1	11.6 \pm 0.1	9.5 \pm 0.1	8.4 \pm 0.1	6.3 \pm 0.1
Gall	5.2 \pm 0.1	21.5 \pm 0.1	19.7 \pm 0.1	18.0 \pm 0.1	14.8 \pm 0.1	12.0 \pm 0.1
NAA						
Normal	3.5 \pm 0.05	12.0 \pm 0.1	9.8 \pm 0.1	8.7 \pm 0.1	7.0 \pm 0.1	6.3 \pm 0.1
Gall	5.2 \pm 0.1	17.6 \pm 0.1	16.5 \pm 0.1	15.1 \pm 0.1	13.0 \pm 0.1	10.5 \pm 0.1
IAA						
Normal	3.5 \pm 0.05	10.5 \pm 0.1	9.5 \pm 0.1	7.7 \pm 0.1	6.0 \pm 0.1	5.2 \pm 0.05
Gall	5.2 \pm 0.1	16.5 \pm 0.1	14.8 \pm 0.1	13.7 \pm 0.1	11.6 \pm 0.1	9.5 \pm 0.1

\pm Standard error

tissues. The highest endogenous auxin level was found in *in vivo* normal and gall tissues followed by tissues grown in medium with DL-tryptophan (5 mg/l), NAA and IAA (10 mg/l), in the decreasing order. There are a number of reports on hyperauxin levels in crown-gall tissues (LOCKE et al. 1938; LINK and EGGERS 1941; KULESCHA and GAUTHERET 1948; NITSCH 1956). KEHR and SMITH (1954) reported that *N. glauca* \times *N. langsdorffii* hybrids showed higher auxin content and a more effective enzyme system for converting tryptophan to auxin than the parents. BAYER (1965) demonstrated that the auxin content was highest in the hybrid, lower in *N. glauca* and lowest in *N. langsdorffii*. Similar results were obtained for *N. longiflora*, *N. debneyi* and their tumor producing hybrid (BAYER and AHUJA 1968). The potentially tumorous combinations showed higher levels of auxin than the parents and non tumorous segregants.

From the findings discussed above, it can be concluded that like crown-gall and genetic tumors, *Zizyphus* gall tissues showed auxin autotrophy and hyperauxinity. In both normal and gall tissues grown in medium having auxin, the endogenous auxin level decreased.

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