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Association of Auxin Protectors, Peroxidase, Indoleacetic Acid Oxidase and Polyphenol Oxidase in *Zizyphus* Gall and Normal Stem Tissues Grown in Culture

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Summary

Zizyphus normal stem tissue extract destroyed IAA when the two were incubated together. On the other hand, with gall tissue extract, IAA destruction occurred only following a lag period which was induced by auxin protectors detected in the gall tissue. The in vivo gall tissue showed no peroxidase and little IAA-oxidase activity for the incubation period tested. Polyphenol oxidase activity and total and *O*-dihydroxyphenols increased with the growth of the gall up to the 30th day. The protector-induced lag in IAA destruction was decreased when higher concentrations of IAA were used in the reaction mixture. Both normal and gall tissues were exposed to effectors such as NAA, IAA, 2,4-D, DL-tryptophan, gibberellic acid, and cycloheximide, each added separately. A differential response of normal and gall tissues to the different growth regulators in terms of activities of peroxidase, IAA oxidase and polyphenol oxidase was esthablished. A general tendency towards a decrease in polyphenol oxidase and an increase in peroxidase and IAA oxidase activities was observed in gall tissue. Both in vivo and in vitro gall tissue showed a higher protein content as compared to normal tissue.

Introduction

The growth autonomy of plant tumor tissues is accompanied by an abnormal hormone metabolism (Stonier 1972; Butcher 1973; Meins 1974; Arya et al. 1975). However, a perusal of literature shows conflicting reports on IAA-catabolism in crown-gall tissues (ref. Bouillene and Gaspar 1970; Stonier 1972). The presence of IAA inactivating enzymes in the normal tissues which were absent in the gall or a decrease in IAA destruction in the latter during gall induction have been reported. Several reports have also indicated that crown-gall had low IAA oxidase activity as compared to normal which resulted in its hyperauxinity. On the other hand, IAA-oxidase inhibitors have also been reported from the gall tissues. We have reported earlier that galls incited by a plant mite, *Eriophyes cernuus* on *Zizyphus jujuba* are of non-self-limiting type and possess tumefacient properties (Tandon and Arya 1976). The *Zizyphus* gall tissue possessed high

Abbreviations: NAA, α-naphthaleneacetic acid; IAA, indole-3-acetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; GA, gibberellic acid; MS, Murashige-Skoog medium; DCP,2,4-dichlorophenol; HRP, horseradish peroxidase

levels of auxin protectors (TANDON and ARYA 1980a). It also showed hyperauxinity and auxin-autotrophy and converted more auxin from DL-tryptophan incorporated in the culture medium as compared to the normal tissue (TANDON and ARYA 1980b).

The subject matter of the present paper deals with the synthesis of high levels of auxin protectors in *Zizyphus* gall tissue possibly through the activity of polyphenol oxidase and the role of auxin protectors in IAA catabolism by affecting the activities of peroxidase and IAA oxidase. An attempt was made to alter the activities of the above-mentioned enzymes in the gall tissue by adding different growth regulators in the culture medium.

Material and Methods

Plant Tissue

Tissue culture of *Zizyphus jujuba* LAMK. normal stem and stem galls incited by *Eriophyes cernuus* MASSEE and also the in vivo tissues of the same were used as experimental material. Both normal and gall tissues were isolated and maintained on modified MURASHIGE and SKOOG (1962) medium as described earlier (TANDON 1976). The *p*H of the medium was adjusted to 5.8 before autoclaving it at 1.06 kg/sq. cm pressure for 20 min. The tissues were grown in dark at 26 ± 2 °C and 55 to 60% relative humidity. The growth regulators used were NAA and IAA (each 10 mg/l), DL-tryptophan (5 mg/l), cycloheximide (1 mg/l) for both normal and gall tissues, 2,4-D (2.5 mg/l for normal and 1 mg/l for gall tissue); and GA_3 (1 mg/l for normal and 0.1 mg/l for gall tissue). Both normal and gall tissues showed optimum growth with these concentrations of the growth regulators incorporated separately in auxin-free MS medium except for cycloheximide which was used in conjunction with 100 mg/l of NAA and showed growth inhibition. NAA treatment was taken as the control, as both normal and gall tissues showed maximum growth with it when compared to other growth regulators. Analytical studies were carried out in i) callus tissues from 0 to 30 d of growth at 5-d intervals in their third and fourth passages of growth subjected to different growth regulators (0 d was the initial d implant from a three week culture), and ii) in vivo gall (from the 10th to the 50th d of growth at 10 d intervals) and normal stem tissues. All the experiments were conducted in triplicate and repeated twice.

Measurement of IAA destruction

Gall tissue extracts ranging from 0.01 mg to 0.5 mg fresh weight equivalent were incubated in 10 ml of IAA reaction mixture containing 10^{-4} M each of DCP, $MnCl_2$ and IAA, and HRP (final concentration 0.25 µg/ml) in 0.02 M phosphate buffer *p*H 6.1. IAA destruction was followed by removing 0.5 ml samples at different times from the reaction mixture. These were mixed with 2 ml of Salkowski reagent, and following a 1 h wait the absorbance was measured at 530 nm (GORDON and WEBER 1951).

Separation of auxin protectors

The auxin protectors from *Zizyphus* gall tissues were separated as described earlier (TANDON and ARYA 1980c). Three auxin protectors, Pr-I (mol. wt. exceeding 2×10^5 daltons), Pr-II (mol. wt. ca. 10^4 daltons) and Pr-III (mol. wt. ca. 2×10^3 daltons) were separated.

Effect of IAA concentration on protector activity

IAA concentration ranging from 0.5×10^{-4} to 2×10^{-4} M in 10 ml of IAA reaction mixture were used to study their effect on the protector-induced lag. The reaction mixture used to assay for auxin protectors consisted of a mixture of 0.05 ml of the protector, 10^{-4} M (final concentration) of each DCP, $MnCl_2$ and IAA; and HRP (at a final concentration of 0.5, 0.2 and 0.1 µg for Pr-I, Pr-II, and Pr-III, respectively). IAA destruction was measured by the method described above.

Tissue extracts

150 mg of the tissues were homogenized in 5 ml of chilled 0.1 M potassium phosphate buffer at *p*H 6 and centrifuged at 5,000 g for 15 min at 0 °C. The following assays were performed in both normal and

gall tissues: (i) 1.11.1. 7 peroxidase activity was determined according to the method as given in the WORTHINGTON ENZYME MANUAL (1972). The rate of decomposition of H_2O_2 by the enzyme with *O*-dianisidine as hydrogen donor was determined spectrophotometrically by measuring the rate of colour development at 460 nm. The activity is expressed as change in absorbance/min/g fresh weight of the tissue; (ii) 1.10.3.1 polyphenol oxidase was measured following Ponting and Joslyn (1948). The activity of the enzyme is expressed as change in absorbance at 420 nm/min/g fresh weight of tissue; and (iii) the activity of IAA-oxidase was measured by the method of Srivastava and van Huystee (1973) with slight modification. In the reaction mixture first DCP was added to 0.1 M phosphate buffer *p*H 6 followed by $MnCl_2$, enzyme extract and then IAA. The total volume of the reaction mixture was kept at 5 ml having 2×10^{-4} M (final concentration) each of DCP, $MnCl_2$ and IAA. The reaction mixture was incubated at 37 °C in a shaking water bath in the dark. After 1 h, 2 ml of Salkowski reagent was added to each tube to terminate the reaction and following a 1 h wait, the absorbance of the mixture was measured at 530 nm. The amount of IAA destroyed was calculated from a standard curve for IAA. The enzyme activity is expressed as mg IAA destroyed/g fresh weight of tissue/h.

Phenolics

Ethanol (30 %) extract of lyophillized tissues was used to estimate the total and *O*-dihydroxyphenols using Folin Ciocalteu and Arnow's reagent, respectively (Mahadevan 1975). Phenolics are presented as mg/g dry weight.

Protein

Protein was estimated by the method of Lowry et al. (1951) and is expressed as μg/g fresh weight of tissue.

Results and Discussion

In vivo *Zizyphus* gall tissue on all growth periods (from 10—50 d) showed less IAA oxidase activity as compared to normal stem tissue (Table 1). As shown in Fig. 1, with gall tissue extract IAA destruction occurred following a lag period. By increasing the

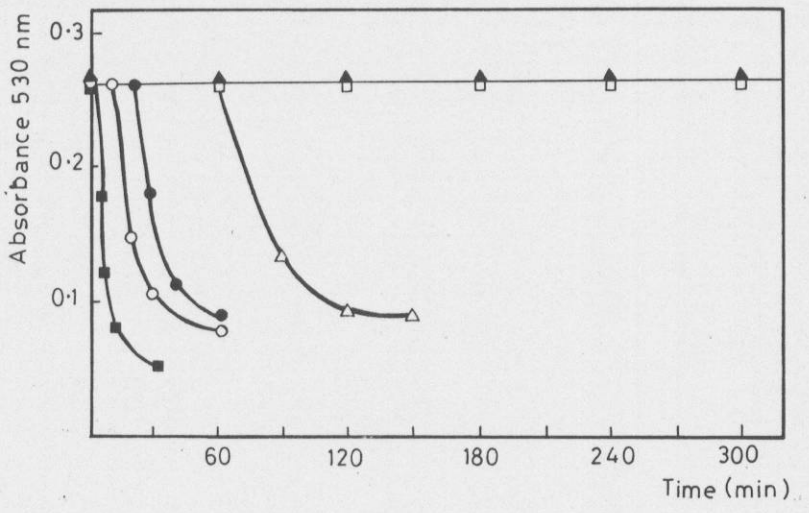

Fig. 1. *Effect of increasing concentrations of gall tissue extract on IAA destruction.*
Fresh weight equivalent of the gall tissue ○: 0.01 mg; ●: 0.05 mg; △: 0.1; ▲: 0.3 mg; □: 0.5 mg; ■: control without gall tissue extract, in 10 ml reaction mixture (see materials and methods). Note the lag period prior to IAA destruction increased with increasing amount of gall tissue extract.

Table 1. *Activities of IAA-oxidase, peroxidase, polyphenol oxidase and contents of total and O-dihydroxyphenols and protein in in vivo normal and gall tissues*

Analysis	Normal	Gall d				
		10	20	30	40	50
IAA-oxidase mg IAA destroyed/g fresh weight/h	1.95 ± 0.01	0.95 ± 0.02	0.68 ± 0.01	0.65 ± 0.01	0.63 ± 0.02	0.63 ± 0.01
Peroxidase Δ A/min/g fresh weight	113.9 ± 5.30	...	...	...	...	...
Polyphenol oxidase Δ A/min/g fresh weight	2.0 ± 0.01	2.2 ± 0.02	2.8 ± 0.02	3.2 ± 0.02	2.6 ± 0.02	2.2 ± 0.02
Total phenols mg/g dry weight	9.8 ± 0.30	12.3 ± 0.10	15.7 ± 0.20	22.9 ± 0.31	20.7 ± 0.20	18.4 ± 0.35
O-dihydroxyphenol mg/g dry weight	5.9 ± 0.07	9.5 ± 0.25	13.3 ± 0.14	20.0 ± 0.20	18.4 ± 0.40	16.1 ± 0.20
Protein mg/g fresh weight	1.9 ± 0.02	2.1 ± 0.03	3.1 ± 0.03	4.7 ± 0.04	5.1 ± 0.03	6.7 ± 0.03

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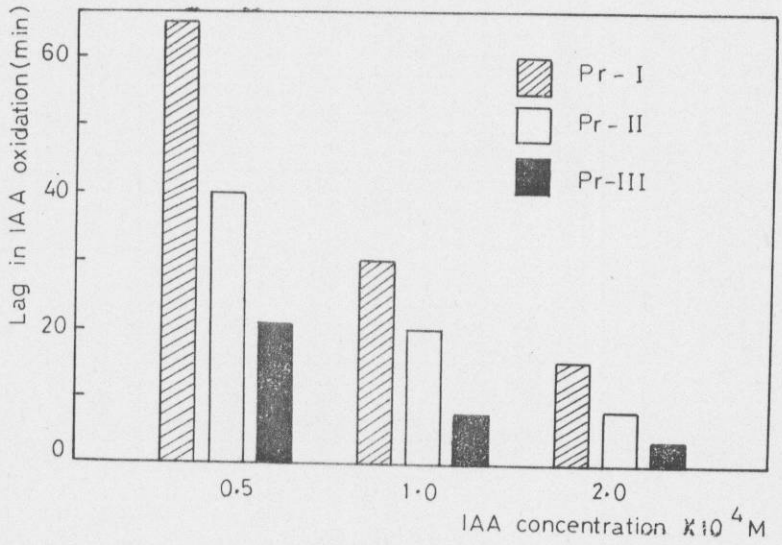

Fig. 2. *Effect of IAA concentration on auxin protector-induced lag in IAA oxidation.* Pr-I, II, III: 3 types of auxin, protectors different in their mol. wts. (see Materials and Methods).

amount of gall tissue extract in the assay mixture, the lag period prior to IAA destruction was increased, ruling out the possibility that gall tissue had less IAA oxidase activity. However, once the oxidation of IAA started, it proceeded at a normal rate. Thus one may ask, "What prevents or inhibits auxin catabolism in *Zizyphus* gall tissue?" The earlier findings of TANDON and ARYA (1980a) showed that high levels of auxin protectors are associated with *Zizyphus* gall tissue. In plant tumor tissues the presence of IAA oxidase inhibitors has been reported by several workers (ref. BOUILLENNE and GASPAR 1970). However, the class of substances isolated in the present studies was not IAA oxidase inhibitor. The auxin protectors isolated from *Zizyphus* gall tissue, prevented peroxidase-catalyzed oxidation of IAA by inducing a lag period. The chemical nature of auxin protectors is *O*-dihydroxyphenol (STONIER et al. 1970; TANDON and ARYA 1980a).

Many naturally occurring compounds such as phenols (both free and bound), coumarins, manganese salts and plant acids can change the activities of peroxidase and IAA-oxidase. Monophenols are regarded as cofactors of IAA-oxidase, while *O*- and *p*-dihydric phenols and polyphenols, as IAA oxidase inhibitors (SCHNEIDER and WIGHTMAN 1974). The enzyme polyphenol oxidase converts monophenols (electron acceptors) to *O*-diphenols (electron donors) to quinones (strong electron acceptors) and thus regulates the level of different phenolics. The in vivo *Zizyphus* gall tissue showed an increase in polyphenol oxidase activity with the growth of the galls up to the 30th d which presumably increased the formation of *O*-dihydroxyphenols (auxin protectors) as shown in Table 1. The gall tissue showed more *O*-dihydroxy and total phenols as compared to the normal.

YAMAZAKI and YAMAZAKI (1973) reported that the reaction of IAA with HRP resulted in the formation of spectroscopically distinct forms of peroxidase known as compounds I, II and III. The protector substances, such as coumarins and phenolic

Table 2. *IAA-oxidase activity: mg IAA destroyed/g fresh weight/h in in vitro normal and gall tissues*

Treatment	Tissue	d						
		0	5	10	15	20	25	30
NAA (control)	Normal	1.8 ± 0.01	2.9 ± 0.03	2.8 ± 0.01	2.6 ± 0.01	2.3 ± 0.02	1.2 ± 0.02	0.5 ± 0.01
	Gall	...	2.0 ± 0.10	1.1 ± 0.05	...	...	...	...
IAA	Normal	3.1 ± 0.02	3.0 ± 0.01	3.3 ± 0.01	3.1 ± 0.02	3.4 ± 0.01	3.0 ± 0.02	3.0 ± 0.01
	Gall	1.9 ± 0.03	2.2 ± 0.05	2.6 ± 0.01	2.7 ± 0.01	2.4 ± 0.02	1.1 ± 0.06	...
2,4-D	Normal	3.3 ± 0.01	3.1 ± 0.01	3.3 ± 0.02	3.5 ± 0.03	3.4 ± 0.01	3.2 ± 0.01	3.0 ± 0.01
	Gall	1.6 ± 0.02	2.3 ± 0.01	2.4 ± 0.02	2.8 ± 0.04	2.1 ± 0.02	1.2 ± 0.01	1.0 ± 0.02
DL-tryptophan	Normal	...	1.2 ± 0.02	2.4 ± 0.01	2.3 ± 0.02	1.8 ± 0.07	...	...
	Gall	...	1.1 ± 0.01	2.2 ± 0.03	2.7 ± 0.01	1.1 ± 0.03	...	...
GA	Normal	3.1 ± 0.01	2.9 ± 0.03	3.0 ± 0.01	3.1 ± 0.01	3.3 ± 0.04	3.0 ± 0.01	2.1 ± 0.01
	Gall	1.0 ± 0.03	2.1 ± 0.01	2.9 ± 0.03	3.1 ± 0.03	1.6 ± 0.05	...	...
Cycloheximide	Normal	0.72 ± 0.01	...	0.33 ± 0.03	2.2 ± 0.01	0.91 ± 0.01	0.54 ± 0.01	0.20 ± 0.03
	Gall	...	...	...	...	...	...	...

± S.E.

derivatives of cinnamic acid reacted preferentially with both compound II and compound III. The protectors were oxidized first and during this period a lag in the IAA oxidation was observed. After the concentration of the protectors reached a low level, IAA was oxidized at a rate close to that of the control lacking protector (Schaeffer et al. 1967; Gelinas 1973).

The scopoletin-inhibited HRP-catalyzed IAA oxidation resulted in rapid destruction of IAA when a tenfold increase in the amount of IAA was made (Serois and Miller 1972). The results shown in Fig. 2 indicate that increasing the concentrations of IAA in the protector assay system decreased the protector-induced lag period prior to IAA oxidation. This led us to incorporate various growth regulators separately in the culture medium and investigate their effect on peroxidase, IAA oxidase and polyphenol oxidase activities. The normal tissue showed more IAA oxidase activity as compared to gall tissue grown in culture with various growth regulators (Table 2). In the gall tissue, with cycloheximide, on all growth periods, no IAA oxidase activity could be detected for the incubation period tested. This was also true for NAA treatment excepting 5th and 10th day. However, with IAA and GA, IAA oxidase activity was detected throughout the growth period except on 30th d, and 25th and 30th d, respectively. In both normal and gall tissues with DL-tryptophan in the medium, IAA oxidase activity could not be observed on 0, 25th, and 30th d, of growth. With IAA, 2,4-D and GA treatments the activity of IAA oxidase was more as compared to other growth regulators. In both normal and gall tissues in culture, a decrease in auxin content has been reported with NAA, IAA and DL-tryptophan treatments (Tandon and Arya 1980b). The IAA oxidase assay in *Zizyphus* gall tissue was done in crude extracts i.e., containing both the enzyme and protector. Bouillene and Gaspar (1970) showed a high peroxidase and IAA oxidase activity in purified extracts of *Impatients* crown-gall tissue free from inhibitors. Thus, it may be possible for *Zizyphus* gall tissue to contain more enzyme and yet destroy less auxin.

The gall tissue showed more peroxidase activity, as compared to normal, under the influence of all growth regulators tested except for 2,4-D treatment (Table 3). Morel and Démétriadès (1955) reported that IAA and 2,4-D enhanced the activity of peroxidase and polyphenol oxidase in cultured Jerusalem artichoke. On the other hand, Cosma et al. (1976) reported that 2,4-D exerts a strong decreasing effect on peroxidase activity in *Arabidopsis* tissues in culture. Recently by ^{3}H-leucine incorporation it has been shown that IAA and 2,4-D stimulate peroxidase activity in mung bean cotyledons by several fold (Dendsay and Chaudhury 1979). It was also reported that increased peroxidase activity did not involve fresh synthesis of enzyme, but alterations in charge on the enzyme molecules resulted in its enhanced catalytic efficiency.

Zizyphus gall tissue contained less activity of polyphenol oxidase as compared to the normal tissue under the influence of NAA, IAA, 2,4-D and DL-tryptophan (Table 4). With GA and cycloheximide a reverse picture was obtained. *Zizyphus* gall tissue contained more protein per g of fresh weight than did the normal tissue under the influence of various growth regulators (Table 5). In vivo gall tissue also showed a similar pattern (Table 1). The rapid growth of *Zizyphus* gall tissue which is not in coordination with the

Table 3. *Peroxidase activity: Δ A/min/g fresh weight in in vitro normal and gall tissues*

Treatment	Tissue	d						
		0	5	10	15	20	25	30
NAA	Normal	50.8 ± 3.20	61.3 ± 2.80	60.9 ± 1.40	58.9 ± 0.80	53.9 ± 1.20	49.7 ± 1.50	54.9 ± 0.95
(control)	Gall	112.7 ± 5.00	93.7 ± 2.60	97.3 ± 1.50	101.3 ± 1.70	111.3 ± 1.80	113.3 ± 2.00	103.6 ± 4.50
IAA	Normal	128.3 ± 2.60	78.7 ± 3.10	98.3 ± 2.00	113.7 ± 2.50	130.3 ± 2.50	106.3 ± 3.10	96.3 ± 4.70
	Gall	130.5 ± 4.80	120.9 ± 3.00	105.6 ± 2.90	110.9 ± 1.80	123.7 ± 3.20	157.3 ± 1.50	160.7 ± 3.20
2,4-D	Normal	63.4 ± 1.60	60.0 ± 1.20	62.0 ± 0.80	74.7 ± 3.10	69.3 ± 1.70	57.7 ± 2.10	63.3 ± 1.10
	Gall	38.5 ± 2.80	48.7 ± 1.60	52.3 ± 1.50	56.7 ± 2.20	43.3 ± 3.10	33.7 ± 1.70	54.0 ± 2.30
DL-tryptophan	Normal	122.7 ± 1.70	110.3 ± 2.90	139.3 ± 3.20	132.0 ± 1.85	115.7 ± 3.10	129.7 ± 1.30	133.3 ± 5.60
	Gall	146.2 ± 4.30	125.8 ± 5.50	143.3 ± 1.90	155.9 ± 3.10	163.3 ± 2.90	131.0 ± 4.10	160.3 ± 2.20
GA	Normal	117.3 ± 3.30	96.7 ± 1.85	100.5 ± 3.10	109.0 ± 2.20	121.0 ± 5.00	113.7 ± 3.10	122.7 ± 2.45
	Gall	144.9 ± 1.70	100.3 ± 3.30	112.3 ± 1.85	125.7 ± 3.18	148.7 ± 1.94	121.3 ± 5.30	131.7 ± 2.84
Cycoheximide	Normal	41.0 ± 2.75	43.3 ± 1.30	48.3 ± 2.70	63.3 ± 1.58	47.0 ± 2.92	35.0 ± 3.10	39.0 ± 1.64
	Gall	89.5 ± 1.60	124.7 ± 1.50	122.3 ± 1.78	116.0 ± 2.50	98.3 ± 3.50	72.6 ± 1.76	46.3 ± 2.30

Table 4. *Polyphenol oxidase activity: Δ A/min/g fresh weight in in vitro normal and gall tissues*

Treatment	Tissue	d						
		0	5	10	15	20	25	30
NAA	Normal	1.66 ± 0.03	1.28 ± 0.01	1.39 ± 0.01	1.43 ± 0.04	1.59 ± 0.01	1.93 ± 0.07	1.07 ± 0.15
(control)	Gall	1.49 ± 0.07	1.23 ± 0.02	1.20 ± 0.01	1.11 ± 0.07	1.01 ± 0.05	1.00 ± 0.03	0.97 ± 0.05
IAA	Normal	1.34 ± 0.07	1.60 ± 0.01	1.53 ± 0.04	1.40 ± 0.03	1.28 ± 0.10	1.47 ± 0.07	1.67 ± 0.09
	Gall	1.23 ± 0.03	1.43 ± 0.07	1.53 ± 0.01	1.30 ± 0.03	1.20 ± 0.01	1.67 ± 0.09	1.06 ± 0.10
2,4-D	Normal	1.64 ± 0.05	1.73 ± 0.01	1.53 ± 0.09	1.33 ± 0.03	1.60 ± 0.03	1.87 ± 0.13	1.34 ± 0.09
	Gall	1.58 ± 0.01	1.50 ± 0.02	1.46 ± 0.07	1.27 ± 0.01	1.53 ± 0.07	1.73 ± 0.03	1.12 ± 0.78
DL-tryptophan	Normal	1.67 ± 0.02	1.73 ± 0.01	1.53 ± 0.05	1.56 ± 0.01	1.73 ± 0.15	1.40 ± 0.03	1.20 ± 0.05
	Gall	1.13 ± 0.01	1.60 ± 0.02	1.47 ± 0.01	1.39 ± 0.03	1.27 ± 0.04	1.07 ± 0.09	0.90 ± 0.01
GA	Normal	1.12 ± 0.03	1.60 ± 0.18	1.20 ± 0.09	1.07 ± 0.01	0.97 ± 0.02	1.30 ± 0.05	1.13 ± 0.03
	Gall	1.37 ± 0.05	1.77 ± 0.09	1.47 ± 0.02	1.40 ± 0.03	1.13 ± 0.01	1.60 ± 0.09	1.33 ± 0.04
Cycloheximide	Normal	1.47 ± 0.07	1.37 ± 0.01	1.30 ± 0.03	1.17 ± 0.10	1.33 ± 0.04	1.60 ± 0.03	1.07 ± 0.01
	Gall	2.0 ± 0.18	1.53 ± 0.04	1.60 ± 0.01	1.73 ± 0.03	1.97 ± 0.07	2.07 ± 0.03	2.27 ± 0.10

± S.E.

Table 5. *Protein content: µg/g fresh weight in in vitro normal and gall tissues*

Treatment	Tissue	d						
		0	5	10	15	20	25	30
NAA	Normal	133.3 ± 5.30	66.6 ± 4.20	73.0 ± 3.10	93.7 ± 5.10	100.5 ± 3.75	165.0 ± 6.18	92.3 ± 4.70
(Control)	Gall	182.4 ± 6.74	73.0 ± 5.90	130.8 ± 6.75	160.2 ± 8.25	182.6 ± 4.50	206.0 ± 7.31	138.5 ± 2.78
IAA	Normal	181.2 ± 5.50	62.0 ± 2.90	112.5 ± 4.70	130.7 ± 5.50	174.4 ± 7.91	208.9 ± 9.30	101.7 ± 4.15
	Gall	197.2 ± 6.75	74.7 ± 4.85	132.5 ± 6.50	169.6 ± 8.18	195.0 ± 5.75	228.8 ± 8.75	136.7 ± 5.15
2,4-D	Normal	170.5 ± 5.75	66.7 ± 5.00	125.6 ± 6.18	146.0 ± 5.72	160.8 ± 3.80	185.5 ± 5.90	101.3 ± 7.35
	Gall	219.4 ± 7.75	79.3 ± 3.18	140.0 ± 6.20	160.6 ± 4.35	215.6 ± 5.72	232.9 ± 7.18	108.3 ± 4.48
DL-tryptophan	Normal	176.2 ± 5.15	43.4 ± 1.90	91.5 ± 2.75	121.5 ± 5.15	161.9 ± 4.38	191.3 ± 4.71	110.8 ± 5.30
	Gall	230.3 ± 6.00	85.0 ± 3.50	107.1 ± 4.00	151.7 ± 5.70	213.8 ± 6.78	250.0 ± 7.00	216.6 ± 8.38
GA	Normal	186.8 ± 5.57	80.3 ± 3.90	128.5 ± 7.30	155.7 ± 4.50	183.8 ± 6.55	196.9 ± 7.30	46.7 ± 2.75
	Gall	216.5 ± 9.70	90.0 ± 4.75	132.5 ± 8.50	186.3 ± 6.50	212.5 ± 5.30	218.8 ± 4.90	74.9 ± 2.75
Cycloheximide	Normal	139.8 ± 6.31	23.3 ± 1.05	38.8 ± 1.50	82.8 ± 3.50	111.3 ± 6.78	185.6 ± 4.30	141.3 ± 6.58
	Gall	210.3 ± 8.60	26.2 ± 1.75	58.8 ± 2.75	99.1 ± 4.70	186.3 ± 4.90	233.1 ± 6.75	158.6 ± 8.30

± S.E.

growth of adjoining normal cells could possibly be explained on the basis of greater protein synthesis. The higher rate of protein synthesis in gall tissues as compared to the corresponding normal tissues of *Parthenocisssus* (KLEIN 1952), common beet root (SCOTT et al. 1962) and bean hypocotyl (NOVAK and GALSTON 1975) has been reported. With cycloheximide treatment the growth of both *Zizyphus* gall and normal tissues and protein synthesis was inhibited, but the inhibitory effect on the activities of peroxidase and polyphenol oxidase was not significant. This is consistent with the findings on peanut cells grown in suspension culture (VAN HUYSTEE and TURCON 1973).

The analysis of in vivo gall tissue showed that the polyphenol oxidase activity increased with the growth of the gall up to the 30th d and subsequently decreased when the gall attained considerable growth, presumably to check the conversion of protector substances to quinones. In general, the activity of polyphenol oxidase was inversely proportional to the activities of peroxidase and IAA oxidase. It was interesting to note that the activity of polyphenol oxidase in the gall tissue decreased when different growth regulators were incorporated into the medium. An increase in peroxidase and IAA oxidase activity was also observed. This supports our earlier observation that the incorporation of growth regulators into the culture medium decreased the auxin content in the gall tissue (TANDON and ARYA 1980b) and it also suggests the possibility of a negative feedback mechanism of auxin operating in the *Zizyphus* gall tissue.

As discussed above, it is quite clear that polyphenol oxidase is the key enzyme in *Zizyphus* gall tissue that regulates the level of phenolic compounds (auxin protectors) which in turn affect the activities of peroxidase and IAA oxidase responsible for IAA destruction.

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