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Effect of Growth Regulators on Carbohydrate Metabolism of *Zizyphus jujuba* Gall and Normal Stem Tissues in Culture

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Summary

Both normal and gall tissues of *Zizyphus jujuba* were cultured on MS medium with NAA, IAA 2,4-D, DL-tryptophan, GA, and cycloheximide incorporated separately in the medium. With all these growth regulators, the gall tissue contained more total carbohydrates and reducing sugars, the only exception being DL-tryptophan, where the normal tissue showed more reducing sugars Normal tissue showed more α-amylase (EC 3.2.1.1.) activity as compared to gall tissue with all the growth regulators except for IAA and 2,4-D treatments. There was an abrupt increase in α-amylase activity from 15th to 20th day of growth in both normal and gall tissues with all the growth regulators tested. Differential response of normal and gall tissues in culture to growth regulators was established. *In vivo*, gall tissue contained more total carbohydrates, reducing sugars, and activity of α-amylase as compared to normal tissue.

Introduction

Earlier studies on carbohydrate metabolism of cultured cells and tissues were mainly concerned with finding the suitable carbon source for their growth. Recently, considerable efforts are being made to study the uptake, utilization, and metabolism of carbohydrates in cultured cells and tissues (see Maretzki et al. 1974). There are a few interesting reports in literature on increase in total carbohydrates in tissue culture of *Ipomoea* sp. (Rose et al. 1972) and *Saccharum* sp. (Maretzki et al. 1974); initial increase and then considerable decrease of reducing sugars in callus cultures of *Nicotiana tabacum* (Thorpe and Meier 1974); and on synthesis and utilization of starch by tobacco callus cultures as influenced by cytokinin (Thorpe and Meier 1972). Presence of α-amylase in the media after the growth of *Rumex acetosa* virus tumor tissue (Brakke and Nickell 1951), tobacco crown gall tissue (Jaspars and Veldstra 1965) and presence of both α- and β-amylase in the spent medium after *Saccharum* sp. growth (Maretzki et al. 1971) have been reported.

In our previous work (Tandon et al. 1975) the effect of various carbon sources on growth of both normal and gall tissues of *Zizyphus* was described. The present paper deals with the influence of growth regulators on the accumulation of sugars and synthesis of intracellular α-amylase.

Abbreviations: NAA, α-naphthaleneacetic acid; IAA, indole-3-acetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; GA, gibberellic acid.

Material and Methods

The tissue cultures of *Zizyphus jujuba* LAMK. stem galls incited by *Eriophyes cernuus* MASSEE and normal stem, 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 elsewhere (TANDON 1976). A brief account of the cultural conditions is as follows. The *p*H of the medium was adjusted to 5.8 before autoclaving the medium at 1.06 kg/sq cm pressure for 20 min. The tissues were grown on MS medium in a dark growth chamber maintained at 26 ± 2 °C and 55 to 60 percent relative humidity. The growth regulators used were: NAA and IAA (10.0 mg/l), DL-tryptophan (5.0 mg/l) and cycloheximide (1.0 mg/l) for both normal and gall tissues; 2,4-D (2.5 mg/l for normal and 1.0 mg/l for gall tissue); and GA (1.0 mg/l for normal and 0.1 mg/l for gall tissue). Both normal and gall tissues showed optimum growth with the mentioned concentrations of the growth regulators incorporated separately in auxin-free MS medium except for cycloheximide which was used in conjuction of 10.0 mg/l of NAA and showed growth inhibition. NAA treatment was taken as control as both normal and gall tissues showed maximum growth with it as compared to other growth regulators tested. Analytical studies were carried out in i) callus tissues upto 30 days of growth at 5-day interval in their third and fourth passages of growth subjected to different growth regulators, and ii) *in vivo* gall (from 10th to 50th day of growth at 10-day interval) and normal stem tissues.

For estimations of water soluble carbohydrates and activity of α-amylase (EC 3.2.1.1.), 200 mg tissue was homogenized in 5 ml chilled distilled water. For estimation of reducing sugars 100 mg of tissue was extracted in 5 ml 80% ethanol. The homogenates were centrifuged at 5,000 · g for 15 min at 0 °C. The total carbohydrates and reducing sugars were estimated by the methods of PLUMMER (1971) and SOMOGYI (1945), respectively. α-amylase activity was assayed using modified method of SHUSTER and GIFFORD (1962). The reaction mixture comprised 1 ml 0.067% starch in 0.06 M KH_2PO_4 and 2 ml suitably diluted enzyme solution. The reaction mixture was incubated for 5 min at 25 °C. The reaction was stopped by adding 1 ml of 10% trichloroacetic acid. One ml of iodine — HCl solution (60 mg KI and 6 mg I_2 in 100 ml of 0.05 N HCl) was added to the reaction mixture. The color developed due to unhydrolyzed starch was measured at 620 nm.

Results and Discussion

As shown in Table 1 the total water soluble carbohydrates, alcohol soluble reducing sugars, and activity of α-amylase increased with the growth of *in vivo* gall tissues. However, the values obtained for normal stem tissue were much less as compared to gall.

The growth regulators influence plant tissues in culture in terms of their growth promotion or inhibition which in turn is associated with metabolic changes. GORIS (1948) reported the influence of auxin on the rate of sugar depletion when callus cultures were transferred to sugar free medium. On the other hand sugar accumulation in tissues in response to addition of auxin to a sugar containing medium was also reported (BOOTH et al. 1962). As shown in Table 2, with NAA and 2,4-D in the medium the amount of total carbohydrates in both normal and gall tissues increased upto 15th day of growth, then subsequently declined. A reverse picture for reducing sugars (Table 3) and α-amylase activity (Table 4) was obtained. Increase in the total carbohydrates in the first 48 h in *Ipomoea* sp. culture (ROSE et al. 1972) and in *Saccharum* sp. during linear growth phase (MARETZKI et al. 1974) has been reported. THORPE and MEIER (1974) reported an initial increase of reducing sugars in *Nicotiana tabacum* cultures and then about 50% decrease by 10th day of growth. With IAA, DL-tryptophan, GA, and cyclo-

Table 1. Analysis of total water- soluble carbohydrates, reducing sugars, and activity of α-amylase in vivo of normal and gall tissues

Analysis	Normal	Gall Days				
		10	20	30	40	50
Total water soluble carbohydrates mg/g fresh wt.	9.8 ± 0.1	10.7 ± 0.2	12.7 ± 0.2	16.0 ± 0.1	32.8 ± 0.2	60.3 ± 0.3
Alcohol soluble reducing sugars mg/g fresh wt.	3.0 ± 0.2	6.1 ± 0.1	7.8 ± 0.1	10.5 ± 0.1	20.1 ± 0.2	21.3 ± 0.2
α-amylase mg starch hydrolysed/g fresh wt.	10.7 ± 0.2	12.7 ± 0.3	20.0 ± 0.2	21.3 ± 0.2	23.0 ± 0.2	25.0 ± 0.2

± Standard error

heximide in the MS medium, the contents of total carbohydrates decreased on 5th day, increased upto 15th day and then declined subsequently. In contrast to this, opposite values for reducing sugars and α-amylase were obtained. However, a marked decrease in contents of reducing sugars in both normal and gall tissues was observed with IAA treatment. The gall tissue contained more total carbohydrates and reducing sugars with all the growth regulators tested on all growth periods with an exception of DL-tryptophan where normal tissue contained more reducing sugars (Table 3). The general findings of the level of total carbohydrates and reducing sugars in *Zizyphus* normal and gall tissues are consistent with the results on single cell clones of stem and *Phylloxera* leaf gall of grape vine (Warick and Hildebrandt 1967). Kaneko (1967) reported increased total soluble sugars in tuber callus as compared to crown gall of Jerusalem artichoke. However, he obtained a reverse picture for reducing sugars.

There are several reports on release of α-amylase into the medium by plant tissue cultures. Nickell and Burkholder (1950) and Brakke and Nickell (1955) reported that sorrel virus tumor tissue secreted excess of sugars used for the growth of the tissues. The release of both α- and β-amylase by callus cultures of *Rubus fruiticosus* and by crown gall cultures of *Nicotiana tabacum* (Karstens and de Meester-Mangercats 1960) and secretion of amylase by normal callus cultures of *Juniperus communis* (Constabel 1963) have been reported.

During present studies the α-amylase activity in normal and gall tissues was influenced by various growth regulators. In contrast to control the gall tissue with 2,4-D and IAA treatment showed higher α-amylase activity as compared to normal tissue. GA, DL-tryptophan, IAA, and cycloheximide induced more production of α-amylase on 5th day of growth in both normal and gall tissues. However, on subsequent days of growth it showed a pattern comparable to control. Activation of α-amylase synthesis by GA in barley whole seeds and in embryoless endosperm was reported by Verbeek et al. (1969).

Table 2. *Contents of total water soluble carbohydrates: mg/g fresh wt. in normal and gall tissue cultures*

Treatment	Tissue	Days						
		0	5	10	15	20	25	30
NAA (control)	Normal	10.7 ± 0.1	12.0 ± 0.2	13.8 ± 0.2	15.9 ± 0.2	12.1 ± 0.2	9.3 ± 0.1	2.5 ± 0.05
	Gall	12.3 ± 0.2	14.5 ± 0.2	18.9 ± 0.2	20.8 ± 0.3	13.0 ± 0.2	12.0 ± 0.2	8.6 ± 0.05
IAA	Normal	12.8 ± 0.2	9.2 ± 0.1	11.1 ± 0.1	15.5 ± 0.2	13.5 ± 0.2	11.4 ± 0.2	1.9 ± 0.05
	Gall	13.8 ± 0.2	9.3 ± 0.2	13.4 ± 0.2	17.1 ± 0.2	14.9 ± 0.2	12.6 ± 0.2	8.7 ± 0.1
2,4-D	Normal	11.1 ± 0.1	11.6 ± 0.1	12.1 ± 0.2	16.3 ± 0.2	11.8 ± 0.1	10.5 ± 0.1	1.8 ± 0.05
	Gall	11.8 ± 0.1	13.3 ± 0.2	15.1 ± 0.2	18.1 ± 0.2	12.6 ± 0.2	11.1 ± 0.2	6.4 ± 0.05
DL-tryptophan	Normal	12.3 ± 0.2	9.8 ± 0.1	11.5 ± 0.2	14.1 ± 0.2	13.6 ± 0.2	10.5 ± 0.1	4.4 ± 0.05
	Gall	13.5 ± 0.2	11.3 ± 0.2	12.4 ± 0.2	15.5 ± 0.2	13.7 ± 0.2	11.9 ± 0.2	6.5 ± 0.05
GA	Normal	14.0 ± 0.2	9.5 ± 0.1	13.8 ± 0.2	15.6 ± 0.2	13.0 ± 0.2	12.1 ± 0.2	5.1 ± 0.05
	Gall	14.9 ± 0.2	10.9 ± 0.1	15.0 ± 0.2	17.3 ± 0.2	14.8 ± 0.2	13.6 ± 0.2	5.5 ± 0.05
Cycloheximide	Normal	13.3 ± 0.2	8.6 ± 0.1	10.2 ± 0.1	13.7 ± 0.2	13.0 ± 0.2	10.9 ± 0.1	5.0 ± 0.05
	Gall	14.8 ± 0.2	10.7 ± 0.1	12.8 ± 0.2	17.5 ± 0.3	17.1 ± 0.2	12.4 ± 0.2	5.5 ± 0.04

± Standard error

Table 3. *Contents of alcohol soluble reducing sugars: mg/g fresh wt. in normal and gall tissue cultures*

Treatment	Tissue	Days						
		0	5	10	15	20	25	30
NAA (control)	Normal	15.3 ± 0.2	10.6 ± 0.1	8.3 ± 0.1	6.0 ± 0.1	14.9 ± 0.2	15.7 ± 0.2	17.5 ± 0.2
	Gall	28.4 ± 0.3	17.4 ± 0.2	15.9 ± 0.2	11.8 ± 0.1	26.5 ± 0.2	27.9 ± 0.2	28.9 ± 0.2
IAA	Normal	1.8 ± 0.04	3.3 ± 0.05	3.0 ± 0.05	1.9 ± 0.05	5.7 ± 0.05	5.8 ± 0.1	5.8 ± 0.1
	Gall	7.8 ± 0.1	9.0 ± 0.1	8.2 ± 0.1	6.4 ± 0.1	8.6 ± 0.1	12.8 ± 0.2	26.7 ± 0.2
2,4-D	Normal	13.3 ± 0.2	9.6 ± 0.1	7.8 ± 0.1	5.8 ± 0.1	14.1 ± 0.2	15.3 ± 0.2	16.7 ± 0.2
	Gall	14.6 ± 0.2	10.8 ± 0.2	8.2 ± 0.1	6.7 ± 0.1	15.3 ± 0.2	15.8 ± 0.3	17.6 ± 0.2
DL-tryptophan	Normal	14.0 ± 0.2	14.4 ± 0.2	11.3 ± 0.2	10.2 ± 0.1	13.3 ± 0.2	15.8 ± 0.2	27.9 ± 0.2
	Gall	11.2 ± 0.2	14.0 ± 0.2	8.7 ± 0.1	8.5 ± 0.1	13.5 ± 0.2	13.8 ± 0.2	13.8 ± 0.2
GA	Normal	12.2 ± 0.2	12.8 ± 0.2	9.3 ± 0.1	7.2 ± 0.1	12.7 ± 0.2	13.2 ± 0.2	13.8 ± 0.2
	Gall	16.5 ± 0.2	18.7 ± 0.3	10.6 ± 0.1	10.2 ± 0.1	15.4 ± 0.3	17.0 ± 0.2	21.0 ± 0.2
Cycloheximide	Normal	11.3 ± 0.2	12.3 ± 0.2	10.3 ± 0.1	7.6 ± 0.1	11.3 ± 0.1	12.6 ± 0.2	14.1 ± 0.2
	Gall	12.0 ± 0.2	13.4 ± 0.2	11.4 ± 0.2	9.5 ± 0.1	12.1 ± 0.2	25.6 ± 0.3	26.8 ± 0.3

± Standard error

Table 4. *α-amylase activity: mg starch hydrolysed/g fresh wt. in normal and gall tissue cultures*

Treatment	Tissue	Days						
		0	5	10	15	20	25	30
NAA (control)	Normal	7.8 ± 0.1	7.3 ± 0.1	6.8 ± 0.1	5.6 ± 0.1	9.6 ± 0.2	6.0 ± 0.1	5.3 ± 0.1
	Gall	6.5 ± 0.1	6.4 ± 0.1	6.0 ± 0.1	5.0 ± 0.1	7.5 ± 0.2	5.0 ± 0.1	3.3 ± 0.05
IAA	Normal	4.4 ± 0.05	6.0 ± 0.1	4.3 ± 0.05	3.8 ± 0.04	7.9 ± 0.1	5.4 ± 0.1	2.3 ± 0.04
	Gall	6.1 ± 0.1	7.1 ± 0.1	6.0 ± 0.1	5.8 ± 0.1	8.3 ± 0.2	5.8 ± 0.1	4.6 ± 0.04
2,4-D	Normal	6.7 ± 0.1	6.1 ± 0.1	4.9 ± 0.1	3.8 ± 0.05	8.1 ± 0.2	5.3 ± 0.1	2.5 ± 0.05
	Gall	7.8 ± 0.1	6.7 ± 0.1	5.6 ± 0.1	4.5 ± 0.1	9.2 ± 0.2	6.3 ± 0.1	3.8 ± 0.05
DL-tryptophan	Normal	6.1 ± 0.1	6.8 ± 0.1	5.3 ± 0.1	3.3 ± 0.05	8.5 ± 0.2	3.6 ± 0.05	2.1 ± 0.05
	Gall	4.8 ± 0.04	6.8 ± 0.1	4.5 ± 0.1	3.5 ± 0.05	9.3 ± 0.2	3.8 ± 0.05	2.8 ± 0.05
GA	Normal	6.6 ± 0.1	7.0 ± 0.1	6.5 ± 0.2	5.6 ± 0.1	7.6 ± 0.1	5.6 ± 0.05	3.6 ± 0.1
	Gall	6.0 ± 0.1	7.0 ± 0.1	4.3 ± 0.1	3.5 ± 0.05	7.1 ± 0.1	4.3 ± 0.05	2.0 ± 0.05
Cycloheximide	Normal	5.8 ± 0.1	7.0 ± 0.1	5.8 ± 0.1	4.6 ± 0.1	8.5 ± 0.1	4.1 ± 0.04	2.5 ± 0.05
	Gall	4.3 ± 0.05	6.0 ± 0.1	5.1 ± 0.1	3.3 ± 0.05	7.3 ± 0.1	2.2 ± 0.04	1.6 ± 0.05

± Standard error

In contrast to normal tissue *Zizyphus* gall tissue both *in vivo* and *in vitro* showed hyperauxinity (Arya et al. 1975). There existed a correlation between α-amylase and IAA oxidase activity of the normal and gall tissues. With the decrease in the IAA oxidase activity in the gall tissue (our unpublihed data) auxin content increased which in turn increased more synthesis of α-amylase. Involvement of IAA in increased α-amylase evolution in barley seedlings was reported by Verbeek et al. (1973). These authors further reported that the decrease in α-amylase was due to lowering of endogenous level of auxin because peroxidase and IAA oxidase activity increased. In *Zizyphus* normal and gall tissues an integral association of growth regulators with the activity of α-amylase and sugar accumulation was found. There existed a differential response of normal and gall tissues to some growth regulators incorporated into the medium.

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