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CARBOHYDRATE METABOLISM IN *MUSA PARADISIACA*

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Key Word Index—*Musa paradisiaca*; Musocene; banana; glucose; fructose; sucrose; starch; sucrose synthetase; sucrose phosphate synthetase; acid- and neutral invertase; ADPG-pyrophosphorylase; starch phosphorylase; β-amylase; hexokinase; glucosephosphate isomerase; acid- and alkaline phosphatase.

Abstract—Considerable variations exist in the content of glucose, fructose, sucrose, starch and protein and in the activities of enzymes involved in carbohydrate metabolism between different parts of the banana plant (*Musa paradisiaca*). Sucrose synthetase is present in the highest concentration in rootstock and fruit pulp, and sucrose phosphate synthetase in the pseudostem. The highest ratio of the activity of sucrose phosphate synthetase to sucrose synthetase is found in leaves. Acid invertase is present in leaves, leaf-sheath and fruit pulp and is not demonstrable in rootstock and pseudostem. Neutral invertase activity is high in pseudostem and leaf-sheath. Starch phosphorylase is largely concentrated in fruit pulp and rootstock. The maximum activity of ATP:D-phosphoglucose (ADPG) pyrophosphorylase is found in rootstock. β-Amylase is not demonstrable in rootstock and is largely concentrated in leaf-sheath. Hexokinase is most active in rootstock and the lowest in leaves. Acid phosphatase and alkaline phosphatase activity is highest in fruit pulp and pseudostem. Glucosephosphate isomerase is most active in the rootstock and lowest in the leaves.

INTRODUCTION

BANANA is one of the important commercial fruit crops of the world. Despite evidence for the synthesis and degradation of starch in the developing and ripening banana fruit,[1–4] only a beginning has been made in the evaluation of the enzymatic mechanisms involved in these transformations.[5–7] As far as the present authors are aware, no study exists on the inter-relationship between the metabolism of the fruit and the rest of the plant. Study of the enzymic activities in the banana plant must take into account the interference by phenolics.[8] In the present paper the distribution of sugars and starch and the activities of enzymes involved in sucrose–starch interconversion in various parts of the banana plant have been studied in an attempt to understand the overall carbohydrate metabolism of the plant.

RESULTS

Sugar, Starch, Protein and Dry Weight Content in Different Parts of the Banana Plant

The results of paper chromatographic analysis of ethanol-soluble carbohydrate fraction

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and chemical analysis of total reducing sugar and starch in different parts of the banana plant are recorded in Table 1. Also reported are the contents of dry weight and protein.

TABLE 1. DRY WEIGHT, PROTEIN AND SUGAR CONTENTS OF VARIOUS PARTS OF THE BANANA PLANT

Tissue	Dry wt (mg/g fr. wt)	Protein (mg/g dry wt)	Total reducing sugars	Glucose	Fructose	Sucrose	Starch
				(mg/g dry wt)			
Leaves	244	48	16	8	7	16	11
Leaf-sheath	57	21	365	242	109	33	143
Rootstock	213	6	14	6	6	4	250
Lower pseudostem	46	29	344	233	109	34	94
Upper pseudostem	60	17	353	243	109	33	55
Fruit pulp	113	22	19	8	9	9	120

Expressed on a dry weight basis, rootstock had the highest starch content, followed by leaf-sheath, fruit pulp, and lower- and upper-pseudostem and, finally, the leaves. The starch content of the rootstock was 23-fold higher than in leaves. Considering the bulk of the rootstock, it will be evident that this organ functions as the reservoir of starch in the banana plant. It was significant to note that although the fruit pulp was at an early stage of maturity, considerable amounts of starch were present.

The major sugars present were glucose, fructose and sucrose. The contents of the reducing sugars in leaves, rootstock and fruit pulp did not differ significantly. Reducing sugars were concentrated in the leaf-sheath and pseudostem, in which they were about 22- to 25-fold higher than in leaves, rootstock and fruit pulp. Glucose and fructose were present in almost equal amounts in leaves, rootstock and fruit pulp. These sugars were concentrated in leaf-sheath and the lower- and upper-pseudostem. The content of glucose in these parts of the banana plant was about 30-fold higher than in leaves, rootstock and fruit pulp, whereas the fructose content was about 15-fold higher. Leaf-sheath and the lower- and upper-pseudostem were distinctive in having glucose in about 2-fold higher concentration than fructose; in the other tissues studied, the two hexoses were present in nearly equivalent amounts.

Sucrose was present in the highest amount in leaf-sheath and the lower- and upper-pseudostem, the content being about 8-fold higher than in rootstock, which had the lowest content. Fruit pulp had about 2-fold higher content of sucrose compared to the rootstock. Leaves had a 4-fold higher content of sucrose than the rootstock, but it had only one quarter the content of the leaf-sheath. The dry solid content fluctuated in the different parts of the plant. The content was maximum in leaves, followed by rootstock, fruit pulp, upper pseudostem, leaf-sheath and lower pseudostem. There was a 5-fold difference between the maximum and the minimum dry solid content. The maximum protein content (in the leaves) and the minimum (in the rootstock), calculated on the basis of dry weight, showed an 8-fold variation. It was significant that the protein content in the pseudostem was nearly the same as that of the pulp.

Enzyme Activities in Different Parts of the Banana Plant

In view of the variation in dry solid content and, more so, of protein among the different parts of the banana plant, the analytical data for enzyme activity are best interpreted in terms of tissue protein when comparisons are made among tissues. As reported elsewhere,[8]

phenolics were distributed to different extents in the various parts of the banana plant; these might inactivate or inhibit enzymes to varying extent on cell rupture. To overcome this interference, all media used for tissue dispersion contained cysteine and EDTA, and when necessary, also polyvinylpyrrolidone (PVP) or Triton X100. The optimum composition of the dispersion medium had been established for each enzyme and each tissue sample.[8] The values for enzyme activity (as also the data for tissue constituents reported in Table 1) were reproducible when the determinations were conducted on 2 other banana plants; the data reported are those obtained in one of the three experiments.

Enzymes which Metabolize Sucrose

Sucrose synthetase (E.C. 2.4.1.13, UDP-glucose: D-fructose 2-glucosyltransferase) and sucrose phosphate synthetase (E.C. 2.4.1.14, UDP-glucose: D-fructose 6-phosphate 2-glucosyltransferase) were present in all parts of the banana plant. Sucrose synthetase activity was maximum in the non-photosynthetic tissues, rootstock followed by fruit pulp; the activity in the former was about 15-fold that in leaves (Table 2). Sucrose phosphate synthetase showed a 6-fold difference between the maximum activity (in the lower pseudostem) and the minimum (in the leaves). Banana leaves had a ratio of 2·4 for sucrose phosphate synthetase to sucrose synthetase activity. The corresponding ratio for rootstock was 0·27, for fruit pulp 0·34 and for leaf-sheath and pseudostem 1.

TABLE 2. DISTRIBUTION OF ENZYME ACTIVITIES IN DIFFERENT PARTS OF THE BANANA PLANT: ENZYMES WHICH METABOLIZE SUCROSE

Tissue	Sucrose synthetase	Sucrose phosphate synthetase	Invertase	
			Acid	Neutral
		(units/mg protein)		
Leaves	0·47†	1·15*	0·41‡	0·45†
	(5·20)	(13·40)	(4·75)	(5·20)
Leaf-sheath	2·86*	3·25†	1·59‡	9·60‡
	(3·35)	(3·80)	(1·87)	(11·30)
Rootstock	7·25†	1·96*	Nil	1·53†
	(8·85)	(2·40)		(1·87)
Lower pseudostem	3·54‡	5·00†	Nil	10·26†
	(4·75)	(6·70)		(13·75)
Upper pseudostem	5·60‡	4·40†	Nil	10·75†
	(5·60)	(4·40)		(10·75)
Fruit pulp	6·92†	2·34*	1·95‡	2·31‡
	(16·80)	(5·70)	(4·75)	(5·65)

* Basal medium. 0·05 M Tris–HCl buffer, pH 7·2, 0·02 M freshly neutralized cysteine hydrochloride and 0·01 M EDTA.

† Basal medium + 1% (w/v) PVP.

‡ Basal medium + 1% (v/v) Triton X100.

Figures in parenthesis represent enzyme activity in units/g fr. wt. When PVP was incorporated into the dispersion medium for enzyme activity determinations, the values for protein were those determined in homogenates in basal medium. Other details were as in text.

Acid invertase (E.C. 3.2.1.26, β-fructofuranoside fructohydrolase) activity was present only in leaves, leaf-sheath and fruit pulp, whereas neutral invertase (E.C. 3.2.1.26, β-fructofuranoside fructohydrolase) was present in all tissues examined. Neutral invertase activity was concentrated in the conducting regions of the plant, namely, pseudostem and leaf-sheath. The rootstock contained a seventh of the activity of upper pseudostem. The lowest neutral invertase activity was in leaves.

Enzymes of Starch Metabolism

ADPG pyrophosphorylase (E.C. 2.7.7, ATP: α-D-glucose 1-phosphate adenylyltransferase) activity was mainly in the rootstock, followed by pseudostem, fruit pulp and leaf-sheath. The activity in leaves was lowest and was only *ca.* 2% of that in rootstock. Starch phosphorylase (E.C. 2.4.1.1, α-1,4-glucan: orthophosphate glycosylyltransferase) was most active in fruit pulp followed by rootstock (Table 3). Leaves contained the lowest concentration of the enzyme which was about 20% of that in fruit pulp. β-Amylase (E.C. 3.2.1.2, β-glucan maltohydrolase) activity was maximum in leaf-sheath followed by pseudostem. Fruit pulp and leaves had very low activity whereas no activity could be demonstrated in the rootstock.

TABLE 3. DISTRIBUTION OF ENZYME ACTIVITIES IN DIFFERENT PARTS OF THE BANANA PLANT: ENZYMES INVOLVED IN STARCH SYNTHESIS AND BREAKDOWN

Tissue	ADPG–pyrophosphorylase	Starch phosphorylase (units/mg protein)	β-Amylase
Leaves	0·38*	0·74†	0·02*
	(4·50)	(8·64)	(0·24)
Leaf-sheath	8·54*	1·97†	1·93*
	(9·99)	(2·31)	(2·25)
Rootstock	18·77*	2·82†	Nil
	(22·91)	(3·46)	
Lower pseudostem	12·27*	1·29‡	1·02†
	(16·45)	(1·73)	(1·37)
Upper pseudostem	14·52*	1·18‡	1·03*
	(14·52)	(1·18)	(1·03)
Fruit pulp	10·55*	3·55†	0·16†
	(25·64)	(8·65)	(0·38)

* Basal medium. 0·05 M Tris–HCl buffer, pH 7·2, 0·02 M freshly neutralized cysteine hydrochloride and 0·01 M EDTA.

† Basal medium + 1% (w/v) PVP.

‡ Basal medium + 1% (v/v) Triton X100.

Figures in parenthesis represent enzyme activity in units per g fr. wt. Other details were as in Table 2 and text.

Enzymes involved in Hexose Metabolism

Hexokinase (E.C. 2.7.1.1., ATP: D-hexose 6-phosphotransferase) activity was maximum in rootstock and minimum in leaves (Table 4). The activity in rootstock was about 20-fold higher than in leaves. Glucosephosphate isomerase (E.C. 5.3.1.9, D-glucose 6-phosphate ketol-isomerase) was most active in rootstock followed by pseudostem, fruit pulp and leaf-sheath. The lowest activity, which was observed in leaves, was about 10% of that in the roostock.

Acid phosphatase (E.C. 3.1.3.2, orthophoshpric monoester phosphohydrolase) and alkaline phosphatase (E.C. 3.1.3.1, orthophosphoric monoester phosphohydrolase) (assayed against β-glycerophosphate) were most active in fruit pulp and pseudostem. Rootstock and leaves contained low acid phosphatase activity. Alkaline phosphatase activity could not be demonstrated in the rootstock and leaf-sheath.

DISCUSSION

Sucrose translocated to non-photosynthetic tissues from banana leaves may be metabolized by two pathways. By the first, it may be hydrolyzed to glucose and fructose by invertase and thereby the available carbon released in the form of utilizable hexoses. However, this process results in the loss of the high free energy of hydrolysis of the glucosidic linkage of sucrose. Banana rootstock contained no acid invertase and low neutral invertase activity.

TABLE 4. DISTRIBUTION OF ENZYME ACTIVITIES IN DIFFERENT PARTS OF THE BANANA PLANT: ENZYMES INVOLVED IN HEXOSE METABOLISM

Tissue	Hexokinase	Glucosephosphate isomerase	Phosphatase Acid	Phosphatase Alkaline
		(units/mg protein)		
Leaves	0·52‡	2·23†	0·55‡	0·16†
	(6·1)	(26·1)	(6·54)	(1·84)
Leaf-sheath	1·28†	10·9†	2·37†	Nil
	(1·5)	(12·8)	(2·77)	
Rootstock	11·56‡	22·8‡	0·70†	Nil
	(14·1)	(27·8)	(0·90)	
Lower pseudostem	1·86‡	14·3†	2·03‡	0·62*
	(2·5)	(19·2)	(2·72)	(0·93)
Upper pseudostem	2·50‡	18·2*	2·72‡	1·78†
	(2·5)	(18·2)	(2·72)	(1·78)
Fruit pulp	1·5‡	13·2‡	3·41‡	1·14†
	(3·8)	(32·1)	(8·30)	(2·76)

* Basal medium. 0·05 M Tris–HCl buffer, pH 7·2, 0·02 M freshly neutralized cysteine hydrochloride and 0·01 M EDTA.
† Basal medium + 1% (w/v) PVP.
‡ Basal medium + 1% (v/v) Triton X100.
Figures in parentheses represent enzyme activity in units per g fr. wt. Other details were as in Table 2 and text.

Its content of sucrose and of glucose and fructose was comparatively low. The fruit pulp had the highest acid invertase activity and in addition had significant neutral invertase activity. Since there was no accumulation of hexoses in this tissue, the hexoses are presumably rapidly utilized after priming by kinase action. The pseudostem with the highest neutral invertase activity was rich in sucrose and in hexoses. The leaf-sheath was distinguished by the high activity of both forms of invertase, so that in this tissue sucrose may be readily hydrolyzed. In conformity with this hypothesis, hexoses occurred in high concentration in the sheath, although the tissue had a high sucrose content. The high invertase activity in the pseudostem supported the concept of its association with rapidly growing tissues.[9–11] By the second of the two pathways, sucrose may be converted by sucrose synthetase to nucleoside diphosphate glucose and fructose, thus conserving the energy of the sucrose bond in the form of nucleoside diphosphate glucose. Since sucrose synthetase catalyses

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sucrose cleavage much more rapidly than sucrose synthesis at physiological pH levels,[12,13] sucrose synthetase would convert sucrose to nucleoside diphosphate glucose, which is ultimately used in starch formation. Sucrose synthetase was maximally active in rootstock and fruit pulp and had significant activity in the pseudostem and in the leaf-sheath. In agreement with this, the greatest amount of starch was present in the rootstock followed by leaf-sheath and fruit pulp. If ADPG and not UDPG functions as the glucosyl donor for starch synthesis in the banana plant, the high level of ADPG-pyrophosphorylase (assayed in the direction of synthesis) found in the present experiment was to be anticipated. The major function of starch phosphorylase *in vivo* is believed to be in the degradation of starch. The starch accumulated in the leaf-sheath and pseudostem may be mobilized by β-amylase. However the complete absence of this enzyme in the rootstock suggests that in this tissue, which functions as the main store of starch, the mobilization of this polysaccharide is mediated through the action of starch phosphorylase. Supporting this view, the rootstock had a high phosphorylase activity. However, it is not clear why the maximum phosphorylase activity was found in the fruit pulp, in which starch accumulation was expected to take place progressively. Disregarding the occasional reports of the unprimed starch synthesis, though confirmed recently by the Hawker *et al.*,[14] it may be assumed that the primer needed for initiating the polysaccharide synthesis is furnished by the synthetic action of starch phosphorylase. This would account for the presence of significant activity in all the starch accumulating tissues, the leaf-sheath, pseudostem and the fruit pulp.

The high ratio of sucrose phosphate synthetase to sucrose synthetase in banana leaves and the low ratio in rootstock and fruit pulp suggest that sucrose phosphate synthetase is involved in the photosynthetic formation of sucrose, and sucrose synthetase in its utilization. Delmer and Albersheim[15] also observed high sucrose phosphate synthetase and low sucrose synthetase activity in photosynthetic tissue. Hawker[16] found that in developing cotyledons of broad bean and developing endosperm of maize, in which conversion of sucrose to reducing sugars or sugar nucleotides was the predominant reaction involving sucrose, there was high activity of sucrose synthetase and relatively low activity of sucrose phosphate synthetase.

EXPERIMENTAL

Plant tissues. Banana plants (*Musa paradisiaca*) were cut at ground level when the inflorescence emerged and the first four hands of the primary fruit bunch were exposed. The skin of the fruit was discarded, the flesh sliced longitudinally and the central core consisting of small seeds discarded. The pseudostem obtained after removal of the leaf-sheath measured 3·5 m in length. The upper 1 m was designated the 'upper pseudostem' and the lower 1 m 'the lower pseudostem'. The leaf-sheath used for analysis was the one immediately surrounding the entire length of the pseudostem. The 3 leaves immediately preceding the inflorescence (and including the atypical leaf preceding the flower) were used after discarding the midribs. The rootstock was dug out of the ground, washed free of soil and the outer layer (2 cm thick) was discarded.

Carbohydrate analysis. The various parts of the banana plant (20 g samples) were homogenized separately with 100 ml of 90% EtOH, immediately boiled and refluxed for 6 hr. The suspension was filtered and the residue extracted with 100 ml of 80% EtOH. The extracts were combined and concentrated under vacuum at 40°. The samples were analysed for total reducing sugars by the method of Nelson[17] as modified by Somogyi.[18] Aliquots were chromatographed in duplicate on Whatman No. 1 paper (descending) using *n*-

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BuOH–HOAc–H_2O (4:1:5) as solvent.[19] Sugars were detected by aniline–diphenylamine reagent[20] and identified by comparing with standard sugars. The area of sugars in the sample which was not sprayed were marked, and the sugars extracted with H_2O at 75–80°.[21] Fructose and sucrose were determined by the resorcinol method[22] and glucose by the method of Nelson[17] as modified by Somogyi.[18] The residue after EtOH extraction was extracted with 40% followed by 20% and 10% perchloric acid and the extracts combined. Starch was precipitated with I_2–KI–EtOH according to Pucher *et al.*[23] and determined by the phenolsulfuric acid method of Montgomery.[24] Soluble starch (B.D.H., AR) was used as standard.

Preparation of homogenates and composition of dispersion media were as reported earlier.[8] The basal grinding medium consisted of 0·05 M Tris–HCl buffer, pH 7·2, 0·02 M freshly neutralized cysteine hydrochloride and 0·01 M EDTA. The other media were prepared by supplementation of the basal media either with 1% PVP (w/v), MW 40000, or 1% Triton X100 (w/v). About 90–95% of total protein of the various tissues was extracted in the homogenates.

Enzyme assays. The assays of sucrose synthetase, sucrose phosphate synthetase, acid and neutral invertases, starch phosphorylases, β-amylase, acid and alkaline phosphatase and glucosephosphate isomerase are described earlier.[8] Hexokinase was assayed according to Sols,[25] with slight modification. To centrifuge tubes containing 0·05 ml potassium phosphate buffer (0·2 M, pH 8·0), 0·1 ml Tris-HCl buffer (0·2 M, pH 8·0), 0·05 ml $MgSO_4$ (0·1 M), 0·1 ml ATP (0·02 M), 0·05 ml NaF (0·1 M) and 0·05 ml fructose (0·02 M) was added 0·1 ml of the homogenate and the mixture incubated for 30 min at 30°. The reaction was stopped by Somogyi's Ba–Zn–deproteinization procedure.[18] The control tubes received fructose after deproteinization of the reaction. After centrifugation at 750 *g* for 15 min, the residual free sugar was measured in aliquots according to the procedure of Roe and Papadopoulos.[22] A unit of enzyme was defined as the amount causing the disappearance of 1 μmol of fructose under the assay conditions. The assay system for ADPG–pyrophosphorylase, based on that of Feinfold *et al.*[26] with slight modification, consisted of 0·15 ml Tris–HCl buffer (1·5 M, pH 7·9), 0·03 ml $MgSO_4$ (0·05 M), 0·02 ml bovine serum albumin (10 mg/ml), 0·05 ml ATP (0·02 M), 0·05 ml NaF (0·5 M), 0·05 ml glucose-1-phosphate (0·02 M), enzyme and H_2O to a total-vol. of 0·5 ml incubated for 30 min at 37°. The reaction was started by the addition of enzyme and terminated by the addition of 0·5 ml of chilled trichloroacetic acid (TCA) (10%). The control tubes received the enzyme after TCA addition. To adsorb nucleotides, 0·5 ml of Norit A suspension (10%) was added to each tube and after storing for 15 min at 0–5° centrifuged at 600 *g* for 15 min. Orthophosphate was determined in aliquots of the supernatant after hydrolysis with 1 N H_2SO_4 for 10 min at the temp. of boiling H_2O by the method of Fiske and Subbarow.[27] The enzyme activity was calculated from the increase of acid-hydrolysable phosphate (difference between phosphate of pyrophosphate and glucose-1-phosphate). One unit of pyrophosphorylase activity was defined as the amount causing the formation of 1 μmol of non-nucleotide acid labile phosphate under conditions of assay.

Protein was estimated in TCA precipitates according to Lowry *et al.*[28] using the Folin–Ciocalteu reagent as modified by Khanna *et al.*[29] Interference by starch was eliminated by washing with perchloric acid (15, 10 and 5% respectively).

Estimation of orthophosphate in homogenates of various parts of the banana plant prepared in basal medium was according to the method of Fiske and Subbarow[27] after deproteinization with TCA.

Dry weight determination. 10 g of each tissue was dried first at 90° for 2 hr, followed by 65° till a constant weight was obtained.

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