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Changes in glucose, fructose and saccharose metabolism in tobacco plants infected with potato virus Y

M. ŠINDELÁŘOVÁ, L. ŠINDELÁŘ and L. BURKETOVÁ

Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Na Karlovce 1a, CZ-160 00 Prague 6, Czech Republic

Abstract

The content of glucose, fructose and saccharose as well as changes in the activities of enzymes involved in their biosynthesis and degradation were studied in tobacco plants infected with potato virus Y (necrotic strain) during the acute-infection period. Over the first part of this period, accumulation of saccharose, glucose and fructose was observed concurrently with decreased activities of the enzymes metabolizing saccharose, glucose and fructose (saccharases, saccharose synthase and hexokinases) and enhancement in the activities of enzymes synthesizing these carbohydrates (saccharosephosphate synthase, glucose-6-phosphate and/or fructose-6-phosphate phosphatases). The subsequent period was characterised by a reduction in both phosphatases that (together with just slightly raised saccharosephosphate synthase) could hardly produce enough sugars for the highly stimulated enzymes such as saccharases, saccharose synthase, and both kinases. Presumably for this reason, the previously increased content of sugars was considerably reduced to the level of control plants. The activities of glucokinase, fructokinase, saccharases and saccharose synthase were strongly increased at the culmination of virus multiplication and negatively correlated with the content of free glucose, fructose and saccharose.

Additional key words: hexokinases, *Nicotiana tabacum* L., phosphatases, saccharases, saccharosephosphate synthase, saccharose synthase.

Introduction

The metabolism of saccharose, glucose and fructose is a frequently studied topic. Saccharose plays an especially important role in plant metabolism as a major end

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Abbreviations: F6P - fructose-6-phosphate; G6P - glucose-6-phosphate; PVY - potato virus Y; SS - saccharose synthase; SPS - saccharosephosphate synthase.

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Fax: (+420) 2 24310113; e-mail: sindelarova@ueb.cas.cz

product of photosynthesis, a main form of translocated carbon and the chief storage saccharide besides starch. The role of sugars in the expression and the repression of specific genes has been examined, *e.g.*, by Jang and Sheen (1994), Taylor *et al.* (1995) and Winters *et al.* (1995). Developmental changes in the concentration of sugars, in their metabolites and in enzyme activities were observed by Hubbard and Pharr (1992), Merlo *et al.* (1993) and Alaoui-Sosé *et al.* (1996). The purification and properties of saccharose synthase isoenzymes was reported by Ross and Davies (1992), Buczynski *et al.* (1993) and Sowokinos *et al.* (1993) while their regulation was studied by Cheikh and Brenner (1992), Cheikh *et al.* (1992) and Del Mar Sola *et al.* (1994).

Saccharose is broken down to glucose and fructose either hydrolytically by saccharase (invertase) or non-hydrolytically by saccharose synthase (SS). The later mode of catalysis leads to the formation of fructose and uridinediphosphate (UDP) glucose. The synthesis of saccharose is primarily catalysed by the joint action of saccharosephosphate synthase (SPS) and saccharosephosphate phosphatase (Emes and Neuhaus 1997, Schaffer and Petreikov 1997). Though the primary role of SS has been postulated to be the breakdown of incoming saccharose in starch-storing sink organs (Bhullar *et al.* 1985, Delmer and Albersheim 1970), a synthetic role for SS has also been postulated in the tubers of *Helianthus tuberosus* (Keller *et al.* 1988). According to Clausen *et al.* (1985), SS activity in leaves is closely regulated by saccharose export which, in turn, depends on the strength of the sink region. The physiological function of SS in the leaves is still not clearly understood.

Turnover of sugars in plant tissues infected with virus is not quite clear. Free sugars are accumulated in virus-infected tissues during the acute period of infection (Kapur *et al.* 1974, Šindelář and Makovcová 1974, *etc.*), the content of which, however, decreases rapidly at the beginning of the chronic period of infection as a consequence of enhanced respiration rate, reduced rate of photosynthesis (Makovcová *et al.* 1980, Técsi *et al.* 1994, Šindelářová *et al.* 1997), decreased phosphatase activity (Wolffgang and Keck 1959, Šindelář and Makovcová 1974) and a sharp increase in saccharase and hexokinase activities (Makovcová *et al.* 1980, Makovcová and Šindelář 1981). No changes or slightly enhanced activity of enzymes of the glycolytic pathway in virus-infected tissues were reported by Solymosy and Farkas (1963), Huth (1973), and Makovcová and Šindelář (1981). In contrast, substantial changes in the reaction rate of this pathway, related presumably to changes in energy demands, were observed at a chronic period of infection in cucumber infected with cucumber mosaic virus (Makovcová *et al.* 1980).

Changes in utilization of free and transportable sugars during infection are still not clearly understood. We therefore studied saccharose, glucose, and fructose contents in tobacco during the acute period of PVY-infection together with activities of saccharase, glucokinase, fructokinase, glucose-6-phosphate phosphatase, fructose-6-phosphate phosphatase, saccharose synthase, saccharosephosphate synthase and UDP-glucose pyrophosphorylase.

Materials and methods

Plants: Two month old tobacco (*Nicotiana tabacum* L., cv. Samsun) plants were grown in pots containing soil, at a 16-h photoperiod, irradiance of 100 μmol m^{-2} s^{-1} and an average temperature of 25°C. The lower leaf, approximately 5 cm length, was mechanically inoculated with purified potato virus Y^N (necrotic strain of PVY; Leiser and Richter 1978) at a concentration of 100 μg cm^{-3}; the corresponding leaves of control plants were mock-inoculated with distilled water. The day of inoculation was designated as day zero (0 day post inoculation - 0 d.p.i.).

Preparation of homogenate: The inoculated leaf, and three leaves above it were used for analysis. They were quickly frozen in liquid nitrogen and stored at -70 °C. With respect to the possibility of diurnal fluctuations in enzyme activity and sugar content (Cheikh and Brenner 1992), the leaf samples were collected at the same time at the end of the dark period.

Homogenates were prepared from samples of leaf tissues by grinding them in a mortar with fine silica sand, 10 % (m/m) insoluble polyvinylpyrrolidone and TEMM buffer (20 mM Tris-HCl buffer, 1 mM EDTA, 2.5 mM $MgCl_2$, 30 mM 2-mercapto-ethanol, pH 7.0) in a ratio of 1:5 (m/v). The resulting homogenate was squeezed through Miracloth and nylon sieve 100 mesh and centrifuged for 10 min at 20 000 *g*. Preparation and storage of the crude homogenates were carried out at 0 to 4 °C (activity of the enzymes did not change for more than 5 h).

Determination of enzyme activities: Hexokinase (EC 2.7.1.1) and fructokinase (EC 2.7.1.4) activities were estimated according to glucose or fructose phosphorylation determined spectrophotometrically (*Beckman DU 6, Beckman Instruments*, Irvine, USA) at 340 nm on the basis of $NADP^+$ reduction in the presence of an excess of glucose-6-phosphate dehydrogenase. The assay mixture (1 cm^3) contained 0.1 M Tris-HCl buffer pH 8.0, 5 μmol glucose, 2.5 μmol $MgCl_2$, 60 μmol KCl, 0.5 μmol $NADP^+$, 2.5 μmol ATP, 1 U glucose-6-phosphate dehydrogenase, and 0.05 to 0.1 cm^3 of homogenate. Fructose phosphorylation was determined similarly; the assay mixture contained 50 μmol of fructose instead of glucose and additionally 1.5 U phosphogluco-isomerase (Turner *et al.* 1977).

For determination of hexose-6-phosphate phosphohydrolase (EC 3.1.3.9) (glucose-6-phosphate phosphatase and fructose-6-phosphate phosphatase) activities, the assay mixture (0.3 cm^3) contained 0.1 cm^3 80 mM glucose-6-phosphate (fructose-6-phosphate), 0.1 cm^3 100 mM citrate buffer, pH 6.5, and 0.1 cm^3 homogenate (Harper 1963).

The activity of saccharase (invertase) (EC 3.2.1.26) was measured in the assay mixture (1 cm^3) containing TEMM buffer (pH 6.4, resp. 8.0), 0.1 cm^3 homogenate and 100 μmol saccharose (Fahrendorf and Beck 1990), the activity of saccharose synthase (EC 2.4.1.13) in the assay mixture (0.16 cm^3) containing 0.01 cm^3 50 mM uridine diphosphoglucose, 0.04 cm^3 100 mM fructose, 0.01 cm^3 100 mM Tris-HCl buffer, pH 7.2, and 0.1 cm^3 homogenate (Cardini *et al.* 1955), and the activity of saccharosephosphate synthase (EC 2.4.1.14) in the assay mixture (0.16 cm^3)

containing 0.01 cm^3 50 mM uridine diphosphoglucose, 0.04 cm^3 100 mM fructose-6-phosphate, 0.01 cm^3 100 mM Tris-HCl buffer, pH 7.2, and 0.1 cm^3 homogenate (Cardini *et al.* 1955).

For determination of UDPG-pyrophosphorylase (EC 2.7.7.9) activity the assay mixture (1 cm^3) contained TEMM bufer (pH 7.5), 1 μmol uridine diphosphoglucose, 1 U glucose-6-phosphate dehydrogenase, 2 μmol glucose-1,6-bisphosphate, 0.1 cm^3 homogenate, 20 μmol $K_4P_2O_7$ and 0.5 μmol $NADP^+$ (Villar-Palasi and Larner 1960).

Enzyme activities were determined at 25 °C, and at their respective pH optima. The enzymatic reactions were initiated by addition of enzyme substrate, and rates were linear for more than 1 h.

Determination of proteins, glucose, fructose and saccharose content: Protein content was determined according to Bradford (1976) using bovine serum albumin as a standard. Glucose and fructose was determined according to Klotzsch and Bergmeyer (1963) with hexokinase, phosphoglucoisomerase and glucose-6-phosphate dehydrogenase after their extraction with 80 % hot ethyl alcohol. Saccharose was determined with saccharase, hexokinase, phosphoglucoisomerase and glucose-6-phosphate dehydrogenase according to Bergmeyer and Klotzsch (1963).

Determination of PVY content: The PVY content was determined by the quantitative DAS-ELISA method (Clark and Adams 1977) with rabbit anti-PVY antibodies and alkaline phosphatase labelled antibodies prepared from our isolates of PVY (necrotic strain).

Statistical treatment and chemicals: The results presented in tables are arithmetical means ± SE of 3 - 7 determinations in four independent experiments. The *t*-test was employed to characterise significance of differences. Chemicals were purchased from *Sigma Chemical Company* (St. Louis, USA).

Results and discussion

We found a close connection between the content of saccharose and the enzymes involved in its turnover (Fig. 1*C,D*, Table 2). At the first period of PVY multiplication (Fig. 1*A*) (from 1 to 4 d.p.i.) and also at 8 and 9 d.p.i., the saccharose content was greatly increased as well as the activity of SPS, in contrast to decreased activities of SS, UDPG-pyrophosphorylase and both saccharases (vacuolar "acid" saccharase of pH optimum 6.4 and cytosolic "alkaline" saccharase of pH optimum 8.0, Schnarrenberger 1990). Conversely, at the culmination of PVY (from 5 to 7 d.p.i.), the content of saccharose was considerably decreased, evidently as a consequence of increased activities of the enzymes under study. Even the enhanced content of SPS observed was insufficient to counterbalance these substantial changes in saccharose content.

Similar relationships were found between the changes in glucose and/or fructose content and the activities of both phosphatases and kinases (Fig. 1*A,B*, Table 1) in

acute period of virus infection. During the first part (from 1 to 4 d.p.i.), accumulation of both the sugars in tissue was observed concurrently with increased activity of phosphatases and decreased activity of hexokinases. During the second part (from 5 to 9 d.p.i.), the only slightly enhanced activities of phosphatases were evidently inefficient in production of carbohydrates for highly stimulated kinases so that the primarily increased contents of carbohydrates were reduced even below the values of healthy control plants. During intensive biosynthesis of virus, the enhanced activities of both hexokinases and phosphoglucoisomerase maintain a higher supply of intermediates for the oxidative pentose phosphate pathway involved in biosynthesis of PVY (Šindelář 1986, Šindelář and Šindelářová 1987a,b, Šindelářová *et al.* 1997).

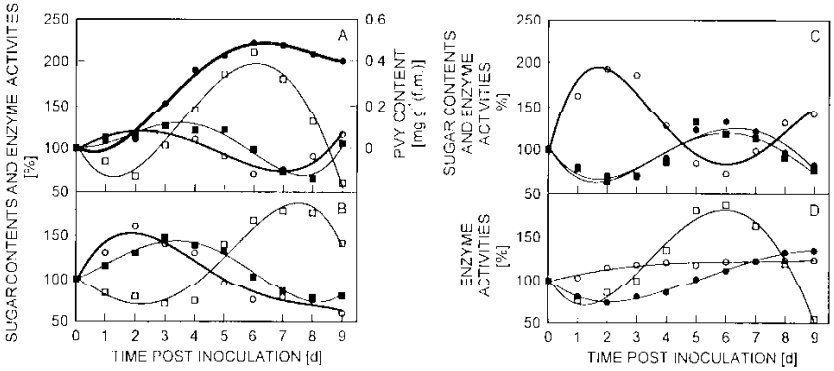

Fig. 1. *A* - PVY multiplication curve (*closed circles*), glucose content (*open circles*), activity of glucokinase (*open squares*), and glucose-6-phosphate phosphatase (*closed squares*); *B* - fructose content (*open circles*), activity of fructokinase (*open squares*) and fructose 6 phosphate phosphatase (*closed squares*); C - saccharose content (*open circles*), activity of saccharase with pH optimum 6.4 (*closed circles*) and 8.0 (*closed squares*); D - saccharosephosphate synthase (*open circles*), UDP-glucose phosphorylase (*closed circles*) and saccharose synthase (*open squares*). All contents and activities are expressed in % of healthy controls.

Negative linear correlations were observed between the contents of the sugars under study and the activities of the enzymes involved in their metabolic utilization. When the sugar contents and the enzyme activities were expressed as a percentage of healthy control, the following correlation coefficients were found:

r = -0.723*** (n = 36) for content of glucose and activity of glucokinase,
r = -0.933*** (n = 29) for content of fructose and activity of fructokinase,
r = 0.892*** (n = 32) for content of saccharose and activity of "acid" saccharase, or
r = -0.901*** (n = 32) for saccharose and "alkaline" saccharase, and
r = -0.876*** (n = 32) for content of saccharose and activity of saccharose synthase.

The protein content in homogenates of virus-infected tissues did not differ significantly from that of healthy ones and therefore the data are not presented.

Table 1. Glucose and fructose content [μg g^{-1}(f.m.)] and activity of glucokinase, fructokinase, glucose-6-phosphate phosphatase and fructose-6-phosphate phosphatase [nmol g^{-1}(f.m.) min^{-1}] in homogenate from leaf tissues of healthy [H] and PVY infected [Y] tobacco.

p.i. [d]		Glucose	Glucokinase	G6P phosphatase	Fructose	Fructokinase	F6P phosphatase
0	H	10.22±1.15	20.90±1.42	14.47±1.56	29.06±2.42	19.29±1.34	12.36±1.33
	Y	-	-	-	-	-	-
1	H	11.12±1.02	14.47±1.03	12.26±1.13	32.11±2.86	16.08±1.32	10.22±0.96
	Y	12.23±1.09	12.54±1.03*	13.80±1.09	39.16±3.88*	13.34±0.89*	12.08±1.11
2	H	12.04±1.33	8.04±0.76	6.22±0.74	34.89±3.72	12.86±1.33	6.04±0.63
	Y	13.89±1.12*	6.43±0.54**	7.37±0.65*	55.16±4.83***	9.97±0.87**	7.75±0.69**
3	H	26.16±2.64	8.04±0.76	5.63±0.47	71.74±5.89	11.25±0.98	6.86±0.74
	Y	32.89±3.69**	8.52±0.75	6.96±0.51**	101.58±7.97***	7.88±0.64**	9.54±0.89**
4	H	36.23±4.23	9.65±0.84	6.15±0.54	110.93±9.59	9.66±0.73	5.01±0.59
	Y	40.90±3.86	13.83±1.22**	7.32±0.62*	144.11±9.03**	7.07±0.56**	6.44±0.52**
5	H	40.02±3.62	12.86±1.23	6.31±0.66	154.15±8.03	12.86±1.31	6.11±0.47
	Y	36.85±2.73	23.79±2.12***	7.60±0.71*	148.02±8.11	16.40±1.34*	7.60±0.66**
6	H	43.22±4.03	17.68±1.32	9.14±0.86	179.78±9.98	16.08±1.48	8.34±0.92
	Y	31.42±2.87**	44.69±3.28***	8.91±0.71	139.18±8.86**	30.23±2.88***	8.52±0.80
7	H	34.86±2.46	16.08±1.54	8.92±0.97	140.13±7.96	14.47±1.23	7.16±0.81
	Y	26.17±2.54**	28.78±2.32***	6.63±0.54**	110.13±6.02**	25.72±2.33***	6.26±0.72*
8	H	23.34±2.04	12.86±1.07	6.11±0.54	103.81±6.98	11.25±0.89	5.72±0.47
	Y	21.43±1.87*	16.72±1.45***	4.19±0.37**	79.64±6.55**	20.10±1.79***	4.56±0.39*
9	H	18.66±1.28	17.69±1.47	8.92±0.94	98.04±6.65	17.70±1.44	8.68±0.65
	Y	21.68±1.31	11.09±0.89***	9.63±0.88	57.56±5.63***	24.92±2.06***	7.22±0.60*

The difference is statistically significant: * at $0.01 \leq P < 0.05$; ** - at $P < 0.01$; and *** - at $P \leq 0.001$.

We conclude from the above results that in the first part of the period monitored the decreased activities of enzymes metabolising saccharose, glucose and fructose (saccharases, saccharose synthase and hexokinases) and the enhanced activities of enzymes synthesising carbohydrates (saccharosephosphate synthase, glucose-6-phosphate and fructose-6-phosphate phosphatases) support the accumulation of saccharose, glucose and fructose. Afterwards, only slightly increased activities of saccharosephosphate synthase and both phosphatases are evidently inefficient in production of carbohydrates for the highly stimulated saccharases, saccharose synthase and both kinases so that the previously increased content of carbohydrates is considerably reduced to the rate of control plants. In addition, the glucose, fructose and saccharose contents and the activity of saccharose synthase, both saccharases and kinases are negatively linearly correlated. Therefore we suppose that the content of carbohydrates is preferentially determined by the rate of their metabolic utilization in PVY infected tobacco leaves.

Table 2. Saccharose content [μg g^{-1}(f.m.)] and activities of saccharase, saccharose synthase saccharosephosphate synthase and UDP-glucose pyrophosphorylase [nmol g^{-1}(f.m.) min^{-1}] in homogenate from leaf tissues of healthy [H] and PVY infected [Y] tobacco.

d.p.i.		Saccharose	Saccharase (pH 6.4)	(pH 8.0)	Saccharose synthase	Saccharosephos-phate synthase	UDPG-pyro-phosphorylase
0	H	35.11±3.14	21.70±1.93	22.07±2.09	8.44±0.75	4.22±0.36	12.36±1.33
	Y	-	-	-	-	-	-
1	H	30.04±2.82	17.68±1.45	16.08±1.22	8.10±0.59	4.01±0.28	10.17±1.05
	Y	41.28±4.09**	14.15±1.07*	13.02±1.22*	7.29±0.46	4.25±0.31	9.25±0.86
2	H	26.13±2.81	12.06±1.21	12.85±1.05	7.28±0.44	3.87±0.22	7.56±0.92
	Y	58.88±6.11***	8.36±0.67**	8.04±0.73**	6.33±0.37	4.57±0.29	5.49±0.46**
3	H	37.68±2.90	11.58±1.03	11.25±0.89	12.37±1.22	6.16±0.64	6.02±0.76
	Y	66.76±4.97***	8.04±0.67**	8.36±0.75**	12.49±1.16	7.39±0.69*	4.82±0.34**
4	H	47.15±4.32	9.81±0.78	9.65±0.69	13.77±1.03	7.56±0.71	6.12±0.61
	Y	73.87±6.56***	8.84±0.75	8.36±0.68*	19.00±1.26*	9.37±0.86**	5.59±0.41*
5	H	64.04±5.32	11.09±1.00	11.25±1.04	15.26±1.14	7.02±0.62	7.56±0.68
	Y	61.40±5.43	13.83±1.24*	12.86±1.22	28.38±2.36**	8.85±0.73**	7.79±0.63
6	H	77.02±6.85	14.79±1.37	14.47±1.32	16.35±1.56	5.73±0.47	9.14±0.81
	Y	42.17±4.11***	20.42±1.87**	22.35±2.04**	31.88±2.86***	7.22±0.66**	10.64±0.89
7	H	83.08±6.12	16.40±1.29	16.08±1.43	16.14±1.22	4.86±0.46	8.00±0.65
	Y	79.09±5.26	19.77±1.42*	18.17±1.47	26.63±2.28***	6.17±0.54**	10.22±0.99*
8	H	85.24±7.33	15.27±1.31	14.47±4.32	17.21±1.61	4.37±0.39	7.98±0.54
	Y	113.22±8.56**	14.47±1.21	12.86±1.03	21.16±1.72*	5.59±0.36**	10.87±1.03**
9	H	90.61±7.68	16.72±1.37	17.68±1.48	17.11±1.33	3.66±0.28	12.41±0.92
	Y	129.29±8.78***	13.99±1.23**	13.34±1.21**	9.92±0.89	4.68±0.31**	17.08±1.48**

The difference is statistically significant: * at $0.01 \leq P < 0.05$; ** - at $P < 0.01$; and *** - at $P \leq 0.001$.

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