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Hexokinases of tobacco leaves: changes in the cytosolic and non-cytosolic isozyme complexes induced by tobacco mosaic virus infection

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Abstract

Changes in the cytosolic (soluble) and the non-cytosolic (particulate) isozyme composition of hexokinases and in their properties were studied by ion exchange chromatography on DEAE cellulose after the subcellular fractionation both in the healthy and the tobacco mosaic virus (TMV) infected tobacco leaves. Three main isozyme complexes were obtained: one particulate fraction (the particulate hexokinase phosphorylating both glucose and fructose, EC 2.7.1.1), and two soluble fractions (the soluble hexokinase phosphorylating both the glucose and the fructose, and the soluble fructokinase, which phosphorylates primarily fructose, EC 2.7.1.4). The total fructokinase activities were nearly twice higher than the total glucokinase activities (188.6 % of glucokinase activity in healthy plants and 181.3 % in infected plants). The total particulate glucokinase activity was increased to 120.6 % and the fructokinase to 118.9 % in TMV infected tissue when compared with healthy control. The similar pattern of activity was observed for soluble hexokinase isozymes - the sum of soluble glucokinase activity was increased to 175.4 % and of fructokinase activity to 131.2 % in TMV infected tissue. The isozymes isolated both from the healthy control and TMV-infected leaves had the similar elution profiles, displayed Michaelis-Menten kinetics, showed the identical profiles of pH optima and were Mg^{2+} dependent with the highest enzyme activity at equimolar Mg^{2+} and ATP concentration.

Additional key words: fructokinase, glucokinase, *Nicotiana tabacum*.

Introduction

A complex of hexokinases forms the first regulatory step determining the rate of glycolysis and the oxidative pentose phosphate pathway in the utilization of free, transport and storage sugars. Function of hexokinases in the cell compartments is not fully clear; some of the problems in interpreting earlier studies of hexokinases and fructokinases arise because little is known about metabolic fluxes and conditions in the tissue involved, and because little attention has been paid to the possibility that the enzyme forms may be expressed in an organ- or development-specific manner. Several studies have shown that plant tissue typically contain a spectrum of three to five different hexose-phosphorylating activities, which are distributed between soluble and particle fractions (Turner and Turner 1980, Kruger 1990).

The functional significance of their different forms is only partly understood. In most cases, some of the isozymes are more or less specific for fructose. These so-termed fructokinases are probably important during sucrose mobilization. The comprehensiveness, in addition, can be due to differing subcellular locations. Most studies have found several soluble isozymes. A hexokinase has also been described in the stroma of castor-bean leucoplasts (Miernik and Dennis 1983) and glucokinase and fructokinase have been attributed to spinach chloroplasts (Schnarrenberger 1990). Hexokinases of spinach leaves have been reported on the outer membrane of mitochondria (Baldus *et al.* 1981, Galina *et al.* 2000, Da Silva *et al.* 2001) and on the outer membrane of chloroplasts (Stitt *et al.* 1978, Wiese *et al.* 1999). However, enzymes bound on the outer side of organelles are also functionally located in the cytosolic metabolic compartment.

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Abbreviations: DEAE - diethylaminoethyl; FK - fructokinases; GK - glucokinases; HK - hexokinases; TMV - tobacco mosaic virus.
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Mitochondrial hexokinases are relatively loosely bound to the outer membrane. The properties and composition of the applied extraction and washing media are of great importance, because under unfavourable conditions they may cause extensive solubilization or activation of hexokinases even during the isolation of intact mitochondria (Šindelářová and Šindelář 1988).

As pointed out by Taylor (1997) and Umemura *et al.* (1998), knowledge of the subcellular distribution of hexokinases in plants may help to elucidate the components in the signal transduction pathways triggered by hexokinase phosphorylation of fructose and glucose (Sheen *et al.* 1999, Smeekens 2000, Xiao *et al.* 2000, Rolland *et al.* 2001).

The content and activities of particulate and soluble isozyme complexes are decreased with the age of leaf tissue. Total hexokinase activity of particulate and soluble isozyme complexes considerably decrease during ageing of tobacco plants and reach the minimum between 90 and 100 d after sowing, since then it does not change (Šindelářová and Šindelář 1997/98).

Hexokinase catalyses the ATP-dependent conversion of hexoses to hexose-6-phosphate and ADP. Several hexokinases with tissue specificity or developmentally regulated expression have been described in plants (Renz *et al.* 1993, Galina *et al.* 1995). Different genes encoding hexokinases (Dai *et al.* 1995, Jang *et al.* 1997) and a fructokinase (Smith *et al.* 1993) have been cloned. From the viewpoint of metabolic flux, the hexokinase reaction plays a key role in the removal of free hexoses from the cytosol, channelling the carbons to either glycolysis or oxidative pentosephosphate pathway depending on cellular demands (Gibson 2000).

Only in the past years has the existence of sugar-regulated gene expression in plants become apparent (Graham *et al.* 1994, Koch 1996, Jang *et al.* 1997, Dai *et al.* 1999). Although hexokinase is commonly known as a glycolytic enzyme, it has been implicated as a glucose sensor that mediates the repression of genes involved in photosynthesis (Jang and Sheen 1994), the glyoxylate cycle (Graham *et al.* 1994) and synthesis of α-amylase (Umemura *et al.* 1998).

In virus-infected plants free sugars are accumulated during the acute period of infection (*e.g.* Kapur *et al.* 1974, Šindelář and Makovcová 1974), the content of which, however, decreases at a high rate at the beginning of the chronic period of infection as a consequence of enhanced respiration rate and 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). Šindelář *et al.* (1999) observed the activities of glucokinase and fructokinase were strongly increased at the culmination of virus multiplication (in the acute phase of infection) and negatively correlated with the decreased contents of glucose and fructose in the tobacco plants infected with PVY.

In this paper, we present the changes in the content and characteristics of particulate and soluble isozyme complexes determined by ion exchange chromatography on DEAE cellulose. The hexokinase isozymes of the healthy and TMV infected tobacco leaves have been compared.

Materials and methods

Plants cultivation and virus inoculation: Tobacco plants (*Nicotiana tabacum* L. cv. Samsun) grown under constant conditions in soil, at an irradiance of 60 μmol m^{-2} s^{-1}, 16-h photoperiod, and average temperature of 25 °C, were used for hexokinases isolation. Two leaves of the bottom insertion (approximately 5 cm long) were mechanically inoculated with purified tobacco mosaic virus (TMV, common strain) (Gooding and Hebert 1967) at a concentration of 100 μg cm^{-3}. Corresponding leaves of control plants were mock-inoculated with distilled water. The samples of upper 2 non-inoculated leaves from 10 mock-inoculated or infected plants (leaves infected systemically) were collected on the 20th day post inoculation and used for hexokinases isolation. The day of inoculation was designated as day zero (0 day post inoculation = 0 dpi TMV).

Preparation of homogenate and ion-exchange chromatography: Tobacco leaves (12 g) from the mock-inoculated healthy control or TMV-infected plant were carefully homogenized at 0 to 4 °C in mortar with the 60 cm^3 IMT isolation medium (with the strict and consistent control of pH values during the isolation procedure) according to Šindelářová and Šindelář (1988). IMT contained 1 mM TES-KOH (pH 8.0), 0.3 M mannitol, 1 mM EDTA, 5 mM $MgCl_2$, plus (added just before extraction) 50 mM 2-mercaptoethanol, 1 mM benzamidine, 1 mM ε-aminocaproic acid, 0.5 mM phenylmethylsulphonyl fluoride and 2 % (m/m) insoluble polyvinylpyrrolidone (*Polyclar AT*). The homogenate was filtered through four layers of *Miracloth* and one layer of nylon net with 100 μm meshes and centrifuged (15 000 *g*, 15 min). The pellet ("particle fraction") was washed with

10 cm^3 IMT, centrifuged, resuspended in 5 cm^3 buffer A (50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 1 mM EDTA and 30 mM 2-mercaptoethanol) and quickly frozen at -80 °C. Combined supernatants were centrifuged at 105 000 *g* for 90 min and supernatant was precipitated by solid $(NH_4)_2SO_4$. The precipitate formed at 30 % to 80 % salt saturation ("soluble fraction") was collected by centrifugation, dissolved in 5 cm^3 buffer B (the same composition as buffer A, but with 20 mM Tris-HCl), desalted by centrifugation method through *Sephadex G-25 Fine* and applied to a column of *DE-52* cellulose with 35 cm^3 bed volume previously equilibrated with buffer B. After washing with one bed volume of buffer B, the isozymes were eluted with 60 cm^3 of a linear gradient of Tris-HCl between 20 and 100 mM, followed by 240 cm^3 of a linear gradient of KCl between 0 and 500 mM in 100 mM buffer C (the same composition as buffer A, but with 100 mM Tris-HCl). Fractions of 6 cm^3 were collected, desalted by centrifugation passage through *Sephadex G-25 Fine* and assayed for enzyme activity. All operations were performed at 0 - 4 °C.

The Triton X-100 was added to the "particle fraction" to the final concentration 1 % and the suspension was stirred for 1 h in an ice bath. The supernatant was centrifuged (20 000 *g*, 10 min), then applied to a column of *DE-52* cellulose as described above, with the difference that all buffers contained 1 % Triton X-100. Active fractions were combined, desalted by centrifugation passage through *Sephadex G-25 Fine* and assayed for enzyme activity. All operations were done at 0 - 4 °C.

Determination of enzyme activities: Enzyme activities were determined at 25 °C. Glucose phosphorylation was determined spectrophotometrically (*Helios* type, *Unicam*, Cambridge, UK) 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 100 μmol of 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 of glucose-6-phosphate dehydrogenase, and from 0.05 to 0.1 cm^3 of enzyme solution. Fructose phosphorylation was determined similarly; the assay mixture contained 50 μmol of fructose and in addition 1.5 U of glucosephosphate isomerase (Šindelář *et al.* 1999).

Determination of TMV content: TMV content were determined by the quantitative DAS-ELISA method (Clark and Adams 1977) using rabbit anti-viral antibodies and alkaline phosphatase labeled antibodies. The amount of virus was estimated from calibration curve of purified viruses with use of computer software described in Mančal (1987).

Statistical treatment and chemicals: The results presented in tables are arithmetical means ± SE of 3 - 7 determinations in 4 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

The high increase in the total activities of glucokinase and fructokinase at the culmination of virus multiplication (in the acute phase of infection) and negative correlation with the decreased contents of glucose and fructose were observed in the tobacco plants infected with PVY (Šindelář *et al.* 1999). To elucidate the involvement of specific hexokinase isozymes in the increase caused by viral infection, the subcelular fractionation of organelles and separation of their proteins on DEAE-cellulose were performed in the healthy and TMV-infected tobacco leaves on the 20th dpi. Three main isozyme complexes were obtained: one particulate complex (the particulate hexokinase phosphorylating both glucose and fructose, fractions 25 - 51), and two soluble complexes: the soluble isozyme complex group I (fractions 25 - 41, phosphorylating both glucose and fructose, EC 2.7.1.1), and the soluble isozyme complex group II (fractions 42 - 53, the soluble fructokinase, which phosphorylates primarily fructose). The profiles of hexokinase activities of the TMV-infected tobacco leaves differed from the healthy ones only in the elution areas not in the position of specific peaks (Fig. 1).

The total fructokinase activity both of the healthy and the infected leaves was nearly twice higher than the total glucokinase activity (Table 1). The total glucokinase activity of healthy control was 687.9 nmol g^{-1}(f.m.) min^{-1} and the total fructokinase activity was 1297.3 nmol g^{-1}(f.m.) min^{-1}, which represented 188.6 % of glucokinase activity. In infected leaves, the total glucokinase activity made up 891.7 nmol g^{-1}(f.m.) min^{-1} while the total fructokinase activity displayed 1617.0 nmol g^{-1}(f.m.) min^{-1}, thus representing 181.3 % of glucokinase activity. The soluble fructokinase complexes predominantly contribute to this difference, as the soluble fructokinase activity is more than five times higher than the total soluble glucokinase activity in healthy leaves and four times higher in the TMV-infected tobacco leaves.

The ratios of particulate and soluble isozyme complexes were different in the healthy and the TMV-infected leaf tissue (Table 1). In healthy leaf tissues, the total glucokinase activity made up 687.9 nmol g^{-1}(f.m.) min^{-1} with particulate isozyme

83.5 %. In TMV-infected leaves, the total glucokinase activity was considerably higher (891.7 nmol g^{-1}(f.m.) min^{-1}) but only 77.7 % of which represented the activity of particulate isozyme. The total fructokinase activity of healthy leaves was 1297.3 nmol g^{-1}(f.m.) min^{-1} and 53.0 % of which represented the activity of particulate isozyme. In TMV-infected leaves, only 50.6 % of total fructokinase activity (1617.0 nmol g^{-1}(f.m.) min^{-1}) represented particulate isozyme.

The TMV infection considerably increased hexokinase activities of all studied hexokinase complexes. In the healthy control, the main peak of particulate glucokinase activity amount to 525.7 nmol g^{-1}(f.m.) min^{-1}, in contrast to 637.7 nmol g^{-1}(f.m.) min^{-1} found in TMV infected tissue, which represents 121.3 % of the healthy control. Similarly, the activity of particulate fructokinase of infected leaves was increased to 817.7 nmol g^{-1}(f.m.) min^{-1} (118.9 %) when compared with 688.0 nmol g^{-1}(f.m.) min^{-1}

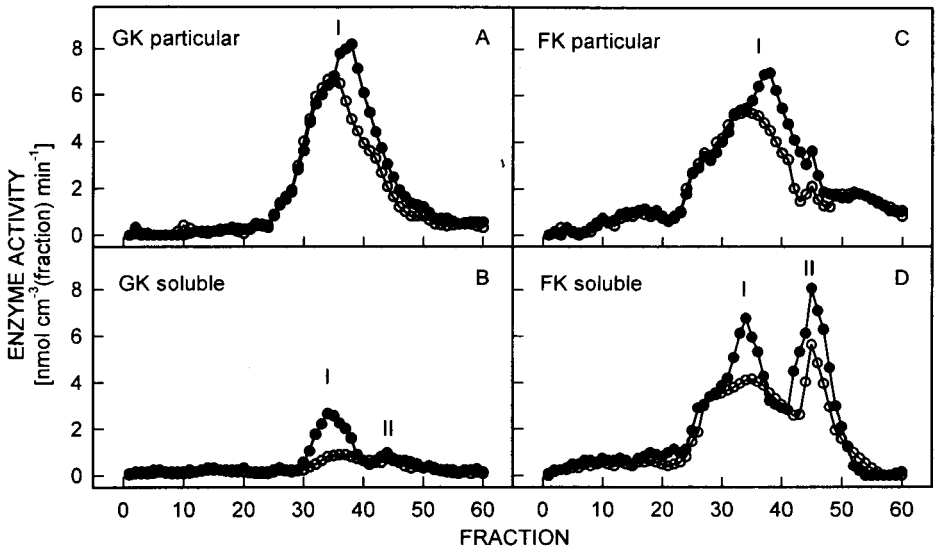

Fig. 1. The elution profiles of the particular and the soluble glucokinases (GK) and fructokinases (FK) obtained from healthy (*open circles*) and TMV-infected (*closed circles*) leaf tissues. The Roman numeral represents the soluble isozyme complexes group I and group II.

Table 1. Glucokinase and fructokinase activity [nmol g^{-1}(f.m.) min^{-1}] of the total, particular and soluble isozymes of the healthy and the TMV infected tobacco leaf tissues (ion exchange chromatography on DEAE cellulose; activity of enzyme/isozyme was determined by the integration of eluting profile areas). Comparison of the glucokinase (GK) and the fructokinase (FK) isozyme activity are expressed in % of total activity and in % of healthy control. Means ± SE, * - the difference is statistically significant at $0.01 \leq P < 0.05$ and ** - $P < 0.01$.

Enzyme/isozyme	Fraction	Healthy activity	[%]	TMV infected activity	[%]	[% of healthy]
GK total	1 - 60	687.9 ± 44.6	100.0	891.7 ± 53.4	100.0	129.6*
GK particular total	1 - 60	574.3 ± 31.6	83.5	692.5 ± 35.3	77.7	120.6*
GK soluble total	1 - 60	113.6 ± 8.7	16.5	199.2 ± 11.4	22.3	175.4**
GK particular	25 - 51	525.7 ± 30.5	76.4	637.7 ± 31.3	71.5	121.3*
GK soluble I	25 - 41	53.5 ± 6.2	7.8	119.9 ± 8.1	13.4	224.1**
GK soluble II	42 - 53	31.8 ± 2.1	4.6	40.5 ± 3.1	4.5	127.4*
FK total	1 - 60	1297.3 ± 68.4	100.0	1617.0 ± 70.9	100.0	124.6*
FK particular total	1 - 60	688.0 ± 29.3	53.0	817.7 ± 33.1	50.6	118.9*
FK soluble total	1 - 60	609.3 ± 34.9	47.0	799.3 ± 37.3	49.4	131.2**
FK particular	25 - 51	545.8 ± 26.2	42.1	644.8 ± 30.3	39.9	118.1*
FK soluble I	25 - 41	342.0 ± 15.5	26.4	409.6 ± 22.7	25.3	119.8*
FK soluble II	42 - 53	197.5 ± 11.4	15.2	292.8 ± 17.2	18.1	148.3**

of healthy control. The same pattern of activity was observed for soluble hexokinase isozymes. The total soluble glucokinase activities 113.6 nmol g^{-1}(f.m.) min^{-1} found in the healthy plants increased to 199.2 nmol g^{-1}(f.m.) min^{-1} in TMV infected tissue (175.4 % of healthy control). Especially, the soluble glucokinase I (fractions 25 - 41) was increased up to 224.1 % when compared with healthy control. The enhancement of total soluble fructokinase activity from 609.3 (healthy control) to 799.3 nmol g^{-1}(f.m.) min^{-1} (TMV infected leaves) representing 131.2 % of healthy control was observed as well. In contrast to the glucokinase activity the soluble fruktokinase II (fractions 42 - 53) was more increased (to 148.3 %) (Table 1).

All three followed hexokinase complexes were increased in TMV infected leaves but the difference in the total specific hexokinase activity was predominantly due to the increase of the soluble fruktokinases, followed by the particular fructokinases, the particular glucokinases and the least soluble glucokinases (FK_{sol} > FK_{part} > GK_{part} > GK_{sol}).

Hexokinases represent the first control point in the utilization of free and transport saccharides by *via* the glycolytic and the oxidative pentose phosphate pathways. Their increased activities decrease the content of glucose and fructose (Šindelář *et al.* 1999) and produce more glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P) which are extensively metabolised by the increased intensity of the oxidative pentose phosphate pathway and could serve for the biosynthesis of the nucleoside precursors needed for virus RNA synthesis. The glycolytic pathway is not obviously involved in this process with regard no changes in virus-infected tissues (Makovcová and Šindelář 1981). In addition, the degradation of free sugars through the oxidative pentose phosphate pathway is supported by decreased phosphatase activities (Wolffgang and Keck 1959, Šindelář and Makovcová 1974), which dephosphorylate the G6P and F6P to glucose and fructose.

All the studied isozyme complexes isolated both from the healthy control and the TMV-infected plants showed the similar profiles of pH optimum in Tris-HCl buffer solutions within the pH range of 7.0 to 9.2 (Fig. 2). All the complexes were Mg^{2+} dependent with the highest enzyme activity at equimolar Mg^{2+} and ATP concentration (data not shown). The isozyme complexes of the healthy control and the TMV-infected plants as well, had similar elution profiles and were released from the column at similar KCl concentrations.

Presumably, the TMV infection of tobacco leaves triggered the increase of some hexokinase isozymes but not the change of isozymes composition.

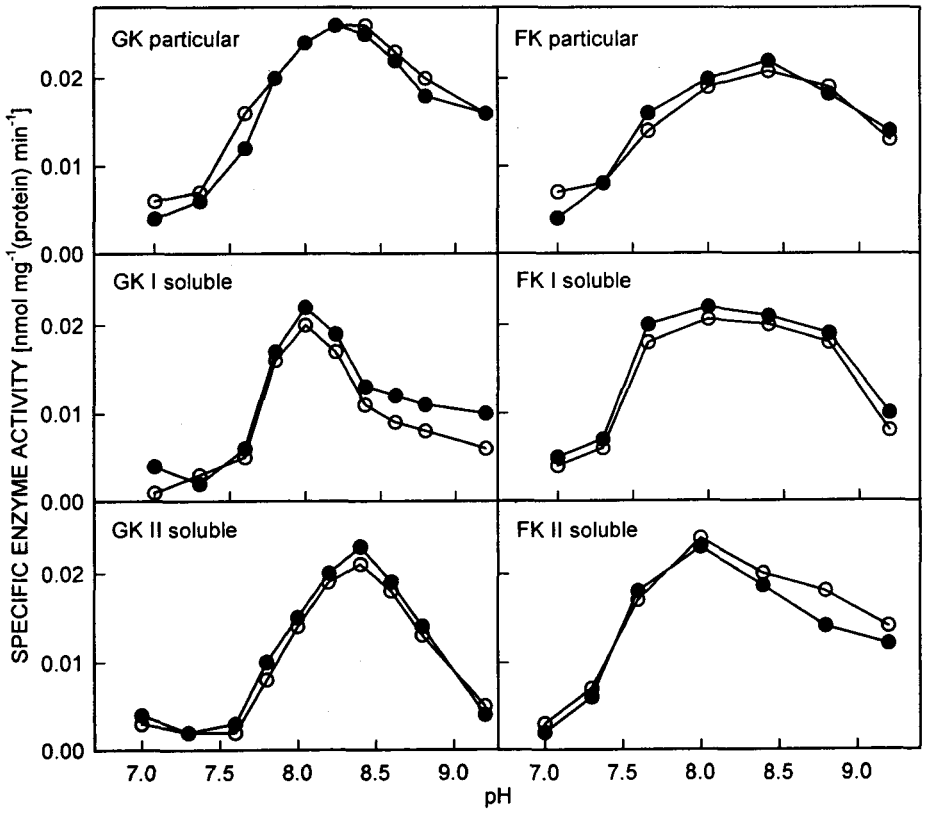

Fig. 2. The effect of pH values (Tris-HCl buffer) on the activities of the particular and the soluble glucokinases (GK) and fructokinases (FK) obtained from healthy (*open circles*) and TMV-infected (*closed circles*) leaf tissues.

References

Baldus, B., Kelly, G. J., Latzko, E.: Hexokinases of spinach leaves. - Phytochemistry **20**: 1811-1814, 1981.

Clark, M.F., Adams, A.N.: Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. - J. gen. Virol. **34**: 473-483, 1977.

Dai, N., Schaffer, A.A., Petreikov, M., Granot, D.: *Arabidopsis thaliana* hexokinase cDNA isolated by complementation of yeast cells. - Plant Physiol. **108**: 879-880, 1995.

Dai, N., Schaffer, A.A., Petreikov, M., Shahak, Y., Giller, Y., Ratner, K., Levine, A., Granot, D.: Overexpression of *Arabidopsis* hexokinase in tomato plants inhibits growth, reduces photosynthesis and induces rapid senescence. - Plant Cell **11**: 1253-1266, 1999.

Da Silva, W.S., Rezende, G.L., Galina, A.: Subcellular distribution and kinetic properties of cytosolic and non-cytosolic hexokinases in maize seedling roots: implications for hexose phosphorylation. - J. exp. Bot. **52**: 1191-1201, 2001.

Galina, A., Da Silva, W.S.: Hexokinase activity alters sugar-nucleotide formation in maize root homogenates. - Phytochemistry **53**: 29-37, 2000.

Galina, A., Reis, M., Albuquerque, M.C., Puyou, A.G., Puyou, M.T., de Meis, L.: Different properties of the mitochondrial and cytosolic hexokinases in maize roots. - Biochem. J. **309**: 105-112, 1995.

Gibson, S.I.: Plant sugar-response pathways. Part of a complex regulatory web. - Plant Physiol. **124**: 1532-1539, 2000.

Gooding, G.V., Hebert, T.T.: A simple technique for purification of tobacco mosaic virus in large quantities. - Phytopathology **57**: 1285, 1967.

Graham, I.A., Denby, K.J., Leaver, C.J.: Carbon catabolite repression regulates glyoxylate cycle gene expression in cucumber. - Plant Cell **6**: 761-772, 1994.

Jang, J.-C., Leon, P., Zhou, L., Sheen, J.: Hexokinase as a sugar sensor in higher plants. - Plant Cell **9**: 5-19, 1997.

Jang, J.-C., Sheen, J.: Sugar sensing in higher plants. - Plant Cell **6**: 1665-1679, 1994.

Kapur, S.P., Gumpf, D.J., Weathers, L.G.: Some effects of metabolic changes induced in „Etrog" citron by three isolates of exocortis virus. - Phytopathology **64**: 196-201, 1974.

Koch, K.E.: Carbohydrate-modulated gene expression in plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **47**: 509-540, 1996.

Kruger, N.J.: Carbohydrate synthesis and degradation. In: Dennis, D.T., Turpin, D.M. (ed.): Plant Physiology, Biochemistry and Molecular Biology. Pp. 268-292. Longman, Harlow 1990.

Makovcová, O., Šindelář, L.: The effect of 2,4-dichloro-phenoxyacetic acid on the metabolic utilization of free carbohydrates in cucumber mosaic virus infected cucumber plants. - Biol. Plant. **23**: 465-468, 1981.

Makovcová, O., Šindelář, L., Hanušová, M.: [Sucrose metabolism in the leaves of cucumber infected with cucumber mosaic virus, as related to yields.] - Ochrana Rost. (Prague) **16**: 263-269, 1980. [In Czech.]

Mančal, P.: Metody Enzymové Imunoanalýzy. [Methods of Enzyme Immunoassay.] - ÚSOL, Prague 1987. [In Czech.]

Miernyk, J.A., Dennis, D.T.: Mitochondrial, plastid, and cytosolic isozyme complexes of hexokinase from developing endosperm of *Ricinus communis*. - Arch. Biochem. Biophys. **226**: 458-468, 1983.

Renz, A., Merlo, L., Stitt, M.: Partial purification from potato tubers of three fructokinases and three hexokinases which show differing organ and developmental specificity. - Planta **190**: 156-165, 1993.

Roland, F., Winderickx, J., Thevelein, J.M.: Glucose-sensing mechanisms in eukaryotic cells. - Trends Biochem. Sci. **26**: 310-317, 2001.

Schnarrenberger, C.: Characterization and compartmentation, in green leaves, of hexokinases with different specifities for glucose, fructose, and mannose and for nucleoside triphosphates. - Planta **181**: 249-255, 1990.

Sheen, J., Zhou, L., Jang, J.-C.: Sugars as signaling molecules. - Curr. Opinion Plant Biol. **2**: 410-418, 1999.

Šindelář, L., Makovcová, O.: Beziehungen zwischen der Phosphatasen-Aktivität und dem Gehalt der freien Zucker bei durch die Strichelkrankheit der Kartoffeln infiziertem *N. tabacum* cv. "Samsun". - Biol. Plant. **16**: 376-381, 1974.

Šindelář, L., Šindelářová, M., Burketová, L.: Changes in glucose, fructose and saccharose metabolism in tobacco plants infected with potato virus Y. - Biol. Plant. **42**: 431-439, 1999.

Šindelářová, M., Šindelář, L.: Hexokinases of tobacco leaves: Subcellular localization and characterization. - Biol. Plant. **30**: 275-284, 1988.

Šindelářová, M., Šindelář, L.: Hexokinases of tobacco leaves: Influence of plant age on particulate and soluble isozyme composition. - Biol. Plant. **40**: 469-474, 1997/1998.

Šindelářová, M., Šindelář, L., Burketová, L.: Dynamic changes in the activities of glucose-6-phosphate dehydrogenase, ribulose bisphosphate carboxylase, and ribonuclease in tobacco leaves, leaf discs, and mesophyll protoplasts in relation to TMV multiplication. - Physiol. mol. Plant Pathol. **51**: 99-109, 1997.

Smeekens, S.: Sugar-induced signal transduction in plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **51**: 49-81, 2000.

Smith, S.B., Taylor, M.A., Burch, L.R., Davies, H.V.: Primary structure and characterization of a cDNA clone of fructokinase from potato *(Solanum tuberosum* L. cv. Record). - Plant Physiol. **102**: 95-106, 1993.

Stitt, M., Bulpin, P.V., apRees, T.: Pathway of starch breakdown in photosynthetic tissues of *Pisum sativum*. - Biochim. biophys. Acta **544**: 200-214, 1978.

Taylor, C.B.: Sweet sensations. - Plant Cell **9**: 1-4, 1997.

Técsi, L.I., Maule, A.J., Smith, A.M., Leegood, R.C.: Metabolic alterations in cotyledons of *Cucurbita pepo* infected by cucumber mosaic virus. - J. exp. Bot. **45**: 1541-1551, 1994.

Turner, J.F., Turner, D.H.: The regulation of glycolysis and the pentose phosphate pathway. - In: Davies, D.D. (ed.): The Biochemistry of Plants. Vol. 2. Pp. 279-316. Academic Press, New York - London - Toronto - Sydney - San Francisco 1980.

Umemura, T., Perata, P., Futsuhara, Y., Yamaguchi, J.: Sugar sensing and a-amylase gene repression in rice embryos. - Planta **204**: 420-428, 1998.

Wiese, A., Groner, F., Sonnewald, U., Deppner, H., Lerchi, J.,

Hebbeker, U., Flügge, U.-I., Weber, A.: Spinach hexokinase I is located in the outer envelope membrane of plastids. - Fed. eur. biochem. Soc. Lett. **461**: 13-18, 1999.

Wolffgang, H., Keck, A.: Untersuchungen über den Stoffwechsel viruskranker Pflanzen. I. Die Phosphatase-Aktivität in *Nicotiana tabacum* L. var. Samsun nach Infektion mit TMV. - Phytopathol. Z. **34**: 57-65, 1959.

Xiao, W., Sheen, J., Jang, J.-C.: The role of hexokinase in plant sugar signal transduction and growth and development. - Plant mol. Biol. **44**: 451-461, 2000.