BIOLOGIA PLANTARUM 45 (4): 575-580, 2002

Glucose-6-phosphate dehydrogenase/6-phosphogluconate dehydrogenase ratio and the glucose-6-phosphate, 6-phosphogluconate and fructose-6-phosphate contents in tobacco plants infected with potato virus Y

L. ŠINDELÁŘ and M. ŠINDELÁŘOVÁ

Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Na Karlovce 1a, CZ-16000 Praha 6, Czech Republic

Abstract

The ratio of activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (G6P DH/6PG DH), and the contents of glucose-6-phosphate (G6P), 6-phosphogluconate (6PG) and fructose-6-phosphate (F6P) were studied at various stages of potato virus Y (PVY) multiplication in *Nicotiana tabacum* cv. Samsun. G6P DH/6PG DH increased through the experiment from 0.42 to 0.53 in leaves of healthy tobacco, and up to 0.59 in PVY systemically infected leaves. However, these ratios in the ruptured protoplast preparations, and the chloroplast and cytosol fractions of healthy protoplasts were similar to that from infected ones. The ratio lower than 1, found in the healthy and/or PVY-infected leaf tissues and in the infected protoplasts as well, confirms the assumption that G6P DH is the control enzyme of oxidative pentosephosphate pathway not only in the healthy but also in the infected plants. The contents of G6P, 6PG and F6P in the period of the highest PVY multiplication were strongly decreased (to 30 - 50 % when compared with control healthy leaves) and were negatively correlated with the G6P DH and 6PG DH activities.

Additional key words: protoplast, chloroplast, cytosol, regulation, oxidative pentosephosphate pathway, *Nicotiana tabacum*.

Introduction

It has long been recognized that the plant has the capacity to metabolize free, storage and transport saccharides through the pentose phosphate cycle, the pathway representing a functionally significant alternative route for saccharides utilization. The oxidative pentose phosphate pathway metabolizes glucose-6-phosphate to ribose-5-phosphate necessary for the *de novo* biosynthesis of purine and pyrimidine nucleotides of viral RNA. Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: $NADP^+$ oxidoreductase, EC 1.1.1.49; G6P DH) and 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate: $NADP^+$ oxidoreductase, EC 1.1.1.44; 6PG DH) play an important role in the regulation of the flux through this pathway at the level of its first enzyme G6P DH. This together with 6-phosphogluconolactonase hydrolysing 6-phospho-D-gluconate lactone to 6-phospho-D-gluconate forms a suitable irreversible enzyme system (Turner and Turner 1980). The physiological significance of this enzymatic regulation during virus-RNA replication is given by the variable requirements of NADPH necessary for pentoses essential for nucleotide synthesis. G6P DH and 6PG DH catalyse the two-stage, $NADP^+$ dependent, oxidation of glucose-6-phosphate, thereby constituting the direct oxidative pathway of saccharide metabolism.

G6P DH and 6PG DH are present in plant tissues in the form of two main isozyme complexes, one of which is located in chloroplasts and the other in cytosol (Eichhorn and Corbus 1988, Wendt *et al.* 2000). Both isozymes show similar properties: nearly the same pH optima, relative molecular mass, kinetic constants K_M and V_{max}, *etc.* (Schnarrenberger *et al.* 1973). Mechanisms of their coarse and fine regulation by some intermediates ($NADP^+$, NADPH, AMP, ADP, erythrose-4-phosphate, phosphoenolpyruvate) and the effect of light (inhibition) or darkness (activation) are partially known (Heber *et al.*

Received 7 September 2001, *accepted* 1 February 2002.

Abbreviations: F6P - fructose-6-phosphate; G6P - glucose-6-phosphate; G6P DH - glucose-6-phosphate dehydrogenase; 6PG - 6-phosphogluconate; 6PG DH - 6-phosphogluconate dehydrogenase; p.i. - post inoculation; PVY - potato virus Y.

Acknowledgements: This work was supported by grant No. 522/99/1264 of the Grant Agency of the Czech Republic.

Fax: (+420) 2 24310109; e-mail: sindelarova@ueb.cas.cz

1967, Anderson *et al.* 1974, Ashihara and Komamine 1974, Anderson and Duggan 1976, Ben-Bassat and Anderson 1981, Srivastava and Anderson 1983, Fickenscher and Scheibe 1986, Graeve *et al.* 1994, Rogoshin 1996, Signorini *et al.* 1995, Wendenroth *et al.* 1997).

Close connection between increased activities of G6P DH and 6PG DH and the contents of viruses was reported by Tien and Tang (1963), Merrett and Sunderland (1967), Simons and Ross (1971), Huth (1973). Our earlier studies also confirmed such an increased activity of the oxidative pentosephosphate pathway including ribosephosphate pyrophosphokinase, the key enzyme of purine and pyrimidine biosynthesis pathway (Šindelář 1986, Šindelář and Šindelářová 1987a,b, Šindelářová *et al.* 1997, 1998, Šindelář *et al.* 1999).

This paper presents the study of regulative changes in the activities of G6P DH and 6PG DH, G6P DH/6PG DH ratio and the content of G6P, 6PG and F6P found in tobacco leaf tissues and mesophyll protoplasts upon potato virus Y infection.

Materials and methods

Plant cultivation and virus inoculation: Two-month-old tobacco (*Nicotiana tabacum* L. cv. Samsun) plants grown under constant conditions in Hoagland No. 3 nutrient solution, at an irradiance of 100 μmol m^{-2} s^{-1} (16-h photoperiod) and average temperature of 25 °C, were used in experiments. The lower leaf, approximately 5 cm in 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 leaf of control plant was mock-inoculated with distilled water. The day of inoculation was day zero (0 d p.i.).

Preparation of homogenate: Crude homogenate was prepared from the systemically infected and/or corresponding control leaves by grinding in a mortar with fine silica sand, 10 % (m/m) insoluble polyvinyl-pyrrolidone 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 of protoplasts and the fractionation of cell organelles: Protoplasts were prepared 7 d p.i. according to Šindelářová and Šindelář (1994). Their disintegration and subcellular fractionation was performed according the methods described in Šindelář *et al.* (1999). Ruptured protoplasts were named the crude homogenate S_0. The pellet (P_1) containing the substantial part of chloroplasts was obtained by 5 min centrifugation at 1 000 *g* and was resuspended in TEMM medium. Supernatant was centrifuged for 15 min at 15 000 *g* and the supernatant (S_{15}) was used in experiments. The fractions were used for estimation of total protein content, chlorophyll content and $NADP^+$-triosephosphate dehydrogenase (markers of chloroplasts), phosphoenol-pyruvate carboxylase (marker of cytosol), G6P DH, and 6PG DH activities. All steps of these procedures were carried out at a temperature from 0 to 4 °C. Under these conditions the activities of the enzymes did not change for more than 5 h.

The number of protoplasts was determined in a haemocytometer and the number of viable protoplasts by staining with methylene blue according to Hooley and McCarthy (1980). The percentage of infected protoplasts was determined by an immunoenzymatical method according to Šindelář and Šindelářová (1994).

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

Determination of chlorophyll, G6P, F6P and 6PG content and enzyme activities: Chlorophyll content was determined according to Arnon (1949).

D-glucose-6-phosphate and D-fructose-6-phosphate were determined by using glucose-6-phosphate dehydrogenase and phosphoglucose isomerase (Pohorst 1963a) and D-6-phosphogluconate by using 6-phosphogluconate dehydrogenase (Pohorst 1963b) after extraction of these metabolites by 0.6 M $HClO_4$.

G6P DH (EC 1.1.1.49) and 6PG DH (EC 1.1.1.44) activities were determined spectrophotometrically, NADPH generation was monitored at 340 nm (Šindelář 1986, Šindelář and Šindelářová 1987a). Phosphoenol-pyruvate carboxylase activity (EC 4.1.1.31) was determined according to Downton and Slatyer (1971), $NADP^+$-triosephosphate dehydrogenase activity (EC 1.2.1.9) according to Heber *et al.* (1963).

Statistical treatment: The results are presented as arithmetical means ± standard deviation of the mean of 3 to 7 determinations in 3 independent experiments. The *t*-test was employed to characterise the differences.

Chemicals: Protoplasts releasing enzymes were obtained from *Serva* (Heidelberg, Germany), alkaline phosphatase from *Boehringer* (Heisenhofen, Germany) and all other fine biochemicals were purchased from *Sigma Chemical Company* (St. Louis, USA).

Results and discussion

The G6P DH and 6PG DH activities of leaf tissue are significantly increased through the period of PVY multiplication (when compared with leaf tissues of control tobacco) (Table 1, Fig. 1) and the courses of these curves correlate with the multiplication curve of the

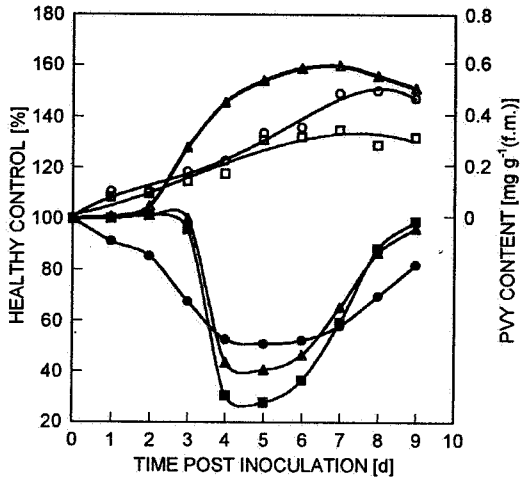

Fig. 1. The G6P (*closed circles*), 6PG (*closed squares*) and F6P (*closed triangles*) contents and G6P DH (*open circles*) and 6PG DH (*open squares*) activities in PVY infected leaf tissues of *Nicotiana tabacum* L. cv. Samsun expressed in % of healthy control. PVY content - *open triangles*.

PVY^N (G6P DH: $r = 0.983^{***}$ for $n = 43$; 6PG DH: $r = 0.971^{***}$ for $n = 32$). This is in good agreement with the results obtained by Tien and Tang (1963), Merrett and Sunderland (1967), Simons and Ross (1971), Huth (1973) and with our previous studies (Šindelář *et al.* 1990, 1999, Šindelářová and Šindelář 1991, Šindelářová *et al.* 1997).

In accordance with the increased G6P DH and 6PG DH activity (3 - 7 d p.i.) in infected leaves of tobacco, the strong decrease to 30 - 50 % of G6P, 6PG and F6P, the substrates of oxidative pentosephosphate pathway, was found in the period of the culmination of multiplication curve of PVY (Table 2, Fig. 1).

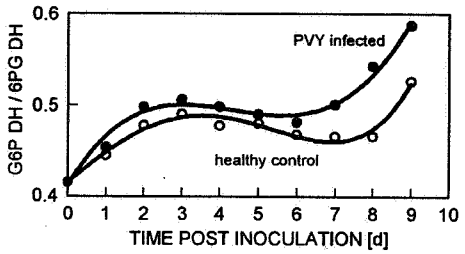

Fig. 2. The G6P DH / 6PG DH activity ratio in healthy and PVY infected leaf tissues of *Nicotiana tabacum* L. cv. Samsun.

It seems to be generally accepted that the rate of the oxidative pentosephosphate pathway is regulated at the level of its first enzyme, glucose-6-phosphate dehydrogenase (Turner and Turner 1980). The activity of this enzyme seems to be determined either by the ratio $NADPH/NADP^+$ or by its specific effector ribulose-1,5-bisphosphate in the chloroplasts (Turner and Turner 1980). To provide reliable efficiency for regulation of the pathway, the higher content of G6P DH than 6PG DH must be established, thereby the ratio of G6P DH activity to 6PG DH must be lower than 1. This regulative mechanism has evidently a common validity because of its observation also in animal tissue (Farnararo and Bruni 1982). Our results obtained at leaf tissue confirm this (Fig. 2). In spite of the significant increase of both enzyme activities in infected tissues, the G6P DH/6PG DH ratio was only insignificantly changed.

Table 1. Glucose-6-phosphate dehydrogenase (G6P DH) and 6-phosphogluconate dehydrogenase (6PG DH) activities in healthy and PVY systemically infected tobacco leaves (* - the differences statistically significant at $0.01 \le P < 0.05$; ** - at $0.001 \le P < 0.01$).

Time [d p.i.]	G6P DH [nmol g^{-1}(f.m.) min^{-1}]		6PG DH [nmol g^{-1}(f.m.) min^{-1}]	
	healthy	infected	healthy	infected
0	55.07 ± 1.41	55.07 ± 1.32	132.62 ± 1.87	132.62 ± 1.96
1	60.01 ± 1.53	66.31 ± 1.51	134.81 ± 2.15	146.04 ± 1.87
2	64.94 ± 1.55	74.25 ± 1.63	136.04 ± 2.08	149.19 ± 1.90
3	64.94 ± 1.43	76.58 ± 1.57*	132.48 ± 2.11	151.39 ± 2.06**
4	63.71 ± 1.38	77.82 ± 1.46**	133.44 ± 2.06	156.18 ± 2.12**
5	63.43 ± 1.40	84.26 ± 1.61**	132.21 ± 2.10	172.07 ± 2.41**
6	68.64 ± 1.51	92.61 ± 1.83**	146.73 ± 2.12	192.49 ± 2.37**
7	82.20 ± 1.73	121.79 ± 1.92**	176.59 ± 2.46	243.59 ± 2.57**
8	90.97 ± 1.86	135.63 ± 1.96**	195.36 ± 2.65	250.03 ± 2.48**
9	102.20 ± 1.91	149.33 ± 1.91**	194.27 ± 2.54	254.41 ± 2.39**

Table 2. Glucose-6-phosphate, 6-phosphogluconate and fructose-6-phosphate in healthy and PVY systemically infected tobacco leaves (* - the differences statistically significant at $0.01 \leq P < 0.05$; ** - at $0.001 \leq P < 0.01$).

Time [d p.i.]	Glucose-6-phosphate [nmol g^{-1}(f.m.)] healthy	infected	6-Phosphogluconate [nmol g^{-1}(f.m.)] healthy	infected	Fructose-6-phosphate [nmol g^{-1}(f.m.)] healthy	infected
0	15.0 ± 0.36	15.0 ± 0.28	1.50 ± 0.03	1.50 ± 0.03	3.53 ± 0.05	3.53 ± 0.05
1	16.8 ± 0.41	15.3 ± 0.31	1.91 ± 0.04	1.91 ± 0.03	3.54 ± 0.05	3.54 ± 0.05
2	27.0 ± 0.43	23.1 ± 0.33**	2.07 ± 0.04	2.76 ± 0.04	7.97 ± 0.06	7.14 ± 0.06
3	37.3 ± 0.46	25.3 ± 0.40**	2.38 ± 0.04	2.76 ± 0.04	8.67 ± 0.06	7.15 ± 0.06*
4	67.5 ± 0.87	35.5 ± 0.63**	6.91 ± 0.05	2.07 ± 0.03**	24.76 ± 0.16	10.61 ± 0.07**
5	142.7 ± 1.19	71.4 ± 0.71**	7.60 ± 0.06	2.07 ± 0.03**	35.37 ± 0.23	14.15 ± 0.06**
6	156.0 ± 1.27	80.0 ± 0.76**	7.00 ± 0.05	2.50 ± 0.04**	33.00 ± 0.26	15.00 ± 0.07**
7	137.0 ± 1.25	78.0 ± 0.71**	6.00 ± 0.05	3.50 ± 0.04**	28.00 ± 0.22	18.00 ± 0.07**
8	108.0 ± 1.17	74.3 ± 0.64**	5.53 ± 0.04	4.84 ± 0.04*	24.76 ± 0.20	21.22 ± 0.12*
9	105.0 ± 1.22	85.0 ± 0.69**	5.00 ± 0.04	4.90 ± 0.05	22.00 ± 0.16	21.00 ± 0.11

Table 3. G6P DH, 6PG DH activity [nmol (10^6 living protoplasts)$^{-1}$ min^{-1}] and G6P DH/6PG DH ratio in protoplasts and cell organelles from healthy and PVY infected tobacco leaves (7 d p.i.). The symbols S_0, P_1 and S_{15} represent the ruptured protoplast preparation, the 1 000 *g* pellet (chloroplasts) and 15 000 *g* supernatant (cytosol), respectively, (* - the differences statistically significant at $0.01 \leq P < 0.05$; ** - at $0.001 \leq P < 0.01$).

Fractions	Healthy protoplasts G6P DH	6PG DH	G6P DH/6PG DH	Infected protoplasts G6P DH	6PG DH	G6P DH/6PG DH
S_0	3.40 ± 0.02	8.95 ± 0.05	0.38	4.08 ± 0.04*	10.20 ± 0.12**	0.40
P_1	0.47 ± 0.01	2.40 ± 0.02	0.20	0.86 ± 0.01**	4.29 ± 0.03**	0.20
S_{15}	2.72 ± 0.02	7.16 ± 0.03	0.38	3.45 ± 0.03*	8.21 ± 0.08*	0.42

To verify this finding, the ratio of G6P DH/6PG DH was also studied in chloroplast and cytosol fractions derived from the healthy and PVY-infected tobacco protoplasts. Protoplasts were prepared from leaves on 7 d p.i. when the multiplication curve of PVY and the curves of activities of G6P DH and 6PG DH culminated (Fig. 1). Protoplasts contained 4.83 μg PVY per 10^6 living protoplasts and 57.1 ± 9.7 % of the protoplasts were infected. Almost all intact chloroplasts (chlorophyll and $NADP^+$-triosephosphate dehydrogenase were used as markers) occurred in P_1 pellet (healthy - more than 94 %, infected - more than 92 % of total chloroplasts). Supernatant S_{15} contained more than 96 %, (or 93 % in infected protoplasts) of the cytosol as estimated by using phosphoenolpyruvate carboxylase as marker. The ratio of G6P DH/6PG DH activity was not significantly changed in ruptured protoplast preparations (S_0), chloroplasts (P_1) and cytosol (S_{15}) by PVY infection (Table 3).

Conclusion: The value of G6P DH/6PG DH ratio lower than 1, found in the healthy and PVY-infected leaf tissues and also in infected protoplasts, is a biochemical feature which confirm the assumption that G6P DH is the control enzyme of oxidative pentosephosphate pathway regulation not only in healthy but also in infected plants. The content of G6P, 6PG and F6P in the period of the highest PVY multiplication was strongly decreased to 30 - 50 % when compared with control healthy leaves and was negatively correlated with the G6P DH and 6PG DH activity.

References

Anderson, L.E., Duggan, J.X.: Light modulation of glucose-6-phosphate dehydrogenase. - Plant Physiol. **58**: 135-139, 1976.

Anderson, L.E., Ng, T.L., Park, K.Y.: Inactivation of pea leaf chloroplastic and cytoplasmic glucose-6-phosphate dehydrogenases by light and dithiothreitol. - Plant Physiol. **53**: 835-839, 1974.

Arnon, D.I.: Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-5, 1949.

Atkinson, D.E.: The energy charge of the adenylate pool as a regulatory parameter. Interaction of feedback modifiers. - Biochemistry **7**: 4030-4034, 1968.

Ashihara, H., Komamine, A.: Regulation of the activities of some enzymes of the pentose phosphate pathway in *Phaseolus mungo*. - Z. Pflanzenphysiol. **74**: 130-142, 1974.

Ben-Bassat, D., Anderson, L.E.: Light induced release of bound glucose-6-phosphate dehydrogenase to the stroma in pea chloroplasts. - Plant Physiol. **68**: 279-283, 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.

Downton, L., Slatyer, R.O.: Variation in levels of some leaf enzymes. - Planta **96**: 1-12, 1971.

Eichhorn, M., Corbus, B.: Metabolic role of glucose-6-phosphate dehydrogenase in photoautotrophic organisms. - Biochem. Physiol. Pflanz. **183**: 449-475, 1988.

Farnararo, M., Bruni, P.: Changes in the G6PDH/6PGDH ratio in the chick brain during development. - Experientia **38**: 1042-1043, 1982.

Fickenscher, K., Scheibe, R.: Purification and properties of the cytoplasmic glucose-6-phosphate dehydrogenase from pea leaves. - Arch. Biochem. Biophys. **247**: 393-402, 1986.

Graeve, K., von Schaeven, A., Scheibe, R.: Purification, characterization, and sDNA sequence of glucose-6-phosphate dehydrogenase from potato (*Solanum tuberosum* L.). - Plant J. **5**: 353-361, 1994.

Heber, V., Hudson, M.A., Hallier, U.M.: Zur intrazellulären Verteilung von Enzymen und Substraten in der Blattzelle. - Z. Naturforsch. **22b**: 1200-1215, 1967.

Heber, V., Pon, N.G., Heber, M.: Localization of carboxydismutase and triose phosphate dehydrogenase in chloroplasts. - Plant Physiol. **38**: 355-360, 1963.

Hooley, R., McCarthy, D.: Extracts from virus infected hypersensitive tobacco leaves are detrimental to protoplast survival. - Physiol. Plant Pathol. **16**: 25-38, 1980.

Huth, W.: Das Verhalten einiger Enzyme des Kohlenhydratstoffwechsels in Kartoffel-X-Virus-kranken Tabakpflanzen. - Phytopathol. Z. **77**: 117-124, 1973.

Leiser, R., Richter, J.: Reinigung und einige Eigenschaften des Kartoffel-Y-Virus. - Arch. Phytopathol. Pflanz.-Schutz **14**: 337-350, 1978.

Merrett, M.J., Sunderland, D.W.: The metabolism of ^{14}C specifically labeled glucose in leaves showing TMV-induced local necrotic lesions. - Physiol. Plant. **20**: 593-599, 1967.

Pohorst, H.-J.: D-glucose-6-phosphate and D-fructose-6-phosphate. Determination with glucose-6-phosphate dehydrogenase and phosphoglucose isomerase. - In: Bergmeyer, H.U. (ed.): Methods of Enzymatic Analysis. - Verlag Chemie GmbH, Weinheim; Academic Press, New York - London, 1963a.

Pohorst, H.-J.: D-6-phosphogluconate. - In: Bergmeyer, H.U. (ed.): Methods of Enzymatic Analysis. - Verlag Chemie GmbH, Weinheim; Academic Press, New York - London, 1963b.

Rogoshin, V.V.: Possible mechanisms of regulating glucose-6-phosphate dehydrogenase activity by an excess of substrate and coenzyme. - Bioorg. Khim. **22**: 575-579, 1996.

Schnarrenberger, C., Oeser, A., Tolbert, N.E.: Two isoenzymes each of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in spinach leaves. - Arch. Biochem. Biophys. **154**: 438-448, 1973.

Signorini, M., Bregoli, A.M., Caselli, L., Bergamini, C.M.: Purification and properties of 6-phosphogluconate dehydrogenase from beet leaves. - Biochem. mol. Biol. Int. **35**: 669-675, 1995.

Simons, T.J., Ross, A.F.: Metabolic changes associated with systemic induced resistance to tobacco mosaic virus in 'Samsun NN' tobacco. - Phytopathology **61**: 293-300, 1971.

Šindelář, L.: Changes in the activity of glucose-6-phosphate dehydrogenase and some problems relating to its regulation in tobacco plants infected with potato virus Y. - Biol. Plant. **28**: 440-448, 1986.

Šindelář, L., Šindelářová, M.: Changes in the activity of phosphogluconate dehydrogenase and its regulation in tobacco infected with PVY. - Biol. Plant. **29**: 150-154, 1987a.

Šindelář, L., Šindelářová, M.: Changes in ribulosephosphate isomerase and ribosephophate pyrophosphokinase activities in tobacco infected with PVY. - Biol. Plant. **29**: 468-472, 1987b.

Šindelář, L., Šindelářová, M.: Effect of 2,4-dichlorophenoxyacetic acid on tobacco mosaic virus multiplication in tobacco protoplasts and leaf discs. - J. Plant Physiol. **144**: 620-622, 1994.

Šindelář, L., Šindelářová, M., Burketová, L.: Changes in activity of glucose-6-phosphate and 6-phosphogluconate dehydrogenase isozymes upon potato virus Y infection in tobacco leaf tissues and protoplasts. - Plant Physiol. Biochem. **37**: 195-201, 1999.

Šindelář, L., Šindelářová, M., Čeřovská, N., Hanušová, M.: Changes in ribonuclease and glucose-6-phosphate dehydrogenase activities during PVY-RNA biosynthesis in potato leaf discs. - Biol. Plant. **32**: 119-127, 1990.

Šindelářová, M., Šindelář, L.: Subcellular localization of glucose-6-phosphate dehydrogenase in tobacco mesophyll protoplasts. - Biol. Plant. **33**: 150-155, 1991.

Šindelářová, M., Šindelář, L.: Subcellular localization of ribonuclease isoenzymes in tobacco mesophyll protoplasts and their changes induced by infection of PVY. - Biol. Plant. **36**: 461-467, 1994.

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.

Šindelářová, M., Šindelář, L., Burketová, L., Táborský, V., Kazda, J.: Potato virus-Y multiplication in susceptible tobacco cultivar and transgenic breeding line producing coat protein mRNA. - Biol. Plant. **41**: 565-573, 1998.

Srivastava, D.K., Anderson, L.E.: Isolation and characterization of light and dithiothreitol-modulatable glucose-6-phosphate dehydrogenase from pea chloroplasts. - Biochim. biophys. Acta **724**: 359-369, 1983.

Tien, P., Tang, P.S.: The role of host respiration in tobacco mosaic virus multiplication. - Acta virol. **7**: 368-373, 1963.

Turner, J.F., Turner, D.H.: The regulation of glycolysis and pentose phosphate pathway. - In: Davies, D.D. (ed.): The Biochemistry of Plants. - Academic Press, New York 1980.

Wendenroth, I., Scheibe, R., von Schaewen, A.: Identification of the cysteine residues involved in redox modification of plant plastidic glucose-6-phosphate dehydrogenase. - J. biol. Chem. **272**: 26985-26990, 1997.

Wendt, U.K., Wenderoth, I., Tegeler, A., von Schaewen, A.: Molecular characterization of a novel glucose-6-phosphate dehydrogenase from potato (*Solanum tuberosum* L.). - Plant J. **23**: 723-733, 2000.