BIOLOGIA PLANTARUM 42 (3): 423-430, 1999

# Changes in ribonuclease and glucose-6-phosphate dehydrogenase activities induced by beet necrotic yellow vein virus in sugar beet

L. BURKETOVÁ, M. SINDELÁŘOVÁ, P. RYŠÁNEK and L. ŠINDELÁŘ

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

**Abstract**

Activities of host ribonucleases and glucose-6-phosphate dehydrogenase were studied in three cultivars (Monosvalof, Steffi and Rimini) of sugar beet differing in their resistance to beet necrotic yellow vein virus (BNYVV). No differences were found in the susceptibility of cultivars to BNYVV between mechanically inoculated and *Polymyxa betae* (a natural fungal vector of the virus) infected plants, but the culmination of reproduction curves of BNYVV in mechanically inoculated plants was observed one week earlier than in plants inoculated by means of *P. betae*. The activities of ribonucleases corresponded with virus multiplication. In roots, activities of ribonucleases reached a maximum at day 7; in leaves, maximum activity was found at day 21 in cv. Monosvalof, and at day 14 in cv. Steffi. The relatively resistant cultivar Rimini showed much lower activities. The activity of glucose-6-phosphate dehydrogenase was only slightly increased at the time of culmination of the BNYVV reproduction curve in cvs. Monosvalof and Steffi.

*Additional key words*: *Beta vulgaris*, oxidative pentose phosphate pathway, *Polymyxa betae*.

## Introduction

Modulation of plant ribonuclease (RNase) activities is connected with plant development and different biotic or abiotic stresses, these enzymes being supposed to play a role in the regulation of RNA levels and turnover. Their role in senescence, gametophytic self-incompatibility or their changes caused by plant growth regulators, heat shock, light, $O_2$ deprivation or fungal elicitors have being previously reported

*Received* 5 February 1999, *accepted* 15 April 1999.

*Abbreviations*: BNYVV - beet necrotic yellow vein virus; TMV - tobacco mosaic virus; PVY - potato virus Y; RNase - ribonuclease; rRNA - ribosomal ribonucleic acid; G6PDH - glucose-6-phosphate dehydrogenase.

*Acknowledgement*: This research was supported by the Grant Agency of the Czech Republic (grant No. 522/96/1412).

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

(Abbler and Green 1996, McKeon *et al.* 1991, Fennoy *et al.* 1997).

Increased RNase activities during virus infections have been were intensively studied (*e.g.* Diener 1961, Reddi 1959, Lusso and Kuć 1995, Burketová 1995, Šindelářová *et al.* 1997) and a quantitative relationship between the content of the virus and the activity of this complex of enzymes was expressed by a linear correlation (Šindelář *et al.* 1988, 1990, Šindelářová *et al.* 1997). These findings indicate the possible role of host ribonucleases in virus multiplication by the utilization of nucleoside precursors for viral RNA biosynthesis (Šindelář *et al.* 1990, Šindelářová *et al.* 1990).

In addition to ribonucleases, a reductive pentose phosphate pathway (during photosynthesis) and/or an oxidative pentose phosphate pathway (mainly in the dark), are another possible source of intermediates required for viral RNA biosynthesis to be considered. Changes in activities of the enzymes involved in the oxidative pentose phosphate pathway induced by viral infections were investigated, *e.g.*, by Solymosy and Farkas (1962), Simons and Ross (1971) and Huth (1973). The linear correlation between the activity of glucose-6-phosphate dehydrogenase (G6PDH, rate-limiting enzyme of this pathway) and the reproduction curve of the virus was found in *Nicotiana tabacum* cv. Samsun infected with potato virus Y (PVY) (Šindelář 1986) and TMV (Šindelářová *et al.* 1997). Increased activity of G6PDH was also found in potato plants infected with PVY (Šindelář *et al.* 1990, Burketová 1995). These key enzymes could be used as markers of resistance to virus multiplication in tobacco (TMV) and potato (PVY) (Šindelář *et al.* 1990, Burketová 1995).

Beet necrotic yellow vein virus (BNYVV) causes an important disease of sugar beet (*Beta vulgaris* L.) called rhizomania. This disease is characterised by massive lateral proliferation of rootlets on the main root resulting in severe stunting and consequent reduction in the sugar content. Yield reduction is caused by lack of a well-developed taproot (Harveson and Rush 1993). Beet necrotic yellow vein virus contains four species of single-stranded RNA. In field conditions, the virus is transmitted by a soil-borne fungus *Polymyxa betae* Keskin *(Plasmodiophoraceae)* infecting lateral roots (Tamada 1975, Putz 1977). Root infection occurs by means of zoospores that originate from zoosporangia or cystosori. During the multiplicative phase of *P. betae*, a plasmodium is formed within the plant cell and can become contaminated with BNYVV. The plasmodium then differentiates into either a zoosporangium, giving rise to new zoospores, or thick-walled resting spores called cystosori, which can survive for many years in the soil (Harveson and Rush 1993).

The objective of the present study was to elucidate the participation of the pathways mentioned in the reproduction of beet necrotic yellow vein virus, which multiplies prevalently in roots, and to determine their contingent connection with plant resistance.

## Materials and methods

**Plants:** Three cultivars differing in their resistance to rhizomania were used. Seeds of *Beta vulgaris* L. susceptible cv. Monosvalöf, and partly resistant cvs. Steffi and

Rimini were surface sterilised in commercial hypochlorite (dilution 1:10, for 10 min) and sown into moist sand. After one week, seedlings were transplanted to pots filled with perlite, and watered regularly with half strength Steiner (1984) nutrient solution. After a further two weeks, plants were inoculated with BNYVV. All experiments were carried out in a 16-h photoperiod (photon flux density of 60 μmol $m^{-2}$ $s^{-1}$) and a temperature of 24 °C.

**Pathogens and inoculation:** Soil infested with *Polymyxa betae* Keskin containing BNYVV was collected from Erstein region in Alsace (France). Two different types of inoculation were used. In the first system, the plants were inoculated by means of *P. betae* zoospores directly in the infested soil. In the second system, omitting the fungus, sugar beet plants were mechanically inoculated by vortexing the roots with inoculum and carborundum for 45 s as described Koenig *et al.* (1991). The inoculum was obtained grinding the roots of BNYVV infected sugar beets in 0.01 M HEPES buffer, pH 6.5, in the ratio 1:10 (m/v) and passing the homogenate through a bacterial filter (0.45 μm; *Milipore Corporation*, Bedford, USA) to remove contingent zoospores and cystosori. Control plants were treated with HEPES buffer in the same way. Mechanically infected plants were cultivated in perlite in Steiner solution as described above.

**Sample collection and homogenate preparation:** Both root and leaf samples represented by 10 plants were collected at pre-determined intervals. Part of sample (0.5 g) was frozen at -18 °C and stored for quantitative analysis of BNYVV by DAS-ELISA. The enzyme activities, protein and chlorophyll contents were measured immediately. The homogenate for their determination was prepared by grinding fresh tissue at 0 - 4 °C with a mortar and pestle in 20 mM Tris-HCl buffer (pH 7.0) containing 1 mM EDTA, 30 mM 2-mercaptoethanol, 2.5 mM $MgCl_2$, 10 % insoluble polyvinylpyrrolidone (*Polyclar AT* saturated for 30 min with extraction buffer) and fine silica sand in a ratio 1:5 (m/v). For the estimation of enzyme activities and protein content, the extract was centrifuged for 10 min at 20 000 *g*.

**Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity** was determined according to Brown and Wray (1968). The rate of NADPH generation was monitored spectrophotometrically at 340 nm. The assay mixture (0.3 $cm^3$) contained 25 μmol Tris-HCl buffer (pH 8.0, pH optimum of the enzyme), 0.625 μmol $MgCl_2$, 1.25 μmol $NADP^+$, 1.25 μmol glucose-6-phosphate, and 50 $mm^3$ of homogenate (10 times diluted by 100 mM Tris-HCl buffer with 12.5 mM $MgCl_2$, pH 8.0).

**Ribonuclease activity** was measured according to Cheo (1971). The assay mixture consisted of 0.3 $cm^3$ of yeast RNA (1.17 mg in 1 $cm^3$ of 0.15 M acetate buffer, pH 5.5, which was RNase pH optimum) and 50 $mm^3$ of homogenate. After 30 - 60 min incubation, enzyme reaction was stopped by addition of 3 $cm^3$ precipitation solution. The mixture was left for 1 h at 0 °C, then centrifuged for 10 min at 5 000 *g*. The amount of degraded RNA in the supernatant was spectrophotometrically determined at 260 nm. One unit of RNase activity was arbitrarily characterized as a change in absorbance by the value of 1.0 per hour.

**Chlorophyll content** was estimated spectrophotometrically at 652 nm according to Arnon (1949).

**The content of BNYVV** was determined by double antibody sandwich ELISA (DAS-ELISA) (Clark and Adams 1977) using rabbit IgG and conjugate with alkaline phosphatase (*Boehringer Mannheim*, Wien, Austria and/or *Loewe Biochemica*, Sauerlach, Germany). Absorbance was measured at 405 nm.

## Results and discussion

The course of BNYVV infection, expressed by reproduction curves of the virus, depended on the type of inoculation. In mechanically infected plants, the virus reproduced more quickly and reached the maximum values from 14 to 21 d after inoculation in the susceptible cultivar Monosvalof (Fig. 1*A*). In plants infected with BNYVV *via P. betae*, the culmination of the reproduction curve was recorded one week later (Fig. 1*B*). In the late stages of the infection (from day 21 in mechanically inoculated, from day 28 in *P. betae* infected plants), the BNYVV reproduction curves slightly declined in susceptible cultivars. This was probably caused by a decreasing portion of BNYVV infected roots in the sample, because of the lower mass of fine lateral roots which contain the highest amounts of BNYVV and/or by lowered viability of rhizomania diseased plants.

The highest content of BNYVV was found in the susceptible cultivar Monosvalof. In the relatively resistant cultivar Rimini, the virus multiplied to a lesser extent. The same effect was evident following both types of inoculation, indicating that the resistance to rhizomania in the cultivars studied is based less on resistance to the vector than to virus multiplication. Virus was not detected in leaves, neither in mechanically inoculated nor in *P. betae* infected plants of any cultivars, by ELISA during the course of the experiment (data not shown).

In spite of the fact, that virus multiplies exclusively in roots, the photosynthetic apparatus was affected in cultivars displaying higher contents of BNYVV, *e.g.*, chlorophyll content (Table 1) in mechanically infected plants of cultivars Monosvalöf and Steffi was decreased within the whole period of the experiment in comparison with healthy control plants (statistically significant by pair *t*-test).

The most pronounced changes were detected in the activities of ribonucleases, which corresponded with virus multiplication. In mechanically inoculated roots (Fig. 1*A*), the activity of RNase dramatically increased immediately after inoculation and reached maximum values of 184.2 % in the susceptible cv. Monosvalöf and 161.2 % in relatively resistant cv. Steffi at day 7. In roots of plants inoculated *via P. betae*, maximum RNase activity was found at day 14 (244.0 %) in cv. Monosvalöf, and at day 21 (169.3 %) in cv. Steffi. In resistant cultivar Rimini, RNase activities were enhanced only moderately to 125 % at day 28.

In leaves, conversely, a decrease in RNases activities was observed followed by their increase. In mechanically inoculated plants, maximum activity was found at day 21 in cv. Monosvalöf (155.0 %) and at day 14 in cv. Steffi (135.4 % of healthy

control). Relatively resistant cultivar Rimini showed much lower activity (105.2 %) which was not statistically significant. In plants inoculated *via P. betae*, RNase activities gradually increased to 198.2 % at day 28 in cv. Monosvalöf and at day 21 to 169.0 % in cv. Steffi. In resistant cv. Rimini, the activity reached only 125.1 % and the difference between infected and control plants was not statistically significant.

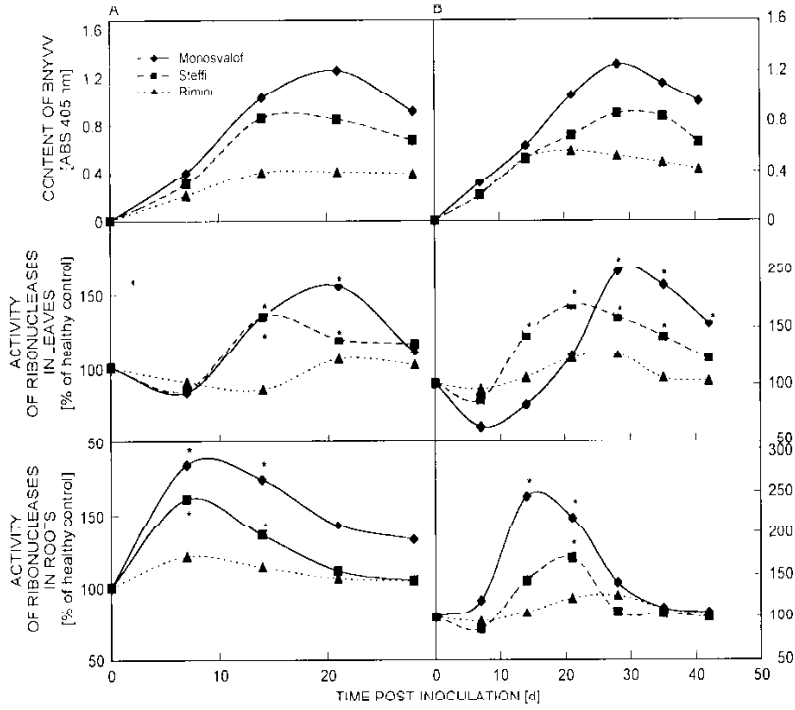


Fig. 1. Reproduction curves of BNYVV in roots determined by ELISA, and RNase activities in roots and leaves of mechanically inoculated (*A*) and *P. betae* inoculated (*B*) sugar beet plants of cultivars differing in their resistance to BNYVV (susceptible - cv. Monosvalöf, relatively resistant - cvs. Steffi, Rimini), expressed in % of healthy control. *Asterisks* indicate significant (*t*-test, $P < 0.05$) differences between BNYVV infected and healthy plants.

Table 1. Changes in chlorophyll content [mg $g^{-1}$(f.m.)] in sugar beet cultivars mechanically inoculated with BNYVV measured at day 7, 14, 21 and 28 post inoculation (d.p.i.). Means + SE, $n$ = 10)

| d.p.i. | Monosvalöf healthy | BNYVV | Steffi healthy | BNYVV | Rimini healthy | BNYVV |
|---|---|---|---|---|---|---|
| 7 | 1.51 + 0.09 | 1.30 ± 0.05 | 1.44 + 0.09 | 1.19 ± 0.08 | 1.38 ± 0.10 | 1.20 ± 0.08 |
| 14 | 1.41 + 0.04 | 1.33 ± 0.04 | 1.50 ± 0.08 | 1.40 ± 0.10 | 1.46 + 0.09 | 1.29 ± 0.07 |
| 21 | 1.27 + 0.12 | 1.15 ± 0.07 | 1.33 + 0.07 | 1.27 ± 0.05 | 1.18 ± 0.05 | 1.24 ± 0.05 |
| 28 | 1.28 + 0.08 | 1.20 ± 0.06 | 1.24 + 0.12 | 1.20 ± 0.09 | 1.23 + 0.03 | 1.36 ± 0.03 |

With the exception of leaves of cv. Monosvalöf cultivated in *P. betae* infested soil, RNase activities both in roots and leaves decreased at the end of the experiment and the difference between BNYVV infected and healthy plants was not significant. The same was found both in plants inoculated mechanically and by *P. betae*.

The RNase activity in leaves corresponded with the reproduction curves of BNYVV, whereas in roots it increased mainly in the initial stages of infection.

The changes in activity of G6PDH were not as pronounced as the changes in activity of RNases. In mechanically inoculated plants (Fig. 2), statistically significant increases were found both in leaves and roots of susceptible cv. Monosvalöf (119 and 115 %, respectively) and in leaves of partly resistant cv. Steffi (116 %) at the time of the culmination of BNYVV reproduction curve. A similar course was detected in *P. betae* infected plants (data not shown).

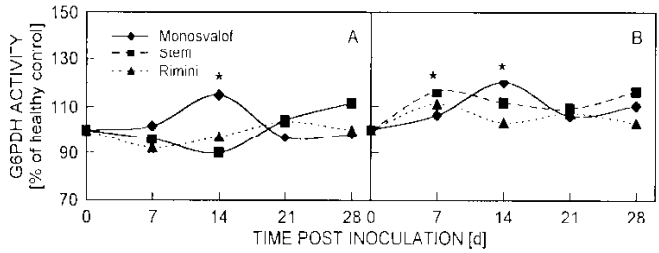


Fig. 2. Glucose-6-phosphate dehydrogenase (G6PDH) activity in roots (*A*) and leaves (*B*) of mechanically inoculated sugar beet plants of three cultivars differing in their resistance to BNYVV expressed in % of healthy control. *Asterisks* indicate significant ($P < 0.05$) differences between BNYVV infected and healthy plants.

In roots of mechanically inoculated plants of cvs. Monosvalöf and Steffi, the content of proteins was increased at the time of maximum BNYVV reproduction (Table 2). Statistically significant differences were detected at day 21 and 28 in cv. Monosvalöf and at day 21 in cv. Steffi. In cv. Rimini no significant changes were detected. No changes were observed in protein content in leaves of any cultivar. Gel electrophoresis (SDS-PAGE) did not reveal new protein bands, with the exception of the band corresponding to BNYVV coat protein in infected roots (data not shown), which indicates only quantitative protein changes.

Similarly to our previous experiments (Šindelář *et al.* 1990, Šindelářová *et al.* 1997, Burketová 1995), virus infection increased activities of both RNase and G6PDH, though, to a different extent. In cultivars exhibiting a high content of BNYVV (Monosvalof and Steffi), the activities of RNase increased first in roots just after their mechanical inoculation, followed by a significant increase of the activity in leaves. In contrast, the activity of G6PDH reached only lower values (116 - 118 %) when compared to healthy control. This indicates, that the way of degrading host RNAs by ribonucleases plays a more important role in the multiplication of BNYVV than the oxidative pentose phosphate cycle. The same trend was observed in potato plants infected with PVY (Burketová 1995). In sugar beet plants inoculated *via*

*P. betae*, reproduction of BNYVV as well as changes in enzyme activities started later. Probably, less rhizodermal cells were infected at initial stages of infection until zoosporangia were formed and new zoospores released.

Table 2. Changes in protein content [mg $g^{-1}$(f.m.)] in sugar beet cultivars mechanically inoculated with BNYVV measured at day 7, 14, 21 and 28 post inoculation (d.p.i.). Means ± SE, $n = 10$)

| | d.p.i. | Monosvalöf healthy | BNYVV | Steffi healthy | BNYVV | Rimini healthy | BNYVV |
|---|---|---|---|---|---|---|---|
| Roots | 7 | 1.40 ± 0.07 | 1.22 ± 0.05 | 1.34 ± 0.13 | 1.24 ± 0.07 | 1.43 ± 0.15 | 1.58 ± 0.13 |
| | 14 | 1.70 ± 0.05 | 1.86 ± 0.06 | 1.77 ± 0.08 | 1.92 ± 0.05 | 1.88 ± 0.09 | 1.99 ± 0.06 |
| | 21 | 1.87 ± 0.08 | 2.23± 0.09** | 1.89 ± 0.06 | 2.27 ± 0.09** | 2.03 ± 0.11 | 2.00 ± 0.09 |
| | 28 | 2.00 ± 0.05 | 2.22 ± 0.08* | 2.05 ± 0.12 | 2.04 ± 0.08 | 1.94 ± 0.08 | 1.88 ± 0.12 |
| Leaves | 7 | 3.62 ± 0.32 | 3.85 ± 0.43 | 3.61 ± 0.27 | 3.63 ± 0.24 | 4.00 ± 0.25 | 3.96 ± 0.19 |
| | 14 | 4.56 ± 0.41 | 4.62 ± 0.34 | 5.34 ± 0.40 | 5.23 ± 0.38 | 5.01 ± 0.31 | 5.14 ± 0.17 |
| | 21 | 4.87 ± 0.46 | 4.79 ± 0.44 | 4.84 ± 0.41 | 5.06 ± 0.53 | 4.64 ± 0.31 | 4.84 ± 0.35 |
| | 28 | 4.85 ± 0.10 | 4.98 ± 0.19 | 5.54 ± 0.35 | 6.01 ± 0.54 | 5.59 ± 0.31 | 5.81 ± 0.39 |

* - the difference is significant at $0.01 \leq P < 0.05$; ** - at $P < 0.01$.

All these results lead to the conclusion that the degradation of host RNAs by RNases could participate in BNYVV - RNA biosynthesis, while the and oxidative pentose phosphate pathway plays a less important role in this process.

## References


Abler, M.L., Green, P.J.: Control of mRNA stability in higher plants. - Plant mol. Biol. **32**: 63-78, 1996.

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-15, 1949.

Brown, A.P., Wray, J.L.: Correlated changes of some enzyme activities and cofactor and substrate contents of pea cotyledon tissue during germination. - Biochem. J. **108**: 437-444, 1968.

Burketová, L.: Changes in glucose-6-phosphate dehydrogenase and ribonucleases activities during PVY-RNA biosynthesis in infected potato plants. - Biol. Plant. **37**: 423-428, 1995.

Cheo, P.C.: Effect in different plant species of continuous light and dark treatment on tobacco mosaic virus replication capacity. - Virology **46**: 256-261, 1971.

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**: 475-483, 1977.

Diener, T.D.: Virus infection and other factors affecting RNase activity of plant leaves. - Virology **14**: 177-189, 1961.

Fennoy S.L., Jayachandran, S., Bailey-Serres, J.: RNase activities are reduced concomitantly with conservation of total cellular RNA and ribosomes in $O_2$-deprived seedlings roots of maize. - Plant Physiol. **115**: 1109-1117, 1997.

Harveson, R.M., Rush, C.M.: A simple method for field and greenhouse inoculation of *Polymyxa betae* and beet necrotic yellow vein virus. - Phytopathology **83**: 1216-1219, 1993.

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

Koenig, R., Jarausch, W., Commandeur, U., Burgemeister, W., Gehrke, M., Luddecke, P.: Effect of recombinant beet necrotic yellow vein virus with different RNA compositions on mechanically inoculated sugarbeets. - J. gen. Virol. **72**: 2243-2256, 1991.

Lusso, M., Kué, L.: Increased activities of ribonuclease and protease after challenge in tobacco plants with induced systemic resistance. - Physiol. mol. Plant Pathol. **47**: 419-428, 1995.

McKeon, T.W., Lyman, M.L., Prestamo, G.: Purification and characterization of 2 ribonuclease from developing tomato fruit. - Arch. Biochem. Biophys. **290**: 303-311, 1991.

Putz, C.: Composition and structure of beet necrotic yellow vein virus. - J. gen. Virol. **35**: 397-401, 1977.

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.

Steiner, A.A.: The universal nutrient solution. - In: Proceedings of the Sixth International Congress on Soilless Cultures. Pp. 633-650. Pudoc, Wageningen 1984.

Šindelář, L.: The changes of activities of glucose-6-phosphate dehydrogenase and some problems of its regulation in potato Y-virus infected tobacco. - Biol. Plant. **28**: 440-448, 1986.

Šindelář, L., Šindelářová, M., Hanušová, M.: [Some metabolic changes in susceptible and resistant tobacco varieties caused by infection with the tobacco mosaic virus.] - Ochrana Rost. (Praha) **24**: 259-261, 1988. [In Czech.]

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., Čeřovská, N., Hanušová, M.: [Changes in the metabolic pathways of PVY-RNA biosynthesis in tobacco leaf disks.] - Ochrana Rost. (Praha) **26**: 81-88, 1990. [In Czech.]

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. - Physiol. mol. Plant Pathol. **51**: 99-109, 1997.

Solymosy, F., Farkas, G.L.: Metabolic characteristics at the enzymatic level of tobacco tissues exhibiting acquired local resistance to viral infection. - Virology **21**: 210-221, 1963.

Tamada, T.: Beet Necrotic Yellow Vein Virus. - In: CMI/AAB Descriptions of Plant Viruses, No. 144, 1975.