PHOTOSYNTHETICA 41 (3): 357-363, 2003

# Photosynthesis and activity of phospho*enol*pyruvate carboxylase in *Nicotiana tabacum* L. leaves infected by *Potato virus A* and *Potato virus Y*

H. RYŠLAVÁ[*], K. MÜLLER[*], Š. SEMORÁDOVÁ[**,***], H. SYNKOVÁ[**], and N. ČEŘOVSKÁ[**]

*Department of Biochemistry, Faculty of Sciences, Charles University, Hlavova 2030, CZ-128 40 Praha 2, Czech Republic*[*]
*Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Na Karlovce 1a, CZ-160 00 Praha 6, Czech Republic*[**]
*Department of Plant Physiology and Anatomy, Faculty of Sciences, Charles University, Viničná 5, CZ-128 40 Praha 2, Czech Republic*[***]

## Abstract

The influence of viral infection caused by two different potyviruses, *Potato virus Y* (PVY) *and Potato virus A* (PVA) on plant metabolism and photosynthetic apparatus of *Nicotiana tabacum* L. cv. Samsun and cv. Petit Havana SR1 was studied. The main stress was focused on the activities of phospho*enol*pyruvate carboxylase (PEPC), NADP-malic enzyme (NADP-ME), and pyruvate phosphate dikinase (PPDK). The analysis of the presence of viral proteins, enzyme activities, and different photosynthetic parameters showed the time dependent progress of viral infection and NADP-ME and PEPC activities. PVY caused significant response, while PVA affected both tobacco cultivars only slightly. Viral infection, namely PVY, affected more negatively photosynthetic apparatus of cv. Petit Havana SR1 than cv. Samsun.

*Additional key words*: chlorophyll fluorescence; NADP-malic enzyme; photosystem 2; pyruvate phosphate dikinase; stomatal conductance; transpiration rate.

## Introduction

Virus-infected plants often show strong morphological and physiological alterations, with symptoms such as chlorosis and necrosis associated with changes in chloroplast structure and function. The severity of chlorotic symptoms seems to correlate with the presence of viral proteins inside the plastids (Reinero and Beachy 1989). Plant viruses reduce photosynthesis and increase susceptibility to photoinhibition (Rahoutei *et al.* 2000). Similarly as under various abiotic stresses, photosystem 2 (PS2) electron transport is often limited and energy-dissipating mechanisms are reduced. The mechanism of the viral infection on PS 2 remains unclear (Balachandran *et al.* 1994a). Irradiance stress may play an important role in host-virus interaction that results in clearly defined visible symptoms (van Kooten *et al.* 1990, Balachandran and Osmond 1994). Destruction of chloroplasts in affected leaf areas was also accompanied by severe reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase content (Balachandran *et al.* 1994b).

Saccharide synthesis and nitrate assimilation are closely related. The alterations in any of them caused by various stress factors or changing environment require alterations in the other one (Scheible *et al.* 2000). This adjustment operates *via* phospho*enol*pyruvate carboxylase (PEPC). In leaves of $C_3$ plants and in non-photosynthetic tissues PEPC is active in several anaplerotic reactions to replenish the intermediates of the Krebs cycle and to provide carbon skeletons to sustain the synthesis of amino acids during $NH_4^+$ assimilation. Together with NADP-malic enzyme it is involved in the fine regulation of cytoplasmic pH and compensates for alkalisation of the cytoplasm during nitrate reduction (Latzko and Kelly 1983, Manh *et al.* 1993). PEPC capacity increases in *Phaseolus vulgaris* grown at zinc toxic concentrations (Vangrosveld and Clijsters 1994) and in *Cucumis sativus* under iron deficiency (De Nisi and Zocchi 2000), and also under salt stress (Thomas *et al.* 1992). Nothing is known about the effect of any viral infection on PEPC

*Received* 27 March 2003, *accepted* 1 August 2003.
Fax: 420-224 310 113, e-mail: synkova@ueb.cas.cz
*Abbreviations*: $g_s$, total stomatal conductance; NADP-ME, NADP-malic enzyme; $P_N$, net photosynthetic rate; PEPC, phospho*enol*-pyruvate carboxylase; PPDK, pyruvate phosphate dikinase; PVA, *Potato virus A*; PVY, *Potato virus* $Y^{NTN}$; SR1, cv. Petit Havana SR1.
*Acknowledgements*: This work was supported by the research projects of the Grant Agency of Charles University No.134/2001/B-Bio/PrF, the Grant Agency of the Czech Republic No. 206/03/0310, and Z5 038 910.

and related enzymes. Pyruvate phosphate dikinase (PPDK) is abundant in leaves of $C_4$ and CAM plants where it is responsible for the production of phospho*enol*-pyruvate. However, there is only a little information about its role and expression in $C_3$ plants (Moons *et al.* 1998).

Aphid-transmitted potyviruses (genus *Potyvirus*) are the largest group of plant viruses. *Potato virus Y* (PVY) is one of the most damaging plant viruses particularly because of the economical importance of its plant host species. PVY is highly variable due to a wide host range. Generally, PVY potato strains are subdivided into three main groups, *i.e*. $PVY^O$, $PVY^C$, and $PVY^N$ (de Bokx and Huttinga 1981). However, during the last few decades, new strain variants have been reported, such as the tuber necrosis strain ($PVY^{NTN}$). Isolates of $PVY^{NTN}$ are associated with potato tuber necrotic ringspot disease (Beczner *et al.* 1984). The disease is characterised by a superficial necrosis on tubers, which occurs at harvest, and often develops during storage. PVY isolates inducing necrosis on tubers belong to the $PVY^N$ subgroup according to their reactions on *Nicotiana tabacum.*

*Potato virus A* (PVA) is one of the potyviruses discovered earlier. It occurs world-wide, infects many potato cultivars, and can cause about 40 % losses of yield (Bartels 1971). The isolates of $PVY^{NTN}$ and PVA have the capacity to infect tobacco plants systemically. The main difference between both viruses is in symptom occurrence. $PVY^{NTN}$ causes the vein necrosis, but in some isolates of tobacco also induces leaf distortion and stem necrosis (*e.g*. Lebanon). The symptoms of PVA infection are very mild diffuse mottle and vein clearing. There is no information available about their effects on photosynthetic apparatus or plant metabolism, though they are believed to occur mainly in the cytoplasm (Shukla *et al.* 1994).

We studied the effect of biotic stress caused by plant viruses on the activity of PEPC and closely co-operating enzymes such as NADP-malic enzyme (NADP-ME) and pyruvate phosphate dikinase (PPDK). We also analysed various photosynthetic parameters to determine if there is any correlation in the time course of viral infection and its effects on photosynthetic apparatus.

## Materials and methods

**Plants**: Tobacco plants (*Nicotiana tabacum* L. cv. Samsun and cv. Petit Havana SR1) were grown and infected in a greenhouse from September till December under 22/18 °C day/night temperatures. Day irradiance [overall integrated mid-values were *ca.* 500 μmol(quantum) $m^{-2}$ $s^{-1}$] was prolonged by the additional irradiation [PPFD *ca*. 200 μmol(quantum) $m^{-2}$ $s^{-1}$] to 16 h. Seeds were sown in pots with sand and plantlets were transferred to soil after 3 weeks. Five-week old plants were mechanically inoculated with PVY or PVA on the adaxial surface of the bottom of mature leaves. Virus isolates of $PVY^{NTN}$ (Lebanon) and PVA (*Lichte Industriae*) were provided by Dr. P. Dědič (Institute of Potato Research, Havlíčkův Brod, Czech Republic).

The extent of viral infection was determined by DAS-ELISA (Clark and Adams 1977) in homogenates of the leaves of infected plants using polyclonal or monoclonal antibodies raised against the respective pathogens (Filigarová *et al.* 1994, Čeřovská 1998). Leaf samples for determination of virus content and enzyme activities were collected each 2–3 d from the mature leaves, which newly developed above those used for inoculation. The material was frozen in liquid $N_2$ and stored at –75 °C.

**Photosynthetic parameters**: Chlorophyll (Chl) *a* fluorescence kinetics was measured on the adaxial surface of detached leaves after a 15-min dark period with the *PAM* Chlorophyll Fluorometer (*Walz*, Effeltrich, Germany) at room temperature and ambient $CO_2$ concentration. Measuring beam intensity was 0.35 μmol $m^{-2}$ $s^{-1}$, actinic irradiance 200 μmol $m^{-2}$ $s^{-1}$. Seven-hundred-ms saturated flashes of "white light" (2 500 μmol $m^{-2}$ $s^{-1}$) were applied at the beginning of measurement and after 300 s. Data sampling, control, and calculation were performed with the *DA 100 Data Acquisition System* (*Walz*, Effeltrich, Germany). The nomenclature of van Kooten and Snel (1990) and Osmond *et al*. (1993) was used.

Net photosynthetic rate ($P_N$), transpiration rate ($E$), and stomatal conductance ($g_s$) were measured using the gas exchange system *LCA-4* (*ADC BioScientific*, Hoddesdon, England) at a temperature of 25 °C, irradiance of 750 μmol $m^{-2}$ $s^{-1}$, relative humidity of 50 %, and $CO_2$ concentration of 350 μmol $mol^{-1}$.

**Enzyme activity assays**: Samples (1 g of fresh leaf) were homogenised in 3 $cm^3$ of extraction buffer containing 100 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, 1 mM EDTA, and 5 mM $MgCl_2$. The homogenate was centrifuged at 250 rps at 4 °C for 15 min. The supernatant was immediately used for enzyme activity measurements.

The PEPC (E.C. 4.1.1.31) assay mixture contained 100 mM Tris-HCl buffer (pH 8.1), 5 mM $NaHCO_3$, 2 mM $MgCl_2$, 2 mM PEP, and 0.2 mM NADH in total volume of 1 $cm^3$ (Slack and Hatch 1967). The PPDK (E.C. 2.7.9.1.) assay mixture contained 100 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 5 mM $NaHCO_3$, 2 mM pyruvate, 2 mM $K_2HPO_4$, 1 mM ATP, and 0.2 mM NADH in a total volume of 1 $cm^3$ (Aoyagi and Bassham 1983). The NADP-ME (E.C. 1.1.1.40.) assay mixture contained 100 mM Tris-HCl (pH 7.4), 10 mM malate, 2 mM $MgCl_2$, and 0.2 mM NADP in total volume of 1 $cm^3$ (Iglesias and Andreo 1990). PEPC, PPDK, and NADP-ME activities were monitored as absorbance changes at 340 nm at 25 °C and activities were calculated [μmol(substrate)

$kg^{-1}$(fresh leaf) $s^{-1}$].

Soluble proteins were determined according to Lowry (1951) with bovine serum albumin as a standard.

**Statistical analysis**: Data were obtained from three independent series of experiments. Statistical analysis was done by the ANOVA test. In case of the DAS-ELISA the most typical time course was presented.

## Results

**Symptoms and time course of viral infection**: The time course of viral infection was followed along with changes in photosynthetic parameters and enzyme activities in infected and control plants. The relative content of virus in tobacco leaves (Fig. 1) was measured from the 4th until the 18th d after inoculation. The maximal content of both viruses was found about 5 or 6 d after inoculation and was more or less constant further on. PVY infected tobacco leaves accumulated much more viral proteins than PVA infected plants. There was no significant difference in the progress of virosis between both cultivars (Fig. 1*A,B*). However, the severity of symptoms and accumulation of viruses was much stronger in PVY than in PVA infected plants.

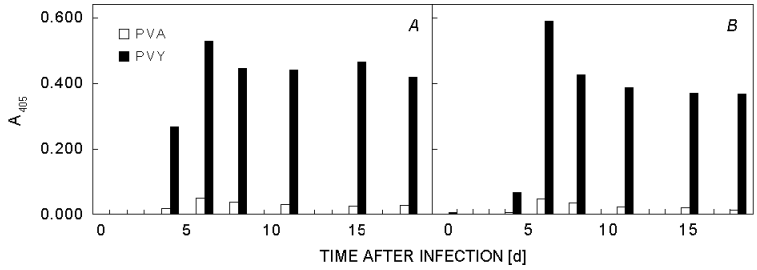


Fig. 1. Time course of infection with PVA and PVY in tobacco plants. The content of viruses was determined with DAS-ELISA with *p*-nitrophenylphosphate as enzyme substrate. Absorbance at 405 nm ($A_{405}$) corresponds to virus content. *A*: cv. Samsun; *B*: cv. SR1.

**Photosynthesis and Chl *a* fluorescence** were measured at two stages of viral infection and with respect to different viruses used for inoculation. Samples were taken in stage I, *i.e.* when the first visible symptoms of virosis appeared on the leaves, which was about 5 d after inoculation by PVY and 7 d after inoculation by PVA; stage II was about 15 d after inoculation by PVY and 18 d after inoculation by PVA. The first mature symptomatic leaf was used for the measurements.

Gas exchange parameters showed significant differences between both tobacco cultivars undergoing viral infection (Fig. 2). Generally, $P_N$ was higher in Samsun than in SR1 and plants were more negatively affected by PVY than by PVA. $P_N$ even increased in both PVA infected cultivars, while significant decline was found in PVY infected SR1 (Fig. 2*A*). Total stomatal conductance ($g_s$) decreased in both PVA and PVY infected SR1. The increase of $g_s$ found in PVY infected Samsun at the stage I was followed by significant decrease at the later stage of infection (Fig. 2*B*) contrary to non-infected and PVA infected plants. *E* followed the same course as $g_s$ with an exception of PVA infected SR1, which exhibited pronounced increase at the later stage of infection (values not shown).

Chl *a* fluorescence kinetic parameters showed statistically significant differences between both tobacco cultivars infected by PVA and PVY. Similarly as gasometric parameters, SR1 was more negatively affected than Samsun. Maximal photochemical efficiency of PS2 ($F_v/F_m$) and quantum yield ($\Phi_2$, not shown) were significantly lower in SR1 than in Samsun, particularly at the

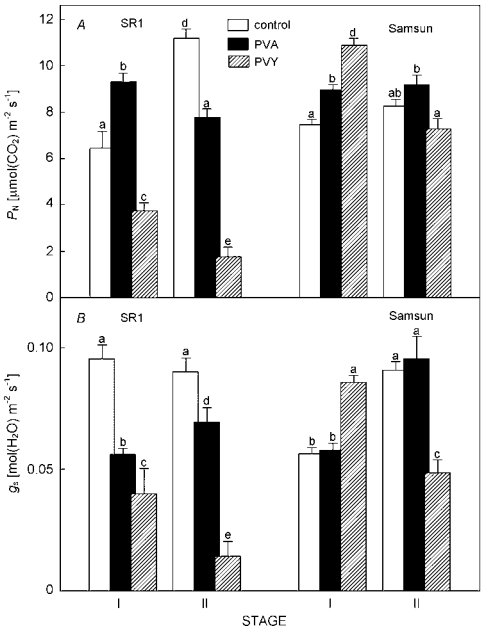


Fig. 2. Net photosynthetic rate ($P_N$; *A*) and stomatal conductance ($g_s$; *B*) of tobacco cv. Samsun and SR1 infected by PVY or PVA at two different stages of infection. Stage I, *i.e.* first visible symptoms of virosis appear on the leaves (about 5 d after inoculation by PVY and 7 d after inoculation by PVA). Stage II (about 15 d after inoculation by PVY and 18 d after inoculation by PVA). Means ± S.E. Different *lower-case letters* mark statistically significant differences at $p = 0.05$.

final stage of infection (Fig. 3*A*). PVY affected SR1 more than PVA, which was demonstrated by serious decline in $F_v/F_m$. SR1 exhibited lower non-photochemical quenching in control and both infected types in contrast to Samsun, where a decrease was found only in PVA infected plants at final stage of infection (Fig. 3*B*). The differences in the reduction status of $Q_A$ $(1 - q_P)$ between both cultivars were small and mostly insignificant, although some increase was found in control SR1 and a decrease in both infected SR1 at later stage of infection contrary to Samsun (Fig. 3*C*). Vitality index (Rfd) declined in both infected cultivars, although more pronounced decrease was found in SR1 and in PVY infected plants (Fig. 3*D*).

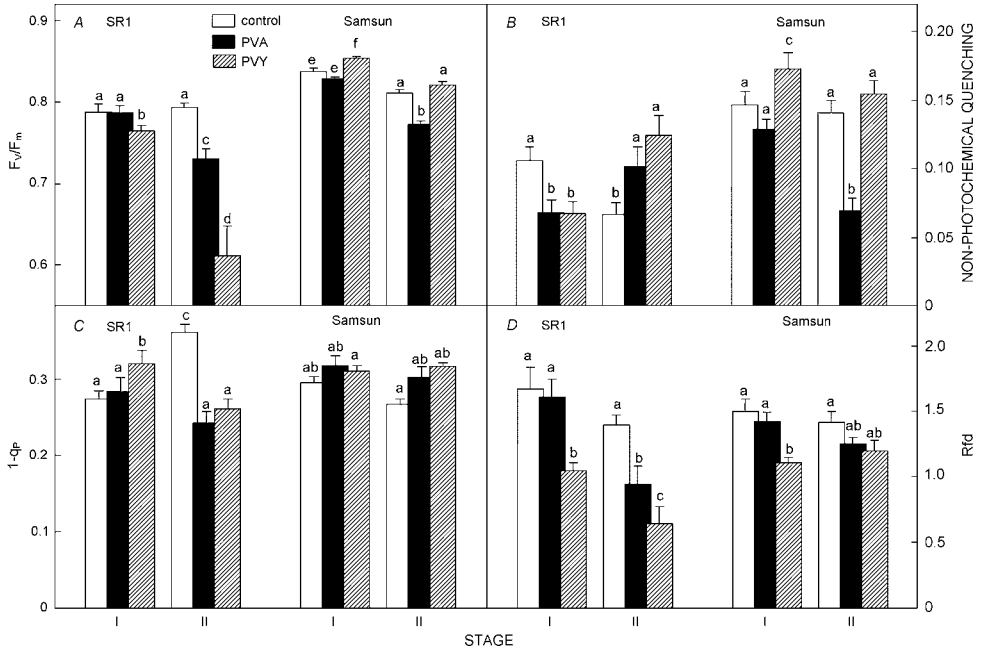


Fig. 3. Parameters of chlorophyll *a* fluorescence kinetics of tobacco cv. Samsun and SR1 infected by PVY or PVA at two different stages of infection. Stage I, *i.e.* first visible symptoms of virosis appear on the leaves (about 5 d after inoculation by PVY and 7 d after inoculation by PVA). Stage II (about 15 d after inoculation by PVY and 18 d after inoculation by PVA). Means ± S.E. Different *lower-case letters* mark statistically significant differences at $p = 0.05$.

**Enzyme activities**: The time courses of activities of PEPC, NADP-ME, and PPDK (Figs. 4 and 5) which were expressed as a percentage of activity of control healthy plants [calculated per fresh leaf matter] showed significant differences in PVY (*A,C,E*) and PVA (*B,D,F*) infected plants.

PVY infection caused an increase in the activity of all three enzymes. NADP-ME was the most sensitive to viral infection, the enzyme activity was 5 times higher in Samsun and 6-fold in SR1 as compared to control plants. The activities of PEPC and PPDK were enhanced 2–3 times in both cultivars (Fig. 4 and 5, *A* and *E*).

In contrast to PVY, PVA infection affected enzyme activities insignificantly (Figs. 4 and 5, *B* and *F*), although a moderate increase of activities was observed. No significant difference between both tobacco cultivars was found.

## Discussion

In contrast to abiotic stresses, a much more difficult task is to follow the metabolic response of plants to biotic stress, particularly due to problems with quantification of the stress factor. We used two types of viruses from the same genus which differ in the severity of their symptoms under the same conditions in tobacco (Bartels 1954, Beczner *et al.* 1984). Thus, PVY with its massive accumulation in tobacco cells seems to be a better candidate for stronger response than PVA under conditions of our experiment.

**Photosynthesis**: Photosynthetic apparatus of plants infected with PVY was more severely affected than that infected with PVA. The significant decline in $P_N$ in PVY infected SR1 was caused by stomata closure probably due to PVY action (see $g_s$, Fig. 2*B*). The decline in $P_N$ could be partly caused also by inhibition of PS2 function as shown by decline in $F_v/F_m$ and Rfd (Fig. 3*A,D*). The involvement of specific viral proteins in inhibition of the photosynthetic electron transport has been proposed frequently. Thus, in tobacco mosaic virus infected plants,

viral coat protein was essentially bound to the PS2 complex (Hodgson *et al.* 1989, Banerjee *et al.* 1995) and considered responsible for decreased PS2 electron transport. The severity of effects depends probably upon the virus type, particularly if the virus enters chloroplasts. However, intracellular virus location is not preciously known. Hlaváčková *et al.* (2002) reported (*a*) a decrease in $P_N$ and damage to the acceptor side of PS2, (*b*) a faster $Q_A$ re-oxidation in the remaining undamaged centres of PS2 caused by plum pox potyvirus in *Nicotiana benthamiana*. The latter virus was only detected in the cytoplasm. Rahoutei *et al.* (2000) showed that in pepper mild mottle virus and paprika mild mottle virus infected *Nicotiana,* both PS2 electron transport and non-photochemical quenching were affected to a different extent depending upon the virus, whereas photosystem 1 electron transport was not affected. Moreover, we found that tobacco cv. Samsun seemed to be more resistant to PVY infection, namely PS2 was less affected than SR1. More efficient non-photochemical energy dissipation seems to protect the photosynthetic apparatus of Samsun against photodamage (Figs. 2 and 3).

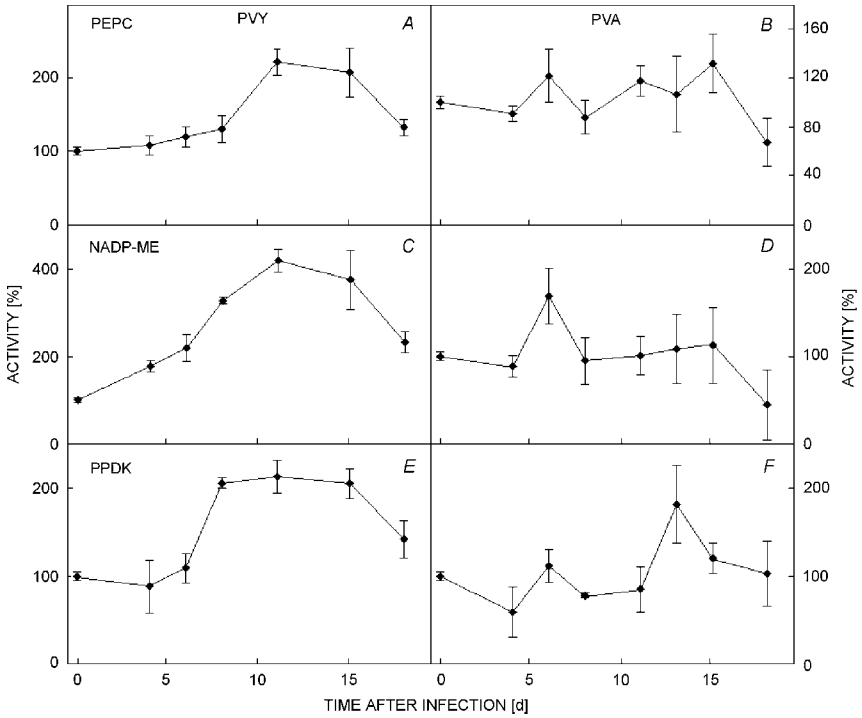


Fig. 4. Activities of PEPC, NADP-ME, and PPDK from tobacco leaves of cv. Samsun 4–18 d after inoculation by PVY (*A, C, E*) or by PVA (*B, D, F*) calculated per fresh mass. The activity of each sample is calculated as percentage of the non-infected control, where 100 % of PEPC is 1.40±0.17 μmol $s^{-1}$ $kg^{-1}$ (*A, B*), 0.50 ±0.08 μmol $s^{-1}$ $kg^{-1}$ of NADP-ME (*C, D*) and 0.58±0.01 μmol $s^{-1}$ $kg^{-1}$ of PPDK (*E, F*). Means ± S.E. In each repetition of the experiment, the activity was measured in triplicate.

**Enzymes of anaplerotic pathways**: PVY infected tobacco plants responded with enhanced activities of enzymes that catalyse anaplerotic metabolic pathways, PEPC, NADP-ME, and PPDK. The response to PVA infection was far less evident. PVA protein accumulation was much lower and interferred probably less with the metabolism of the host plant cell than PVY, which may have larger source and energy demands for its multiplication. This may cause larger involvement of anaplerotic metabolic pathways to fulfil such a need. However, it is very difficult to find any threshold point where the need is so urgent to involve additional metabolic pathways that then respond by apparent increase in respective enzyme activities.

The experiment showed that PEPC activity was only enhanced in tobacco undergoing PVY viral infection (Figs. 4 and 5). This corresponds with facts that increased activities of PEPC were found in plants with $C_3$ metabolism under abiotic or nutrition stress. Higher activity of PEPC was detected in phosphorus-deficient roots of *Lupinus albus* (Johnson *et al.* 1994). De Nisi and Zocchi (2000) found 4-fold increase of PEPC activity with altered kinetic properties of the enzyme in roots of cucumber under iron deficiency. The synthesis of malate and PEPC activity was also affected by osmotic stress caused by mannitol (Asai *et al.* 2000).

PEPC, NADP-ME, and PPDK play a key role in $C_4$ and CAM metabolism. This could represent an adaptation to "stress" conditions, *e.g.* to high temperature, high irradiance, water deficit, *etc*. Plants with $C_3$-CAM intermediate metabolism can switch their metabolic pathway to CAM under water deficit or salt stress (Taybi and

Cushman 1999).

Gradual dehydration, cold stress, osmotic stress, and high salt concentrations induce PPDK protein accumulation in rice roots but not in shoots. However, heat shock did not induce PPDK protein accumulation (Moons *et al.* 1998).

NADP-ME showed the most sensitive response to viral infection in our experiments (Figs. 4 and 5). In $C_4$ plants, this decarboxylating enzyme releases $CO_2$ from four carbon compounds into the Calvin cycle. However, increased activities of this enzyme were found also in tobacco stems before flowering, where it releases $CO_2$ from malate (Hilbert and Quick 2002). Reducing equivalent NADPH is another product of the reaction catalysed by NADP-ME, which is used for many synthetic reactions including specific defence compounds, phytoallexins (Dixon 2001). NADPH of this origin may be utilised for reduction of glutathione, which is directly involved in an inactivation of active oxygen species (Sanz *et al.* 1997, Dat *et al.* 1998). Maize roots treated with cellulase, fungal elicitors, jasmonate, and under hypoxic conditions responded by an increase of NADP-ME

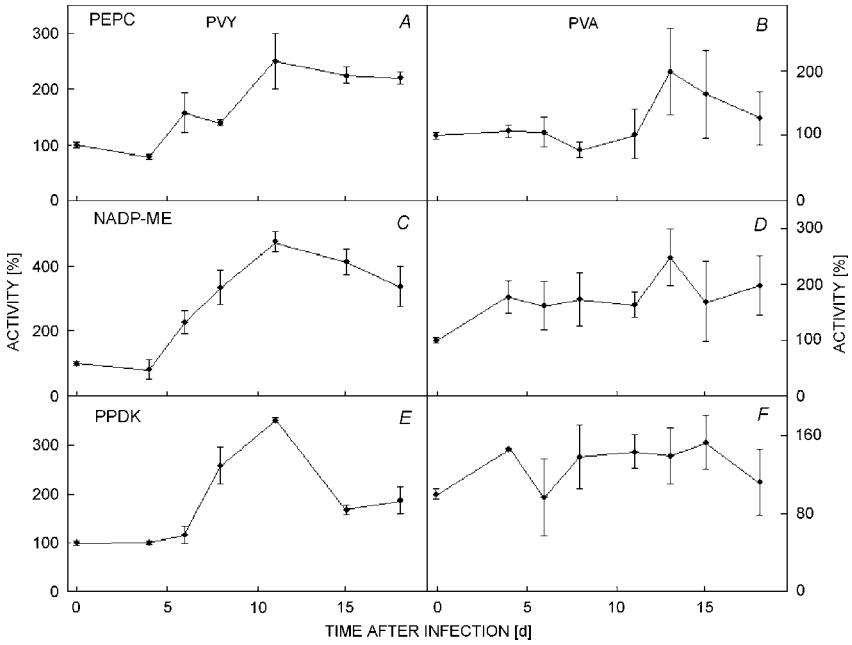


Fig. 5. Activities of PEPC, NADP-ME, and PPDK from tobacco leaves of cv. Petit Havana SR1 4–18 d after inoculation by PVY (*A*, *C*, *E*) or by PVA (*B*, *D*, *F*) calculated per fresh mass. The activity of each sample is calculated as percentage of the non-infected control, where 100 % activity of PEPC is 1. 40±0.13 μmol $s^{-1}$ $kg^{-1}$ (A, B), 0.58±0.16 μmol $s^{-1}$ $kg^{-1}$ of NADP-ME (C, D), and 0.50±0.06 μmol $s^{-1}$ $kg^{-1}$ of PPDK (*E*, *F*). Means ± S.E. In each repetition of the experiment, the activity was measured in triplicate.

activity, protein, and mRNA amounts (Maurino *et al.* 2001).

In our experiment, plants infected by PVY closed their stomata and lowered photosynthesis, which is the important source of reducing equivalents and ATP. NADP-ME could help to overcome this situation, when $CO_2$ availability was restricted by stomata closure, by releasing $CO_2$ from malate, and may also compensate for increased imbalance in NADPH.

In conclusion, our results show that the enhancement of PEPC and NADP-ME activities correlates with the development of viral infection and reduction of photosynthesis, particularly in PVY infected tobacco. In contrast to this, PVA caused only negligible changes in photosynthesis and intermediary metabolism of infected plants. No dramatic differences were found in susceptibility of both studied cultivars of tobacco.

## References

Aoyagi, K., Bassham, J.A.: Pyruvate orthophosphate dikinase in wheat leaves. – Plant Physiol. **73:** 853-854, 1983.

Asai, N., Nakaima, N., Tamaoki, M., Kamada, H., Kondo, N.: Role of malate synthesis mediated by phosphoenolpyruvate carboxylase in guard cell in the regulation of stomatal movement. – Plant Cell Physiol. **41**: 10-15, 2000.

Balachandran, S., Osmond, C.B.: Susceptibility of tobacco leaves to photoinhibition following infection with two strains of tobacco mosaic virus under different light and nitrogen nutrition regimes. – Plant Physiol. **104**: 1051-1057, 1994.

Balachandran, S., Osmond, C.B., Daley, P.F.: Diagnosis of the earliest strain-specific interactions between tobacco mosaic virus and chloroplasts of tobacco leaves *in vivo* by means of chlorophyll fluorescence imaging. – Plant Physiol. **104**: 1059-

1065, 1994a.

Balachandran, S., Osmond, C.B., Makino, A.: Effect of two strains of tobacco mosaic virus on photosynthetic characteristics and nitrogen partitioning in leaves of *Nicotiana tabacum* cv. Xanthi during photo-acclimation under two nitrogen nutrition regimes. – Plant Physiol. **104**: 1043-1050, 1994b.

Banerjee, N., Wang, J.Y., Zaitlin, M.: A single nucleotide change in the coat protein gene of tobacco mosaic virus is involved in the induction of severe chlorosis. – Virology **207**: 234-239, 1995.

Bartels, R.: Potato virus A. – CMI/AAB Descriptions of Plant Viruses No. 54, 1971.

Beczner, L., Horváth, H., Romhanyi, L., Forster, H.: Etiology of tuber ringspot disease in potato. – Potato Res. **27**: 339-351, 1984.

Čeřovská, N.: Production of monoclonal antibodies to potato virus $Y^{NTN}$ strain and their use for strain differentiation. – Plant Pathol. **47**: 505-509, 1998.

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.

Dat, J.F., Foyer, C.H., Scott, I.A.: Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings. – Plant Physiol. **118**: 1455-1461, 1998.

DeBokx, J.A., Huttinga, H.: Potato virus Y. – CMI/AAB Descriptions of Plant Viruses, No. 242, 1981.

De Nisi, P., Zocchi, G.: Phosphoenolpyruvate carboxylase in cucumber (*Cucumis sativum* L.) roots under iron deficiency: activity and kinetic characterization. – J. exp. Bot. **51**: 1903-1909, 2000.

Dixon, R.A.: Natural products and plant disease resistance. – Nature **411**: 843-847, 2001.

Filigarová, M., Čeřovská, N., Franěk, F., Dědič, P.: Reactivity of monoclonal antibodies to potato virus A in various types of enzyme linked immunosorbent assay. – Potato Res. **37**: 135-147, 1994.

Hilbert, J.M., Quick, W.P.: Characteristics of $C_4$ photosynthesis in stems and petioles of $C_3$ flowering plants. – Nature **415**: 451-454, 2002.

Hlaváčková, V., Špundová, M., Nauš, J., Navrátil, M., Kouřil, R., Kaňa, R.: Mechanical wounding caused by inoculation influences the photosynthetic response of *Nicotiana benthamiana* plants to plum pox potyvirus. – Photosynthetica **40**: 269-277, 2002.

Hodgson, R.A.J., Beachy, N.R., Pakrasi, B.H.: Selective inhibition of photosystem II spinach by tobacco mosaic virus: an effect of the viral coat protein. – FEBS Lett. **245**: 267-270, 1989.

Iglesias, A.A., Andreo, C.S.: Kinetic and structural properties of NADP-malic enzyme from sugarcane leaves. – Plant Physiol. **92**: 66–72, 1990.

Johnson, J.F., Allan, D.I., Vance, C.P.: Phosphorus stress-induced proteoid roots show altered metabolism in *Lupinus albus*. – Plant Physiol. **104**: 657-665, 1994.

Latzko, E., Kelly, G.J.: The many-faceted function of phosphoenolpyruvate carboxylase in $C_3$ plants. – Physiol. vèg. **21**: 805-815, 1983.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent. – J. biol. Chem. **193**: 265-275, 1951.

Manh, C.T., Bismuth, E., Boutin, J.P., Provot, M., Champigny, M.L.: Metabolite effectors for short-term nitrogen-dependent enhancement of phosphoenolpyruvate carboxylase activity and decrease of net sucrose synthesis in wheat leaves. – Physiol. Plant. **89**: 460-466, 1993.

Maurino, V.G., Saigo, M., Andreo, C.S., Drincovich M.F.: Non-photosynthetic, malic enzyme from maize: a constitutively expressed enzyme that responds to plant defence inducers. – Plant mol. Biol. **45**: 409-420, 2001.

Moons, A., Valcke, R., Van Montagu, M.: Low-oxygen stress and watter deficit induce cytosolic pyruvate orthophosphate dikinase (PPDK) expression in roots of rice, a $C_3$ plant. – Plant J. **15**: 89-98, 1998.

Osmond, C.B., Ramus, J., Levavasseur, G., Franklin, L.A., Henley, W.J.: Fluorescence quenching during photosynthesis and photoinhibition of *Ulva rotundata* Blid. – Planta **190**: 97-106, 1993.

Rahoutei, J., Garcia-Luque, I., Baron, M.: Inhibition of photosynthesis by viral infection: Effect on PSII structure and function. – Physiol. Plant. **110**: 286-292, 2000.

Reinero, A., Beachy, R.N.: Reduced photosystem II activity and accumulation of viral coat protein in chloroplasts of leaves infected with tobacco mosaic virus. – Plant Physiol. **89**: 111-116, 1989.

Sanz, N., Diez-Fernandez, C., Valverde, A.M., Lorenzo, M., Benito, M., Cascales, M.: Malic enzyme and glucose 6-phosphate dehydrogenase gene expression increases in rat liver cirrhogenesis. – Brit. J. Cancer **75**: 487-492, 1997.

Scheible, W.R., Krapp, A., Stitt, M.: Reciprocal diurnal changes of phospoenolpyruvate carboxylase expression and cytosolic pyruvate kinase, citrate synthase and NADP-isocitrate dehydrogenase expression regulate organic acid metabolism during nitrate assimilation in tobacco leaves. – Plant Cell Environ. **23**: 1155-1167, 2000.

Shukla, D., Ward, C.W., Brunt, A.A. (ed.): The Potyviridae. – CAB International, Wallingford 1994.

Slack, C.R., Hatch, M.D.: Comparative studies on the activity of carboxylases and other enzymes in relation to the new pathway of photosynthetic carbon dioxide fixation in tropical grasses. – Biochem. J. **103**: 660–665, 1967.

Taybi, T., Cushman, J.C.: Signaling events leading to crassulacean acid metabolism induction in the common ice plant. – Plant Physiol. **121**: 545-556, 1999.

Thomas, J.C., De Armond, R.L., Bohnert, H.J.: Influence of NaCl on growth, proline and phosphoenolpyruvate carboxyase levels in *Mesembryanthemum crystallinum* suspension cultures. – Plant Physiol. **98**: 626-631, 1992.

Vangrosveld, J., Clijsters, H.: Toxic effects of metals. – In: Farago, M. (ed.): Plants and the Chemical Elements. Biochemistry, Uptake, Tolerance and Toxicity. Pp. 150-177. VCH Weidham, New York – Basel 1994.

van Kooten, O., Meurs, C., van Loon, L.C.: Photosynthetic electron transport in tobacco leaves infected with tobacco mosaic virus. – Physiol. Plant. **80**: 446-452, 1990.

van Kooten, O., Snel, J.F.H.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. – Photosynth. Res. **25**: 147-150, 1990.