EFFECT OF *POTATO VIRUS Y* ON THE ACTIVITIES OF ANTIOXIDANT AND ANAPLEROTIC ENZYMES IN *NICOTIANA TABACUM* L. TRANSGENIC PLANTS TRANSFORMED WITH THE GENE FOR P3 PROTEIN

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Summary. The response of transgenic *Nicotiana tabacum* L. plants carrying the gene for potyviral non-structural P3 protein to *Potato virus Y* (strain NTN) infection was not significantly different from the wild type. The activities of antioxidant enzymes catalase (CAT), glutathion reductase (GR), ascorbate peroxidase (APOD) and superoxid dismutase (SOD), determined in systemically infected leaves at early stages of the infection did not change except for the transient increase in CAT and GR activities in the wild type infected plants compared to the healthy ones. At the stages, when severe symptoms of the infection developed on the leaves and the virus content reached its maximum, a significant increase in the activities of several enzymes of anaplerotic metabolic pathways, NADP-dependent malic enzyme (NADP-ME), phosphoenolpyruvate carboxylase

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(PEPC), and pyruvate orthophosphate dikinase (PPDK) in transgenic plants was found, similarly as in the wild type.

Key words: antioxidant enzymes, NADP-malic enzyme, PEPC, PPDK, *Potato virus Y*.

***Abbreviations*:** AOS-activated oxygen species, APOD-ascorbate peroxidase, CAT- catalase, DAS-ELISA-Double Antibody Sandwich Enzyme Linked Immuno Sorbent Assay, FW-fresh weight, GR-glutathion reductase, SOD-superoxid dismutase, NADP-ME-NADP-dependent malic enzyme, OAA-oxaloacetace, P3 plants-*Nicotiana tabacum* L, cv. Petit Havana, SR1 transformed with gene of protein P3 from *Potato virus A*, PEP-phosphoenolpyruvate, PEPC-phosphoenolpyruvate carboxylase, PPDK-pyruvate orthophosphate dikinase, PVY-*Potato virus Y*, SR1 plants-*Nicotiana tabacum* L, cv. Petit Havana, SR1, TMV-*tobacco mosaic virus.*

INTRODUCTION

Plants must continuously defend themselves against changing and often harmful environmental conditions. One of the factors which affect plants in their environment, is biotic stress that results from a battery of potential pathogens such as fungi, bacteria, nematodes, viroids and viruses (Dangl and Jones, 2001) Viral diseases are one of the main causes for decreased crop productivity world wide (Arias et al., 2003). *Potato virus Y*, strain NTN (PVY^{NTN}) invoking necrotic lesions on the host plant *Nicotiana tabacum* L., is a member of the genus *Potyvirus* (family *Potyviridae*), the largest and most destructive group of plant viruses (Shukla et al., 1994). Although plants respond to pathogens in a variety of ways, their resistance always correlates with the activation of diverse sets of certain defence mechanisms. The response involves transcriptional activation of numerous defence-related genes, opening of ion channels, modification of protein phosphorylation status, and activation of preformed enzymes to undertake specific modifications of primary and secondary metabolism. In addition, a range of secondary signalling molecules is generated to ensure coordination

of the defence response both temporally and spatially, resulting in rapid containment of the pathogen (Hammond-Kosack and Jones, 2000).

Plant-virus interaction may result in a host hypersensitive response or in systemic symptoms (Arias et al., 2003). One of the earliest responses of plant cells to pathogens is the production of activated oxygen species (AOS) (Mehdy et al., 1996). The typical AOS detected are superoxide radicals ($O_2^{\bullet}$) and hydrogen peroxide (H_2O_2) (Hammond-Kosack and Jones, 2000). AOS play a crucial role during pathogenesis. They are involved in the hypersensitive response typical for plant-pathogen incompatible interactions. They can limit the spread of pathogen by strengthening plant cell walls and/or by killing pathogens directly (Dat et al., 2000). However, AOS act as cytotoxic compounds, too. Plants have evolved complex antioxidant systems in order to protect cellular membranes and organelles from the damaging effects of AOS (Lee and Lee, 2000). Antioxidant enzymes and metabolites are located in different plant cell compartments to fulfil their protective function. The key enzymes, superoxide dismutases (EC 1.15.1.1; SODs), are a family of metalloenzymes catalyzing the dismutation of $O_2^{\bullet}$ to H_2O_2. SODs can be found in chloroplasts, mitochondria, peroxisomes, and in cytoplasm. Catalases (EC 1.11.1.6; CATs), heme proteins that catalyze the removal of H_2O_2, are located in peroxisomes. Enzymes and metabolites of the ascorbate-glutathione cycle (ascorbate peroxidase (APOD), EC 1.11.1.11; glutathione reductase (GR), EC 1.8.1.7; monodehydroascorbate reductase (DHAR), EC 1.6.5.4), which is important in H_2O_2 scavenging, are located in organelles and cytoplasm (Bartosz, 1997; Dat et al., 2000; Lee and Lee, 2000). Antioxidant enzymes were often studied at sites of attempted pathogen attack and in connection with immediate responses of invaded cells (Wojtaszek, 1997). The activity of SOD and guaiacol peroxidase increased under blight conditions (caused by *Phytophthora colocasiae*) in taro (*Colocasia esculenta* L. Schott) (Sahoo et al., 2007). Simultaneous overexpression of both CuZn SOD and APOD in transgenic tall fescue plants confers increased tolerance to a wide range of abiotic stresses (methyl viologen, H_2O_2 and heavy metals) (Lee et al., 2007). Enhanced activities of peroxidase, APOD, SOD and CAT were found in rice in response to infection caused by *Rhizoctonia solani* (Paranidharan et al., 2003).

Our present study focused on systemic responses at later infection stages. Anaplerotic enzymes can redirect the flux of intermediates in primary and secondary metabolism, thus regulating plant metabolism under stress conditions. NADP-malic enzyme (NADP-ME; EC 1.1.1.40), phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31), and pyruvate orthophosphate dikinase (PPDK; EC 2.7.9.1) are known as photosynthetic enzymes in C4 plants (e.g. maize, sorghum), but in C3 plants (i.e. tobacco) these enzymes play diverse functions. One of them, in addition to maintaining intracellular pH, is production of reduction equivalent NADPH. Generally, the demand for NADPH is probably higher in infected cells especially for anabolic processes requiring NADPH, such as biosynthesis of lipids, protein turnover and synthesis of specific plant defence compounds as phytoalexins (Edwards and Andreo, 1992). Transgenic plants containing viral proteins can modulate the sensitivity of the plant to viral infection due to gene silencing. It is well documented for viral coat protein (Goldbach et al., 2003). The function of potyviral non-structural P3 protein remains unknown. Immunological studies showed that it was localized either in the cytoplasm or in the nucleus of infected cells (Langenberg and Zhang, 1997; Rodríguez-Cerezo et al., 1993). P3 is believed to play a role in virus replication (Guo et al., 2001; Merits et al., 1998), in cell-to-cell movement (Johansen et al., 2001), as a protease cofactor (Riechmann et al., 1992). In addition, it can play an important role in some highly specific process, e.g. in the virus-host interaction. Although the functions of the P3 protein in the potyviral infection are not clear, interactions with products of plant resistance genes have been indirectly demonstrated (Jenner et al., 2003; Johansen et al., 2001). Therefore, it is possible that P3 protein affects some enzymes included in plant defence reactions.

The aim of the present work was to study the effect of viral infection on the activities of antioxidant and anaplerotic enzymes in upper, systemically infected leaves of transgenic *Nicotiana tabacum* L. plants transformed with the non-structural P3 gene of *Potato virus A* (PVA) in comparison with the wild type.

MATERIALS AND METHODS

Plant material

Transgenic plants of *Nicotiana tabacum* L. cv. Petit Havana SR1 with introduced one copy of the PVA *P3* gen (P3 plants) were prepared in the Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia (Nováková et al., 2005; Jљubr et al., 2006). These plants as well as the non-transgenic controls-wild type *Nicotiana tabacum* L. cv. Petit Havana SR1 (SR1 plants) were grown in a greenhouse at 22/18 °C day/night temperatures. Seeds were sown in pots with sand and plantlets were transferred to pots with soil after 3 weeks. Leaves of 50 seven-week-old plants were infected with the virus PVY^{NTN}. Virus isolate PVY^{NTN} (Lebanon) was kindly provided by Dr. P. Dědič (Institute of Potato Research, Havlíčkův Brod, Czech Republic) as frozen symptomatic leaves. For virus inoculation, those leaves were homogenized (1/10 w/v) in 0.057 M Na_2HPO_4 buffer (pH 8) and mechanically inoculated on the adaxial surface of the bottom of mature leaves using carborundum mesh 600 as abrasive.

Two groups of controls were used for measuring the activities of the antioxidant enzymes. The first one consisted of 50 healthy, non-inoculated plants while the second one included 50 mock-inoculated (buffer and carborundum) plants. Mixed samples from upper, systemically infected leaves were collected after 2, 12, and 24 h, and then each 3-4 following days from whole mature leaves, placed above those used for the inoculation. Samples were immediately frozen in liquid N_2 and stored at –80 °C. The extent of viral infection was determined by DAS-ELISA (Clark and Adams, 1977) in homogenates of the leaves of infected plants using polyclonal antibodies raised against the respective pathogens (Čeřovská, 1998).

Enzyme activity assays

For enzyme activity assays, 1 g of leaf material was homogenized in 3 ml 100 mM Tris-HCl (pH 7.8) containing 1 mM dithiothreitol, 1 mM EDTA and 5 mM $MgCl_2$ (buffer A); then 0.02 g of polyvinylpolypyrrolidone was added and the homogenate was centrifuged at 23 000 *g* for 15 min at 4 °C.

The supernatant was immediately used for enzyme activity measurements and native electrophoretic separations (applied in 20 % sucrose).

Antioxidant enzymes (CAT, GR, APOD)

Catalase was detected at 240 nm as the rate of decomposition of H_2O_2 as described by Aebi (1984). Glutathione reductase activity was assayed by the oxidation increase of NADPH at 340 nm according to Goldberg and Spooner (1984) Total ascorbate peroxidase activity was determined as the decrease in absorbance of ascorbate at 298 nm by the method of Gerbling et al. (1984).

Enzymes of anaplerotic pathways (NADP-ME, PEPC, PPDK)

The activities of all enzyms were determined spectrophotometrically at 25 ^{o}C by monitoring NAD(P)H production or depletion at 340 nm and activities were calculated as [μmol (substrate/product).min^{-1}.g^{-1} (fresh weight)].

The NADP-ME assay mixture contained 100 mM Tris-HCl buffer (pH 7.4), 10 mM malate, 2 mM $MgCl_2$ and 0.2 mM $NADP^+$ in total volume of 1 ml (Iglesias and Andreo, 1990). The PEPC 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 a total volume of 1ml (Slack and Hatch, 1967). The PPDK assay mixture contained 100 mM Tris-HCl buffer (pH 8.1), 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 ml (Aoyagi and Bassham, 1983).

Electrophoretic separation

Native gel electrophoresis was performed according to Lee and Lee (2000).

Detection of SOD

The patterns and activities of superoxide dismutase (SOD) isoenzymes

were obtained after separation by gradient 7 - 14 % non-denaturing polyacrylamide gel electrophoresis (PAGE). Aliquots of supernatants corresponding to 25 µg of protein per lane were used. SOD isoenzymes were detected *in situ* in the gel by the photochemical nitrobluetetrazolium (NBT) staining method according to Beauchamp and Fridovich (1971). For identification of Cu/Zn-, Mn-, and Fe-SOD isoenzymes the inhibition by 2 mM KCN and 5 mM H_2O_2 was used prior to placing the gels into the staining solution. KCN inhibited Cu/Zn-SOD while H_2O_2 inactivated both Cu/Zn-SOD and Fe-SOD.

Protein content determination

Protein content was determined by the method of Bradford using bovine serum albumin as a standard (Bradford, 1976).

RESULTS

PVY^{NTN} infection

The spread of PVY^{NTN} infection was followed by the development of necrosis and determined by DAS-ELISA. The development of the symptoms was identical in both wild type and transgenic plants. Typical vein necrosis followed by leaf distortion and deformation was observed from the 9^{th} – 12^{th} day of the infection. The relative content of PVY^{NTN} according to DAS-ELISA was rapidly enhanced from the 7^{th} day of the infection in infected transgenic plants and from the 10^{th} day in the wild type plants (Table 1).

Antioxidant enzymes

Firstly, we studied the antioxidant enzymes, GR, APOD, CAT and SOD. These enzymes are known to be involved in an immediate plant defence response. Samples for activity measurements of the antioxidant enzymes were collected (a) during the early stage of the infection (2, 12 and 24 h after inoculation), and (b) when the first visible symptoms of the virus infection appeared on the leaves (10 days after PVY^{NTN} inoculation).

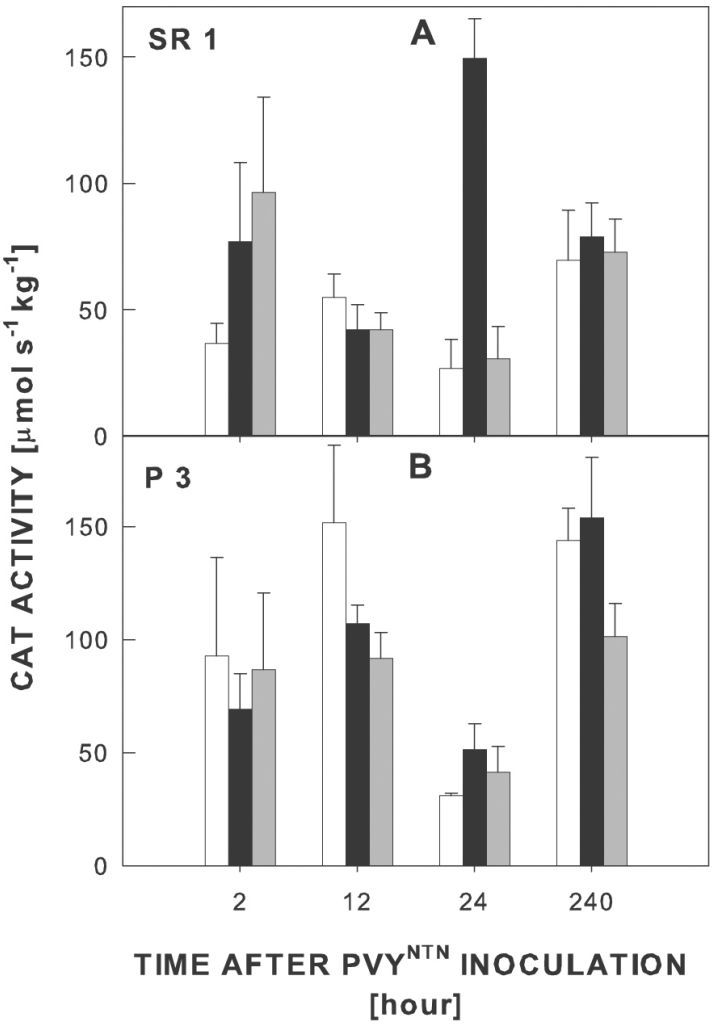

Fig. 1. Activities of CAT from *Nicotiana tabacum* L., SR1 leaves (A) and from leaves of transgenic *Nicotiana tabacum* L., SR1 transformed with the gene for P3 protein (B) 0-240 hours after inoculation by PVY^{NTN} calculated per fresh mass. Activity of control healthy plants - □, activity of mechanically mock-inoculated - ▨, and activity of PVY^{NTN} infected plants - ■. The activities were measured in triplicate. The means ± SE are shown.

The activities of CAT, GR and APOD were measured spectrophotometrically (Fig. 1, 2, 3), whereas the activities of the particular isoforms of SOD were monitored in the gel (Fig. 4).

The changes in the antioxidant enzyme activities at early stages of the infection were insignificant particularly in the transgenic P3 plants infected with PVY^{NTN}. The only exception was CAT activity which was 5-fold higher in the PVY^{NTN} infected SR1 already 24 h after inoculation (Fig. 1A) and 1.5-fold higher in the infected P3 plants compared to the healthy plants (Fig. 1B).

GR activity increased significantly 2 h after the inoculation in PVY^{NTN} infected SR1 (Fig. 2A), while a decrease was observed in P3 infected plants (Fig. 2B). The increase of GR activity was apparent in both types of infected plants (up to 120 %) 10 days after the inoculation (Fig. 2A, B).

APOD activity showed a similar time-course as GR activity. An increase

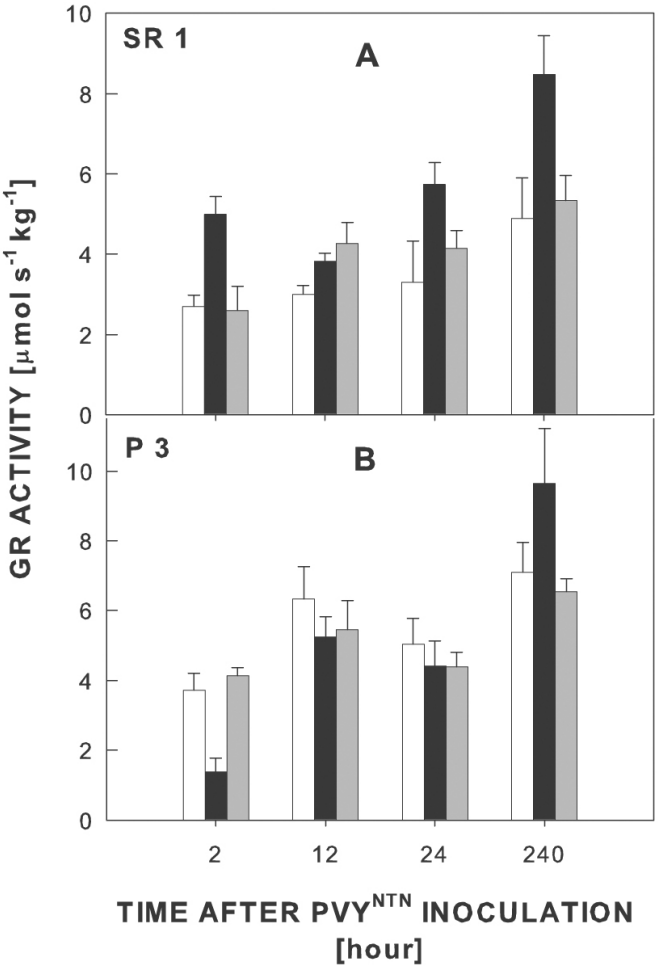

Fig. 2. Activities of GR from *Nicotiana tabacum* L., SR1 leaves (A) and from leaves of transgenic *Nicotiana tabacum* L., SR1 transformed with the gene for P3 protein (B) 0-240 hours after inoculation by PVY^{NTN} calculated per fresh mass. Activity of control healthy plants - □, activity of mechanically mock-inoculated plants - ▨, and activity of PVY^{NTN} infected plants - ■. The activities were measured in triplicate. The means ± SE are shown.

was observed 2 h after the inoculation in the infected SR1 and then 10 days after the inoculation (Fig. 3A), while in P3 plants (Fig. 3B) no significant differences among control, mock-treated and infected plants were found, although generally APOD activity increased in all plants at a later stage of the infection (Fig. 3).

The activity of SOD was specifically detected after non-denaturating electrophoresis (Fig. 4). Total SOD activity represents the combined action of Cu/Zn-, Mn- and Fe-SOD. Using KCN to inhibit Cu/Zn-SOD (Fig. 4C) or H_2O_2 to inactivate both Cu/Zn-SOD and Fe-SOD (Fig. 4D), SOD isoforms were identified. As shown in Fig. 4, one isoform of SOD in SR1 and transgenic P3 plants was identified as Mn-SOD, one isoform as Fe-SOD and two isoforms as Cu/Zn-SOD. No changes in the isoenzyme content between control healthy plants and PVY^{NTN} infected plants, and between transgenic P3 plants and wild type plants were found (Fig. 4). The

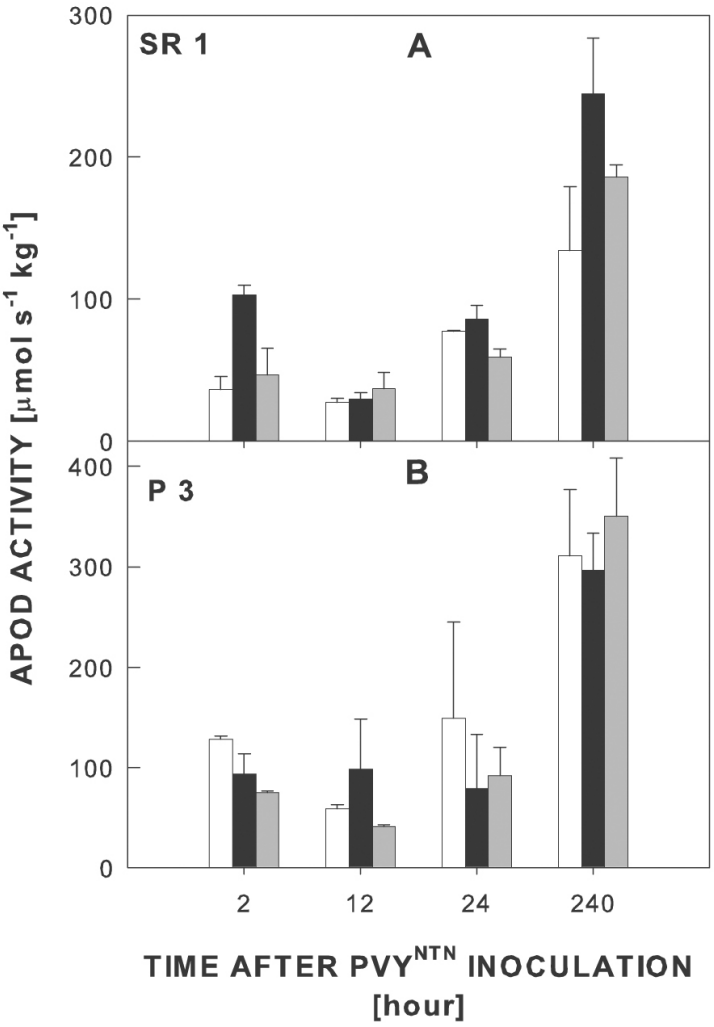

Fig. 3. Activities of APOD from *Nicotiana tabacum* L., SR1 leaves (A) and from leaves of transgenic *Nicotiana tabacum* L., SR1 transformed with the gene for P3 protein (B) 0-240 hours after inoculation by PVY^{NTN} calculated per fresh mass. Activity of control healthy plants - □, activity of mechanically mock-inoculated plants - ▨, and activity of PVY^{NTN} infected plants - ■. The activities were measured in triplicate. The means ± SE are shown.

difference was apparent between the 10th day of the infection, when the activity of SOD was much higher than on the 2nd day of PVY^{NTN} infection (Fig. 4A, B). It could be related to senescence of the plant.

Enzymes of anaplerotic pathways

We focused our attention on NADP-ME, PEPC, and PPDK, the enzymes of anaplerotic metabolic pathways, which seem to play a very important role in plants under stress conditions (Ryšlavá et al. 2003). The time course of enzymatic activities in control (healthy), PVY^{NTN} infected and transgenic plants during the 25-d period of infection is shown in Fig.5. NADP-ME, PEPC and PPDK significantly increased from the 10th day of PVY^{NTN} infection in leaves of infected SR1 and transgenic P3 plants as compared to the healthy controls (Fig. 5). The highest activities of NADP-

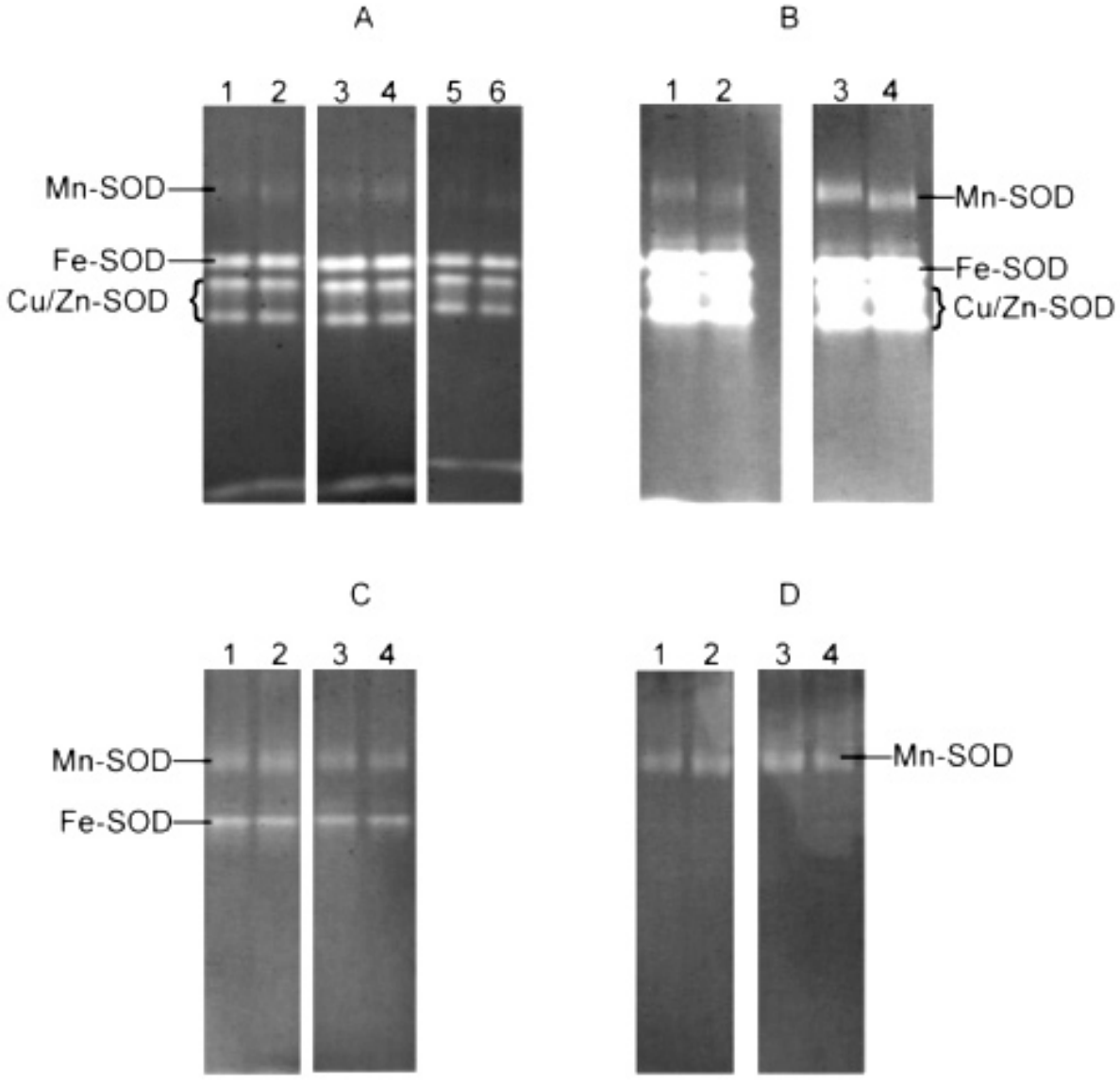

Fig. 4. Specific detection of SOD after non-denaturating electrophoresis on the 2nd (A) and 10th (B) day of PVY^{NTN} infection and identification of SOD isoforms in the presence of 2 mM KCN which inhibited Cu/Zn-SOD (C) and in the presence of 5 mM H_2O_2 which inactivated both Cu/Zn-SOD and Fe-SOD (D).
1 - healthy control leaves from *Nicotiana tabacum* L., SR1
2 - infected leaves from *Nicotiana tabacum* L., SR1
3 - healthy control leaves from transgenic *Nicotiana tabacum* L., SR1 plants carrying the gene for P3 protein
4 - infected leaves from transgenic *Nicotiana tabacum* L., SR1 plants carrying the gene for P3 protein
5 - mechanically mock-inoculated leaves from *Nicotiana tabacum* L., SR1
6 - mechanically mock-inoculated leaves from *Nicotiana tabacum* L., SR1 plants carrying the gene for P3 protein

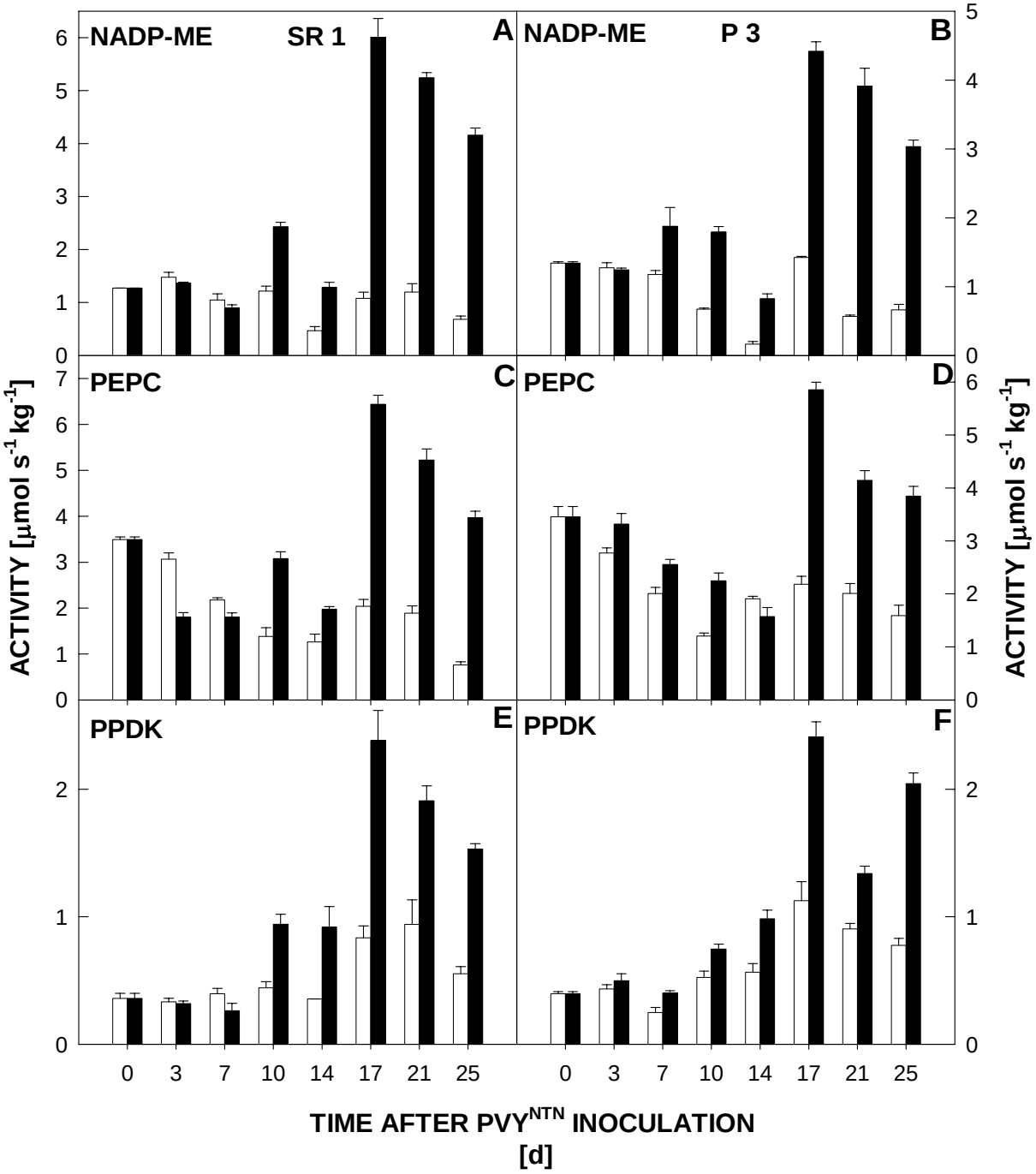

Fig. 5. NADP-malic enzyme, phospho*enol*pyruvate carboxylase and pyruvate orthophosphate dikinase.
Activities of NADP-ME, PEPC and PPDK from *Nicotiana tabacum* L., SR1 leaves (A, C, E) and from leaves of transgenic *Nicotiana tabacum* L., SR1 transformed with gene for protein P3 (B, D, F) 0-25th day after inoculation by PVY^{NTN} calculated per fresh mass. The activity of control healthy plants is shown as □ , the activity of PVY^{NTN} infected plants as ■. The activity was measured in triplicate; S.E. are shown.

	A (405 nm)	
DAY OF PVY^{NTN} INFECTION	*Nicotiana tabacum* L., SR 1	Transgenic *Nicotiana tabacum*- P3
3.	0.010	0.001
7.	0.003	0.214
10.	0.454	0.128
17.	0.389	0.185
21.	0.227	0.112

Table 1. DAS-ELISA. The relative content of virus PVY^{NTN} in infected *Nicotiana tabacum* L., SR1 plants and in infected transgenic *Nicotiana tabacum* L., SR1 with gene for protein P3 was determined by DAS-ELISA with p-nitrophenylphosphate as a substrate. Absorbance at 405 nm corresponds to virus content.

ME, PEPC and PPDK were obtained on days 17, 21 and 25 of the infection in both groups of infected plants (Fig. 5). NADP-ME was the most sensitive enzyme to PVY^{NTN} spread. The activity of NADP-ME was 6 times higher in SR1 plants and also in transgenic P3 plants within the last 8 days of the infection, when severe symptoms of virosis were visible (Fig. 5A, B).

The activity of PEPC increased from 10th day of the PVY^{NTN} infection up to 520 % in infected SR1 plants and up to 270 % in infected transgenic P3 plants (Fig. 5C, D). A similar response was observed with PPDK. This enzyme was most enhanced on the 7th and 25th days of the infection. The increase was slightly higher (up to 280 %) in infected SR1 plants (Fig. 5E) than in infected P3 plants (Fig. 5F) (up to 260 %).

DISCUSSION

Virus infection

Tobacco plants transformed with potyviral *P3* gene may be resistant against the homologous virus, as shown by Moreno et al. (1998). The P3 plants used in our experiments showed no resistance against PVA, although the P3 protein was immunologically proved in them (Nováková et al., 2005; 2006). However, a slight infection delay was observed in comparison with

non-transgenic plants (Nováková, personal communication). With PVY^{NTN} we did not detect such a delay but the virus concentration according to ELISA was generally lower in P3 plants than in SR1 plants although no time or severity differences in symptoms formation were recorded.

Antioxidant enzymes

Infection of plants by necrogenic pathogens often results in enhanced protection against secondary infection not only in the inoculated leaves but also systemically, i.e. in the healthy leaves located above the inoculated site. The biochemical mechanisms of this effect are still poorly understood, even when phenomenon of local acquired resistance and systemic acquired resistance have been thoroughly studied for many years (Ryals et al., 1996). In our study, we have focused our attention on several antioxidant enzymes, which play a crucial role in the metabolism of H_2O_2 that seems to play a central role in oxidative burst. It acts as a signal for localized death of challenged cells (i.e. hypersensitive reaction) and as a diffusible signal for the induction of a cellular protectant in adjacent cells (Levine et al., 1994). As we were interested particularly in the systemic response, we followed only the systemic response to the PVY^{NTN} infection in the leaves located above the inoculation site.

Surprisingly, a transient increase of the activities of GR and APOD was observed already 2 h after the inoculation in the infected SR1 plants. This effect was only temporary but significant and it seemed to respond to virus inoculation rather than to mock-treatment. Substantial enhancement was observed 10 d after the inoculation. A transient increase was found also in CAT activity 24 h after the inoculation. Later on, no differences among healthy control, mock-treated and infected plants were observed in CAT activity. This is in agreement with previous findings of other research groups (Hernández et al., 2001; Fodor et al., 1997). They found no changes in tobacco infected by TMV (Fodor et al., 1997) or rather a decrease in CAT activity in apricot infected by *plum pox* virus (Hernández et al., 2001). Fodor et al. (1997) found also a transient decline in APOD, GR, and SOD activities preceding the appearance of symptoms, but a substantial increase of these activities after the onset of necrosis in the inoculated leaves. In

the upper leaves, the glutathione level and the activities of GR and SOD increased 10-14 d after the inoculation concomitantly with the development of systemic acquired resistance. We did not find any substantial changes in SOD isoenzymes pattern evoked by PVY^{NTN} infection, although the activities of all isoenzymes were significantly higher compared with non-infected controls.

In transgenic P3 plants, an early systemic response to the inoculation was not observed and no significant changes in antioxidant enzyme activities were found. Nevertheless, the infected P3 plants exhibited a similar increase in GR activity as SR1 plants 10 days following the inoculation.

Enzymes of anaplerotic metabolic pathways

In the maximum of the infection, when the symptoms were fully developed and the virus proteins were immunochemically detected, enhanced activities of anaplerotic enzymes (NADP-ME, PEPC, PPDK) in the infected plant leaves were found (Fig. 5). These enzymes catalyse reactions connecting primary metabolism and thus redirect the metabolic flow which could be advantageous for the plant under non-physiological or stress conditions. PEPC can connect the metabolism of saccharides and amino acids and proteins. Under stress conditions the glycolytic degradation of storage polysaccharides and the synthesis of proteins including "pathogenesis-related proteins" is enhanced. The reaction catalysed by NADP-ME provides reduction equivalents NADPH for biosynthetic purposes, such as lipids and lignin for fortification of cell wall, specific defence compounds, phytoalexins or substrates for antioxidant enzymes (Edwards and Andreo, 1992). All these functions of NADPH could be important for the plant under viral infection. PPDK catalyses the production of phosphoenolpyruvate from pyruvate and so yields a substrate for PEPC. The described reactions could form a cycle inside one cell analogous to the cycle present in mesophyll and bundle sheeth cells in C_4 plants. The significance of such a cycle in plants under biotic stress caused by viral infection could be the production of NADPH at the expense of ATP and NADH. CO_2 released after malate decarboxylation can be used for photosynthetic fixation via Calvin cycle, which can be important when the stomata are closed.

We can summarize that enhanced activities of the monitored anaplerotic enzymes were found in systemically infected leaves, however, enhanced activities of antioxidant enzymes were measured not only in infected plants, but also in mock-inoculated and healthy plants. These results were obtained for both groups of tobacco plants - control and transgenic plants carrying the gene for P3 protein. The increased metabolic flow through pathways catalyzed by PEPC, NADP-ME and PPDK seemed to be advantageous for plants under biotic stress, while the enhanced activities of the antioxidant enzymes were related more likely to plant senescence.

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