BIOLOGIA PLANTARUM 37 (3): 423-428, 1995

Changes in glucose-6-phosphate dehydrogenase and ribonucleases activities during PVY-RNA biosynthesis in infected potato plants

L. BURKETOVÁ

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

Abstract.

Changes in glucose-6-phosphate dehydrogenase, ribonucleases activities and chlorophyll content were studied in leaves of plants systemically infected by potato virus Y, necrotic strain (PVY^N). Potato cultivars Jara and Adretta differing in resistance to potato virus Y were used. No statistically significant differences were observed between healthy and infected plants of both cultivars in chlorophyll content. Activity of glucose-6-phosphate dehydrogenase slowly increased in connection with virus multiplication and reached 203.4 % of the values of non-infected control in susceptible cv. Jara and 160.4 % in the resistant cv. Adretta. Differences between cultivars were significant from 60 d after inoculation ($P \leq 0.05$). The activity of ribonucleases quickly increased in the initial period of the experiment and then slowly decreased. Their activities reached 195.6 % in susceptible cultivar and 183.5 % in the resistant one. Significant differences ($P \leq 0.01$) between susceptible and resistant cultivars was found from 18 to 35 d after inoculation. The activities of enzymes corresponded to PVY^N multiplication which was since 40 d considerably higher ($P \leq 0.01$) in susceptible cultivar in comparison with the resistant one. Thus the activities of studied enzymes could be considered as markers of resistance of potato cultivars to PVY^N multiplication.

Key words: chlorophyll, potato virus Y, *Solanum tuberosum*.

Introduction

According to previous studies (Šindelář *et al.* 1990, Šindelářová *et al.* 1990), three metabolic ways participate in the utilization of precursors necessary for viral RNA multiplication. Virus RNA can be syntesized either from intermediates of the reductive pentose phosphate pathway during photosynthesis or by the oxidative pentose pathway that uses both free and storage carbohydrates (mainly in the dark)

Received 30 September 1994, *accepted* 20 March 1995.

and/or from nucleotides released from the host rRNA by ribonucleases. The preference of individual metabolic ways depends on the particular host-virus combination and environmental conditions (Šindelář *et al.* 1987).

Changes in activities of enzymes involved in the oxidative pentose phosphate pathway induced by viral infections were systematically investigated (*e.g.* Solymosy and Farkas 1962, Simons and Ross 1971, Huth 1973). The linear correlation between the activity of glucose-6-phosphate dehydrogenase (rate-limiting enzyme of this pathway) and the reproduction curve of PVY was found in *Nicotiana tabacum* cv. Samsun (Šindelář 1986) and in potato (Šindelář *et al.* 1990).

Increased activities of host ribonucleases during virus infections were reported by many authors. However, this increase can be elicited also by other pathogens and even by mechanical injury of plant tissues (Diener 1961). The linear relationship between the increased activity of this complex of enzymes and virus multiplication was established by Šindelářová *et al.* (1990) and Šindelář *et al.* (1988, 1990).

The aim of present paper is to determine the participation of the oxidative pentose pathway and ribonucleases in PVY^N-RNA biosynthesis in whole potato plants. The use of the cultivars with different resistance to PVY should contribute to the explanation of metabolic bases of plant resistance to virus multiplication.

Materials and methods

Experimental plants: Two cultivars of potato (*Solanum tuberosum* L.) differing in their relative resistance to the potato virus Y (susceptible cv. Jara and resistant cv. Adretta) were used for the experiments.

The isolate of potato virus Y, necrotic strain (PVY^N) was kindly provided by Dr. Nohejl from the Potato Research Institute at Havlíčkův Brod.

***In vitro* cultivation and induction of tuberization:** Nodal segments of *in vitro* cultivated potato plants on Murashige and Skoog (1962) basal medium (temperature 22 °C, photoperiod 16 h, irradiance 30 µmol m^{-2} s^{-1}) were placed on tuber-inducing medium which consisted of MS medium supplemented with 8 % (m/v) agar. After 4 weeks plants were transferred to tuber inducing regime (14 - 16 °C, dark) for 3 - 6 months and then minitubers were harvested and stored at 4 °C.

Plant cultivation and inoculation: Potato minitubers were cultivated in pots with soil in a glasshouse. After 4 - 6 weeks one leaf of medium insertion of each plant was dusted with carborundum and mechanically inoculated with sap from PVY infected *Nicotiana tabacum* L. cv. Samsun plants. The sap was obtained by grinding leaves in 0.01M potassium phosphate buffer, pH 7.0, in ratio 1:10 (m/v). Control leaves were treated with distilled water in the same way.

Samples collection and homogenates preparation: Samples of systemically infected leaves (0.5 g) were collected. The enzyme activities and chlorophyll content were immediately measured. For the detection of PVY^N the leaf tissue samples (0.5 g) were frozen at -18°C and stored until the analysis by DAS-ELISA.

Crude homogenates for determination of enzyme activities and chlorophyll content was prepared by grinding fresh leaf tissue at 0 - 4°C with a mortar and pestle in 20mM Tris-HCl buffer (pH 7.0) containing 1 mM EDTA, 30 mM 2-mercapto-ethanol, 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 estimation of enzyme activities extract was centrifuged for 10 min at 20 000 *g*.

Determination of enzymes activities and chlorophyll content: 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 (*Beckman, DU-6*). 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 0.05 cm^3 of homogenate (11 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 300 µl 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 0.05 cm^3 of homogenate. After 30 - 60 min incubation ezymatic reaction was stopped by addition of 3 cm^3 precipitation solution, the mixture was left for 1 h at 0 °C, centrifuged for 10 min at 5 000 *g* and the amount of degradated RNA in the supernatant was spectrophotometrically determined at 260 nm. One unit of RNase activity was arbitrarily characterized as a change in 260 nm absorbance by the value of 1000 per hour.

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

PVY content: The double antibody sandwich microplate ELISA (DAS-ELISA) procedure (Clark and Adams 1977) was used. IgG and conjugate with alkaline phosphatase were obtained from Institut für Phytopathologie, Aschersleben, Germany. Optimum dilution of gamma globulins for coating plates was 1:1000 (v/v) and 1:500 (v/v) for the conjugate.

Leaf tissue samples were ground with a mortar and pestle in extraction buffer PBS-Tween with 2 % polyvinyl pyrrolidone of M_r 25 000 (*Serva Feinbiochemica GmbH*, Heidelberg, Germany) and 0.2% Ovalbumin (*Sigma Chemical Co*., St. Louis, USA) with fine silica sand in ratio 1:10 (m/v). The same buffer was used for dilution of conjugate. P-nitrophenyl phosphate (*Serva*) in 10% dietanolamine buffer (pH 9.8) was used as a substrate and absorbance values at 405 nm were measured.

Curves expressing enzyme activities, chlorophyll and virus contents were obtained by means of the method of the least squares in the form of a polynom:

$$y = a_1 + a_2x + a_3x^2 + ... a_nx^{n-1}$$

using the data obtained in three independent experiments. The levels of statistical significance are expressed by *P* level obtained in *t*-test.

Results

The virus content within the plants of both cultivars gradually increased. PVY^N synthesis was since 40 d significantly higher ($P \leq 0.01$) in susceptible cv. Jara in comparison with the resistant cv. Adretta (Fig. 1.).

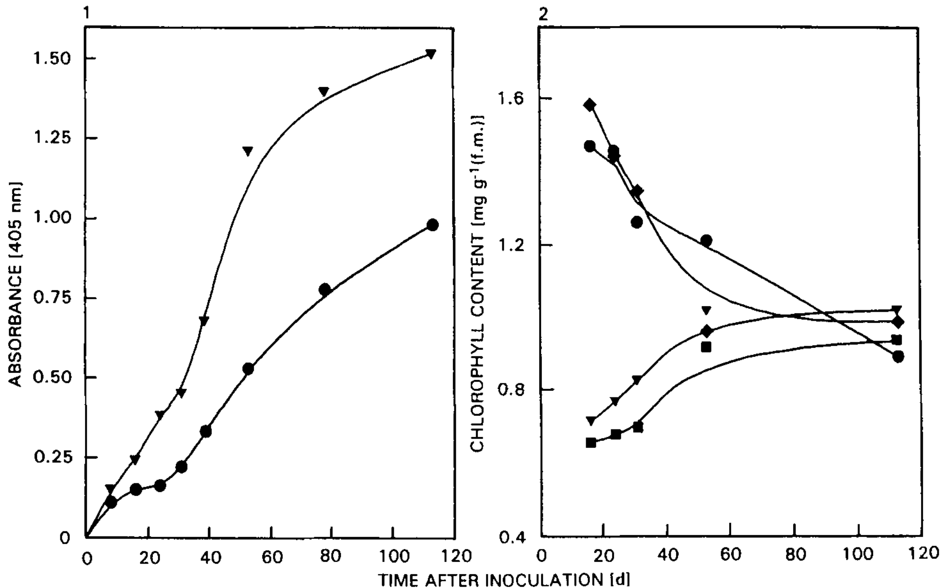

Fig. 1. Reproduction curve of potato virus Y, necrotic strain, in systemically infected leaves of resistant cv. Adretta (*circles*) and susceptible cv. Jara (*triangles*) determined by ELISA.

Fig. 2. Chlorophyll content in the noninfected control and potato virus Y infected plants of both resistant cv. Adretta (*rhombs* - control, *circles* - infected) and susceptible cv. Jara (*squares* - control, *triangles* - infected).

No significant changes in *chlorophyll content* occured between the healthy and infected plants (Fig. 2). The differences between cultivars were statistically significant ($P \leq 0.01$) from 18 to 35 d of experiment. In the susceptible cultivar the chlorophyll content gradually increased, on the other hand it dropped to 66.2 % of initial amount in the resistant one.

The glucose-6-phosphate dehydrogenase activity in infected plants was similar to the value of noninfected control during first six weeks (Fig. 3A). After this period activity of this enzyme increased and reflected developing PVY^N infection; the differences between resistant and susceptible cultivar became evident from 60 d after inoculation ($P \leq 0.05$) and the activity reached 203.4 % in susceptible cultivar and 160.4 % in resistant one of the values of non-infected control at the end of the experiment.

Activities of ribonucleases (Fig. 3B) rapidly increased in the period of the acute phase of infection and the differences between cultivars were pronounced two weeks after inoculation. The ribonucleases activities reached 195.6 % of the values of the

non-infected control in susceptible cultivar and 183.5 % in the resistant one. Statistically significant difference ($P \leq 0.01$) between susceptible and resistant cultivar was found out from 18 to 35 d after inoculation.

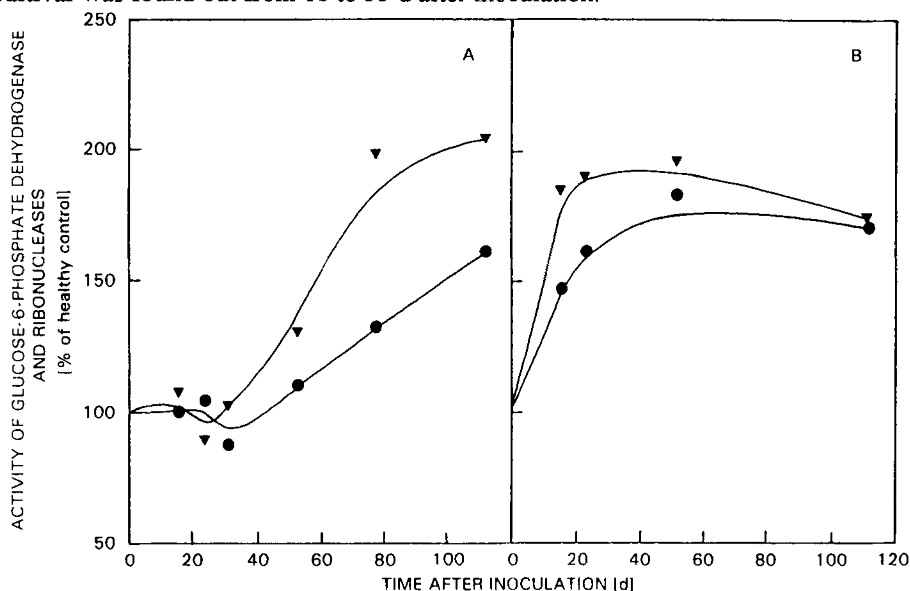

Fig. 3. Ribonucleases (*A*) and glucose-6-phosphate dehydrogenase (*B*) activities in potato virus Y infected leaves of resistant (cv. Adretta - *circles*) and susceptible (cv. Jara - *triangles*) potato cultivars. The results are expressed in % of healthy control.

Discussion

As was mentioned above, some of the metabolic pathways involved in virus RNA multiplication could be preferentially utilized in the particular host-virus combination. Šindelářová *et al.* (1990) concluded that in tobacco discs in light PVY-RNA was initially synthesized by the metabolic way of ribonuclease degradation of host rRNA; soon after the process was continued through the metabolic way of intermediate products of the oxidative pentose cycle utillizing free and storage saccharides. In addition, the intensity of these pathways was higher in PVY^N-infected tissues than in PVY^O infected discs; what corresponded to higher PVY^N content in plant tissues.

The results presented in this paper confirmed the participation of oxidative pentose pathway and host ribonucleases in PVY^N multiplication in whole potato plants.

In view of the fact that no significant changes in chlorophyll content were found it could be considered that PVY^N infection did not affect the photosyntetic apparatus and probably did not influence the reductive phosphate cycle.

The increase of glucose-6-phosphate dehydrogenase and ribonucleases activities proves a close relationship between activity of the enzymes under study and PVY^N content in infected tissues. However, these changes in enzyme activities did not

occur in the same time as was reported by Šindelář *et al.* (1990). While the activity of ribonucleases dramatically raised between 2 and 6 weeks after inoculation, no significant increase in glucose-6-phosphate dehydrogenase activity was found in this time. The same tendency was observed in both resistant and susceptible cultivars.

The data presented above support the conclusions of Šindelář *et al.* (1988) in tobacco cultivars differing in their resistance to tobacco mosaic virus (TMV). The activites both of glucose-6-phosphate dehydrogenase and ribonucleases were markedly increased in susceptible cultivar in comparison with resistant one and strongly reflected the multiplication of PVY^N within the intact potato plants. Activities of these enzymes could be considered as markers of the resistance of potato cultivars to PVY^N multiplication.

References

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.

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.

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

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-497, 1962.

Simons, T.J., Ross, A.F.: Metabolic changes associated with systemic induced resistance to tobacco mosais virus in "Samsun NN" tobacco. - Phytopathology **61**: 293-300, 1971.

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

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**: 259-264, 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.

Šindelář, L., Šindelářová, M., 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., Hanušová, M.: [Changes in ribonuclease and glucose-6-phosphate dehydrogenase activities during PVY-RNA biosynthesis in tobacco leaf discs.] - Ochrana Rost. (Praha) **24**: 87-93, 1988. [In Czech.]

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