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Photosynthetic Pigments in Potato Plants (*Solanum tuberosum* L.) cv. Igor after Primary Infection with Potato Virus Y^{NTN}

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K e y w o r d s : *Solanum tuberosum*, potato virus Y^{NTN}, photosynthetic pigments, high performance liquid chromatography (HPLC).

S u m m a r y

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The aim of this work was to investigate the effect of potato virus Y^{NTN} on photosynthetic pigments of the potato (*Solanum tuberosum* L.) cv. Igor grown in soil. Photosynthetic pigments were analysed in the shoots of infected, mock-inoculated, and healthy (PVY^{NTN}-free) control potato plants. The composition and amounts of photosynthetic pigments were studied 24 hours and 5 days after inoculation, before visible symptoms appeared. Pigments were separated by high performance liquid chromatography (HPLC) and identified by their light absorbance characteristics, hypsochromic shifts and retention times. Compared to the control, a decrease of photosynthetic pigments was detected 5 days after inoculation. The changes were more pronounced in infected than in mock-inoculated plants.

Introduction

A viral infection of a plant can lead to a multitude of symptoms, some immediately apparent and others requiring microscopic examination. Many are accompanied by profound biochemical and physiological changes. When the interaction between the virus and host results in the formation of chlorotic or yellow leaf tissue, there is an almost inevitable associated effect on many aspects of chloroplast ultrastructure and function, the magnitude of which usually correlates with the degree of symptom severity. Photosynthesis is affected in

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various ways. Perhaps the most commonly observed perturbation is a decrease in the net photosynthetic rate. An observed reduction in photosynthetic rates has usually been shown to be accompanied by a decrease in the chlorophyll content of affected leaf tissue. (MANDAHAR 1990). Plant viruses which cause systemic infections may be particularly important as inhibitors of chlorophyll synthesis since they spread continuously during plant growth and development (ŠUTIĆ & SINCLAIR 1990). One of such viruses is the strain of the potato virus Y^N (PVY^N) designated as PVY^{NTN}, which causes potato tuber necrotic ringspot disease (PTNRD). The type of reaction to the infection is specific for each potato cultivar (KUS 1995). Response to infection with PVY^{NTN} also depends greatly on the environment. Secondarily infected plantlets of potato cv. Igor grown in vitro were not severely inhibited and the symptoms were suppressed in comparison to secondarily infected plants grown in soil (DERMASTIA & RAVNIKAR 1995, ANŽLOVAR & al. 1996). This potato cultivar grown in soil, in contrast to that grown in vitro, is highly sensitive to primary infection with PVY^{NTN}. Infected plants develop severe symptoms: mosaics, vein necroses, yellow senescent leaves, and leaf drop or palm tree symptoms. The aim of our work was to follow the effect of PVY^{NTN} on photosynthetic pigments of the potato cv. Igor after different periods of primary infection, before visible symptoms appeared.

Materials and Methods

Potato plants cv. Igor were obtained from the Laboratory for Physiology and Potato Viruses Disease, Unit of M-KŽK Kranj. The plants were multiplied by a stem node segmentation procedure and transferred into soil in a growing chamber with Osram L18 W20 lights and a photoperiod of 16 hours of light at 50-90 μmol m^{-2} s^{-2}. The temperature was 20±2°C in the light and 17±2°C in the dark. The humidity was 70-80%. Plants were grown in May and November.

After 4 to 5 weeks, two to three bottom leaves were mechanically wounded and inoculated with the sap of healthy plants (mock-inoculated) or PVY^{NTN} infected plants. The controls were intact healthy plants. After 24 hours and 5 days of inoculation the shoots were cut, immediately frozen, and lyophilised.

Samples of 50 mg dry weight were homogenised after addition of quartz sand, two drops of bidistilled water and 5 ml of 100% acetone. The pigment extracts were centrifuged at 10000×g at 4°C for 10 minutes. 40 μl of supernatants were used for the analyses.

The pigments were separated with HPLC equipment from Waters. We used Spherisorb ODS-2 column, 250 mm long, 4 mm i.d., 5 μm particle size, and Pelliguard LC-18 Supercosil precolumn 50 mm long, 4 mm i.d., 40 μm particle size. Mobile phase: Solvent A was acetonitrile / bidistilled water (9 / 1, v / v) with 0.1% triethylamine and solvent B was ethyl acetate. The flow rate was 0.8 ml min^{-1}. The gradient was linear from 0% to 100% of solvent B within 25 min., then 100% B for 5 min. Peaks were detected at 445 nm by a Waters™ 996 Photodiode Array spectrophotometer. The system was supervised by the computer program Millenium 2010.

The 0.5 minute fractions were collected and used for identification of pigments as ANŽLOVAR & al. 1996 described. The pigments were identified by comparing published light absorbance characteristics, hypsochromic shifts and retention time (DAVIES 1976, KHALCIK & al. 1986, LICHTENTHALER 1987, GOODWIN & BRITTON 1988, PARRY & HORGAN 1992).

The amounts of pigments were calculated using published extinction coefficients (DAVIES 1976) and conversion factors.

The pigment contents are mean values of three extracts. The Student t-test was used to calculate the levels of significant differences between plants. Symbols used in the figures are: . $p < 0.05$, .. $p < 0.01$, ... $p < 0.001$, I means ± SD.

Results and Discussion

Although the plants were morphologically different in autumn compared to spring, the percentages of individual photosynthetic pigments were similar in both experiments. Six major pigments were identified in all samples: neoxanthin, violaxanthin, lutein, chlorophyll a, chlorophyll b and, β-carotene, representing 2.1%, 1.9%, 7.2%, 20.1%, 64.1% and 3.4% respectively, of total pigment content. In plants grown in autumn the sum of photosynthetic pigments was more than 30% lower than in plants grown in spring (Fig. 1).

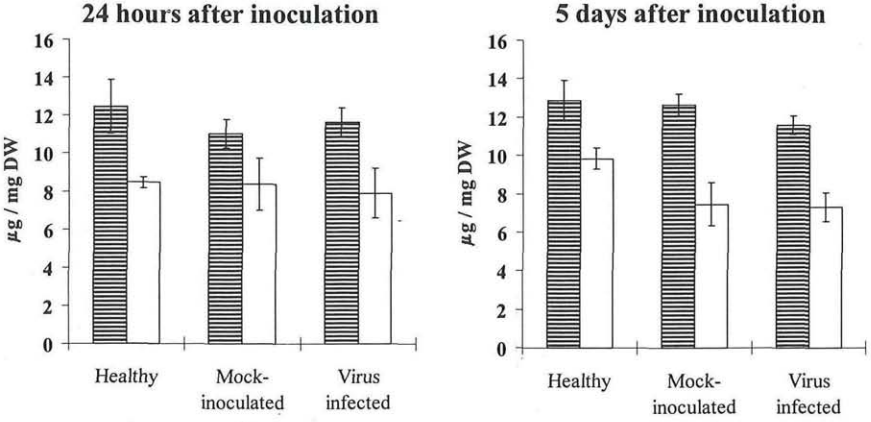

Fig. 1. Sum of photosynthetic pigments 24 hours and 5 days after inoculation (▤ spring, ▭ autumn).

The decreases of chlorophyll a and lutein were observed 24 hours after inoculation in mock-inoculated and virus infected plants in comparison to the control in both experiments, while changes of the absolute amounts of other pigments were variable (Fig. 2).

On the other hand, the trend of decrease of all photosynthetic pigments was found 5 days after inoculation in both mock-inoculated and virus infected plants compared to the control, and was more evident in plants grown in autumn. In virus infected plants the decrease was more pronounced than in mock-inoculated plants (Fig. 3). The results of other studies on the effect of a plant virus infection on carotenoids are contradictory, while generally viral pathogens cause a reduction of chlorophyll (reviewed in ŠUTIĆ & SINCLAIR 1990).

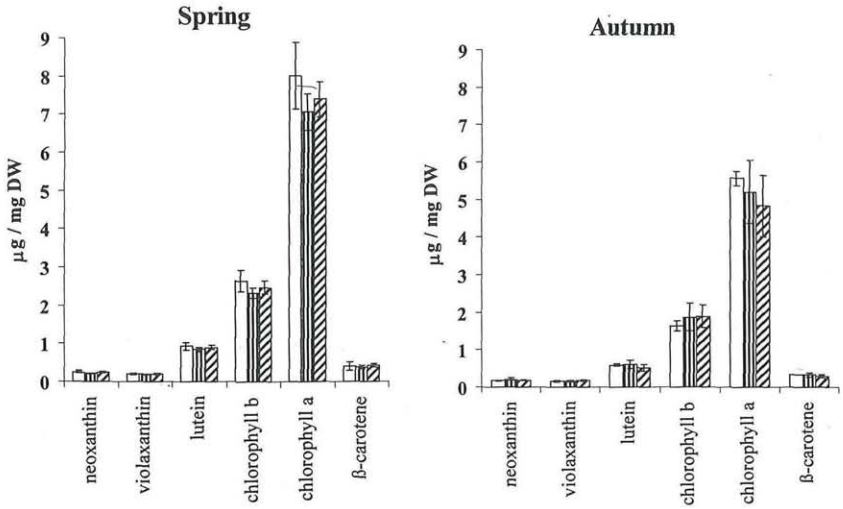

Fig. 2. Changes of photosynthetic pigments 24 hours after inoculation (☐ healthy plants, ▥ mock-inoculated plants, ▨ virus infected plants).

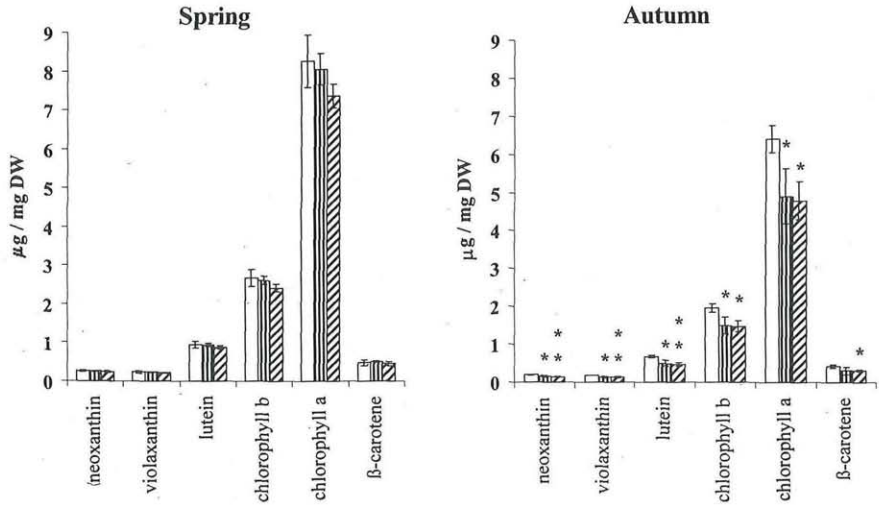

Fig. 3. Changes of photosynthetic pigments 5 days after inoculation (☐ healthy plants, ▥ mock-inoculated plants, ▨ virus infected plants).

The similar decrease of photosynthetic pigments 5 days after inoculation in mock-inoculated and virus infected plants in comparison to the control is probably the result of wounding. Moreover, the more pronounced decrease in virus infected plants compared to mock-inoculated plants could be the consequence of infection, although the symptoms on leaves were not visible. Plants grown in autumn had a lower amount of pigments and the decrease of pigments was more expressed, suggesting that plants with less pigments are more susceptible to different kinds of stress.

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