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Photosynthetic Pigments in Healthy and Virus-infected Potato Plantlets (*Solanum tuberosum* L.) Grown in vitro

By

Sabina ANŽLOVAR*) , Maja KOVAČ**) & Maja RAVNIKAR**)

With 2 Figures

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Summary

ANŽLOVAR S., KOVAČ M. & RAVNIKAR M. 1996. Photosynthetic pigments in healthy and virus-infected potato plantlets (*Solanum tuberosum* L.) grown in vitro. – Phyton (Horn, Austria) 36 (2): 221–230, 2 figures. – English with German summary.

The composition and amounts of leaf photosynthetic pigments of PVY^{NTN} infected and PVY^{NTN} free potato (*Solanum tuberosum* L., cv. Desirée, Igor, and Pentland Squire) grown in vitro, were studied. Pigments were identified by their light-absorbance characteristics, reactions with acid, and HPLC retention times. Their qualitative composition in healthy and infected leaves was similar. The response to the infection was specific for each potato cultivar regarding the morphological changes and changes of photosynthetic pigments. The length of axillary shoots of infected plantlets cv. Desirée and cv. Pentland Squire was inhibited and the amount of photosynthetic pigments per mg of dry weight was diminished when compared to healthy plantlets. In cv. Igor morphological changes were less pronounced and no significant difference in pigment content between healthy and infected plantlets was found (PVY^{NTN}, potato virus Y necrotic strain (tuber necrotic)).

Zusammenfassung

ANŽLOVAR S., KOVAČ M. & RAVNIKAR M. 1996. Photosynthesepigmente von in vitro kultivierten gesunden und virusinfizierten Pflanzen der Kartoffel (*Solanum tuber-*

*) S. ANŽLOVAR, Department of Biology, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia.

**) M. KOVAČ and M. RAVNIKAR, National Institute of Biology, Karlovška 19, 1000 Ljubljana, Slovenia.

osum L.). – Phyton (Horn, Austria) 36 (2): 221–230, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Es wurden bei in vitro kultivierten Kartoffeln (*Solanum tuberosum* L.) die Zusammensetzung und die Gehalte an Photosynthesepigmenten in PVY^{NTN} infizierten und freien Blättern untersucht. Die Pigmente wurden entsprechend ihrer charakteristischen Spektralabsorption, ihren Reaktionen mit Säure und den HPLC-Retentionszeiten identifiziert. Die qualitative Zusammensetzung verhielt sich in gesunden und infizierten Blättern ähnlich. Die Reaktion auf eine Infektion war für jede Kartoffelvarietät spezifisch und betraf morphologische Veränderungen und Veränderungen im Gehalt photosynthetischer Pigmente. Bei den infizierten Pflanzen der cv. Desirée und cv. Pentland Squire war die Länge der Axillarsprosse gehemmt und der Gehalt an photosynthetischen Pigmenten pro mg Trockengewicht im Vergleich zu gesunden Pflanzen vermindert. In der cv. Igor waren morphologische Veränderungen geringer ausgeprägt und es konnte kein signifikanter Unterschied im Pigmentgehalt zwischen gesunden und infizierten Pflanzen gefunden werden.

Introduction

A virus infection can strongly modify growth and development of a plant. Pathological symptoms appear, as a consequence of the modifications of a host metabolism caused by the infection. Plant viruses which cause systemic infections may be particularly important as inhibitors of chlorophyll synthesis, since they spread continuously during plant development and growth (Šutič & Sinclair 1990). One of such viruses is a strain of the potato virus Y^N (PVY^N), designated as PVY^{NTN}, which causes potato tuber necrotic ringspot disease (PTNRD). The disease, which is tuber- sap- and aphid- transmitted, is very harmful and has become widespread in Europe (Le Romancer & al. 1994, Kus 1992). After primary infection PVY^{NTN} evokes severe symptoms: chlorophyll figures, vein necroses, necrotic streaks on petioles and stems, leaf drop or palm tree symptoms. Secondarily infected plants react with distinct, sometimes very pronounced mosaics and more or less expressed crinkles. On tubers of some cultivars the disease induces superficial necrotic areas. The type of reaction, in particular on tubers, is specific for each cultivar (Kus 1992). For our experiments, we chose three cultivars with different reactions to infection by PVY^{NTN} in vivo: cv. Pentland Squire was not inhibited in growth and had mild mosaics on leaves; the same changes were observed in cv. Desirée, but were more pronounced; growth inhibition was most pronounced in cv. Igor where the leaves were pale green. Our previous experiments showed that the response to virus infection of potato plants grown in vitro conditions differed from that grown in vivo (Dermastia & Ravnikar 1995). This result led us to investigate the pigment content and morphological changes of secondarily infected potato plantlets (*Solanum tuberosum* L.) grown in vitro.

Materials and Methods

Potato tubers (*Solanum tuberosum* L.) of three cultivars: Igor, Desirée, and Pentland Squire were obtained from The Laboratory for Physiology and Potato Viruses Disease, Unit of M-KŽK Kranj. They were tested against PVM, PVA, PVS, PVX, PLRV and PVY by ELISA.

Each cultivar had two groups: a healthy group and virus infected plantlets. The latter were cultured from PVY^{NTN} infected stock, infected systemically. Both groups were grown with a stem node segmentation procedure in MS (Murashige and Skoog) medium with 3% sucrose and 0.8% agar at 20 °C, lit with Silvania grolux lights and a photoperiode of 16 hours of light, 8 hours of darkness, at 55-70 μmol m^{-2} s^{-1}. After six to seven weeks of cultivation, the lengths of the axillary shoots and the number of nodes were measured. Leaves were then cut, weighed, immediately frozen, and kept at –20 °C until being analyzed.

Samples of potato leaves (500 mg) were homogenized after addition of $CaCO_3$ and quartz sand. Pigments were extracted with acetone. Cell debris and inorganic matter were removed by filtration with Whatman 1 paper and the samples were reextracted twice. Excess solvent was removed at 30 °C by a rotary evaporator to permit a final concentration of 100 mg of fresh-weight tissue per 1 ml.

The pigment extracts were centrifuged at 10 000 × g at 4 °C for 10 min and 20 μl of the supernatant was injected into the HPLC equipment from LDC / Milton Roy. For pigment separation, we used a partially modified Pfeifhofer's method (Pfeifhofer 1989).

We used a Spherisorb ODS-2 column, 250 mm long, 4 mm i. d., 5 μm particle size and a Pelliguard LC-18 precolumn, 50 mm long, 4 mm i.d., 40 μm particle size. The flow rate was 1 ml min^{-1}. Peaks were detected at 445 nm by an LDC / Milton Roy spectro monitor detector. Mobile phase: A: acetonitrile/bidestilled water/methanol (20/2/1, v/v/v); B: acetone/ethyl acetate (2/1, v/v); gradient elution: linear gradient from 10% to 70% B in A within 21 min., then 70% B until the elution of β-carotene. The typical working pressure was approximately 1.4 kPa.

Fractions corresponding to individual pigments were collected, dried with stream of nitrogen, and the residues dissolved in 0.5 ml ethanol, hexane or petroleum for carotenoids and in acetone for chlorophyll analyses. Pigments were identified by comparing published absorbance spectra and HPLC retention times (Davies 1976, Val & al. 1986, Pfeifhofer 1989, Rivas & al. 1989, Parry & al. 1990, Laznik & Kovač 1995). The absorbance spectra were recorded using a Hewlett Packard diode array spectrophotometer. Further evidence for the identity of these compounds came from their reactions with acid which catalyses the specific isomerisation of 5,6-epoxide groups to 5,8-furanoid groups, resulting in a hypsochromic shift of approx. 20 nm for monoepoxides such as neoxanthin and 40 nm for diepoxides such as violaxanthin (Davies 1976).

Quantification was carried out using published specific extinction coefficients (Davies 1976) and conversion factors used by Kovač & Ravnikar 1994.

The calculated pigment contents were mean values of at least two injections and three extracts obtained from three experiments. Statistical analysis was performed using Student's t-test.

Results and Discussion

Infection with PVY^{NTN} influenced stem elongation, whereas the number of nodes did not change. Infected plantlets were always smaller; the difference in height was most pronounced in cv. Pentland Squire and less in cv. Desirée. In cv. Igor both groups had a similar height (Fig. 1). The percentage of water was higher in healthy leaves; and this was also most pronunced in cv. Pentland Squire, less in cv. Desirée, and least in cv. Igor. (Tab. 2, 3, 4). This indicated that infection with PVY^{NTN} also affects water-gathering mechanisms. Morphological changes were more expressed in cv. Pentland Squire, where infected plantlets also had red stems, probably as a result of anthocyans accumulation. Anthocyans are frequently found to be stress indicators (KRAMER & KOZLOWSKI 1979).

The qualitative composition of photosynthetic pigments was similar in healthy and virus infected leaves for all three cultivars. The chromatographic system clearly separated the six major pigments, neoxanthin, violaxanthin, lutein, chlorophyll b, chlorophyll a, β-carotene, and the five minor components (Fig. 2). Two of them were identified as antheraxanthin and 9′-cis violaxanthin by comparing published absorbance spectra, HPLC retention times, and their hypsochromic shift. Additional confirmation of

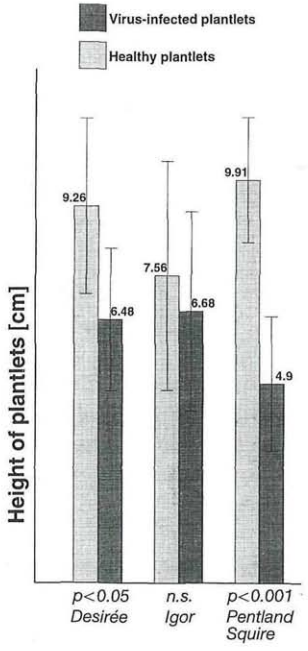

Fig. 1. The height of healthy and infected plantlets.

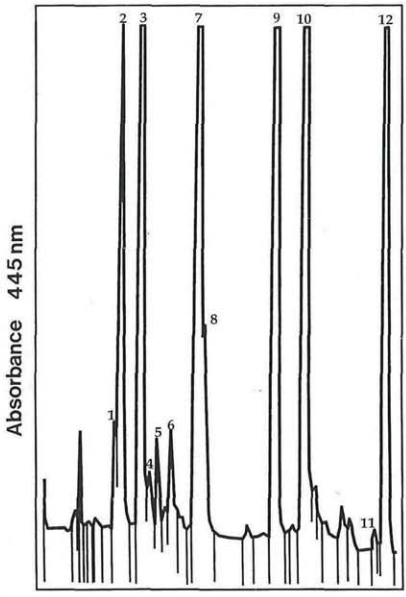

Fig. 2. Representative HPLC chromatogram of photosynthetic pigments extracted from potato leaves. 1, x_1; 2, 9'-cis neoxanthin; 3, all-trans violaxanthin; 4, x_2; 5, 9'-cis violaxanthin; 6, anteraxanthin; 7, lutein; 8, zeaxanthin; 9, chlorophyll b; 10, chlorophyll a; 11, pheophytin a; 12, β-carotene.

Table 1

Chlorophyll and carotenoid contents (ng mg^{-1} fresh and μg mg^{-1} dry weight) in healthy and infected leaves of potato.

Cultivar	Fresh weight		%	Dry weight		%
	healthy	infected	of control	healthy	infected	of control
Desirée						
∑ Chl[a]	895.48	972.93	97.5	13.03	11.12	85.3
∑ Car[b]	154.50	155.13	100.4	2.25	1.77	78.7
Igor						
∑ Chl[a]	1030.18	955.23	92.7	12.40	11.44	92.3
∑ Car[b]	173.46	161.28	93.0	2.09	1.93	92.3
Pentland Squire						
∑ Chl[a]	1282.12	1827.65	142.5	12.58	11.09	88.2
∑ Car[b]	204.16	275.78	135.1	2.00	1.67	83.5

[a] sum of both Chlorophylls

[b] sum of all Carotenoids

Table 2

Pigment content (ng mg^{-1} fresh weight) and pigment relations in healthy and infected leaves of potato cv. Desirée.

Pigments (ng mg^{-1} fresh weight)	Healthy means	±SD	Infected means	±SD	Sig. (t-test)	% of control
Carotenoids[a]						
x_1	4.65	1.47	3.00	1.09	$p<0.01$	64.5
c-Neoxanthin	17.53	4.74	18.29	5.63	n.s.	104.3
t-Violaxanthin	27.87	5.08	22.68	5.01	$p<0.01$	81.4
x_2	0.52	0.15	0.45	0.09	n.s.	86.5
c-Violaxanthin	4.20	1.27	2.89	0.40	$p<0.001$	68.8
Antheraxanthin	2.09	0.30	3.88	1.70	$p<0.001$	185.7
Lutein	65.91	11.97	69.98	16.63	n.s.	106.2
Zeaxanthin	0.73	0.45	0.97	0.29	n.s.	132.9
β-Carotene	30.99	8.05	32.99	8.35	n.s.	106.5
Chlorophylls						
b	225.24	50.50	243.56	53.41	n.s.	108.1
a	670.24	170.70	729.37	161.23	n.s.	108.8
Quotients[b]						
chl/C	5.80		6.27			
chl a/b	2.98		2.99			
X/β-Carotene	3.99		3.70			
Dry weight of leaves (mg g^{-1} fresh weight)	68.7		87.5			

Each value represents the mean of three replicates of three independent experiments

[a] c-, 9'-cis; t-, all-trans;

[b] chl/C = chlorophylls / carotenoids;
chl a/b = chlorophyll a / chlorophyll b
X/β-Carotene = Xantophylls / β-Carotene

9'-cis violaxanthin was obtained by a cis peak in the UV region (DAVIES 1976, PARRY & al. 1990). In view of the hypsochromic shift, and published HPLC retention time (PARRY & HORGAN 1992), the unknown carotenoid x_1 eluted just before 9'-cis neoxanthin could be a neoxanthin isomer.

Like morphological changes, pigment content changes were also cultivar specific. In the infected plantlets of cv. Igor, where morphological changes were less expressed, the amount of photosynthetic pigments per mg of dry weight or fresh weight did not change significantly (Tab. 1, 4). We may conclude that when grown in tissue culture, infection of cv. Igor did not influence pigment synthesis. In the infected leaves of cv. Pentland Squire and Desirée, whose growth was more inhibited, the amount of pigments per mg of dry weight was diminished (Tab. 1). On the other hand, if the pigment content was expressed on a fresh weight basis, no difference

Table 3

Pigment content (ng mg^{-1} fresh weight) and pigment relations in healthy and infected leaves of potato cv. Pentland Squire.

Pigments (ng mg^{-1} fresh weight)	Healthy means	±SD	Infected means	±SD	Sig. (t-test)	% of control
Carotenoids[a]						
x_1	3.69	1.18	4.43	2.07	n.s.	120.1
c-Neoxanthin	24.24	4.73	35.76	5.85	p<0.001	147.5
t-Violaxanthin	34.26	5.90	41.76	4.47	p<0.001	121.9
x_2	0.53	0.26	0.77	0.43	n.s.	145.3
c-Violaxanthin	4.64	1.34	5.27	1.03	n.s.	113.6
Antheraxanthin	3.73	0.71	4.41	2.30	n.s.	118.2
Lutein	89.86	17.22	123.76	11.17	p<0.001	137.7
Zeaxanthin	1.26	0.62	1.51	0.70	n.s.	119.8
β-Carotene	41.95	10.07	58.11	7.70	p<0.001	138.5
Chlorophylls						
b	327.16	69.59	444.84	51.32	p<0.001	136.0
a	954.96	223.61	1382.81	139.87	p<0.001	144.8
Quotients[b]						
chl/C	6.28		6.63			
chl a/b	2.92		3.11			
X/β-Carotene	3.87		3.75			
Dry weight of leaves (mg g^{-1} fresh weight)	101.9		164.8			

Each value represents the mean of three replicates of three independent experiments

[a] c-, 9'-cis; t-, all-trans;

[b] chl/C = chlorophylls / carotenoids;
chl a/b = chlorophyll a / chlorophyll b
X/β-Carotene = Xantophylls / β-Carotene

was found between infected and uninfected plantlets of cv. Desirée, only some minor carotenoid components were diminished (Tab. 1,2). In infected plants of cv. Pentland Squire their amount even increased (Tab. 1,3). These results indicate not only the involvement of virus infection in water absorption, but also in pigment metabolism in these two cultivars. Similar results were found in PVX infected potato plants, where pigment content was also diminished (Tsoglin & al. 1985, Melik-Sarkisov & al. 1987). The reductions in chlorophyll content were also found in some other plant virus (reviewed in Šutič & Sinclair 1990).

The ratios of chlorophyll a/b, xanthophylls/β-carotene and chlorophylls/carotenoids were similar in both groups in all cultivars indicating that these photosynthetic pigments were equally affected by virus infection. An unchanged ratio of chlorophyll a/b had been observed in

Table 4

Pigment content (ng mg^{-1} fresh weight) and pigment relations in healthy and infected leaves of potato cv. Igor.

Pigments (ng mg^{-1} fresh weight)	Healthy means	±SD	Infected means	±SD	Sig. (t-test)	% of control
Carotenoids[a]						
x_1	3.47	1.22	3.21	1.04	n.s.	92.5
c-Neoxanthin	20.50	4.22	18.06	3.02	n.s.	88.1
t-Violaxanthin	29.06	3.90	26.40	2.98	$p<0.05$	90.9
x_2	0.53	0.25	0.66	0.36	n.s.	124.5
c-Violaxanthin	3.72	0.82	3.43	0.58	n.s.	92.2
Antheraxanthin	3.76	1.52	3.34	1.18	n.s.	88.8
Lutein	76.95	14.08	73.37	9.17	n.s.	95.4
Zeaxanthin	0.68	0.46	0.85	0.59	n.s.	125.0
β-Carotene	34.79	7.32	31.97	5.07	n.s.	91.9
Chlorophylls						
b	266.04	55.77	244.05	40.80	n.s.	91.7
a	764.14	173.39	711.17	104.15	n.s.	93.1
Quotients[b]						
chl/C	5.94		5.92			
chl a/b	2.87		2.91			
X/β-Carotene	3.99		4.05			
Dry weight of leaves (mg g^{-1} fresh weight)	83.1		83.5			

Each value represents the mean of three replicates of three independent experiments

[a] c-, 9'-cis; t-, all-trans;

[b] chl/C = chlorophylls / carotenoids;
chl a/b = chlorophyll a / chlorophyll b
X/β-Carotene = Xantophylls / β-Carotene

field grown cassava Manihot esculenta clones infected with cassava mosaic disease (AYANRU & SHARMA 1982).

We can conclude that drastic changes of growth in vitro are accompanied with changes in chlorophyll and carotenoids content. Response to infection with PVY^{NTN} depends greatly on the environment. Plantlets of cv. Igor grown in tissue culture surprisingly were not severely inhibited, whilst in natural conditions they were. Contrary to expectations, cv. Pentland Squire was the most affected in vitro although it is highly resistant in vivo. We know that infected plants of Pentland Squire show no symptoms even though they contain large numbers of viruses. In this cultivar, resistance mechanism were overcome in tissue culture. Changes in virus sensibility are probably due to differences between natural conditions compared to tissue culture. One of the reasons may be also the

changed ratios in plant growth regulators. The growth of potato plantlets in tissue culture induces a specific response of the endogenous plant growth regulators, cytokinins, (DERMASTIA 1993, DERMASTIA & RAVNIKAR 1995) which are thought to be involved in chlorophyll metabolism (FLETCHER & MCCULLAGH 1971, LEW & TSUJI 1982, ARNOLD & FLETCHER 1986). Involvement of viruses in cytokinin metabolism in infected potato plants grown in vivo was also shown (DERMASTIA & al. 1995). To verify this hypothesis, more analyses of plant growth regulators are required.

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