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Ultrastructure of Chloroplasts in Leaves of Potato Plants infected by Potato Virus Y^{NTN}

By

Maruša POMPE-NOVAK*), Mercedes WRISCHER**) and Maja RAVNIKAR*)

With 7 figures

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Summary

POMPE-NOVAK M., WRISCHER M. & RAVNIKAR M. 2001. Ultrastructure of chloroplasts in leaves of potato plants infected by potato virus Y^{NTN}. – *Phyton* (Horn, Austria) 41 (2): 215–226, with 7 figures. – English with German summary.

PVY^{NTN} infection has been shown to influence the ultrastructure of chloroplasts in primary infected potato plants (*Solanum tuberosum* L.) of cultivar Igor grown in vivo and secondary infected plants of cultivar Désirée grown in vitro. The ultrastructure is most affected in spot necrosis, where the changes are part of apoptotic processes. In the parts of inoculated leaves without spot necrosis, a decrease in size of chloroplasts was observed as compared to healthy controls. From the edge to the middle of a spot necrosis, swelling of chloroplasts, loosening of thylakoid structure and changes in optical density of chloroplasts were followed. In the middle of spot necrosis the cytoplasm of the cells was dense, no vacuoles were present in the cells, cells were shrunk, the cell wall was wrinkled and the intracellular spaces were enlarged. An increase in the number of chloroplasts per cell was observed in the green parts of yellow-green leaves as compared to healthy controls. Secondary infected plants grown in vitro had smaller chloroplasts with exvaginations, but the same volume proportion of thylakoids, starch grains and osmophilic globuli as in chloroplasts of healthy plants. In green parts of leaves of primary and secondary infected plants, PVY^{NTN} infection did not cause any visible changes in thylakoid structure, so

*) M. POMPE-NOVAK and M. RAVNIKAR, National institute of biology, Večna pot 111, 1000 Ljubljana, Slovenia.

**) M. WRISCHER, Ruđer Bošković institute, Bijenička 54, 1000 Zagreb, Croatia.

the decreased level of photosynthesis must be a consequence of changes at the biochemical level.

Zusammenfassung

POMPE-NOVAK M., WRISCHER M. & RAVNIKAR M. 2001. Die Chloroplastenultrastruktur von mit dem Kartoffelvirus Y^{NTN} infizierten Blättern. – *Phyton* (Horn, Austria) 41 (2): 215–226, 7 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Es konnte gezeigt werden, dass eine PVY^{NTN}-Infektion die Ultrastruktur von Chloroplasten in primär infizierten Kartoffelpflanzen (*Solanum tuberosum* L.) der Sorten Igor in vivo und sekundär infizierte Pflanzen der Sorten Désirée, welche in vitro gezogen wurde, beeinflusst. Die Ultrastruktur erweist sich als am meisten beeinflusst in den Punktnekrosen, wo die Veränderungen Teil der Abbauvorgänge sind. In jenen Teilen der befallenen Blätter ohne Punktnekrosen konnte im Vergleich zu den Kontrollen eine Verringerung der Chloroplastengröße beobachtet werden. Vom Rand gegen die Mitte der Punktnekrosen zu erfolgte ein Anschwellen der Chloroplasten, Verlust der Thylakoidstrukturen und Änderungen in der Chloroplastendichte. In der Mitte der Punktnekrosen erschien das Plasmalemma der Zellen dicht und die Vakuolen fehlten, die Zellen waren geschrumpft und die Zellwand erschien gerunzelt und die Interzellularräume waren erweitert. Im Vergleich zu den Kontrollen wurde eine Zunahme der Chloroplastenzahl pro Zelle in den grünen Teilen der gelbgrünen Blätter beobachtet. Sekundärinfizierte in vitro gezogene Pflanzen hatten kleinere Chloroplasten mit Ausstülpungen, jedoch dieselben Proportionen von Thylakoiden, Stärkekörnern und osmophilen Globuli wie gesunde Pflanzen. Keine sichtbaren Veränderungen in der Thylakoidstruktur erfolgten in den grünen Teilen der Blätter von primär und sekundär mit PVY^{NTN} infizierten Pflanzen, sodass die verminderte Photosynthese biochemisch bedingt sein mußte.

Introduction

Potato virus Y is a member of the *Potyviridae* group. It causes potato tuber necrotic ringspot disease. Some potato cultivars, for example Igor and Jaerla, are very susceptible to the infection while others, for example Désirée and Pentland squire, are more tolerant. Very few, for example cultivar Sante, are completely resistant (KUS 1995).

In addition to visible symptoms, infection with PVY^{NTN} also causes biochemical and physiological changes in potato plants. In secondary infected potato plants of cultivar Igor grown in vitro the levels of biochemically active cytokinins, which among other roles promote chloroplast development, decrease (DERMASTIA & RAVNIKAR 1996), and the distribution of jasmonic acid changes (PETROVIĆ & al. 1997). 14 days after infection several new proteins appeared and some protein breakdown was observed in cultivars Igor, Désirée and Pentland squire (GRUDEN & al. 2000). In primary infected plants of cultivar Igor grown in vivo and in secondary infected plants of cultivar Désirée grown in vitro an increase in the level of cysteine and aspartic proteinase inhibitors was noticed (POLJŠAK-PRIJATELJ 1998).

In systemically infected potato plants of different cultivars grown in vitro, a decrease in the total amount of photosynthetic pigments per unit of dry weight of leaves was shown (ANŽLOVAR & al. 1996) and a decrease of chlorophyll a 24 hours and of chlorophyll a and b 5 days after primary infection was reported for cultivar Igor (MILAVEC & al. 1999).

Some viruses have been reported to affect chloroplast structure. Rib-gras mosaic virus (RMV) and tobacco mosaic virus (TMV) cause changes in number, size, shape and clumping pattern of chloroplasts as well as of starch grains and osmophilic globules accumulated inside (XU & FENG 1998). Tomato spotted wilt virus (TSWV) causes degenerative changes of chloroplasts, such as swelling, more osmophilic globuli and loosened thylakoid structure (ALMASI & al. 1996). Melon rugose mosaic virus (MRMV) causes the appearance of vesicles at the periphery of chloroplasts, and a tendency of the chloroplasts to aggregate (MAHGOUB & al. 1997). Maize draft mosaic virus (MDMV) causes degenerative changes in chloroplasts including the presence of small vesicles, deformation of membranes, reduction in granal stack height and disappearance of osmophilic globules (CHOI 1996).

On the basis of these reports, completed with the facts that PVY^{NTN} infection lowers the rate of photosynthesis and the content of photosynthetic pigments, that PVY coat protein and the helper component-proteinase were found inside chloroplasts of infected tobacco plants (GUNASINGHE & BERGER 1991) and that PVY was located in chloroplasts of secondary infected potato plants (POLJŠAK-PRJATELJ & RAVNIKAR 1992), we have tested the hypothesis that PVY^{NTN} infection has an influence on the ultrastructure of chloroplasts.

Materials and Methods

Two experimental systems were used: for PVY^{NTN} infection susceptible potato plants, cultivar Igor grown in vivo, and the more tolerant cultivar Désirée grown in vitro. Plants were kept at 18 ± 2 °C, with illumination at $70 \mu\text{M}^{-2}\text{s}^{-1}$ and a photoperiod 16h.

Healthy potato plants (*Solanum tuberosum* L. cultivar Igor) were multiplied by stem node segmentation and transferred into soil in a growth chamber. After 4 weeks four bottom leaves were mechanically inoculated with the sap of healthy plants (mock-inoculated) or of PVY^{NTN} infected plants. Intact healthy plants were taken as controls. 5 and 14 days after inoculation 1mm^2 pieces of the 3rd and 7th leaves were taken.

Single-node cuttings of healthy and PVY^{NTN} secondary infected potato plants (*Solanum tuberosum* L. cultivar Désirée) were grown on modified Murashige-Skoog medium. After 5 weeks of cultivation 1mm^2 pieces of 7th leaf were taken.

Pieces of leaves were fixed in a mixture of 2% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer or in 1% glutaraldehyde in cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated in a series of ethanol solutions and embedded in Agar100, ERL or Araldite. Pieces of cultivar Igor grown in vivo were

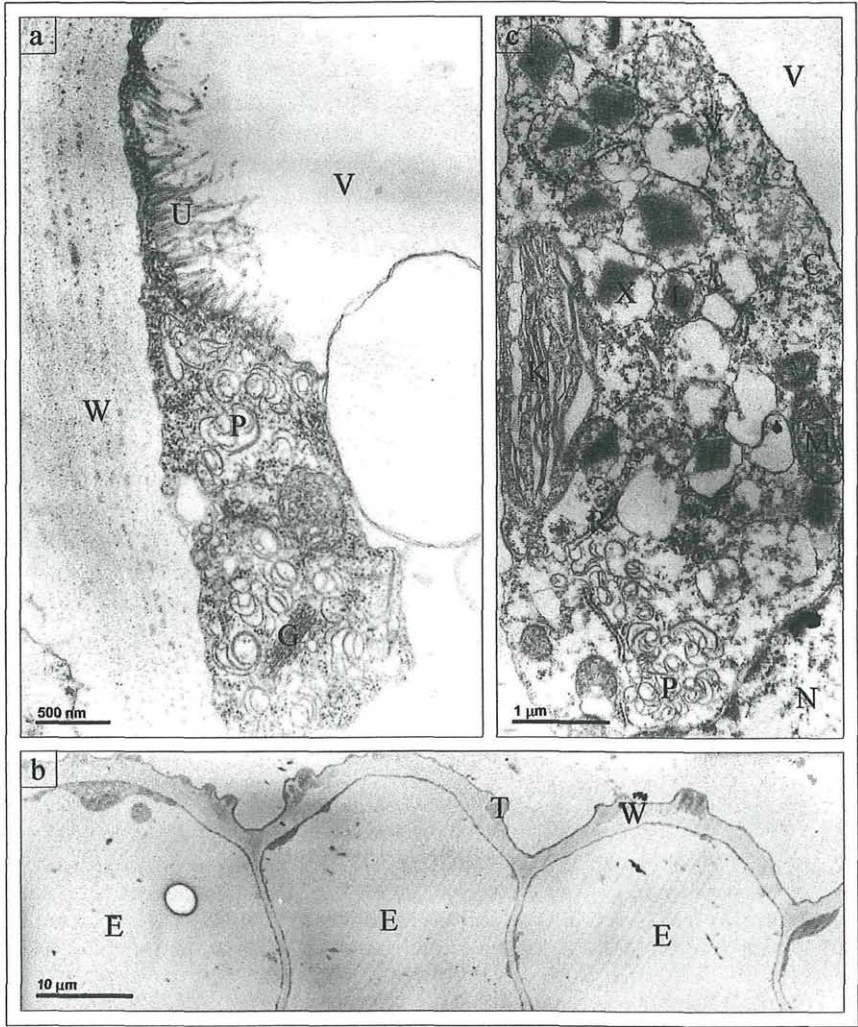


Fig. 1. (a,b) PVY^{NTN} infected stem epidermis cells. (c) PVY^{NTN} infected stem cell three layers beyond epidermis. W – cell wall, V – vacuole, C – cytoplasm, N – nucleus, K – chloroplast, M – mitochondria, R – endoplasmic reticulum, G – Golgi apparatus, X – peroxisomes, L – crystals, P – pinwheels, U – tubuli, T – optically dense thickenings of outer cell wall, E – epidermal cell.

either fixed in paraformaldehyde and glutaraldehyde in phosphate buffer and embedded in Agar100 or fixed in glutaraldehyde in cacodylate buffer and embedded in Araldite. Pieces of cultivar Désirée grown in vitro were fixed in paraformaldehyde and glutaraldehyde in phosphate buffer and embedded in ERL. Different types of fixations made no difference for our experiments.

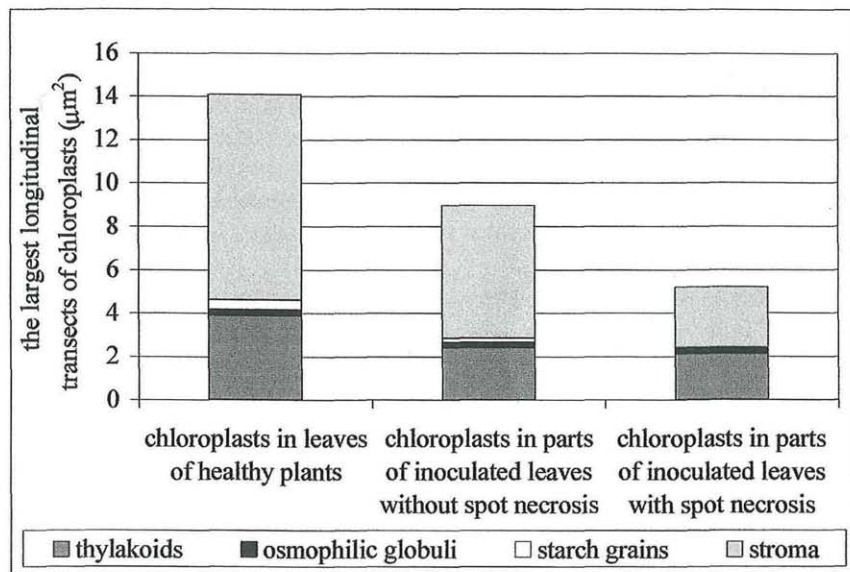


Fig. 2. The largest longitudinal transects of chloroplasts in palisade tissue of healthy and PVY^{NTN} infected potato plants cultivar Igor grown in vivo.

Semithin sections were cut with a glass knife on ultracut and mounted on glass slides. Sections were stained with 0.5% methylene blue in 0.5% borax. Samples were observed by light microscopy.

Ultrathin sections were cut with a diamond knife on ultracut and mounted on copper grids with formvar supporting film. Sections were stained with 3% uranyl acetate and Reynold's lead citrate. Samples were observed with a JEOL-JEM 1200 EX II transmission electron microscope at 80kV.

The numbers of chloroplasts in palisade cells were estimated only relatively in pairs of samples by counting chloroplasts in the largest transects of cells on longitudinal sections. The size of chloroplasts and proportions of thylakoides, starch grains and osmophilic globuli in chloroplasts were estimated by computer picture analysis of the largest longitudinal transects of chloroplasts, or by counting the test points with known associated area (MAYHEW 1992) on the largest longitudinal transects of chloroplasts. Computer picture analysis was done on the basis of counting the pixels of defined gray level corresponding with certain structure. For the largest longitudinal transects of chloroplasts the largest 5 transects of chloroplasts on each of 5 sections of 30 palisade cells were taken for each sample.

For statistical assays the Mann-Whitney U-test was used.

Results and Discussion

In both experimental systems changes in ultrastructure were observed in infected plants. Pinwheels, typical protein cellular inclusions for *Potiviridae*, were found in the cytoplasm of infected cells. Huge amounts of pinwheels were observed in epidermal cells of stem (Fig. 1a), where opti-

cally dense thickenings of outer cell wall were also observed (Fig. 1b). Pinwheels, mitochondria, peroxisomes and chloroplasts were often situated tight together and there were large crystals in the most peroxisomes (Fig. 1c). These changes were most obvious in stem and in leaves or parts of leaves with no visible symptoms, although Hinrichs-Berger & al. reported that the number of mitochondria, microbodies, vesicles and inclusion bodies increased in necrotic reacting cells or in cells close to necrotic vein lesions in PVY⁰ infected potato plants of cultivar Quarta (HINRICHS-BERGER & al. 1999).

Chloroplasts were studied in more detail. In primary infected potato plants of cultivar Igor grown in vivo, chloroplasts in palisade cells of inoculated leaves were compared to chloroplasts in palisade cells of healthy leaves of the same age. In the parts of inoculated leaves without spot necrosis, a decrease in chloroplasts size was observed as compared to healthy controls (Fig. 2, Table 1). Close to the edge of spot necrosis, the chloroplasts were even smaller. From the edge to the middle of spot necrosis, changes in the ultrastructure of chloroplasts can be followed. First, swelling of the outer part of some chloroplasts in a cell occurred (Fig. 3a), later becoming more and more severe and affecting all the chloroplasts in a cell (Fig. 3b), the thylakoid structure loosened (Fig. 3c) and finally, chloroplasts became condensed and very dense optically (Fig. 3d). In the middle of spot necrosis the cytoplasm of the cells was dense, no vacuoles were present in the cells, cells were shrunk, the cell wall was wrinkled and the intracellular spaces were enlarged (Photo 3e), while the diameter of leaves was much reduced in spot necrosis. An electron-opaque cytoplasm with almost no center vacuole and with extremely thick cell wall has been reported for early stages after PVY⁰ infection when lesions just began to appear in very resistant cultivar Pirola, but only for epidermal and parenchymatic cells close to epidermis (HINRICHS-BERGER & al. 1999).

14 days after inoculation chloroplasts in palisade cells of yellow-green systemically infected leaves of potato plants cultivar Igor grown in vivo were compared to chloroplasts in palisade cells of healthy leaves of approximately the same age. Larger number of chloroplasts per cell was observed in the green parts of yellow-green systemically infected leaves as compared to healthy controls (Fig. 4).

Yellow-green systemically infected leaves were also compared with younger leaves without symptoms from the same infected plants. Significantly less chloroplasts were present in young leaves than in green parts of yellow-green leaves (Fig. 5).

Secondary infected potato plants of the more tolerant cultivar Désirée grown in vitro were also investigated. Chloroplasts in palisade cells of PVY^{NTN} infected plants were smaller than in healthy plants, had ex-vaginations, but not statistically different volume proportions of thyla-

Table 1.

Absolute and relative areas of the largest longitudinal transects of chloroplasts in palisade tissue of healthy and PVY^{NTN} infected potato plants cultivar Igor grown in vivo. Average \pm standard error is shown. Statistically significant differences are indicated: ***p < 0.001, **p < 0.01, *p < 0.05. ns – not significant.

	leaves of healthy plants	parts of inoculated		statistical difference between		statistical difference between		statistical difference between	
		leaves without spot necrosis	leaves with spot necrosis	leaves of healthy plants and inoculated leaves without spot necrosis	leaves of healthy plants and inoculated leaves with spot necrosis	leaves of healthy plants and inoculated leaves without spot necrosis	leaves of healthy plants and inoculated leaves with spot necrosis	inoculated leaves without spot necrosis	inoculated leaves with spot necrosis
the area of chloroplasts (μm^2)	14.10 \pm 1.44	8.97 \pm 1.05	5.20 \pm 0.53	*	***	***	***	**	**
the area of thylakoids (μm^2)	3.89 \pm 0.32	2.42 \pm 0.29	2.13 \pm 0.21	**	***	***	***	ns	ns
the area of osmophilic globuli (μm^2)	0.26 \pm 0.07	0.24 \pm 0.06	0.24 \pm 0.06	ns	ns	ns	ns	ns	ns
the number of osmophilic globuli	8.65 \pm 0.77	3.56 \pm 0.62	8.54 \pm 0.92	***	ns	ns	ns	***	***
the number of osmophilic globuli on μm^2 of chloroplast	0.63 \pm 0.04	0.47 \pm 0.10	1.66 \pm 0.15	**	***	***	***	***	***
the area of starch grains (μm^2)	0.46 \pm 0.14	0.20 \pm 0.08	0.03 \pm 0.02	ns	**	**	**	*	*
the area of chloroplasts (%)	100	100	100	ns	ns	ns	ns	ns	ns
the area of thylakoids (%)	29.53 \pm 1.75	28.58 \pm 2.28	39.90 \pm 3.33	ns	ns	ns	ns	ns	ns
the area of osmophilic globuli (%)	1.70 \pm 0.44	2.36 \pm 0.69	4.78 \pm 1.32	ns	ns	ns	ns	ns	ns
the area of starch grains (%)	2.75 \pm 0.75	1.68 \pm 0.65	0.70 \pm 0.50	ns	**	**	**	ns	ns

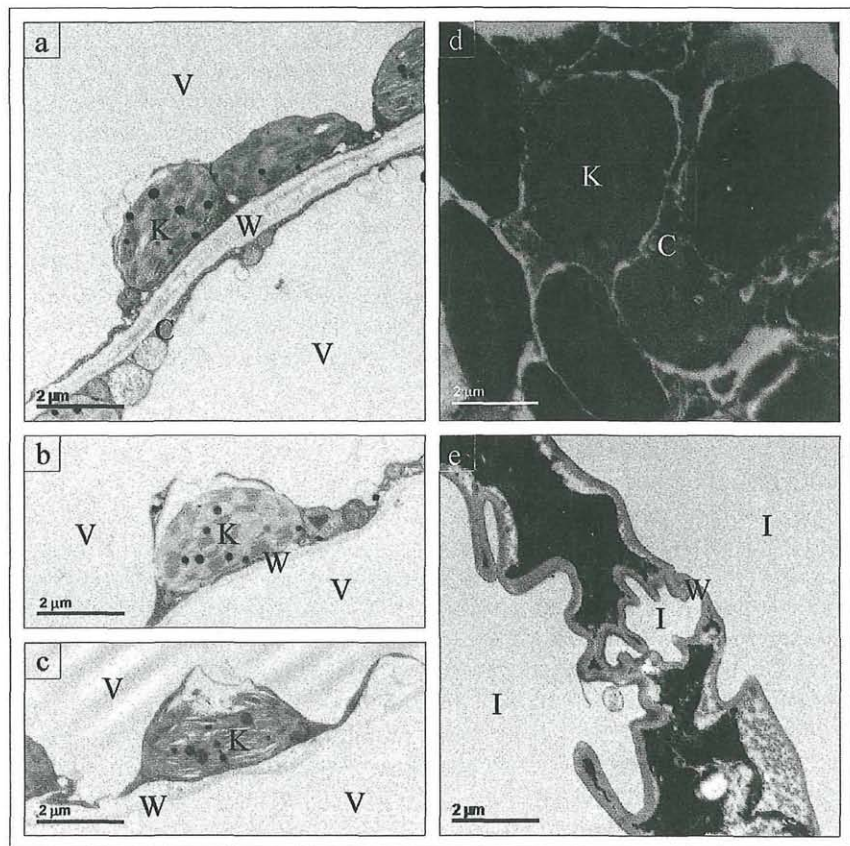


Fig. 3. PVY^{NTN} infected leaf cells in different cell layers from the edge (a) towards the middle (b, c, d, e) of the spot necrosis. W – cell wall, V – vacuole, C – cytoplasm, K – chloroplast, I – intercellular space.

koids, starch grains and osmophilic globuli (Fig. 6, Table 2). The size of osmophilic globuli in chloroplasts of healthy and virus infected plants did not differ, but their number was lower in chloroplasts of infected plants (Fig. 7). The smaller size of chloroplasts in infected plants can be correlated with the fact that the level of biochemically active cytokinins decreases in PVY^{NTN} secondary infected potato plants grown in vitro (DERMASTIA & RAVNIKAR 1996), since cytokinins promote chloroplast development (HORGAN 1984).

These results lead to the conclusion that PVY^{NTN} infection has an influence on the ultrastructure of chloroplasts in primary and secondary infected plants, particularly in spot necrosis, where the changes are part of apoptotic processes.

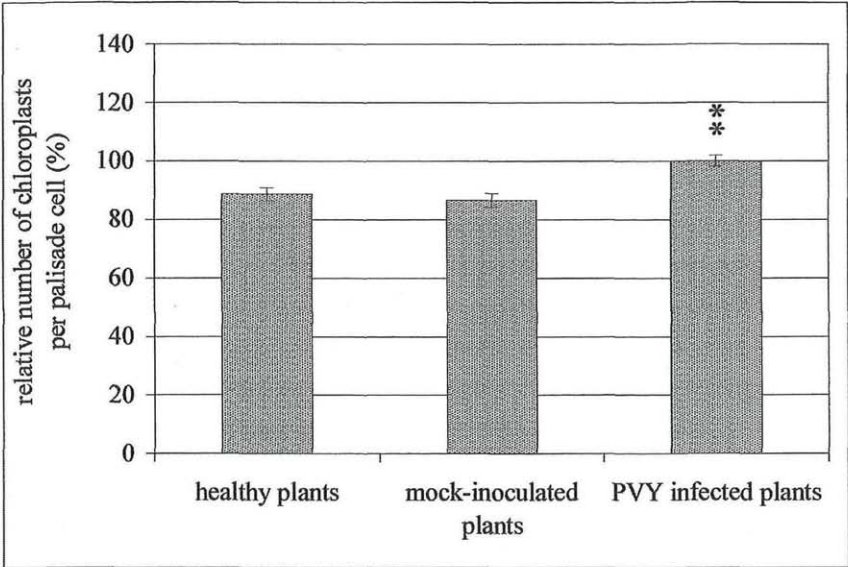


Fig. 4. Relative number of chloroplasts per palisade cell in systemically infected leaves of PVY^{NTN} infected plants and in leaves of the same age from healthy and mock-inoculated plants. Statistical comparison is made between healthy and mock-inoculated plants and between healthy and PVY^{NTN} infected plants. Statistically significant differences are indicated: **p < 0.01. Two times standard error is shown.

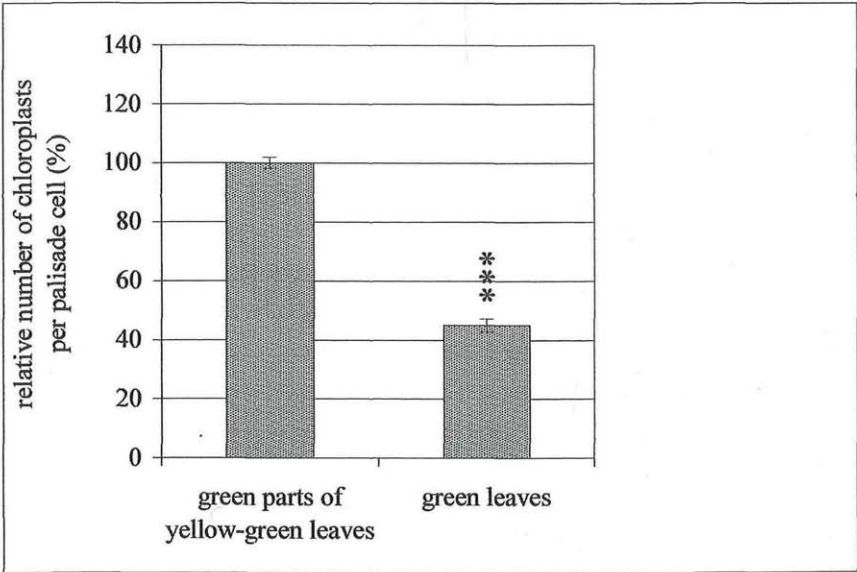


Fig. 5. Relative number of chloroplasts per palisade cell in leaves of different age from PVY^{NTN} infected plants. Statistically significant differences are indicated: ***p < 0.001. Two times standard error is shown.

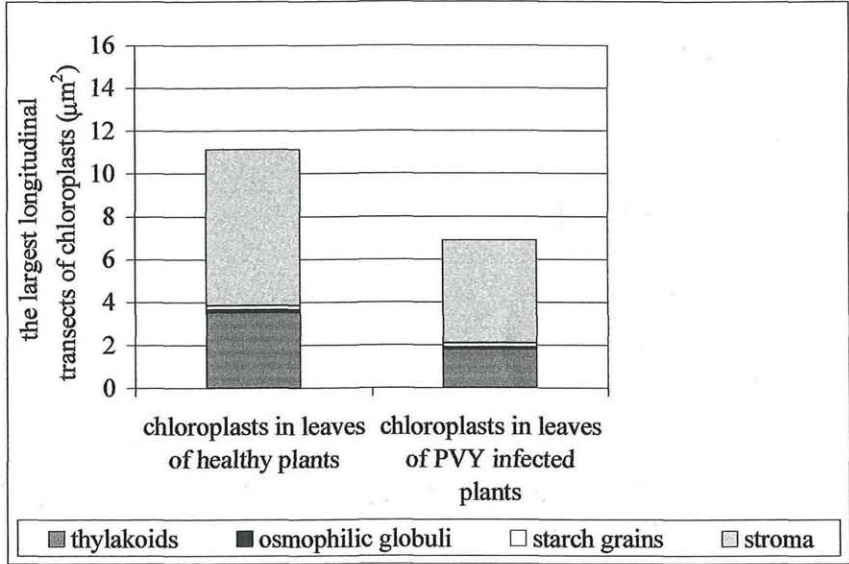


Fig. 6. The largest longitudinal transects of chloroplasts in palisade tissue of healthy and PVY^{NTN} infected potato plants cultivar Désirée grown in vitro.

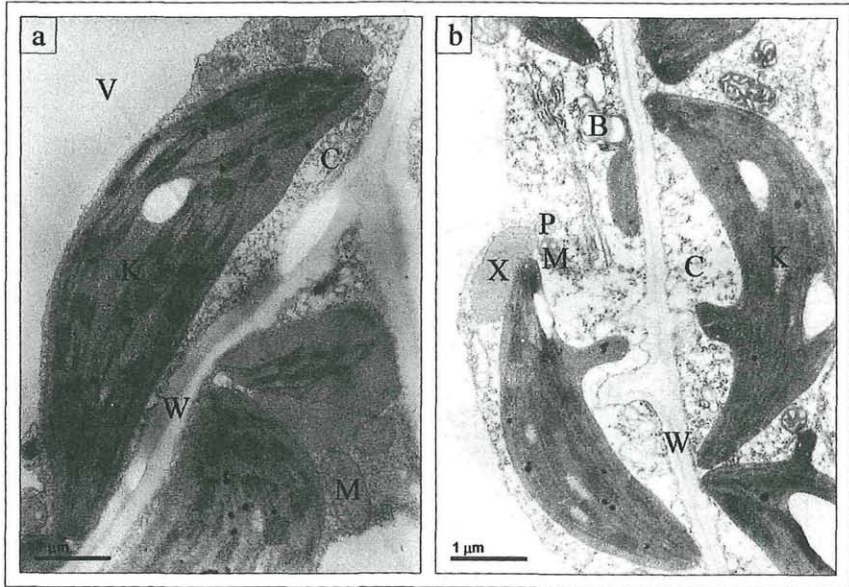


Fig. 7. Chloroplasts in palisade tissue of (a) healthy and (b) PVY^{NTN} infected leaves. W – cell wall, V – vacuole, C – cytoplasm, K – chloroplast, M – mitochondria, X – peroxisomes, P – pinwheels, B – lipid body.

Table 2.

Absolute and relative areas of the largest longitudinal transects of chloroplasts in palisade tissue of healthy and PVY^{NTN} infected potato plants cultivar Désirée grown in vitro. Average \pm standard error is shown. Statistically significant differences are indicated: *** $p < 0.001$, * $p < 0.05$. ns – not significant

	healthy plants	PVY infected plants	the statistical difference
the area of chloroplasts (μm^2)	11.14 \pm 0.43	6.89 \pm 0.41	***
the area of thylakoids (μm^2)	3.54 \pm 0.28	1.81 \pm 0.16	*
the number of osmophilic globuli	16.90 \pm 1.97	10.00 \pm 1.10	*
the area of single osmophilic globul (μm^2)	0.0063 \pm 0.0005	0.0065 \pm 0.0003	ns
the area of osmophilic globuli (μm^2)	0.10 \pm 0.01	0.06 \pm 0.01	*
the number of starch grains	1.60 \pm 0.22	1.25 \pm 0.12	ns
the area of starch grains (μm^2)	0.21 \pm 0.04	0.21 \pm 0.03	ns
the area of chloroplasts (%)	100	100	ns
the area of thylakoids (%)	31.46 \pm 1.68	26.24 \pm 2.00	ns
the area of osmophilic globuli (%)	0.87 \pm 0.10	0.93 \pm 0.08	ns
the area of starch grains (%)	1.00 \pm 0.35	1.57 \pm 0.51	ns

In the green parts of leaves of primary and secondary infected plants, PVY^{NTN} infection does not cause any visible changes on thylakoids, so the decreased level of photosynthesis is likely to be a consequence of changes at the biochemical level, such as changes in carbohydrate metabolism (HERBERS & al. 2000), in synthesis of pathogenesis-related proteins (HUUB & LINTHORST 1991) and in the level of peroxidases, which, according to our recent studies (SEMPRIMOŽNIK 1999), change in early stages of infection.

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Autor(en)/Author(s): Pompe-Novak Marusa, Wirscher Mercedes, Ravnikar Maja

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