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Changes in the photosynthetic functions in leaves of Chinese cabbage infected with turnip yellow mosaic virus

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Virus infection induces changes in host plant metabolic processes, including the most basic one, photosynthesis. Loss of photosynthetic activity, which is frequently reflected to macroscopic symptoms as yellow/green mosaic pattern or chlorosis of leaves, may be the result of decomposition processes or inhibited biosynthesis of some components (Harsányi et al. 2001). In several virus-host interactions the altered CO_2-fixation and starch accumulation, being the secondary consequence of source-sink imbalance, may inhibit gene expression and lead to changes in the pattern and composition of chlorophyll-protein (CP) complexes (Balachandran et al. 1997).

Most studies focused on alterations in the photosystem (PS)II complex. The amount of certain proteins belonging to the oxygen-evolving complex decreased significantly in various virus-plant combinations. Diminished ability of PSII reaction centres for energy capture was reported in virus-infected plants, and it functioned as a photoprotective mechanism to excess irradiance (Rahoutei et al. 1998). Photoprotective mechanisms usually influence also Chl fluorescence.

Certain stress factors alter not only the red and far-red fluorescence emission of Chl *a*, but cause significant changes also in the blue and green region. When excited with UV-A radiation, plant leaves show a fluorescence emission in the visible range with maxima around 440 nm (F440), 520 nm (F520), 690 (F690) and 740 nm (F740) (Stober and Lichtenthaler 1993). The blue-green fluorescence is thought to be primarily due to the accumulation of stress related substances including hydroxycinnamic acid derivatives such as ferulic acid, p-coumaric acid and caffeic acid (Lichtenthaler and Schweiger 1998).

The aim of the present work was to investigate the effects of turnip mosaic *tymovirus* (TYMV) infection on the structural and functional characteristics of the photosynthetic apparatus of Chinese cabbage leaves studying the changes in the pattern and composition of CP complexes and the CO_2-fixation activity. The strength of the stress effects was studied by fluorescence imaging of leaves in the red and in the blue-green spectral regions.

Materials and Methods

Plants and viruses. Chinese cabbage (*Brassica pekinensis* cv. Pach Choi) plants were grown under normal greenhouse conditions. Plants were inoculated with TYMV mechanically. Inoculum was prepared from symptomatic leaves.

Determination of chlorophyll (Chl) content. Samples were cut separately from the green and the yellow area of leaves showing mosaic symptoms. Tissue pieces were homogenised in 80% (v/v) acetone. Absorbance was measured with a Shimadzu UV-2101PC spectrophotometer. Chl content was calculated according to Porra et al. (1989).

Chlorophyll-protein pattern. Isolation of thylakoids and separation of CP complexes by Deriphat polyacrylamide gel electrophoresis (PAGE) using glucosidic detergents for solubilisation were carried out according to Sárvári and Nyitrai (1994). Polypeptide patterns of thylakoids and CP bands (used for their identification) were determined by denaturing PAGE (Laemmli 1970) but in 10-18% gradient gels. Relative ratios of the bands were calculated from the densitograms measured with a Perkin Elmer 554 spectrophotometer equipped with a gel scanner at 671 nm. Absolute values were calculated by dividing the Chl content of one g leaf material according to the ratios obtained from the densitograms.

Fluorescence induction and imaging procedure. F_v/F_m values were determined by PAM fluorometer (Walz, Effeltrich, Germany). For the fluorescence images of leaves a compact flash-lamp fluorescence imaging sytem was used (Lichtenthaler and Babani 2000). The images were sensed at the adaxial (upper) leaf side, and were corrected by the background and by the inhomogeneity of the exciting light.

CO_2-fixation. CO_2-fixation was measured using radioactive isotope labeling (^{14}C) (Láng et al. 1985). CO_2 concentration in the gas phase was 1% (v/v). Leaf disks of the same sizes were cut from the infected and control plants (10-12 leaf disks per samples). Radioactivity of the samples was determined by liquid-scintillation method.

Results and Discussion

Chl content of leaves in infected plants reduced significantly compared to the control plants. In the green part of leaves it decreased to 70% of the control. In the yellow tissues these changes were more pronounced - 37% of the control Chl content remained - in accordance with the observed strong macroscopic symptoms. However, the Chl *a/b* ratio changed only slightly even in the yellow parts.

To investigate how the decreased Chl content affected CP complexes we separated CPs in green gels. Bands 1, 2 and 4 contained differently solubilised PSI particles with (1,2) or

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Table 1. CO_2 fixation in leaf disks of TYMV-infected and control Chinese cabbage plants. Level of significance (n=10-15 samples): * - $p \leq 0{,}001$, or ns - non-significant.

Sample	CO_2 fixation (cpm)	% of control
Chinese cabbage control	58873 ± 5573	100,0
TYMV-infected Chinese cabbage green leaf area	62522 ± 9652^{ns}	106,2
TYMV-infected Chinese cabbage yellow leaf area	49192 ± 8164*	83,6

Table 2. Fluorescence intensity ratios (F440/F690, F440/F740, F690/F740) in control and TYMV-infected Chinese cabbage leaves calculated from the corrected images and F_v/F_m values characterising the maximal quantum efficiency of PSII.

	control	TYMV-infected	in % of control
F440/F690	0.111	0.209	188.3
F440/F740	0.098	0.265	270.4
F690/F740	0.881	1.269	144.0
F_v/F_m	0.83	0.77	92.7

without (4) LHCI. The components of PSII core complexes were present in the bands 3 and 6 (RC és CP47 dimer or monomer forms), in band 5 (RC and CP47, CP43 monomers) and band 10 (CP43 monomer). Oligomer forms of the connecting antennae of PSII (CP29) were present in band 7, monomer forms (CP29, CP26, CP24 together with low amount of solubilised LHCI) in bands 11 and 12. In spite of the non-significant changes in the Chl a/b ratios there were some variations even in the relative pattern of CP complexes. While in the green tissues the amount of all CPs reduced simultaneously with the reduction in the Chl content, the decrease of PSI and LHCII was more pronounced than that of the other CPs in the yellow segments of leaves. Analysing the protein composition of thylakoids, it was found out that mainly the amount of LHCI and LHCII changed. Therefore, TYMV infection affected CPs of light harvesting antennae of both PSs (LHCI and LHCII) similarly to iron deficient cucumber plants (Fodor et al. 1995). This led us to the conclusion that the effect of virus infection on photosynthetic structures is possibly a non-specific one. Loss of apoproteins of CP complexes can be the consequence of the disturbed protein synthesis of the host plant due to the replication processes of *tymovirus,* which is known related to the chloroplasts.

Concerning the activity of the photosynthetic apparatus, CO_2-fixation was about 20% less in the yellow tissues than in the control plants while in the green tissues it was even higher (Table 1). However, this increase in the photosynthetic activity did not prove to be significant. Reduced CO_2 fixation is a frequent symptom in virus infection (Balachandran et al. 1997), which reflects inhibition in the electron transport (see F_v/F_m in Table 2) and in the following steps of the photosynthesis (Calvin cycle).

The effects of virus infection on the activity of the photosynthetic apparatus (red and far-red fluorescence) and on the blue-green fluorescence were investigated by fluorescence imaging (Table 2).

The changes in the fluorescence intensity ratios calculated from the images were more pronounced compared to the F_v/F_m, the maximal quantum efficiency of PSII. The higher values of the blue/red, blue/far red, red/far red ratios were accompanied with decrease of F_v/F_m. Identical observations were made in the case of other virus infections, too. From these results it can be concluded that the higher fluorescence ratios mentioned above, can correlate with the lower PSII activity.

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