PHOTOSYNTHETICA 43 (3): 457-462, 2005

Photosynthetic responses of radish (*Raphanus sativus* var. *longipinnatus*) plants to infection by turnip mosaic virus

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Abstract

Plant growth, chlorophyll (Chl) content, photosynthetic gas exchange, ribulose-1,5-bisphosphate carboxylase (RuBPCO) enzyme activity, and Chl fluorescence in radish (*Raphanus sativus* var. *longipinnatus*) plants were examined after turnip mosaic virus (TuMV) infection. Plant fresh mass, dry mass, Chl content, net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and RuBPCO activity were significantly lower in infected plants after 5 weeks of virus infection as compared to healthy plants. The 5-week virus infection did not induce significant differences in intercellular CO_2 concentration (C_i), photochemical efficiency of photosystem 2, PS2 (F_v/F_m), excitation capture efficiency of open PS2 reaction centres (F_v'/F_m'), effective quantum efficiency of photosystem 2 ($\Delta F/F_m'$), and photochemical quenching (q_P), but non-photochemical quenching (q_N) and alternative electron sink (AES) were significantly enhanced. Thus the decreased plant biomass of TuMV-infected plants might be associated with the decreased photosynthetic activity mainly due to reduced RuBPCO activity.

Additional key words: chlorophyll fluorescence; gas exchange; photosystem 2; photochemical activity; ribulose-1,5-bisphosphate carboxylase/oxygenase.

Introduction

Radish (*Raphanus sativus* var. *longipinnatus*), an important vegetable, is thought to be originally domesticated in China, now cultivated all over the world, especially in eastern Asia (Zhou and Chen 1991). However, the productivity of radish is heavily affected by virus diseases including turnip mosaic virus (TuMV) (Yang *et al.* 1998). TuMV is an RNA virus that belongs to the genus *Potyvirus*, and is transmitted by aphids in a non-persistent manner (Edwardson and Christie 1986). TuMV causes a systemic infection of radish in which plants with viral infection exhibit some typical symptoms including stunting, mosaic, chlorosis, leaf distortion, mottling, malformation, and curling on leaves of radish, as well as malformed flowering stalk and fruit, and brown spots in the roots (Liu *et al*. 1990, Yang *et al*. 1998). Infection with virus reduces radish plant vigour and subsequent root size.

Virus infection negatively influences photosynthesis in many plant species (*e.g.* Funayama *et al.* 1997a,b, Ryšlavá *et al*. 2003). The lower productivity of infected plants may be attributed to the lower photosynthetic rate of chlorotic leaves (Swiech *et al.* 2001). Actually, decrease in photosynthetic rate of infected leaves is often associated with the development of the symptoms (Platt *et al.* 1979, Guo *et al.* 2005). In radish growing field, plant growth is generally limited by virus infection (Yang *et al.* 1998), and fitness of virus-infected plants is frequently lower than that of uninfected plants (Friess and Maillet 1996, Funayama *et al.* 1997a).

Determination of chlorophyll (Chl) *a* fluorescence is a rapid and non-invasive means of exploring aspects of the behaviour of the photosynthetic apparatus during

Received 16 December 2004, *accepted* 7 March 2005.
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Abbreviations: AES – alternative electron sinks; C_i – intercellular CO_2 concentration; CE – CO_2 carboxylation efficiency; Chl – chlorophyll; E – transpiration rate; ETR – apparent electron transport rate; F_v/F_m – photochemical efficiency of PS2; F_v'/F_m' – excitation capture efficiency of open PS2 reaction centres; $\Delta F/F_m'$ – effective quantum efficiency of photosystem 2; g_s – stomatal conductance; PFD – photon flux density; P_N – net photosynthetic rate; q_P – photochemical quenching of chlorophyll fluorescence; q_N – non-photochemical quenching of chlorophyll fluorescence; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; TuMV – turnip mosaic virus; ϕ_{CO2} – quantum efficiency CO_2 assimilation.
Acknowledgements: This work was supported by a grant (No. 30270908) from the National Natural Science Foundation of China.

environmental stress (Leipner *et al.* 1999, Maxwell and Johnson 2000, Sayed 2003). For the virus-infected plants, previous studies of fluorescence parameters have shown contrasting effects. The F_v/F_m ratio did not change in *Eupatorium makinoi* infected by geminivirus (Funayama *et al.* 1997b). In contrast, Ryšlavá *et al.* (2003) and Zhou *et al.* (2004) showed significant decrease in F_v/F_m in *Nicotiana tabacum* after *potato virus* A and *potato virus* Y infection. Similarly, Chia and He (1999) observed a lower F_v/F_m in *Oncidium* after virus infection. This variation might be explained by differences in plant sensitivity or resistance to virus. To better understand the changes in growth and photosynthetic properties after TuMV infection in radish plants, we examined CO_2 assimilation and related parameters, and compared the Chl fluorescence in infected and healthy plants.

Materials and methods

Plants: This experiment was conducted with radish plants (*Raphanus sativus* var. *longipinnatus* cv. Zhedachang) grown for 3 weeks from seeds in pots containing silica : vermiculite (1 : 1) in a greenhouse with temperature of 25/20 °C (day/night) under natural light. Plants were watered daily and received 80 cm^3 of Hoagland solution (Hoagland and Arnon 1938) twice a week. Inoculation was performed by placing 0.5 cm^3 of inoculum of a field TuMV isolate on one newly expanded leaf previously dusted with carborundum (600 mesh) at a four-leaf stage. Six plants or more per treatment (healthy and infected) were used in the following experiments.

Chl content: About 1.0 g of the third leaf tissue from different plants were extracted in 80 % acetone after grinding. Chl *a* and *b* amounts were estimated by a procedure described by Lichtenthaler (1987).

Photosynthetic gas exchange was measured using an open system (*HCM-1000*, *Walz*, Effeltrich, Germany) according to the procedure of Guo *et al.* (2003), using the third completely expanded leaf from the top of each plant. Leaf net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were determined at a temperature of 25 °C, CO_2 concentration of 350 μmol mol^{-1}, 45 % relative humidity, and photon flux density of 800 μmol m^{-1} s^{-1}. Leaf temperature was controlled using a leaf cuvette with a *1010-M* system (*Walz*, Effeltrich, Germany). Photosynthetic gas exchange was measured 1 and 5 weeks after virus infection.

The P_N/C_i response curves were established for infected and healthy plants at a leaf chamber temperature of 25 °C and saturating irradiance (800 μmol m^{-1} s^{-1}). Different intercellular CO_2 concentrations were used in the order: 250, 150, 100, 70, 35, 20, and 250, 320, 430, 525, 638, 780 μmol mol^{-1} (Long and Berancchi 2003), using an external computer control system and CO_2 dosing unit (*1030-D*, *Walz*, Effeltrich, Germany). CO_2 carboxylation efficiency (CE) was estimated for each individual leaf by fitting maximum likelihood regressions to the initial slope and plateau of the P_N/C_i response curves using the methods of Guo *et al.* (2000).

Chl fluorescence was measured with a pulse-modulated fluorometer (*PAM-2000*, *Walz*, Effeltrich, Germany) at room temperature (25 °C). The fluorometer was connected to a computer with the Data Acquisition System (*PAMWIN*, *Walz*, Effeltrich, Germany). Before each measurement, the sample leaf was dark-adapted for 20 min with dark leaf clips (*DLC-8*, *Walz*, Effeltrich, Germany) provided (Demmig *et al.* 1987, Guo *et al.* 2005). To determine the minimal fluorescence F_0, the weak measuring radiation (<0.1 μmol m^{-2} s^{-1}) was turned on and F_0 was recorded. Then the sample leaf was exposed to a 0.8 s saturating flash of approximately 8 000 μmol m^{-2} s^{-1} to obtain the maximal fluorescence yield (F_m). The ratio of variable to maximal fluorescence (F_v/F_m) was calculated automatically. The sample leaf was continuously irradiated with "white actinic light" of 336 μmol m^{-2} s^{-1}. The steady state value of fluorescence (F_s) was thereafter recorded and a second saturating pulse at 8 000 μmol m^{-2} s^{-1} was imposed to determine maximal fluorescence in the light-adapted leaf (F_m'). F_0' was basal fluorescence after far-red irradiation. The photochemical quenching coefficient (q_P), the non-photochemical quenching coefficient (q_N), and $\Delta F/F_m'$ were calculated as: $(F_m' - F_s)/(F_m' - F_0)$, $(F_m - F_m')/(F_m' - F_0)$, and $(F_m' - F_s)/F_m'$, respectively (Genty *et al.* 1989, Krall and Edwards 1992). The alternative electron sinks (AES) were calculated as: $[(F_m' - F_s)/F_m']/ø_{CO2}$ (Ribeiro *et al.* 2003). $ø_{CO2}$ was calculated as: $(P_N + R_D)/(PFD \times 0.84)$ (Edwards and Baker 1993), where R_D is dark respiration rate and 0.84 is the fractional radiation absorbance. All measurements were made between 08:00 and 11:00 and replicated at least six times.

RuBPCO enzyme activity assay: For these measurements, leaves at 5 weeks after TuMV infection were collected and frozen in liquid nitrogen. The frozen leaves were then ground to fine powder in liquid nitrogen and rapidly extracted with 2.0 cm^3 ice cold extraction buffer containing 50 mM bicine, pH 8.0, 20 mM $MgCl_2$, 2 mM phenylmethylsulfonyl fluoride, 50 mM 2-mercaptoethanol, and 30 mg polyvinylpolypyrrolidone (PVPP). The extracts were clarified by centrifugation (10 000×*g* at 4 °C for 2 min) and the initial and total RuBPCO activities were determined according to the procedure described by Parry *et al.* (1997). Soluble protein was determined according to the method of Bradford (1976). The third leaf from top of healthy and virus infected plants were used in this experiment. The measurements were

replicated at least six times.

Measurement of plant biomass, plant height, and leaf area: At the end of the experiment, plants were harvested. The leaves and roots of each plant were separately collected. Leaf area was measured with a leaf area measurement system (*LI-3000*, *LI-COR*, USA). After fresh mass determination, the materials were dried at 80 °C for 48 h, and dry masses of leaves and roots were determined.

Statistical analysis: Experimental data were analyzed by analysis of variance (*ANOVA*) and the significance of the Tukey test was determined at $p<0.05$.

Results

The Chl *a* and *b* contents slightly increased with the growth of plants, irrespective of infected or healthy plants (data not shown). There was no great difference in Chl *a* and *b* contents between infected or healthy plants after the first week of virus infection, however, five weeks after virus infection, the infected plants had a significantly lower Chl *a* content compared to healthy plants. Meanwhile, the Chl *a/b* was not significantly influenced by viral infection (Table 1).

Table 1. Chlorophyll (Chl) content, gas exchange, RuBPCO activity, fluorescence parameters, leaf number, leaf area, and plant height in TuMV infected and healthy plants. Means ± S.E. of six plants. Parameters at same date within a row with the same letter are not significantly different at $p<0.05$ using the Tukey test.

Parameters	1 week		5 weeks	
	Healthy	Infected	Healthy	Infected
Chl *a* [g kg^{-1}(DM)]	9.18±0.37 a	8.57±0.85 a	19.88±2.11 a	12.91±0.68 b
Chl *b* [g kg^{-1}(DM)]	3.39±0.70 a	3.33±0.38 a	11.22±2.44 a	7.67±1.15 a
Chl (*a*+*b*) [g kg^{-1}(DM)]	12.57±2.22 a	11.90±1.23 a	31.11±2.22 a	20.58±2.23 a
Chl *a/b*	2.71±0.07 a	2.57±0.06 a	1.77±0.10 a	1.68±0.09 a
P_N [μmol m^{-2} s^{-1}]	13.33±0.89 a	12.98±1.07 a	17.11±0.68 a	13.15±1.01 b
g_s [mmol m^{-2} s^{-1}]	172.10±19.88 a	174.38±16.71 a	252.62±22.20 a	167.98±29.16 b
E [mmol m^{-2} s^{-1}]	4.45±0.36 a	4.10±0.33 a	6.56±0.24 a	4.87±0.63 b
C_i [μmol mol^{-1}]	258.38±9.18 a	269.52±3.04 a	273.42±11.03 a	253.37±22.48 a
F_v/F_m	0.81±0.03 a	0.80±0.02 a	0.84±0.01 a	0.83±0.01 a
F_v'/F_m'	0.74±0.04 a	0.71±0.05 a	0.75±0.01 a	0.72±0.03 a
$\Delta F/F_m'$	0.59±0.02 a	0.53±0.04 a	0.68±0.02 a	0.59±0.04 a
q_P	0.78±0.02 a	0.77±0.09 a	0.90±0.02 a	0.83±0.03 a
q_N	0.39±0.02 a	0.40±0.03 a	0.40±0.02 b	0.50±0.02 a
AES	15.80±0.32 a	17.03±1.56 a	32.05±1.47 b	40.94±3.60 a
RuBPCO initial activity [mmol kg^{-1}(protein) s^{-1}]			14.00±1.33 a	9.50±0.50 b
RuBPCO total activity [mmol kg^{-1}(protein) s^{-1}]			24.50±1.50 a	18.00±1.17 b
Leaf number [plant^{-1}]			12.34±1.03 a	11.83±0.75 a
Leaf area [cm^2 plant^{-1}]			105.34±8.56 a	94.27±10.95 a
Plant height [cm]			26.53±1.34 a	25.11±2.10 a

The P_N, E, and g_s were significantly inhibited in infected plants 5 weeks after virus infection. However, C_i changed significantly. In comparison with the parameters measured after 5 weeks of virus infection, the differences between infected and healthy plants in the above parameters were not apparent 1 week after infection (Table 1).

The response of P_N to C_i showed that the initial slope (reflecting CO_2 carboxylation efficiency) and saturated P_N (representing ribulose-P_2 regeneration rate) were *ca*. 0.0488 and 450 μmol mol^{-1} in symptomatic leaves of infected plants in contrast to *ca*. 0.0609 and 500 μmol mol^{-1} in healthy plants, respectively (Fig. 1).

After 5 weeks of virus infection, there were significant declines in both the initial and total RuBPCO activity in infected plants, with RuBPCO activity declining by approximately 32 and 26 %, respectively, of the initial and total activity in healthy plants.

No differences between infected and healthy plants in Chl fluorescence parameters F_v/F_m, F_v'/F_m', q_P, q_N, $\Delta F/F_m'$, and AES were observed 1 week after virus infection. However, after 5 weeks of infection, q_N and AES were significantly enhanced in infected plants. F_v/F_m, F_v'/F_m', $\Delta F/F_m'$, and q_P were not significantly affected by virus infection, although a slighter decrease was frequently observed in infected plants (Table 1).

A stunted growth of plants was a typical symptom of virus infection. The plant and root fresh and dry masses in infected plants were significantly reduced compared with healthy plants at the end of experiment, although the differences in leaf fresh and dry masses were not

significant (Fig.2).

Leaf number, leaf area, and plant height were also apparently reduced by virus infection, although this reduction did not reach a significant level (Table 1).

Discussion

The decreased biomass in infected plants may be attributed to the reduced photosynthetic efficiencies (Funayama *et al.* 1997a). The total Chl content and Chl *a* content were significantly decreased in infected plants after 5 weeks of virus infection, which is likely to be reflected by the slight chlorosis of leaves (Table 1). The found decreased total Chl content was not accompanied by the increase in Chl *a/b* ratio. Hence the viral infection did not cause loss of light-harvesting pigment-protein complexes 2 (LHC2), which is in accordance with the re–sults of Funayama *et al.* (1997b) for *Eupatorium makinoi* infected by a gemini virus.

The decrease in P_N induced by TuMV infection in radish was consistent with the findings in some other plants after virus infection (Platt *et al.* 1979, Chia and He 1999, Swiech *et al.* 2001). It contradicted the results of Funayama *et al.* (1997b) that the maximal P_N was not much influenced by virus infection. Funayama *et al.* (1997b) and Sayed (2003) suggest that the reduced P_N may be due to the declined content of Chl per unit leaf area in infected leaves. We also found a correlation of P_N and Chl content per unit leaf mass (Table 1).

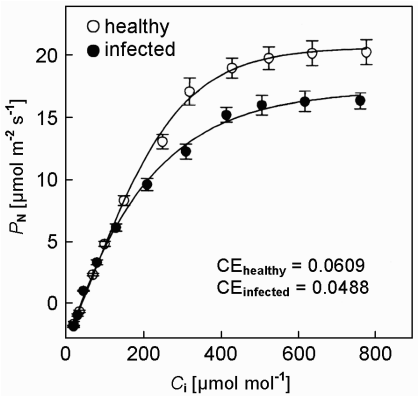

Fig. 1. Response curve of net photosynthetic rate (P_N) to intercellular CO_2 concentration (C_i) in radish leaves of infected (●) and healthy (○) plants 5 weeks after TuMV infection. Means ± S.E. of three replicates.

RuBPCO activity is regulated at different levels (Portis 1995, Zhang and Portis 1999) and it is difficult to state from the *in vivo* measurements which level may be involved in deactivating the enzyme in response to viral infection. However, the effect on RuBPCO activity is apparent from the measurement performed, where a significant decline in RuBPCO activity was observed in leaves of infected plants (Table 1). Furthermore, at high CO_2 (C_i>300 µmol mol^{-1}) (Fig. 1) the low RuBPCO activity in infected plants did not suffice to support the rate of CO_2 assimilation that was limited by the rate of RuBP regeneration, if P_N was not as high as in healthy plants (Fig. 1). By contrast, CO_2 assimilation by healthy plants was much higher than in infected plants at relatively higher C_i. In healthy plants, CO_2 assimilation was saturated at approximately 500 µmol mol^{-1}. However, the curve approached a plateau at lower CO_2 (at about 450 µmol mol^{-1}) in infected plants, indicating that assimilation had become limited by RuBP regeneration (Fig. 1). Therefore, the difference in shape of the CO_2 response curves of radish leaves readily reveals the influence of TuMV infection on RuBPCO activity.

In the present experiment, decrease in P_N in infected leaves was concurrent with the decrease in RuBPCO activity and RuBP regeneration rate measured with equivalent leaves (Fig. 1, Table 1), suggesting that TuMV infection limiting P_N is also likely attributable to the decline in the RuBPCO activity. Therefore, decreased P_N might occur after virus infection and subsequently result in a lower biomass in infected plants (Fig. 2).

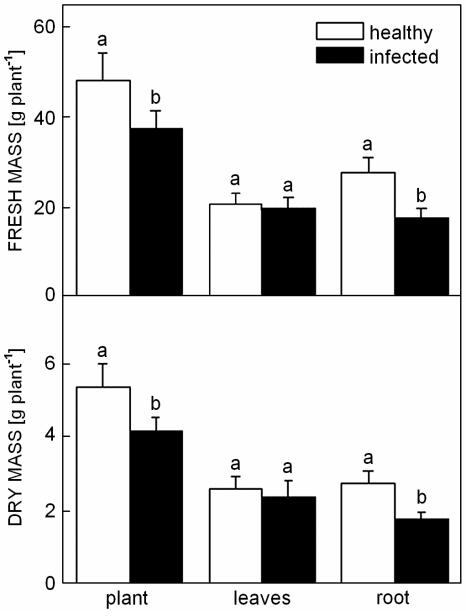

Fig. 2. Biomass of plant, leaves, and roots in infected and healthy plants 5 weeks after TuMV infection. Means ± S.E. of twelve plants. *Different letters* indicate significant differences at p<0.05 using the Turkey test.

g_s and E were also significantly reduced in infected plants after 5 weeks of virus infection, which is consistent with the reports in tobacco (Ryšlavá *et al.* 2003, Guo *et al.* 2005). In infected plants, the reduction in P_N was probably caused by non-stomatal limitation, since a non-significant decreased C_i was accompanied, which reflected (Table 1) the results of Farquhar and Sharkey (1982).

Accordingly, it is further evident that the decrease in RuBPCO activity was likely the result of P_N reduction (Table 1).

In the present experiment, the $\Delta F/F_m'$ was not significantly affected by virus infection, as well as F_v/F_m and F_v'/F_m', even though virus infection led to slight decrease of them (Table 1). However, after 5 weeks of virus infection, the q_N increased significantly in infected plants, indicating the occurrence of the non-radiative energy dissipation, where a larger proportion of absorbed photons may be lost as thermal energy instead of being utilized in photosynthesis (Schreiber *et al*. 1994, Shangguan *et al*. 2000). Such thermal loss is a protective mechanism, reducing the probability of photodamage (Barber and Andersson 1992, Song *et al*. 2003).

Two factors regulate electron flux in leaves by utilizing excitation energy that exceeds the capacity of CO_2 assimilation, the efficiency of excitation energy transfer to photochemically active PS2 reaction centres (*i.e*. non-photochemical quenching), and the ability of PS2 to transfer electrons to acceptors (*i.e*. photochemical quenching). The relative importance of these two quenching components in leaves is not fixed, it can be influenced more or less by conditions of the leaf, although there are additional pathways in chloroplasts (*i.e*. ferredoxin-dependent reduction or NADPH) (Scheibe 1987, Ort and Baker 2002). Reports show that acceleration of alternative electron transport is accompanied with a limitation in CO_2 fixation (Loreto *et al*. 1994, Song *et al*. 2003). The alternative electron sinks are particularly important in plants growing under stress conditions. In such situations, PS2 electron fluxes can be greatly in excess of the requirements for CO_2 assimilation and photorespiration. In the present study, the elevated AES in infected plants implies more electrons regenerated that can contribute to photoprotection when photosynthesis is limited (Foyer and Noctor 2000, Ort and Baker 2002).

In the present experiment, Chl content, Chl fluorescence parameters, gas exchange, and RuBPCO activity were not affected apparently by virus infection as expected and did not differ significantly from those measured in healthy plants before the 5th week of virus infection (data not shown). This indicates the progress of virosis in radish plants is time taking, which is consistent with the results of Ryšlavá *et al.* (2003), Zhou *et al.* (2004), and Guo *et al.* (2005).

Our present results demonstrate that radish after TuMV infection shows a general response similar to that in other plants infected by virus. The reduced plant biomass in infected plants may be due to a reduced photosynthetic rate, which is mainly associated with the reduced RuBPCO activity.

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