# Changes in Photosynthetic Apparatus of Tobacco Leaves in Conditions of Virus Infection and Shortage of Nitrogen

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**Abstract**

Data of the comparative investigation of the viral infection (TMV) and nitrogen starvation in the ratio of chlorophyll a/b, photochemical activity of PS I and PS II, pigment-protein structure of chloroplasts thylakoids and parameters of the fluorescence induction of tobacco leaves are presented. The changes of the structural and functional characteristics of the photosynthetic apparatus testify to negative influence of this factors on the function of both photosystems with primary inhibition of PS II.

**Keywords**: virus infection; *Tobacco mosaic virus* (TMV); stress; *Nicotiana debneyi*; PS I; PS II; chloroplasts; chlorophyll; photosynthesis

## INTRODUCTION

The question about specificity of influence of virus infection on different components of photosynthetic apparatus obtains a special meaning in solving the problem of revealing of various kinds of stresses. It is known from literature sources that membrane system of chloroplasts is a subject for destruction in virus-infected plants. There was shown that some changes in organization of pigment-protein complexes occur. Besides, some authors demonstrated (KOIWA *et al*. 1992) that virus-induced inactivation of photosystem II (PS II) is due to the destruction of its antenna which is represented by light-collecting chlorophyll a/b complex LHC II. Also, some changes in content of proteins of oxygen-emissing PS II complex were revealed (NAIDU *et al*. 1986). There was a hypothesis that different protein complexes of photosynthetic apparatus may degrade at different times and different degree under effect of virus infection (VAN KOOTEN & SNEL 1990). Comparative study of influence of stresses of various origin (*Tobacco mosaic virus* infection and nitrogen shortage) on tobacco plants' photosynthetic apparatus was the aim of this work. Such parameters as induction of chlorophyll fluorescence, photochemical activity of PS I and PS II, ratio of chlorophylls a/b and composition of pigment-protein complexes of tobacco plants were the targets of the research.

## MATERIALS AND METHODS

*Nicotiana debneyi* plants were the object of the research. Both control and experimental plants were grown in sand, in "Tulpe-2" greenhouse in the same light, water and temperature conditions. Plants with visible symptoms of virus infection persisting for 20 days at least were used in experiments. Different kinds of nitrogen nutrition has been applied. Virus inoculation was performed on the stage of two pairs of true leaves. Virus content in infectious mixture was controlled using indirect ELISA (CATTY 1991). Chloroplasts from *Nicotiana debneyi* leaves were extracted in buffer containing 50mM tricyne/NaOH, pH 7.5; 0.4M sucrose; 10mM NaCl; 5mM $MgCl_2$. Electrophoresis of chlorophyll-protein complexes of chloroplasts was carried out in polyacrylamide gel (PAG) (ANDERSON 1980) on GE-2/4 device (Pharmacia, Sweden). Speed of electron transport in chloroplasts on the level of separate photosystems was evaluated by polarographic technique using quantity of emissed (PS II activity) or absorbed (PS I photochemical activity) $O_2$ on universal polarograph OH-105 (Radelicis, Hungary) during the

lighting of chloroplasts with white light of saturating intensity (FUNAYAMA *et al*. 1997). Functional state of photosynthetic apparatus was tested by the data of measurement of luminescence induction on stationary automatic spectrofluorometric device. Following (VAN KOOTEN & SNEL 1990), such parameters as $F_0$, $F_m$ and $F_i$ were measured. Ratios $F_v/F_m$ and $F_{pL} - F_0/F_m - F_o$ were used for evaluation of photoinhibition degree and changes in relative quantity of Qb-non-reductive centres of PS II during the effect of stressors.

## RESULTS

Analysis of chlorophylls a/b ratio in chloroplasts of tobacco leaves demonstrated that nitrogen shortage and virus infection as well induces a decrease of its value (Table 1).

Table 1. Changes in value of chlorophylls a/b ratio in chloroplasts of leaves of tobacco growing in conditions of nitrogen shortage or TMV infection

| Variants of experiment | Value of chlorophylls a/b ratio | |
|---|---|---|
| Control (C) | 2.54 ± 0.01 | 100% |
| -$NH_4NO_3$ (-N) | 2.43 ± 0.02 | 95.6% |
| + TMV | 2.27 ± 0.02 | 89.4% |

Results of comparative analysis of pigment-protein complexes of studied variants are represented in Table 2. The most significant changes revealed after the electrophoretic separation of pigment-protein complexes of chloroplasts of tobacco leaves are in LHCP[2] and CPa complexes. This may cause changes in transfer of light energy from light-collecting pigments to reactive centres of PS II, and therefore be one of the factors of its inactivation. Also, we discovered changes in work of electron transport chain of chloroplasts.

Studied plant variants differed significantly in activity of PS I in chloroplasts, too (Table 4). This data indicates greater resistance of PS I to viral infection and to level of nitrogen nutrition.

Changes in PS II activity (Table 3) revealed during determining of photochemical activity of photosystems in chloroplasts thylakoids were confirmed in experiments on measurement of parameters of luminescence induction of plant leaves. According to obtained data (Table 5), decreasing of nitrogen nutrition leads to serious increasing of $F_0$ and $F_m$ values. Also, parameter $F_m - F_0/F_m = F_v/F_m$ known in literature as an index of quantum yield of PS II (GENTY *et al*. 1989) has been decreasing. This decrease made up 5% in mean. Decreasing of $F_v/F_m$ value was up to 9% in virus-infected plants. Changes of $F_v/F_m$ value were mainly induced by increasing of intensity of initial fluorescence $F_0$.

Table 2. Relative distribution of pigment-protein complexes during electrophoretic separation of thylakoid membranes

| Pigment-protein complexes | Control | | Virus-infected plants | | Nitrogen shortage | |
|---|---|---|---|---|---|---|
| | intensity, rel. un | % | intensity, rel. un | % | intensity, rel. un | % |
| CP1a + CP1 | 168.5 | 30.6 | 165.0 | 30.5 | 164.7 | 31.5 |
| LHCP[1] | 129.3 | 23.5 | 132.0 | 24.4 | 132.8 | 25.4 |
| LHCP[2] | 29.7 | 5.4 | 32.5 | 6.0 | 15.7 | 3.0 |
| Cpa | 30.3 | 5.5 | 19.6 | 3.6 | 15.8 | 3.0 |
| LHCP[3] | 94.6 | 17.2 | 96.8 | 17.9 | 96.7 | 18.5 |
| FP | 93.5 | 17.0 | 95.0 | 17.6 | 97.3 | 18.6 |

C – control plants, V – virus-infected plants, N – nitrogen shortage

Table 3. Activity of PS II in chloroplasts of tobacco leaves due to nitrogen shortage in nutrient medium or to TMV infection

| Variants | McM extr. $O_2$/mg chl. un | % from control |
|---|---|---|
| Control (C) | 100.1 ± 4.6 | 100 |
| -$NH_4NO_3$ (-N) | 53.4 ± 3.8 | 53.4 |
| + TMV | 49.0 ± 1.4 | 49.0 |

Table 4. Activity of PS I in chloroplasts of tobacco leaves due to nitrogen shortage in nutrient medium or to TMV infection

| Variants | McM extr. $O_2$/mg chl. un | % from control |
|---|---|---|
| Control (C) | 519 ± 22.5 | 100 |
| -$NH_4NO_3$ (-N) | 376.6 ± 11.0 | 72.5 |
| + TMV | 314.0 ± 22.2 | 60.4 |

Table 5. Parameters of luminescence induction in tobacco leaves adapted to dark, in different growing conditions

| Variant | $F_0$ | $F_m$ | $F_{pl}$ | $F_v/F_m$ | $F_{pl} - F_0/F_m - F_0$ |
|---|---|---|---|---|---|
| C | 25 ± 5 | 125 ± 5 | 62 ± 6 | 0.83 ± 0.03 | 0.37 ± 0.02 |
| -N | 51 ± 1 | 352 ± 21 | 234 ± 11 | 0.79 ± 0.03 | 0.61 ± 0.03 |
| V | 82 ± 9 | 283 ± 11 | 212 ± 9 | 0.76 ± 0.03 | 0.065 ± 0.03 |

## DISCUSSION

Obtained data did not allowed to reveal clear changes in plant reaction on nitrogen shortage and virus infection. However, some peculiarities of effects of these two types of stressors, such as changes in pigment-proteins LHCP2, CPa, parameters of fluorescence induction (more serious increasing of Fo fluorescence in leaves of virus-infected plants comparing to nitrogen-lacking) let propose that these stress factors cause disruptions on the level of light-collecting complexes and reactive centres at different degree.

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