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CHANGES IN THE SUBMILLI- AND MILLISECOND KINETICS OF DARK RELAXATION OF DELAYED FLUORESCENCE IN TOBACCO LEAVES UNDER CONDITIONS OF BACTERIAL INFECTION BY *PSEUDOMONAS SYRINGAE* PV. *TABACI*

Georgi Milanov[1*], Detelin Stefanov[2], Vassilii Goltsev[1], Rositsa Batchvarova[3]

[1]Faculty of Biology, University of Sofia, 8 Dragan Tsankov Blvd., 1421 Sofia, Bulgaria
[2]Acad. M. Popov Institute of Plant Physiology, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria
[3]Institute of Genetic Engineering, Kostinbrod, Bulgaria

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Summary. The influence of *Pseudomonas syringae* pv. *tabaci* from the first to tenth day after infections on three tobacco cultivars – Nevrokop 1146, transgenic Nevrokop 1146 and Aurea mutant was studied. The kinetics of dark relaxation of delayed fluorescence in the time range from 200 μs to 5.5 ms were investigated. A drop in the quantum yield of delayed fluorescence in the sensitive cultivar Nevrokop 1146 was established. An enhancement of the characteristic times with the development of the infection was observed. In both sensitive cultivars – Nevrokop 1146 and Aurea mutant – more considerable changes were established in the submilliseconds component (300–800 μs). The damages of the photosynthetic apparatus as possible results of bacterial infection were mainly in the acceptor side of photosystem 2 and were connected to the electron transfer between Q_A and Q_B. The damages in the Aurea mutant were more pronounced, while the changes in the transgenic plants from the 1st to the 10th day after infection were weaker. Therefore the infection by *Pseudomonas syringae* pv. *tabaci* did not considerably affect the primary photosynthetic reactions in photosystem 2 of the transgenic plants.

Key words: bacterial infection, decay kinetics, delayed fluorescence, photosynthesis, *Pseudomonas syringae* pv. *tabaci*, tobacco

* Corresponding author, E-mail: Milanov@biofac.uni-sofia.bg

Abbreviations: DF – delayed fluorescence, PS2 – photosystem 2, PQ – plastoquinone pool, Q_A and Q_B – primary and secondary electron acceptors of PS2

Introduction

Wildfire is one of the most widely spread diseases of tobacco plants. It is caused by the bacterium *Pseudomonas syringae* pv. *tabaci*, which produces a tabtoxin (Turner and Debbage, 1982). This toxin is the agent producing characteristic chlorotic halos on the leaves of tobacco plants. The tabtoxin is an inhibitor of glutamine synthetase, which is a key enzyme in the photorespiratory nitrogen cycle. The inhibition of glutamine synthetase leads to accumulation of NH_4 and cell death (Turner and Debbage, 1982). Wildfire disease is one of the main plant protection problems of tobacco breading (Lucas, 1975). Very little success has been achieved using chemical control. An alternative for controlling this plant disease is development of resistant cultivars. The pathogenic toxins might be the most attractive targets for genetic engineering of plant disease resistance (Yoneyama and Anzai, 1993). On this basis transgenic tobacco cultivars have been developed (Batchvarova et al., 1998).

Photosynthesis is the most sensitive process in plants in cases when different stress conditions occur (Baker, 1996). The biotic stress has particular effects on photosynthesis (Osmond et al., 1990; Funayama et al., 1997) and especially on photosystem 2 (Reinero and Beachy, 1989; Hodgson at al., 1989). The symptoms of leaf bacterial infection reflect the interactions between the pathogen (or action of the toxin) and the photosynthetic apparatus. It is known that the tabtoxin destroys chloroplasts and inhibits the glutamine synthetase, as mentioned above, which is a key enzyme in the photorespiratory nitrogen cycle.

There is not much data about the influence of bacterial infection on the electron-transport reactions in the thylakoid membranes, especially on photosystem 2 level. In the present investigation, changes in tobacco leaf photosynthesis due to bacterial infection with *Pseudomonas syringae* pv. *tabaci* were studied using the kinetics of dark relaxation of submilliseconds and milliseconds DF.

Materials and Methods

Plant material and inoculation

Eight-week-old tobacco plants (*Nicotiana tabacum* L.) were used in the experiments. All plants were grown as soil cultures in greenhouse conditions with 120 $\mu mol.m^{-2}.s^{-1}$ photosynthetic photon flux density, 16 h photoperiod, 25/20 °C day/night temperature and 60–80% RH. In the sensitive to wildfire N1146 the symptoms of disease were quickly developed – chlorotic haloses appeared even one day after infection. The

transgenic resistant to wildfire tobacco plants (T1146), carrying ttr-gene, were obtained by transformational experiments according to Horsch et al. (1985), on the basis of N1146 (Batchvarova et al., 1998). Aurea type tobacco plants, obtained from Nevrokop 1146 (Atanassov et al., 1993) were also infected. These chlorophyll deficient mutant plants possessed yellow-green leaves and differed from N1146 and T1146, which had dark green leaves. The investigations were carried out with healthy and infected plants – 1, 5 and 10 days after bacterial inoculation. Eight-week-old tobacco plants with 6 to 8 fully expanded leaves were infected with inoculum prepared from *Pseudomonas syringae* pv. *tabaci* bacteria. They were grown as described by Batchvarova et al. (1998). Inoculum concentration was adjusted spectrophotometrically to 10^8 cells.ml^{-1}. Fifth and sixth fully expanded leaves were pricked with a needle, sprayed with the bacterial suspension and grown at high humidity (95%) in a greenhouse at 25 °C and later used in the assays.

Fluorescence measurements

Chlorophyll luminescence was measured at room temperature using a fluorimeter Fl-2006 (Test, Russia). Signal recordings and calculations were performed on a personal computer using Fl2006 data aquision and software. Many types kinetic components of DF are known, distinguished by their life time: nano-, micro-, submilli-, milli-, deciseconds, seconds, etc (Malkin, 1978; Veselovskii and Veselova, 1990). In our study the kinetics of dark relaxation of submilliseconds and milliseconds DF in different periods of the induction curve were measured (Goltsev and Yordanov, 1997). Each kinetic included 102 points, registered every 50 μs. Decay kinetics were registered on 11 ms, 168 ms and 5.7 s after illuminating the sample. The kinetics of dark relaxation were described as exponential drop, where the fitting procedure was done according the equation: $I_{DF} = L_1.e^{-t/\tau 1} + L_2.e^{-t/\tau 2} + L_3$, where L_1, L_2 and L_3 are the amplitudes of submilliseconds, milliseconds and "slow" (more than 10 ms) components, respectively, and τ_1 and τ_2 are the characteristic times of the fast components.

Results and Discussion

The kinetics of dark relaxation (decay kinetics) in submilli- and milliseconds time domains were analyzed, registered on 11 ms, 168 ms and 5.7 s after switching on of the actinic light. These moments correspond to the characteristic points of the induction curve of DF – I_2, D_2 and I_5. Decay kinetics were registered in the range of 200 μs to 5.5 ms and became approximate to a twoexponential function : $I_{DF} = L_1.e^{-t/\tau 1} + L_2.e^{-t/\tau 2} + L_3$, where L_1, L_2 and L_3 are the amplitudes of submilliseconds, milliseconds and "slow" (more than 10 ms) components, respectively. τ_1 and τ_2 are the characteristic times of the fast components. It is supposed that the emission, falling in the submilliseconds

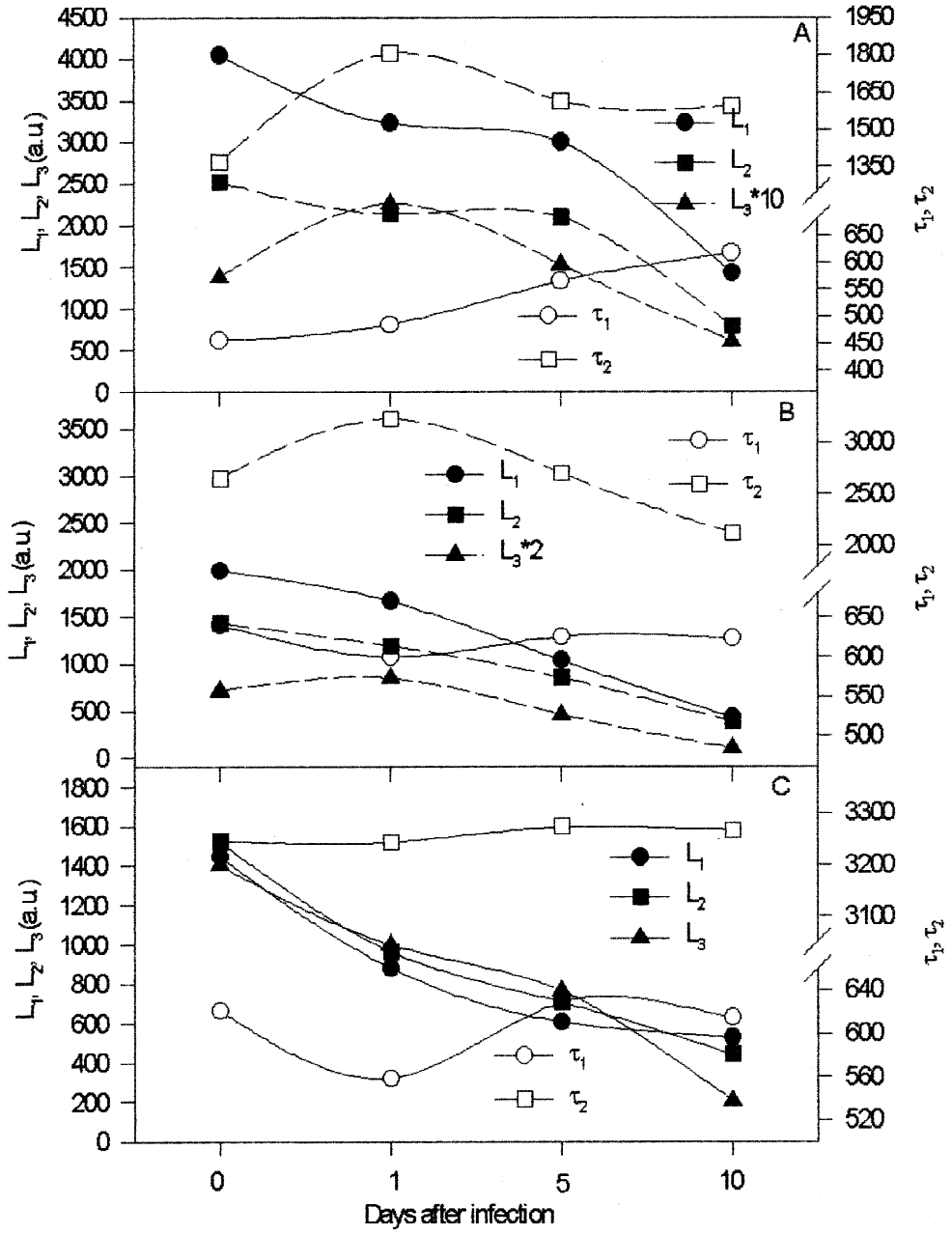

Fig. 1. Changes in the three amplitudes (L_1, L_2 and L_3) and two characteristic times (τ_1 and τ_2) in the maximum I_2 (A), in the minimum D_2 (B) and in the maximum I_5 (C) from the induction curve of DF in leaves of infected sensitive tobacco plants (N1146). Data represent the average of the three separate expertiments and were reproducible to within ±20% SE.

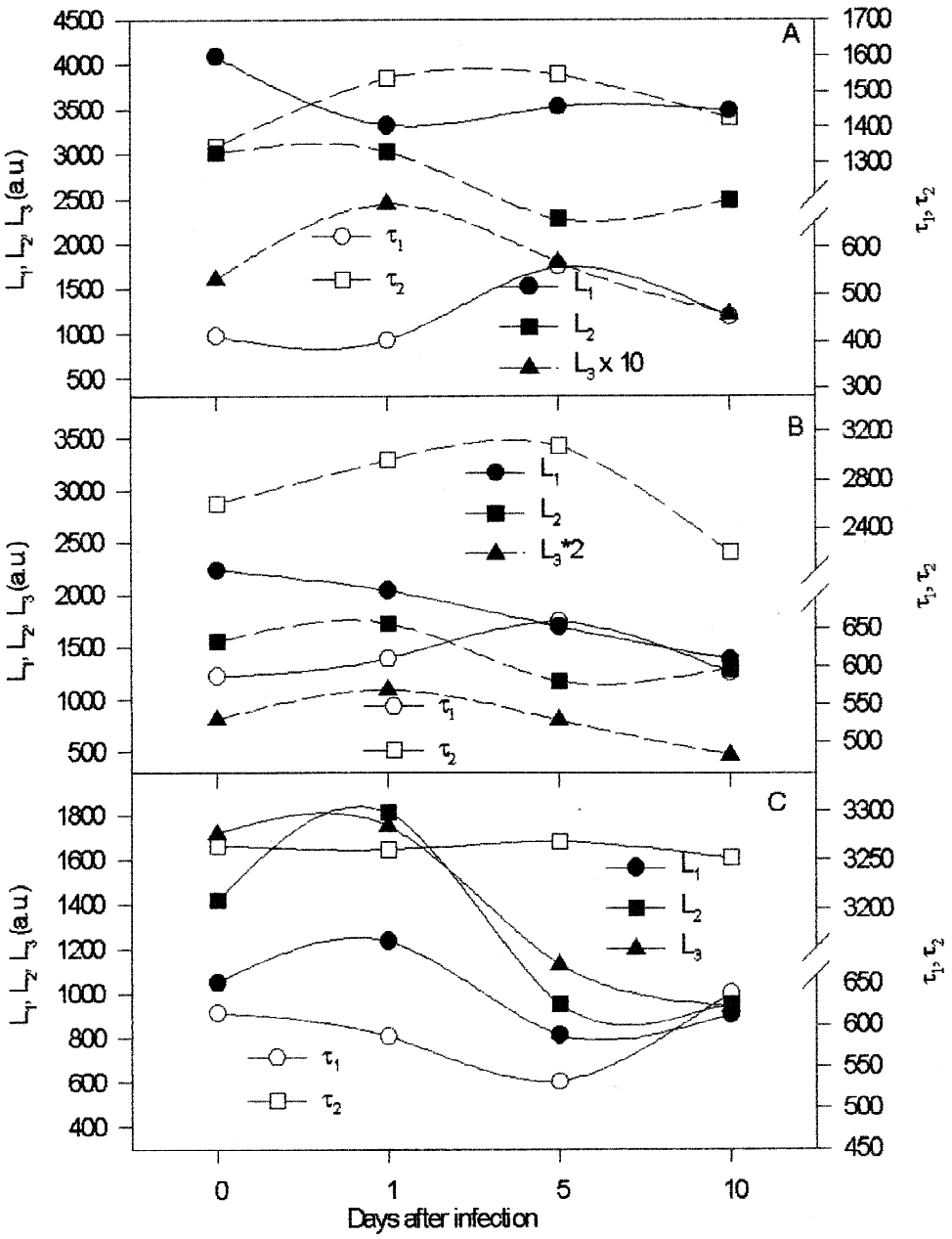

Fig. 2. Changes in the three amplitudes (L_1, L_2 and L_3) and two characteristic times (τ_1 and τ_2) in the maximum I_2 (A), in the minimum D_2 (B) and in the maximum I_5 (C) from the induction curve of DF in leaves of infected transgenic tobacco plants (T1146). Data represent the average of the three separate expertiments and were reproducible to within ±20% SE.

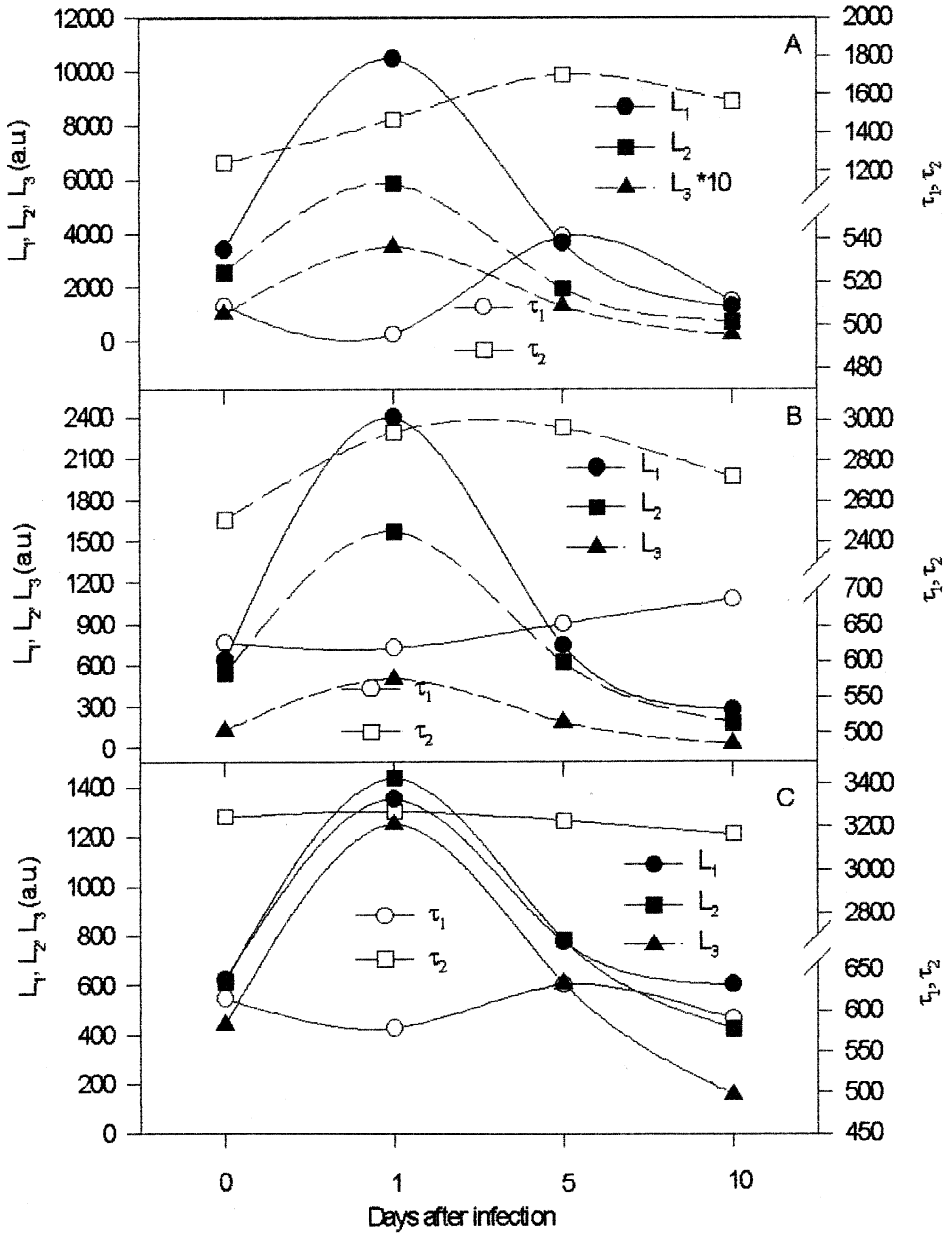

Fig. 3. Changes in the three amplitudes (L_1, L_2 and L_3) and two characteristic times (τ_1 and τ_2) in the maximum I_2 (A), in the minimum D_2 (B) and in the maximum I_5 (C) from the induction curve of DF in leaves of infected Aurea tobacco plants. Data represent the average of the three separate expertiments and were reproducible to within ±20% SE.

domain was related to the recombination of $Z^+P_{680}Q_A^-Q_B^{--}$, as the lifetime was determined by the possibility of recombination of the state and electron transport rate from Q_A^- to Q_B. I_{DF} would be the highest in $Z^+P_{680}Q_A^-Q_B^{--}$ (the lowest possibility of transport from Q_A to Q_B). L_2 and τ_2 represent emission of RC of PS2 in $Z^+P_{680}Q_B^{--}$ state. The amplitude of this light state (L_2) was determined by the quazistationary concentration of the RC of PS2 with the $Q_AQ_B^{--}$ states. The characteristic time (τ_2) was estimated by the oxidation of Q_B^- from the PQ pool. The slower kinetic components of DF were read only as a constant member of the equation – L_3.

A drop in the quantum yield of DF of the sensitive cultivar N1146 was established. An enhancement of the characteristic times was observed with the development of infection. The dynamics of the changes of the amplitudes and characteristic times in the initial stages of infection in N1146 (Fig. 1) and Aurea (Fig. 3) was probably due to appearance of a hypersensitive reaction. The later effects could be a consequence of changes in the reaction centres of PS2 – conformational changes in the proteins in N1146 and alterations in the cross-section of pigment antenna of PS2, especially in Aurea. By comparing the changes of τ_1 in I_2 and D_2 positions we can conclude that the infection changed the rate of electron transport between Q_A and Q_B, but not the recombination of $Q_A^-Z^+$ in the reaction centres of PS2. The acceleration of τ_2 in N1146 five and ten days after infection was related with the insufficient filling of the PQ pool, because of damages in the antenna complex of PS2, leading to decreased influx of energy to the reaction centres of PS2. In both sensitive cultivars – N1146 and Aurea mutant – more considerable changes were established in the submilliseconds component (300−800 μs). Damages of the photosynthetic apparatus as possible results of bacterial infection were mainly in the acceptor side of photosystem 2 and were connected to the electron transfer between Q_A and Q_B. Damages in the Aurea mutant were more pronounced, while changes in the transgenic plants (Fig. 2) from the 1st to the 10th day after infection were smaller. Therefore the infection by *Pseudomonas syringae* pv. *tabaci* did not considerably affect the primary photosynthetic reactions in PS2 in the transgenic plants and we can conclude that these plants revealed increased resistance of the photosynthetic reactions to bacterial infection.

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