St Kliment Ohridski Sofia University
Biological faculty
Department Biophysics and radiobiology
Assoc Prof. Detelin Stefanov PhD

Main scientific interests

Main scientific activity is focused on investigation on biophysics (optical spectroscopy – absorption and prompt and delayed fluorescence; infrared gas analysers – the rate of CO_2 assimilation by leaves; leaf oxygen evolution; photoacoustic spectroscopy) and ecophysiology of photosynthesis (photosynthesis in sun and shade leaves; photosynthesis in resurrect plants such as poikilohydric homoiochlorophyllous plants; effect of abiotic and biotic stresses on photosynthesis) including the use and development of non-invasive methods for evaluation of the changes in photosynthesis in intact leaves.

Following studies with non-invasive techniques are involved in my scientific studies such as:

1. Fluorescence induction curves and related fluorescence parameters for estimation of photosynthetic electron transport activity using of both main approaches for estimation of fluorescence induction phenomena In intact leaves – conventional methods (based to so called JIP test) and pulse modulated techniques – quenching analysis). Main publications involve:

Stefanov D and Terashima I. (2008) Non-photochemical loss in photosystem II in high and low light grown leaves of *Vicia faba* quantified by several fluorescence parameters including L_{NP}, F_0/F_m', a novel parameter. Physiol. Plant. 133: 327-338

Yordanov, I., Goltsev, V., Stefanov, D., Chernev, P., Zaharieva, I., Kirova, M., Gecheva, V., Strasser, R.J. (2008) Preservation of photosynthetic electron transport from senescence-induced inactivation in primary leaves after decapitation and defoliation of bean plants. Journal of Plant Physiology 165: 1954-1963

Goltsev, V., Zaharieva, I., Chernev, P., Kouzmanova, M., Kalaji, H.M., Yordanov, I., Krasteva, V., Alexandrov V., Stefanov D., Allakhverdiev S., Strasser, R.J. (2012) Drought-induced modifications of photosynthetic electron transport in intact leaves: Analysis and use of neural networks as a tool for a rapid non-invasive estimation. Biochimica et Biophysica Acta - Bioenergetics 1817 (8) , pp. 1490-1498 doi:10.1016/j.bbabio.2012.04.018

Denev, I., Stefanov, D., Terashima, I. (2012) Preservation of integrity and activity of Haberlea rhodopensis photosynthetic apparatus during prolonged light deprivation Physiologia Plantarum 146 (1) , pp. 121-128 doi:10.1111/j.1399-3054.2012.01608.x

An example on conventional registration of fluorescence induction and JIP test

Stefanov, D., Petkova, V., Denev, I.D. (2011) Screening for heat tolerance in common bean (Phaseolus vulgaris L.) lines and cultivars using JIP-test. Scientia Horticulturae 128 (1), pp. 1-6 doi:10.1016/j.scienta.2010.12.003

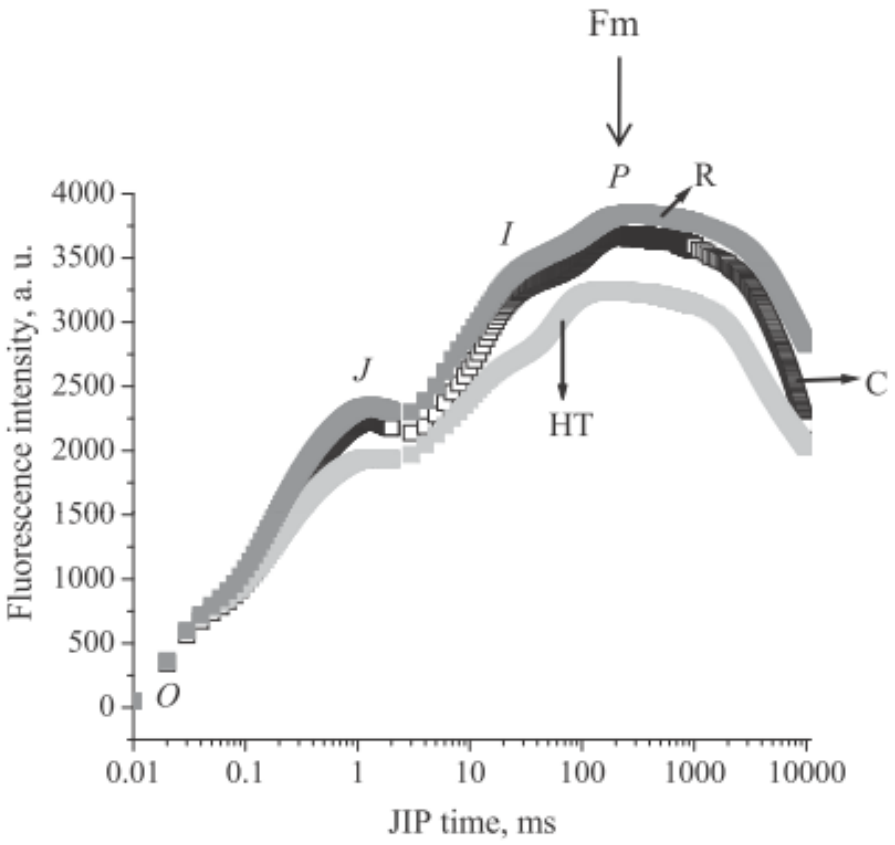

Fig. 1. Typical traces of fluorescence induction curve (*OJIP*-transient) recorded on BBRS17 leaves by PEA fluorometer. The figure presents changes caused by high temperature treatment. The plants were treated during blossoming stage with HT (45° C) for 2 h and returned for 4 h to recover at temperature 23° C. C – control plants at 23° C; HT – high temperature treated plants at 45° C for 2 h; R – HT treated plants returned for recovery for 4 h at 23° C.

An example on pulse amplitude method for registration of fluorescence induction in intact leaves

Stefanov D and Terashima I. (2008) Non-photochemical loss in photosystem II in high and low light grown leaves of *Vicia faba* quantified by several fluorescence parameters including L_{NP}, F_0/F_m', a novel parameter. Physiol. Plant. 133: 327-338

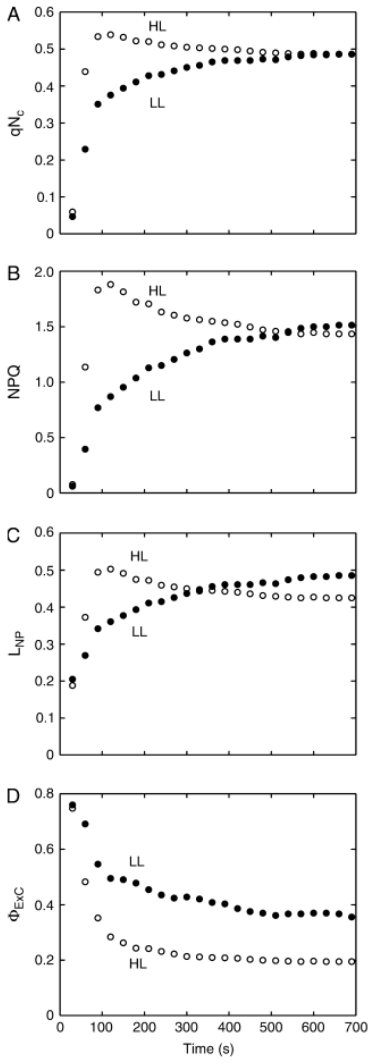

Fig. 1. Induction of qN_C (A), NPQ (B), L_{NP} (C) and Φ_{ExC} (D) in an actinic light of 1180 μmol m^{-2} s^{-1} PFD. ●, HL leaf and ○, LL leaf kept in the complete darkness for 5 min before illumination with actinic light. The leaves were treated in the room light before the dark treatment. Typical traces are shown. qN_C, NPQ, L_{NP} and Φ_{ExC} were calculated using Eqns (28), (15), (11) and (29), respectively, using the data of F_0, F_m, F'_m.

2. The experience on infrared gas analysis by means of infrared gas analysers (LI-COR) - measurements of rate of photosynthesis, light and CO_2 curves and water vapour changes.

Denev, I., Stefanov, D., Terashima, I. (2012) Preservation of integrity and activity of Haberlea rhodopensis photosynthetic apparatus during prolonged light deprivation Physiologia Plantarum 146 (1) , pp. 121-128 doi:10.1111/j.1399-3054.2012.01608.x

Stefanov, D., Yordanov I., Tsonev T. (1996) Effect of thermal stress combined with different irradiance on some photosynthetic characteristics on barley (*Hordeum vulgare L.*) plants. Photosynthetica, 32, 171 -181

An example with pre-darkened Haberlea plant the rate of CO_2 assimilation in intact leaves

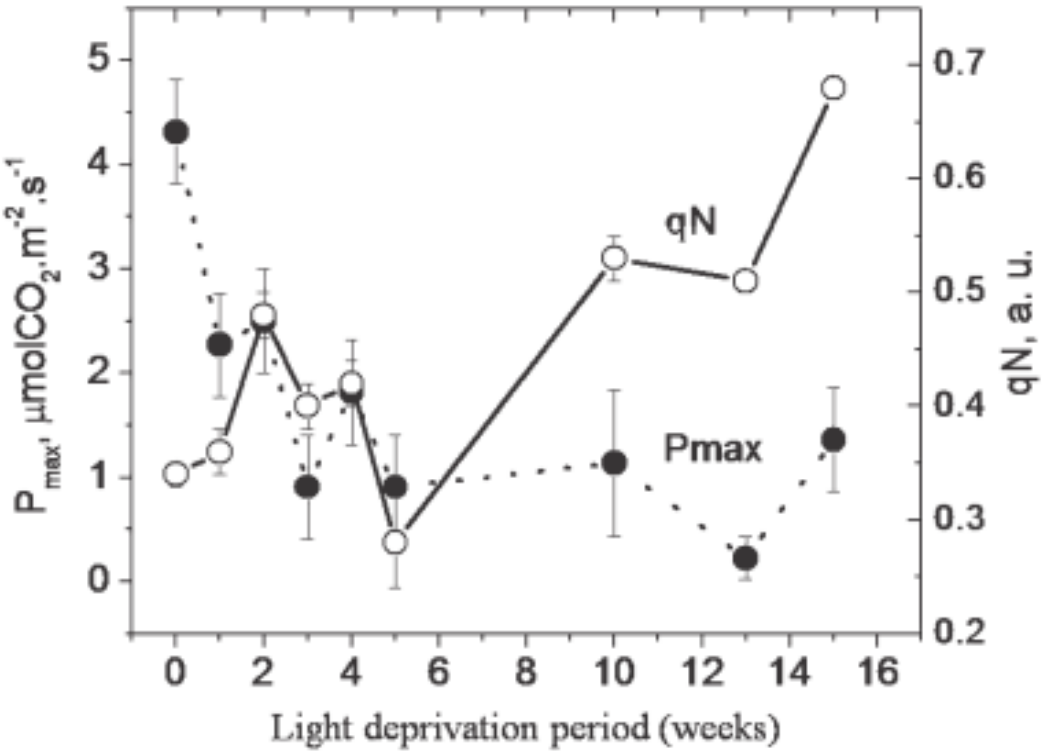

Fig. 7. Dynamics of the changes in non-photochemical quenching, qN (open symbols and straight line) and rate of CO_2 assimilation (closed symbols and dot line) during prolonged dark treatment of *Haberlea rhodopensis* leaves.

3. We were constructed apparatus for photoacoustic measurements - photothermal and photobaric signals after collaboration with colleagues from Physical faculty at Sofia University (Assoc. Prof. T. Velinov)

Christov I., Stefanov D., Velinov T., Goltsev V., Georgieva K., Abracheva P., Genova Y., Christov N. (2007) The symptomless leaf infection with grapevine leafroll associated virus 3 in grown *in vitro* plants as a simple model system for investigation of viral effects on photosynthesis. J. Plant Physiol. 164, 1124 – 1133.

Stefanov, D., T. Tsonev, T. Velinov, I. Yordanov, K. Bransalov, M. Gateshki 1998. High- and low-temperature induced photoinhibition in bean plants characterized by photoacoustic and fluorescence measurements. In: Photoacoustic and Photothermal Phenomena (Ed. F.Scudieri, M. Bertolotti), AIP Press, New York, 643-645 pp.

Stefanov, D., T. Tsonev, T. Velinov, I. Yordanov, K. Bransalov, M. Gateshkii 1998. High- and low-temperature induced photoinhibition in bean plants characterized by data analysis of the drop from R- to S-level of the photothermal signal. The XI th Congr. Fed. Eur. Soc. Plant Physiol., September 7-11, Varna, Bulgaria, 1998.

An example on induction kinetics of the photoacoustic signal

Christov I., Stefanov D., Velinov T., Goltsev V., Georgieva K., Abracheva P., Genova Y., Christov N. (2007) The symptomless leaf infection with grapevine leafroll associated virus 3 in grown *in vitro* plants as a simple model system for investigation of viral effects on photosynthesis. J. Plant Physiol. 164, 1124 – 1133.

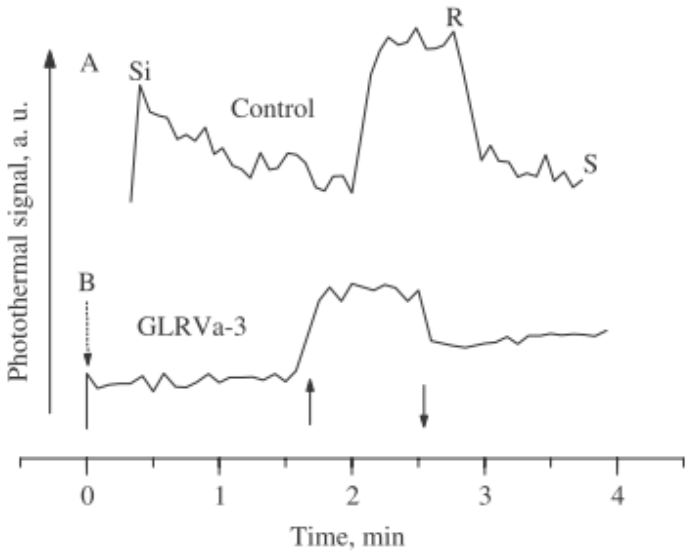

Figure 2. Original traces of the induction kinetics of photothermal signal from healthy and virus-infected in vitro grown plants at room temperature (photothermal signal was measured at 272 Hz). Downward dashed arrow indicates the start of the measurements. Upward and downward solid arrows indicate the positions, at which saturating background light of 1200 μmol m^{-2} s^{-1} is on and off, respectively.

4. Studies on leaf photosynthesis estimated by means of delayed fluorescence - induction curves, and decay kinetics.

Goltsev, V., Zaharieva, I., Chernev, P., Kouzmanova, M., Kalaji, H.M., Yordanov, I., Krasteva, V., Alexandrov V., Stefanov D., Allakhverdiev S., Strasser, R.J. (2012) Drought-induced modifications of photosynthetic electron transport in intact leaves: Analysis and use of neural networks as a tool for a rapid non-invasive estimation. Biochimica et Biophysica Acta - Bioenergetics 1817 (8) , pp. 1490-1498 doi:10.1016/j.bbabio.2012.04.018

Yordanov, I., Goltsev, V., Stefanov, D., Chernev, P., Zaharieva, I., Kirova, M., Gecheva, V., Strasser, R.J. (2008) Preservation of photosynthetic electron transport from senescence-induced inactivation in primary leaves after decapitation and defoliation of bean plants. Journal of Plant Physiology 165: 1954-1963

An example on registration of submillisecond and millisecond delayed fluorescence induction

Yordanov, I., Goltsev, V., Stefanov, D., Chernev, P., Zaharieva, I., Kirova, M., Gecheva, V., Strasser, R.J. (2008) Preservation of photosynthetic electron transport from senescence-induced inactivation in primary leaves after decapitation and defoliation of bean plants. Journal of Plant Physiology 165: 1954-1963

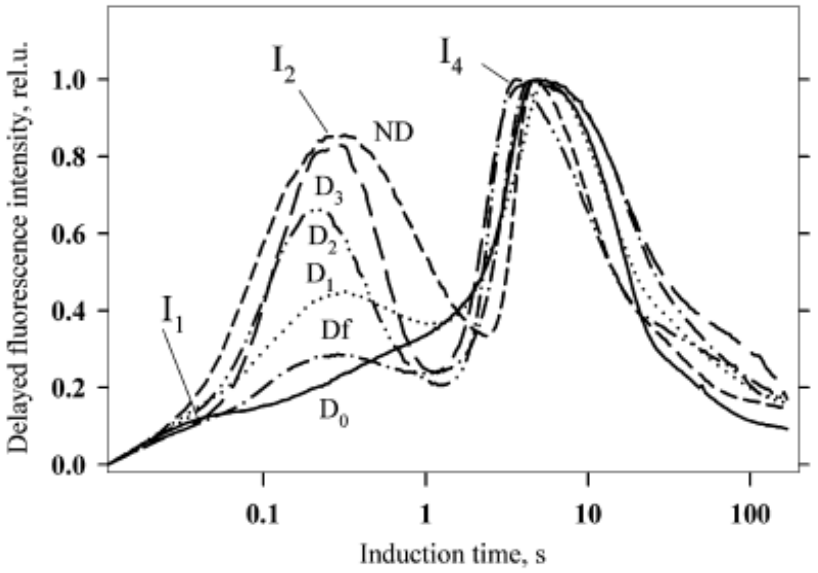

Figure 6. Changes in delayed chlorophyll fluorescence transients measured in primary leaves of 30-d-old bean plants modified and marked according to the experimental scheme (see Figure 1). Leaf disks were dark adapted for 3 min and delayed fluorescence induction curves were recorded for 3 min using the fluorometer FL-2006 ("TEST", Krasnojarsk, Russia) at actinic light intensity 1200 μmol m^{-2} s^{-1} PFD according to Zaharieva and Goltsev (2003). The delayed fluorescence values are normalized to their maximal level.

and temperature curves of prompt and delayed fluorescence

Christov I., Stefanov D., Velinov T., Goltsev V., Georgieva K., Abracheva P., Genova Y., Christov N. (2007) The symptomless leaf infection with grapevine leafroll associated virus 3 in grown *in vitro* plants as a simple model system for investigation of viral effects on photosynthesis. J. Plant Physiol. 164, 1124 – 1133.

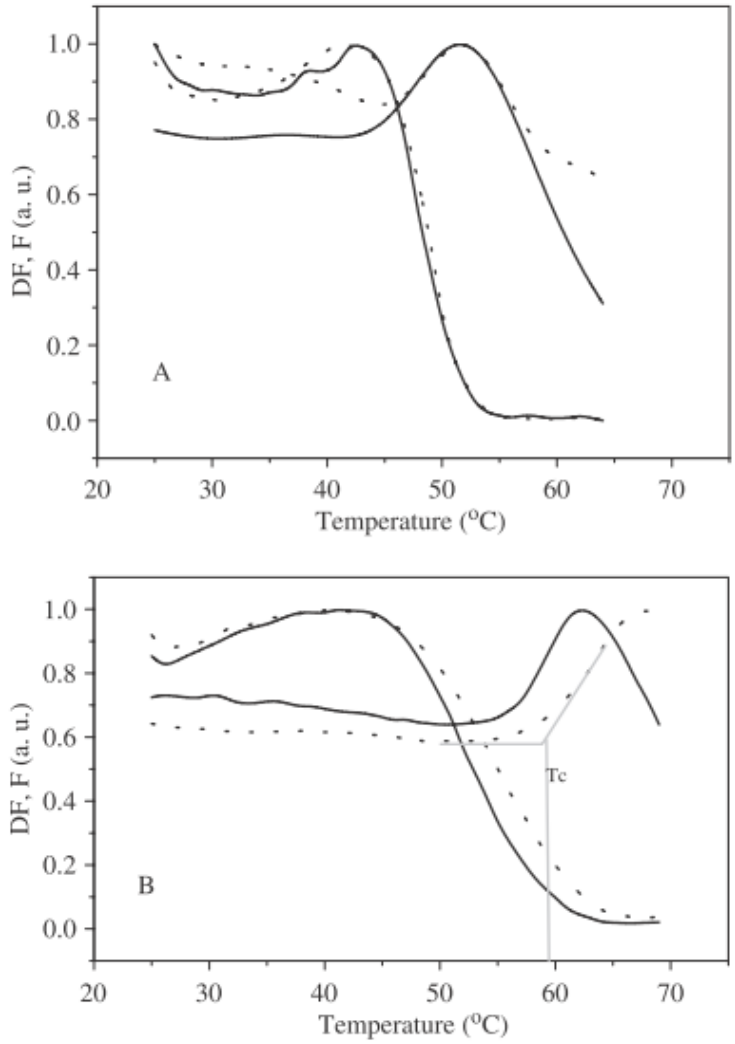

Figure 3. Variable fluorescence, *F*, and delayed fluorescence, DF, curves of healthy (solid lines) and virus-infected (dotted lines) grapevine leaves as a function of temperature in the conditions of low (A) and high (B) actinic light conditions. Temperature curves were measured simultaneously after 6 min irradiation with continuous weak light of 45 μmol $m^{-2} s^{-1}$ PFD (A) and strong light of 1200 μmol $m^{-2} s^{-1}$ PFD (B).

As well as an example with measuring delayed fluorescence in second and minute time domains from:

Stefanov, D., Milanov, G., Lambrev, P., (...), Goltsev, V., Kapchina, V. (2018) Delayed fluorescence measurements show increased S2Q−B charge recombination in PS2 of tobacco pigment-deficient aurea mutant. Comptes Rendus de L'Academie Bulgare des Sciences 71(8), 1052-1061

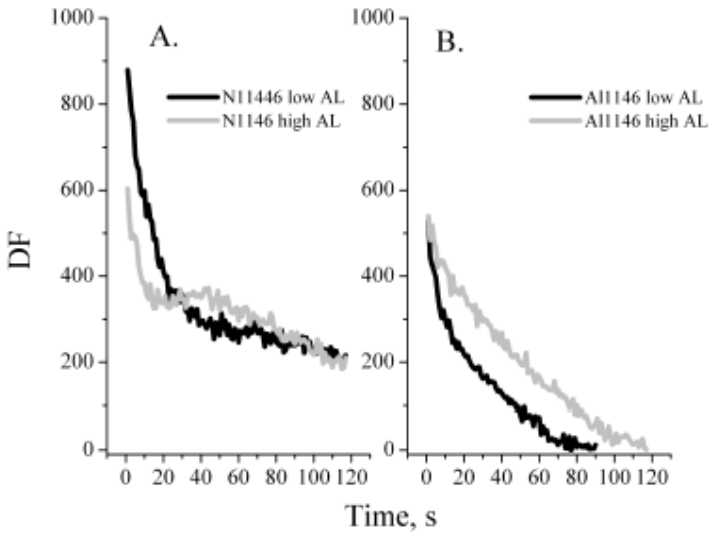

Fig. 1. Second-to-minute time DF decay kinetics in N1146 (A) and A1146 (B) leaf discs pre-illuminated with the low (black lines) and high actinic light (grey lines) conditions. The experimental traces in general represent the averages of three samples (see Material and methods)

5. Experience on transient absorption kinetics at 505, 518, 535 and 830nm.

Yordanov, I., Goltsev, V., Stefanov, D., Chernev, P., Zaharieva, I., Kirova, M., Gecheva, V., Strasser, R.J. (2008) Preservation of photosynthetic electron transport from senescence-induced inactivation in primary leaves after decapitation and defoliation of bean plants. Journal of Plant Physiology 165: 1954-1963

Georgieva K., Maslenkova L., Peeva V., Markovska Y., Stefanov D. and Tuba Z. (2005) Comparative study on the changes in photosynthetic activity of the homoiochlorophyllous desiccation tolerant *Haberlea rhodopensis* and spinach leaves during desiccation Photosynth. Res 85 (2), 191 – 203.

An example on absorption kinetics of the A830 signal

Georgieva K., Maslenkova L., Peeva V., Markovska Y., Stefanov D. and Tuba Z. (2005) Comparative study on the changes in photosynthetic activity of the homoiochlorophyllous desiccation tolerant *Haberlea rhodopensis* and spinach leaves during desiccation Photosynth. Res 85 (2), 191 – 203.

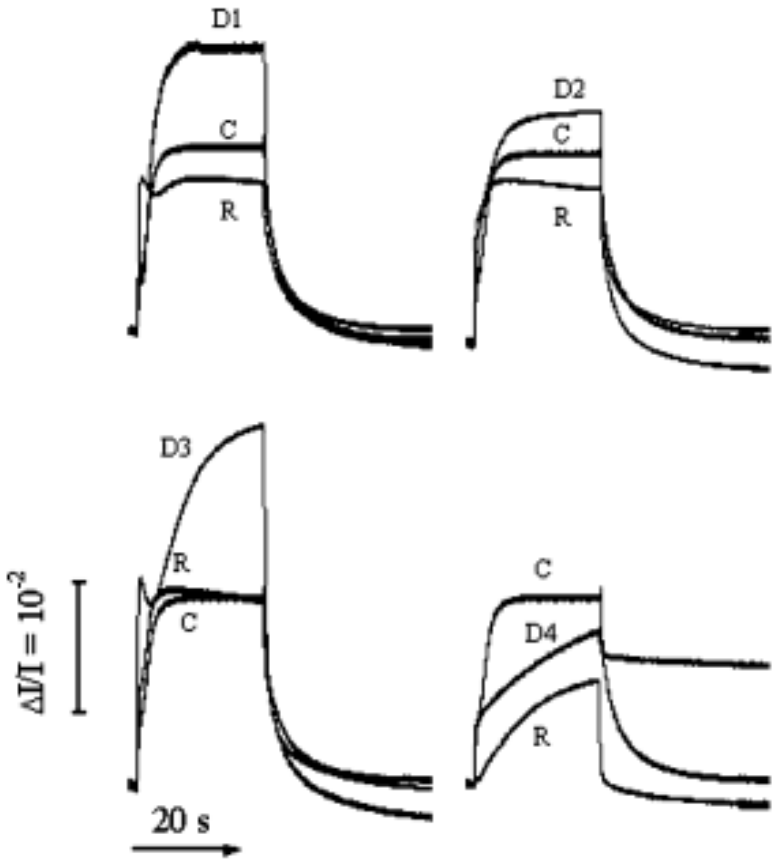

Figure 5. Original traces of P700 oxidation induced by 20 s far-red light in *Haberlea rhodopensis* leaves. Upward and downward arrows show far-red turn on and off, respectively. Each trace is the average for 3–5 separate leaf discs. Δ A/A = 0.02 V. The water status of the leaves was as described in Figure 2.

6. Experience on Clarck type leaf oxygen electrode.

Christov I., Stefanov D., Velinov T., Goltsev V., Georgieva K., Abracheva P., Genova Y., Christov N. (2007) The symptomless leaf infection with grapevine leafroll associated virus 3 in grown *in vitro* plants as a simple model system for investigation of viral effects on photosynthesis. J. Plant Physiol. 164, 1124 – 1133.

7. An experience in HPLC chromatography of leaf pigments.

Stefanov, D., I. Yordanov 1995. High performance liquid chromatography separation of xanthophylls isolated from barley leaves with semi-preparative reversed phase column. Compt. Rend. Bulg. Acad. Sci., 47, 61 - 63.

Stefanov, D., I. Yordanov P. Moskovski 1995. High performance liquid chromatography separation of xanthophylls isolated from barley (*Hordeum vulgare* L.) leaves. Bulg. Chem. Commun. 28 (1), 119 - 123.