PHOTOSYNTHETICA 41 (1): 77-82, 2003

Chlorophyll *a* fluorescence analysis in response to excitation irradiance in bean plants (*Phaseolus vulgaris* L. and *Vigna unguiculata* L. Walp) submitted to high temperature stress

E.S. COSTA, R. BRESSAN-SMITH*, J.G. OLIVEIRA, and E. CAMPOSTRINI

Setor de Fisiologia Vegetal, Universidade Estadual do Norte Fluminense/CCTA/LMGV, Campos dos Goytacazes, RJ, Brazil

Abstract

Bean plants *Phaseolus vulgaris* L. (cv. Carioca and Negro Huasteco) and *Vigna unguiculata* L. Walp (cv. Epace-10) were grown in a growth chamber with a photosynthetic photon flux density of 200 μmol m^{-2} s^{-1} at leaf level and air temperature of 25±1 °C. Fully expanded, first pair leaves of 12-d-old plants were submitted for 90 min to high temperature (25, 30, 35, 40, 45, and 48 °C). Chlorophyll *a* fluorescence parameters (ETR, q_P, q_N, and F_0) were investigated using a modulated fluorimeter at 25 °C during recovery considered here as 48 h after stress induction period. An accentuated decrease in q_P and an increase in q_N at 48 °C in Carioca and Negro Huasteco was not observed in Epace-10. In response to excitation irradiance a great potential for ETR was found in Negro Huasteco at 25 °C, also demonstrated by net photosynthetic rate. At 48 °C ETR was high for Epace-10 while it was equal to zero for Carioca and Negro Huasteco. Tolerance to high temperature observed in Epace-10 provided important information about the adaptative characteristics of *Vigna* cultivars to warm climates.

Additional key words: electron transport rate; net photosynthetic rate; photochemical and non-photochemical quenching.

Introduction

Electron transport is a key regulator in photosynthesis decisive in the control of processes by which plants are able to perceive and modulate their responses to environmental changes (Cleland 1998). The electron transfer in photochemical reactions is sensitive to high temperature and more susceptible than the enzymatic reactions in the chloroplast stroma (Krause and Santarius 1975, Caemmerer 2000). Thus the relative rate of electron transport is an excellent indicator of environmental stress, and is considered one of the first events to register damage to the chloroplast.

A fine control regulates photosynthesis under high temperature and this seems to modulate the balance between the Calvin cycle activities and the photosynthetic electron transport, causing alterations to the different parameters of chlorophyll (Chl) *a* fluorescence (Krause and Weis 1984). Under these conditions, electron transport efficiency decreases, implying an inefficient use of quantum energy by the chloroplast (Schreiber and Bilger 1987). Indeed, if the electron transport capacity decreases, photosynthesis as a whole becomes more susceptible (Oberhuber *et al.* 1993), that is, saturation with photons is achieved more quickly with the sequential occurrence of photoinhibition.

These statements are in line with studies carried out in bean plants susceptible to thermo-inhibition (Pastenes and Horton 1996); these results associated high temperature with decrease in q_P and increase in q_N. Analysis of the quenchings has considerable diagnostic value and has been applied in several studies, including differentiation of leaf physiology in shade and sun, detection of freezing effects, high and low temperature, water stress, and damage caused by herbicides (Bolhàr-Nordenkampf *et al.* 1989).

In the assessment of effects caused by high temperature on the photosynthetic activity, Chl *a* fluorescence may be a safer indicator than net photosynthetic rate (P_N), because it is a practical and precise method. P_N may be influenced by induced stomatal closure caused primarily by heat (Bolhàr-Nordenkampf *et al.* 1989), by abscisic

Received 9 September 2002, *accepted* 24 March 2003.
*Fax: +55 (22) 2726-1549, e-mail: brsmith@uenf.br

Abbreviations: Chl – chlorophyll; ETR – relative electron transport rate; F_0 and F_m – initial and maximum fluorescence, respectively; LHC2 – light-harvesting complex of PS2; P_N – net photosynthetic rate; PPFD, photosynthetic photon flux density; PS – photosystem; Q_A – quinone A; q_N – non photochemical quenching; q_P – photochemical quenching.

acid, and by the dehydration of guard cells. Among the parameters of Chl *a* fluorescence, F_0 (initial fluorescence when all the electron carriers of the PS2 are in the open state) represents the best diagnosis of the effect of high temperature on PS2 activity (Pastenes and Horton 1999). An increase is generally observed between 40 and 45 °C followed by a decrease, representing irreversible damage to the photosynthetic apparatus (Yamane *et al.* 1997, Costa *et al.* 2002).

This study assessed the photochemical activity of photosynthesis under high temperature in bean plants with different high temperature tolerance. The photochemical activity was contrasted by means of F_0, q_P, q_N, and ETR as fluorescence parameters. P_N was used as a measure of carbon assimilation rate.

Materials and methods

Plants: Two *Phaseolus vulgaris* L. cultivars (Carioca and Negro Huasteco) and one *Vigna unguiculata* L. Walp cultivar (Epace-10) were used. These genotypes originated from the Active Germplasm Bank at Universidade Estadual do Norte Fluminense and the National Centre for Bean and Rice Research (CNPAF-EMBRAPA). Bean plants were obtained from seeds placed in a germinator for 8 h of irradiation at temperature of 30 °C and 16 h in the dark at 20 °C. After germination, the plants were rigorously standardised by vigour and root size before transfer to 300 cm^3 plastic pots containing *Plantmax*® organic substrate. After this standardisation, the plants were taken to a growth chamber (*LAB-LINE Instruments*, USA) with eight fluorescent lamps (*F15T12*, 15 W, *General Electric*, USA) providing a PPFD of 200 μmol m^{-2} s^{-1} with a 12-h photoperiod. The temperature and relative humidity were recorded every hour by model *250* probes (*Spectrum Technologies*, USA). The mean temperature during the experiments was 25/22 °C and relative humidity was 48.0/72.5 % day/night, respectively. The plants remained in this condition for 10 or 11 d, enough time for the first pair of leaves to reach maximum growth.

Stress induction: The plants were submitted to high temperature stress in a growth chamber (*JP-100 J, Prolab*, Brazil). Before stress submission, the plants were selected for maximum photochemical efficiency (F_v/F_m). Only those with ratio F_v/F_m between 0.80 and 0.85 were used in the experiment. The plants were exposed for 90 min to temperatures of 25, 30, 35, 40, 45, and 48 (±0.5) °C and for each temperature a group of five plants was used corresponding to five replications. Temperature in the interior of the chamber was monitored constantly by a digital thermopar type thermometer (*Digi-Thermo*, China). During the stress induction period, a panel with four lamps (incandescent crystal, 60 W, *General Electric*, Brazil) was installed inside the chamber supplying a PPFD of 80 μmol m^{-2} s^{-1}. The plant recovery was accompanied by fluorescence parameters 48 h after the stress induction. During the recovery period, the plants remained in the growth chamber under the conditions described initially.

Chl *a* fluorescence and CO_2 assimilation measurements: The Chl *a* fluorescence parameters were obtained at 25 °C with a *MINI-PAM* modulated fluorimeter (*Walz*, Germany). The fluorimeter employs 0.3 s pulses of light-emitting-diode with peak emission at 650 nm. Fluorescence is detected at wavelengths above 710 nm. Heat-filtered "white light" from a halogen lamp serves for actinic irradiation and saturation pulses. The leaves sampled corresponded to the first pair of completely expanded leaves. The variables were obtained following an irradiance response curve and the leaves were adapted to the dark for 30 min preceding the recording of this curve. This curve was obtained with eight consecutive periods of actinic irradiation of increasing PPFD (85, 154, 274, 419, 588, 890, 1 227, and 1 891 μmol m^{-2} s^{-1}) not exceeding 2 min in total. This avoided excessive internal heating of the *MINI-PAM*, which could interfere in the results. Each irradiation lasted about 10 s at the end of which there was a pulse of saturating radiation (6 000 μmol m^{-2} s^{-1}) of 0.3 s duration. At first, one measurement was done without actinic radiation (0 μmol m^{-2} s^{-1}) before beginning the sequence of measurements with increasing actinic radiation. This allowed to measure F_0 and F_m, necessary to define q_P, q_N, and ETR as follows: $q_P = (F'_m - F)/(F'_m - F_0)$; $q_N = (F_m - F'_m)/(F_m - F_0)$. These quenching coefficients were automatically calculated by the *MINI-PAM*. Electron transport rate was estimated as ETR = $\Delta F/F'_m \times PAR \times 0.5 \times$ ETR factor, where $\Delta F/F'_m$ is the effective quantum yield, PAR is photosynthetically active radiation, and ETR factor corresponds to the fraction of incident radiation absorbed by green leaves (value of 0.84) (Schreiber *et al.* 1994).

P_N was measured with an infra-red gas analyser model *LI 6200* (*Licor*, USA). These measurements were taken with a *1/4 L* camera and a *7158XHP* lamp as radiation source (150 W, *Phillips*). Readings were taken at 25±1 °C, the CO_2 concentration of the air was close to 400 μmol mol^{-1}, and PPFD from 0 to 1 800 μmol m^{-2} s^{-1}.

Results and discussion

The irradiance response curve of Chl *a* fluorescence supplies relevant information on the pattern of photosynthesis response and the performance of PS2 in irradiated plants submitted to high temperature. The increase in PPFD caused a decrease in the q_P and an increase in q_N for all the cultivars (Fig. 1). This is an expected response pattern, where the decrease in q_P after the saturating radiation pulse reflects the reduced state of the first stable electron acceptor of PS2, Q_A. The increase in q_N reflects the energy dissipated as heat, due to the increase in the proton gradient between the lumen and the chloroplast stroma. A lesser component of q_N is controlled by the quantity of energy transferred to PS1 by phosphorylation of mobile LHC2 (Bolhàr-Nordenkampf *et al.* 1993).

The quenching values in function of the PPFD generally showed that q_P was higher for the Negro Huasteco and Epace-10 cultivars and lower for Carioca. The q_P indicates the ability of PS2 to use the excitation energy in linear electron transport needed for the photosynthetic assimilation of carbon (Genty *et al.* 1989). After adaptation to the dark, the start of the irradiation curve showed that Q_A is completely oxidised and q_P reaches its maximum value after the first pulse of saturating radiation. However, at the start of the 48 °C curve, q_P was very low for Carioca and Negro Huasteco, an indication of damage in the photosynthetic pigment-protein complexes. This was not observed for Epace-10 (Fig. 1). The low q_P values observed at 45 °C for all the cultivars may be associated to the high initial fluorescence, that is, a major part of the energy had already dissipated in the LHC2 even before reaching the PS2 reaction centre (Table 1).

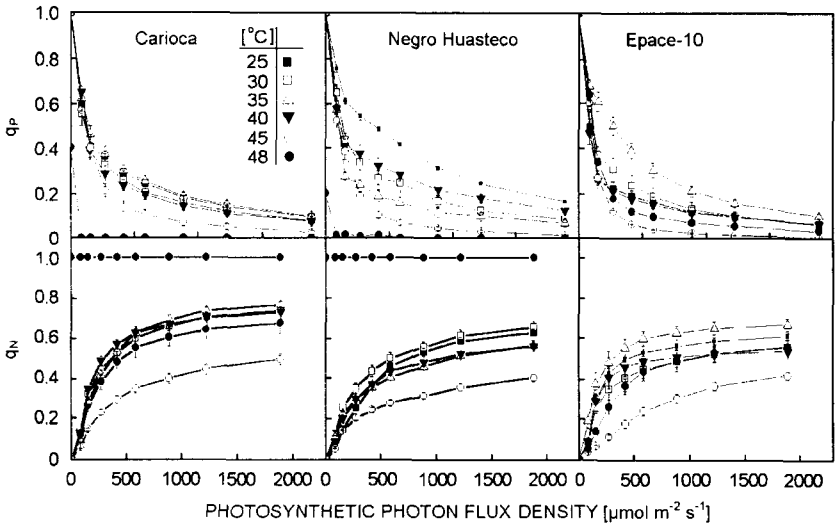

Fig. 1. Photochemical (q_P) and non-photochemical (q_N) quenching in response to PPFD in plants of cvs. Carioca, Negro Huasteco (*P. vulgaris*), and Epace-10 (*V. unguiculata*) submitted to different temperatures, 48 h after stress induction period. Measurements with *MINI-PAM* were done at 25 °C, 30 min after dark adaptation. Means of five replications.

The response pattern for q_N at 45 °C was similar for all the cultivars that showed the lowest values at this temperature. At 48 °C, q_N reached maximum for Carioca and Negro Huasteco at the first pulse of saturating irradiation, showing the occurrence of severe damage to the energy transfer system to PS2. This effect was not observed for Epace-10.

Besides the damaging effects of exposure to 45 °C on the photochemical events in the thylakoids reflected by the increase in F_0 (Table 1), an inhibition of the biochemical reactions in Calvin cycle may also occur and result in reduction of CO_2 fixation. The negative response of P_N to high temperature is related to the stomatal closure in order to avoid excessive water loss, as well as to a possible inactivation of the Calvin cycle. Besides that, other restrictions may become evident with the occurrence of membrane lipid peroxidation followed by an increase in the thylakoid permeability (Raison *et al.* 1982) leading to an extrusion of the chloroplast matrix (Santarius *et al.* 1991). This would be consistent with the results obtained at 45 °C where an alteration in P_N is suggested by the reduction in the electron transport rate (Fig. 2) and decrease in the photochemical quenching (Fig. 1) (Schreiber and Bilger 1987).

ETR increases with PPFD until saturation of the electron carriers. In this study, the greatest rates of electron transport were registered for the Negro Huasteco at 25 and 40 °C (Fig. 2). The Negro Huasteco ETR at 25 °C was two-fold that attained by Carioca and Epace-10. Burkey *et al.* (1996) suggests that the pool of plastocyanin can vary significantly and limit the capacity for photosynthetic electron transport in plant species such as

Table 1. Initial fluorescence (F_0) in plants of the cvs. Carioca, Negro Huasteco (*P. vulgaris*), and Epace-10 (*V. unguiculata*) submitted to 25 (control) and 45 °C, 48 h after stress induction period. Net photosynthetic rate (P_N) was measured at 25 °C under irradiance saturation (1 400 μmol m^{-2} s^{-1}). Measurements with *MINI-PAM* were done at 25 °C, 48 h after stress induction. Means of five replications.

Cultivar	F_0 [relative] control	45 °C	% of control	P_N [μmol(CO_2) m^{-2} s^{-1}]
Carioca	283.80 ± 1.64	412.60 ± 23.74	+45.38	13.530 ± 0.056
Negro Huasteco	266.40 ± 9.20	387.80 ± 18.19	+45.57	15.450 ± 0.030
Epace-10	257.80 ± 7.59	379.00 ± 7.03	+47.01	13.170 ± 0.797

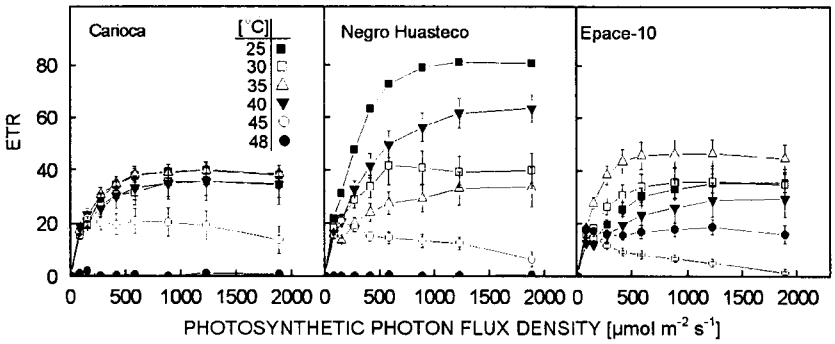

Fig. 2. Relative electron transport rate (ETR) in response to PPFD in plants of the cvs. Carioca, Negro Huasteco (*P. vulgaris*), and Epace-10 (*V. unguiculata*) submitted to 25, 30, 35, 40, 45, and 48° C, 48 h after stress induction period. Measurements with *MINI-PAM* were done at 25 °C, 30 min after dark adaptation. Means of five replications.

soybean and spinach. This may perhaps explain the large differences observed between Negro Huasteco and the two other cultivars studied. The ETR was comparatively low even at the lowest PPFD and 48 °C for Carioca and Negro Huasteco indicating a damage to the electron carriers and consequently photoinhibition of photosynthesis. Furthermore, high temperature promotes a metabolic disturbance leading to oxidative stress by means of ERO's production (Panchuk *et al.* 2002). At 48 °C, an oxidative burst might have occurred in Carioca and Negro Huasteco, damaging chloroplast membranes and followed by inactivation of PS2. In Epace-10, the oxidative burst may have functioned as a signal for the activation of protective mechanisms such as antioxidant enzymes (Noctor and Foyer 1998) and the xanthophyll cycle (Horton *et al.* 1996). Because antioxidant enzymes function as efficient electron scavengers, their activation under high temperature may explain why ETR and q_P were higher for Epace-10 at 48 °C than at 45 °C. This could be partially explained by Lee *et al.* (2000), who affirm that thermostable iso-forms of ascorbate peroxidase are activated by an increase of hydrogen peroxide concentrations and by the expression of HSPs, both induced by high temperature. In addition, the action of the xanthophyll cycle allows the radiation-less dissipation of excess energy in thylakoid lumen, avoiding damage of PS2 (Demmig-Adams and Adams 1996) under high temperature (Havaux and Gruszecki 1993). Nevertheless,

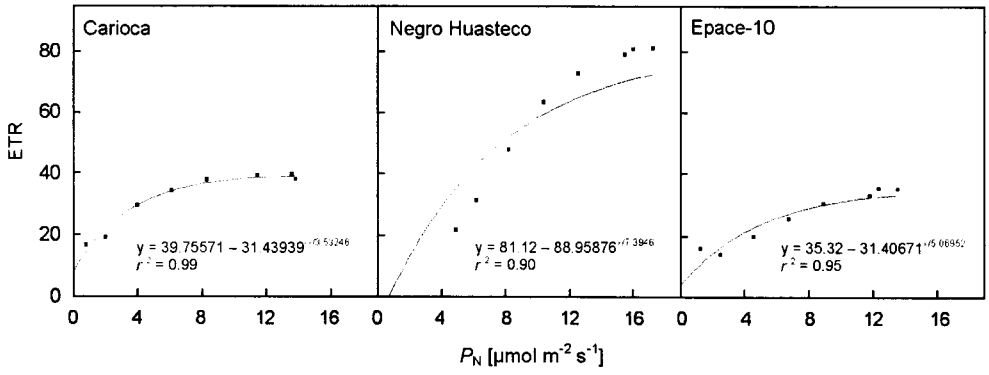

Fig. 3. Correlations between electron transport rate (ETR) and net photosynthetic rate (P_N) in plants of the cvs. Carioca, Negro Huasteco (*P. vulgaris*), and Epace-10 (*V. unguiculata*) under varying PPFD (0–1 800 μmol m^{-2} s^{-1}) at 25 °C.

we could not support the affirmation that Epace-10 photosynthetic apparatus makes a better use of this dissipation system than Carioca and Negro Huasteco.

The irradiance response curves of P_N were obtained at 25 °C with an infrared gas analyser. In this experiment, P_N under saturation (1 400 μmol m^{-2} s^{-1}) was evaluated and the Negro Huasteco showed a tendency to superiority (Table 1). ETR in Negro Huasteco was also superior to that of the other cultivars at 25 °C under saturating irradiance. Hence P_N under saturating irradiance for all cultivars could have a similar tendency to that of ETR as reported by Edwards and Baker (1993). In support to this idea, a correlation was observed between P_N and ETR in all cultivars studied and better explained by an exponential model (Fig. 3).

According to our results, the photosynthetic apparatus of Epace-10 (*V. unguiculata*) presented a greater tolerance to heat stress than that of Carioca and Negro Huasteco (*P. vulgaris*). The two species investigated differed in the capacity to resist to the detrimental effects of high temperature. In the tropics, Carioca is not recommended for cultivation in warm regions with persistent high temperature periods, while Negro Huasteco is considered tolerant to supra-optimum temperature (Masaya and White 1991) and Epace-10 is widely cultivated in warm and dry regions. An important feature is that *V. unguiculata* is highly resistant to intense irradiance, water deficiency, and high temperature (Pimentel *et al.* 1999). Consequently, other morpho-physiological characteristics are probably involved in tolerance capacity.

References

Bolhàr-Nordenkampf, H.R., Long, S.P., Baker, N.R., Öquist, G., Shreiber, U., Lechner, E.G.: Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. – Funct. Ecol. **3**: 497-514, 1989.

Bolhàr-Nordenkampf, H.R., Öquist, G.: Chlorophyll fluorescence as a tool in photosynthesis research. – In: Hall, D.O., Scurlock, J.M.O., Bolhàr-Nordenkampf, H.R., Leegood, R.C., Long, S.P. (ed.): Photosynthesis and Production in a Changing Environment. A Field and Laboratory Manual. Pp. 193-206. Chapman & Hall, London – Glasgow – New York – Tokyo – Melbourne – Madras 1993.

Burkey, O.K., Gizlice, Z., Carter, T.E., Jr.: Genetic variation in soybean photosynthetic electron transport capacity is related to plastocyanin concentration in the chloroplast. – Photosynth. Res. **49**: 141-149, 1996.

Caemmerer, S. von: Biochemical Models of Leaf Photosynthesis. – CSIRO Publishing, Collingwood 2000.

Cleland, E.R.: Voltameric measurement of the plastoquinone redox state in isolated thylakoids. – Photosynth. Res. **58**: 183-192, 1998.

Costa, E.S., Bressan-Smith, R., Oliveira, J.G., Campostrini, E., Pimentel, C.: Photochemical efficiency in bean plants (*Phaseolus vulgaris* L. and *Vigna unguiculata* L. Walp) during recovery from high temperature stress. – Braz. J. Plant Physiol. **14**: 105-110, 2002.

Demmig-Adams, B., Adams, W.W., III: The role of xanthophyll cycle carotenoids in the protection of photosynthesis. – Trends Plant Sci. **1**: 21-26, 1996.

Edwards, G.E., Baker, N.R.: Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? – Photosynth. Res. **37**: 89-102, 1993.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. biophys. Acta **990**: 87-92, 1989.

Havaux, M., Gruszecki, W.I.: Heat-induced and light-induced chlorophyll *a* fluorescence changes in potato leaves containing high or low levels of the carotenoid zeaxanthin: Indications of a regulatory effect of zeaxanthin on thylakoid membrane fluidity. – Photochem. Photobiol. **58**: 607-614, 1993.

Horton, P., Ruban, A.V., Walters, R.G.: Regulation of light harvesting in green plants. – Annu. Rev. Plant Physiol. Plant. mol. Biol. **47**: 655-684, 1996.

Krause, G.H., Santarius, K.A.: Relative permeability of the chloroplast envelope. – Planta **127**: 285-299, 1975.

Krause, G.H., Weis, E.: Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. – Photosynth. Res. **5**: 139-157, 1984.

Lee, B.H., Won, S.H., Lee, H.S., Miyao, M., Chung, W.I., Kim, I.J., Jo, J.: Expression of the chloroplast-localized small heat shock protein by oxidative stress in rice. – Gene **45**: 283-290, 2000.

Masaya, P., White, J.W.: Adaptation to photoperiod and temperature. – In: Van Schoonhoven, A., Voysest, O. (ed.): Common Beans – Research for Crop Improvement. Pp. 445-500. CIAT, Colômbia 1991.

Noctor, G., Foyer, C.H.: Ascorbate and glutathione: Keeping active oxygen under control. – Annu. Rev. Plant Physiol. Plant. mol. Biol. **49**: 249-279, 1998.

Oberhuber, W., Dai, Z.-Y., Edwards, G.E.: Light dependence of quantum yields of Photosystem II and CO_2 fixation in C_3 and C_4 plants. – Photosynth. Res. **35**: 265-274, 1993.

Panchuk, I.I., Volvov, R.A., Schöffl, F.: Heat stress- and heat shock transcription factor-dependent expression and activity of ascorbate peroxidase in *Arabidopsis*. – Plant Physiol. **129**: 838-853, 2002.

Pastenes, C., Horton, P.: Effect of high temperature on photosynthesis in beans. II. Oxygen evolution and chlorophyll fluorescence. – Plant Physiol. **112**: 1245-1251, 1996.

Pastenes, C., Horton, P.: Resistance of photosynthesis to high temperature in two bean varieties (*Phaseolus vulgaris* L.). – Photosynth. Res. **62**: 197-203, 1999.

Pimentel, C., Roy-Macauley, H., Abboud, A.C.S., Diouf, O., Sarr, B.: Effects of irrigation regimes on the water status of cowpea cultivated in the field. – Physiol. mol. Biol. Plants **5**: 153-159, 1999.

Raison, J.K., Roberts, J.K.M., Berry, J.A.: Correlations between the thermal stability of chloroplast (thylakoid) membranes and the composition and fluidity of their polar lipids upon acclimation of the higher plant, *Nerium oleander*, to growth temperature. – Biochim. biophys. Acta **688**: 218-228, 1982.

Santarius, K.A., Exner, M., Thebud-Lassak, R.: Effects of high temperature on the photosynthetic apparatus in isolated

mesophyll protoplasts of *Valirianella locusta* L. Betcke. – Photosynthetica **25**: 17-26, 1991.

Schreiber, U., Bilger, W.: Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. – In: Tenhunen, J.D., Catarino, E.M., Lange, O.L., Oechel, W.C. (ed.): Plant Response to Stress. Pp. 27-53. Springer-Verlag, Berlin – Heidelberg – New York – London – Paris – Tokyo 1987.

Schreiber, U., Bilger, W., Neubauer, C.: Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. – In: Schulze, E.-D., Caldwell, M.M. (ed.): Ecophysiology of Photosynthesis. Pp. 49-70. Springer-Verlag, Berlin 1994.

Yamane, Y., Kashino, Y., Koile, H., Satoh, K.: Increase in the fluorescence F_o level reversible inhibition of Photosystem II reaction center by high-temperature treatments in higher plants. – Photosynth. Res. **52**: 57-64, 1997.