BULG. J. PLANT PHYSIOL., 1995, 21(1), 12–28

THERMAL ACCLIMATION OF THE PHOTOSYNTHETIC APPARATUS DEPENDING ON TEMPERATURE AND DURATION OF TREATMENT

Ivan Yordanov*[1], Vassilii Goltsev[2], Tsonko Tsonev[1], Lidia Kruleva[1]

[1]Acad. M. Popov Insitute of Plant Physiology, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria
[2]Department of Biophysics and Radiobiology, Biological Faculty, Sofia University, 8 Dragan Tzankov Blvd., 1421 Sofia, Bulgaria

Received April 6, 1995

Summary. The ability of young bean plants to increase the thermal tolerance of their photosynthetic apparatus after treatment with different high temperatures or at fixed temperature with different duration was studied. Parameters of chlorophyll fluorescence induction kinetics were used as a criterion to evaluate the photosynthetic activity of primary leaves. The thermosensitivity of photosynthesis was estimated immediately after the acclimation procedure, as well as after additional stress treatment at 55 °C for 5 hours. Exposure of the plants for 5 hours to high temperatures (38, 43, 45 or 47.5 °C) led to a marked increase (2–3 °C) in thermal tolerance of the photosynthetic apparatus. This effect was most obvious in plants exposed at 45 °C for 5 hours. Additional stress caused further development of plant resistance. It was shown that under the experimental conditions used, maximal stability was achieved at sublethal temperatures. The dynamics of temperature acclimation was followed by changes in thermosensitivity of fluorescence parameters after 0.5, 1, 2, 4, and 8 hour's exposure of the plants at 45 °C. It was found that even a very short treatment (30 min) led to well-expressed acclimation allowing the plants to endure additional stress. Thermal stability (T_{50}) of variable fluorescence (F_v/F_m) was unchanged at short-term acclimation (0.5–1 h), but it rose from 45 °C up to 48 °C after 8 h hardening. It was assumed that acclimation effect is realized primarily not on the level of primary photosynthetic reactions, but on the cellular level.

Key words: *Phaseolus vulgaris*, chlorophyll fluorescence, heat acclimation, photosynthesis (photosystem 2).

*Corresponding author

Abbreviations: A – Acclimated plants; AS – Acclimated stressed plants; C – Control plants; CS – Control stressed plants; F_0 – Initial chlorophyll fluorescence; F_m – Maximal chlorophyll fluorescence; F_v – Variable chlorophyll fluorescence; PS1 – Photosystem 1; PS2 – Photosystem 2

Introduction

Under mild stress conditions, plants react by increasing their tolerance to them and sometimes to other treatments (Alexandrov, 1975; Santarius and Müller, 1979, 1981). It is well known that thermal acclimation involves all levels of organisation of living systems. Thermal stress for a definite period of time (10–60 min or more) can imitate the short periods of overheating in a habitat with huge temperature amplitude (Kappen, 1981). An increase in thermal hardening of plants was achieved by a second heat shock, followed by optimal temperature (Alexandrov, 1975; Yordanov, 1981; Yordanov et al., 1986).

The ability of plants to acclimate to contrasting temperature regimes is indeed a hardening at the photosynthetic level (Björkman, 1975; Berry and Raison, 1981). It is connected with a considerable rearrangement of the thylakoid membrane, including adaptive changes in the lipid content and in molecular composition of the pigment system (Armond et al., 1978; Süss and Yordanov, 1986), ensuring an optimal for functioning membrane physical state (of lipid matrix). The protein synthesising apparatus plays also an important role in the process of thermal acclimation as heat shock proteins favour acquirement of heat tolerance (Ougham and Stoddart, 1986; Süss and Yordanov, 1986; Neumann et al., 1989; Kritenko and Titov, 1990). The exogenous cytokinin BAP, stimulating the protein synthesizing system's activity, increases the thermal tolerance of the plants (Titov et al., 1986).

According to Weis (1984), the acclimation of the photosynthetic apparatus to high temperature could be perceived as a long-term response of the leaves, to changes in the temperature regime for several days or weeks. However, significant changes for some hours, in diurnal temperature can often be observed as well.

The mechanism of photosynthetic acclimation to very rapid temperature variations is still poorly understood. Temperature coefficients of different photosynthetic reactions (e.g. primary photosynthetic processes, electron transport, enzyme catalysis) differ significantly. It is therefore assumed that temperature fluctuations act on the balance between them. It is supposed that after a short period of hardening some reversible temperature-induced transitions in the thylakoid structure and in the distribution of absorbed energy in favour of PS1 take place.

Usually it is presumed that the best acclimation to high temperature could be achieved in a temperature interval slightly higher than that, in which a maximal activity of a given process is reached (Alexandrov, 1975), so that even for thermophilic cultivars such as beans it is hardly probable for a process of acclimation to take place

over 40 °C. Some contradictory opinions exist about the connection between the duration of treatment with elevated temperature and the extent of the acclimation effect.

The aim of the present study was to examine the ability of young bean plants to increase the thermal tolerance of their photosynthetic apparatus (monitored by variable fluorescence) after acclimation at different temperatures, or at definite temperature, but of different duration.

Material and Methods

Plant material

Bean plants (*Phaseolus vulgaris* L.), cv. Cheren starozagorski, were grown as water culture in a climatic chamber at 23–26 °C, light intensity 35 $W.m^{-2}$ and a day/night photoperiod 12/12 hours.

Acclimation procedure and temperature treatment

Seven-eight days young plants were divided into two groups: 1) controls (C), growing at 23–26 °C; 2) acclimated plants (A), exposed at the same light conditions, but at different temperature regimes: a) for 5 hours at 38, 43, 45 or 47.5 °C air temperature; b) at 45 °C for a different periods of time – 30 min, 1, 2, 4, and 8 hours.

On the following day a number of control and acclimated plants were subjected to 5-hour heat stress at air temperature either 53°C (in the case of a), or 55°C (in the case of b) at the same growth light regime.

In this way 4 groups of plants were formed: control (C), acclimated (A), control stressed (CS) and acclimated stressed (AS) plants.

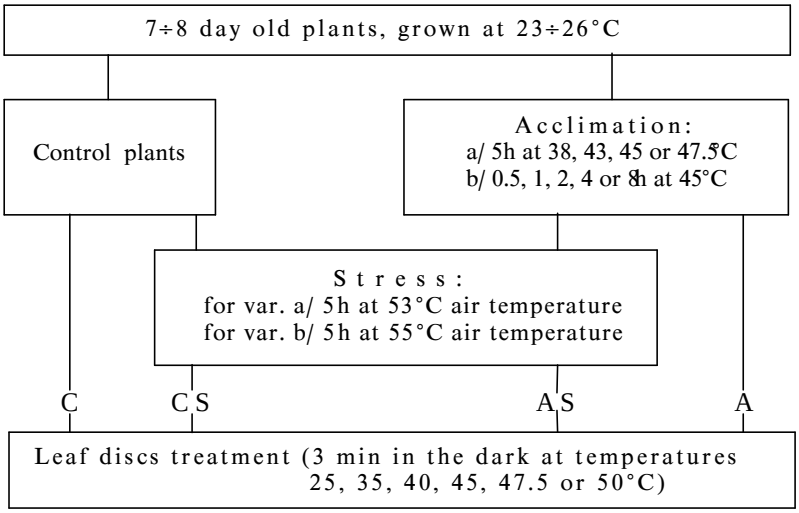
Testing the temperature sensitivity

Leaf's sensitivity was evaluated immediately after the acclimation procedure or after the 5-hour stress treatment on the following day. As a criterion, the changes in chlorophyll fluorescence parameters after 3 min dark incubation of leaf discs at different (25–50 °C) leaf temperatures were used.

All procedures described above are summarized in the Scheme 1.

Measurement of fluorescence

The photosynthetic activity of primary leaves was evaluated by the fast phase (OIDP) of the chlorophyll fluorescence induction kinetics, measured by Chlorophyll Fluorescence Measuring System – PAM (H. Walz, Germany). The induction kinetics of fluorescence was measured at different temperatures after 3 min dark adaptation of leaf discs at the same temperature. The measuring light intensity was 0.75 $\mu mol.m^{-2}.s^{-1}$ and actinic photon flux density – 2250 $\mu mol.m^{-2}.s^{-1}$.



Scheme 1. View of experimental approaches

Results

Heat acclimation at different temperatures

Induction kinetics parameters of control and treated plants are presented in Table 1.

Table 1. Dependence of chlorophyll fluorescence parameters on acclimation temperature. Young been plants were grown for 8 days at 25°C (Control variant), treated once for 5 h at corresponding temperature (38–47.5°C) and additionally on the next day for 5 h at 53°C (Acclimated variant) or exposed directly for 5 h at 53°C (Control stressed variant). The data shown are mean values ± standard errors (SE) from 4–5 experiments and are presented as a percent to control variant values

T°C	Variant	F_0±SE	F_v±SE	F_m±SE	F_v/F_m±SE
38	Controls	100.0±3.0	100.0±1.0	100.0±0.8	100.0±0.8
	Acclimated	88.8±2.9	86.4±2.3	87.0±2.3	99.5±0.5
	Contr. stressed	90.4±3.5	87.2±1.6	87.9±2.1	99.2±0.4
43	Controls	100.0±3.7	100.0±3.2	100.0±3.2	100.0±0.5
	Acclimated	92.8±6.0	90.4±3.9	90.9±4.2	99.5±0.6
	Contr. stressed	130.3±26.3	88.3±2.5	97.2±5.8	91.8±5.2
45	Controls	100.0±1.6	100.0±2.9	100.0±2.5	100.0±0.5
	Acclimated	93.4±3.3	90.1±3.8	90.8±3.7	99.2±0.4
	Contr. stressed	103.9±2.4	90.5±5.9	93.2±5.0	97.0±1.2
47.5	Controls	100.0±3.3	100.0±1.6	100.0±1.9	100.0±0.5
	Acclimated	96.8±4.7	91.2±2.6	92.3±2.9	98.9±0.6
	Contr. stressed	99.6±6.7	89.2±3.7	91.3±4.0	97.7±1.0

Evidently, the initial (F_0) and the variable fluorescence (F_v), measured at 25 °C immediately after the stress treatments vary very slightly for both groups of plants (the differences are not statistically significant), notwithstanding the acclimation temperature. The variations of fluorescence values (mostly for F_0) around these of the control, are most probably due to individual phenotypic differences of the plants. It is noteworthy that the plants not submitted to preliminary acclimation procedure – control stressed plants (CS), were greately damaged. Judging visually, only about 30% of their leaves were not fully damaged. The other 70% were almost dried, could not be used in experiments so the measurements were carried out with the less injured (e.g. more resistant) 30% of leaves. Therefore, data, shown in Tables 1 and 2 about CS plants, included only a small part of this group, and are not representative.

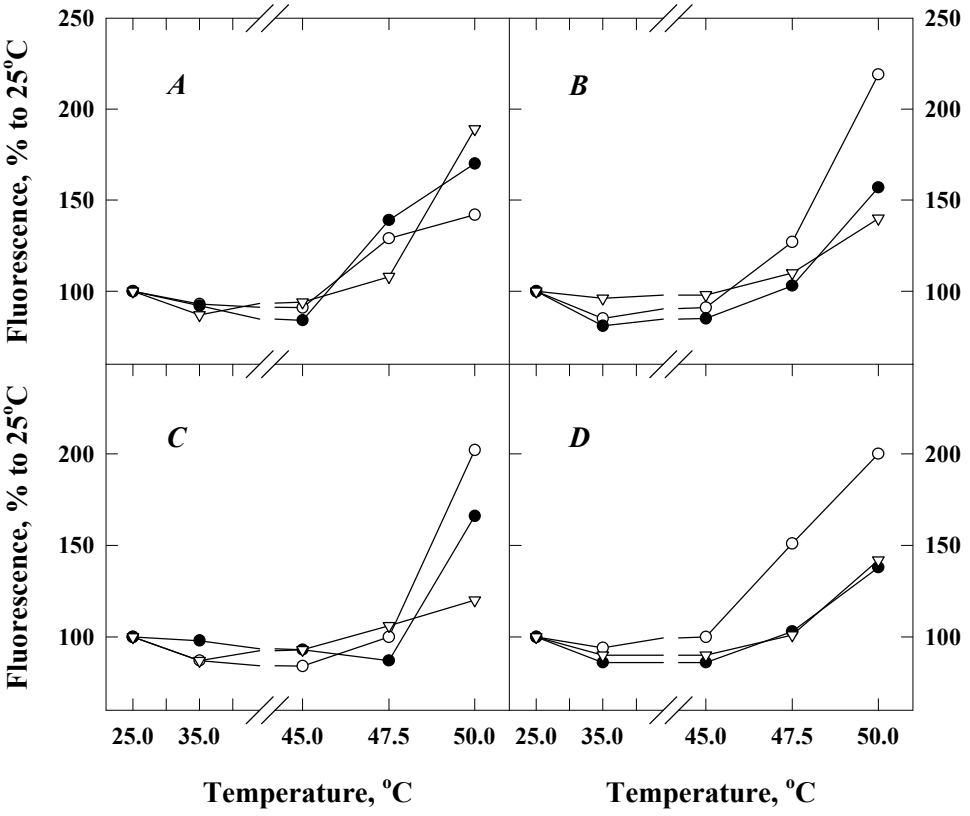


Fig. 1. Temperature dependence of initial chlorophyll fluorescence (F_0) in leaf discs from bean plants subjected to different acclimation procedures. Acclimation was carried out 5 h at different temperatures: 38°C – (*A*), 43°C – (*B*), 45°C – (*C*) and 47.5°C – (*D*). Control and acclimated plants were analyzed either directly (—○— and —●—, respectively) or after 5 h additional stress treatment at 53 °C of acclimated plants (marked —∇—). Leaf discs dark incubation (3 min) and fluorescence kinetics measurement were carried out at the same temperature. Exciting light intensity was 2250 μmol.m^{-2}.s^{-1}. Data are presented in percent to F_0 value measured at 25 °C

Most stable of all fluorescent parameters (least affected by stress treatment) was the F_v/F_m ratio, the maximal deviation not extending 7% (excluding 1 case of CS – 8.2%) of the average control value (0.805±0.008). The difference in temperature sensitivity of the photosynthetic apparatus between plants of the 3 groups showed more significant variations. Changes in F_0, F_m and F_v/F_m, following 3 min incubation of leaf discs at elevated temperature and measured at the same temperature, are presented in Fig. 1, 2 and 3. Two typical intervals: 25–45 °C and 45–50 °C could be distinguished when the temperature dependence of F_0 had been analyzed (Fig. 1, *A*, *B*, *C* and *D*). In the first interval a slight monotonous decline of the fluorescence intensity could be observed, reaching 80% of the control value (the value of this parameter at 25 °C). The courses of the curves, as well as the absolute values of the decrease, are very much alike for all groups of plants. Significant variations appear after plant treatment at 47.5–50 °C. In this temperature range, F_0 intensity sharply rises and the degree of the increase is determined by the physiological state of the plant, modified by acclimation procedures. No acclimation effect could be observed after acclimation with 38 °C and a slight one – after stress treatment (Fig. 1*A*). The maximal value of F_0 at 50 °C increased for all variants.

A more pronounced acclimation effect in connection with this parameter is displayed in plants, heated for 5 hours at 43 °C (Fig. 1*B*). The increase of F_0, induced by 3 min incubation of leaf discs at 50 °C, is lower in acclimated as well as in stressed plants, as compared to the control (62±3, 36±4 and 126±4%, respectively). This means that the acclimated plants exhibit increased stability, which is further developed after 5-hour-treatment at 53 °C on the next day.

Highest stability of F_0 was observed after acclimation with 45 °C (Fig. 1*C*). Immediately after acclimation, the value of F_0 at 50 °C increased twice in the control plants, and only 60±2% in the acclimated plants. The differences were more pronounced after stress treatment and the increase in this group of plants was only with 33±2%. The situation was similar to that in plants, acclimated at 47.5 °C (Fig. 1*D*).

Hence, the enhanced thermotolerance of the photosynthetic apparatus in regard to F_0 is expressed as a shift of its sharp increase to higher temperatures (which has been demonstrated by Schreiber and Berry, 1977; Yordanov and Weis, 1984) and as a result of this – a decrease of initial fluorescence at 50 °C.

An interesting fact is the presence of acclimation effect in CS plants, treated directly at 53 °C for 5 hours (data not shown). Obviously, even this sublethal treatment could provoke a certain increase in the heat tolerance of surviving plants, which endured the stress.

Another important parameter of prompt fluorescence induction kinetics is F_m, representing its maximal effectiveness when all reaction centres in PS2 are closed. The temperature dependence of F_m is presented in Fig. 2. Similarly to Fig. 1, there are (as regard the character of experimental curves) two distinct temperature intervals, too. For all variants in the interval 25–45 °C, a relatively slow monotonous de-

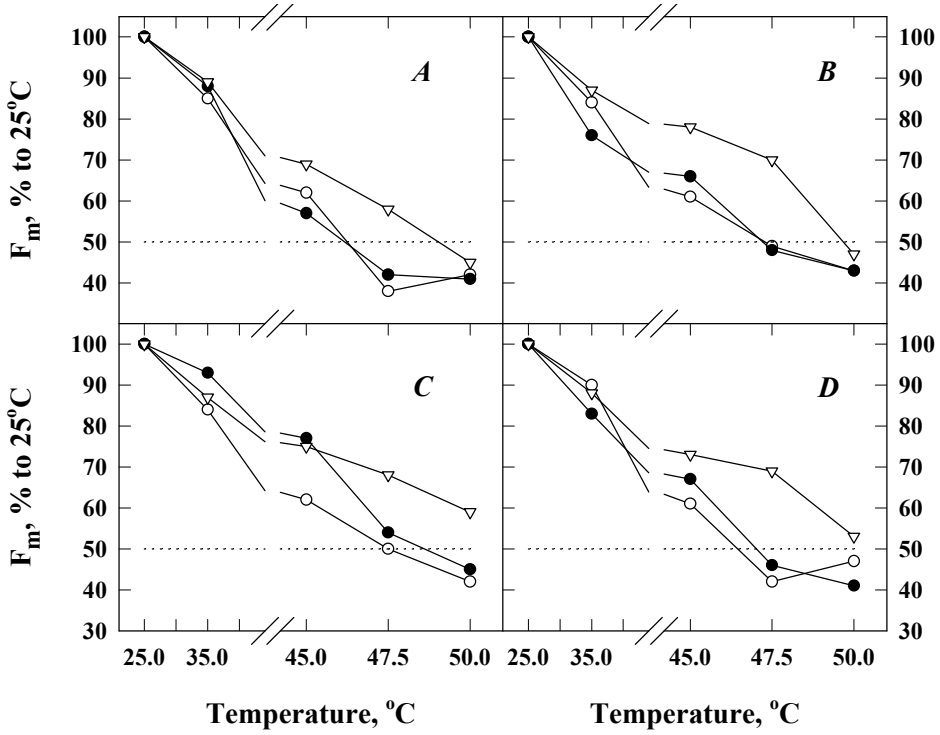


Fig. 2. Temperature dependence of maximal chlorophyll fluorescence (F_m) in leaf discs from bean plants subjected to different acclimation procedures. The conditions of acclimation and measurement procedures as well as the curves symbols are as in Fig. 1. Data are presented in percent to F_m value measured at 25 °C

crease of fluorescence intensity was observed and after 45 °C with the rise of temperature fluorescence sharply decreased. The change of fluorescence in the first interval correlated with the course of F_0 temperature dependence, though the absolute values of the decrease were about twice higher. This means that the drop is due not only to reduced quantum effectivity of F_0, but also to F_v decline, provoked by inhibition of photochemical reactions. A drastic fall of the variable component of induction kinetics was observed in the second temperature interval, which not only compensated the steep temperature dependent increase of F_0, but predetermined the sharp decrease of F_m. Almost all acclimation procedures as well as the stress treatment at 53 °C led to heat tolerance of the photosynthesizing apparatus rise. The thermostability of F_v, as judged by half-inactivation temperature (T_{50}), did not differ significantly from that of the controls when measured immediately after the acclimation procedure. After the additional stress treatment, a visible positive effect of acclimation was observed, expressed in the weaker inhibition of fluorescence, especially in the 45–50 °C interval. In case the effectiveness of acclimation procedures was compared at 38 °C (Fig. 2*A*),

43 °C (Fig. 2*B*), 45 °C (Fig. 2*C*) and 47.5 °C (Fig. 2*D*), a noticeable increase of T_{50} at 45°C was observed. In that case T_{50} showed a shift of 5°C towards higher temperature.

The F_v/F_m ratio is frequently used to characterize the functional state of PS2 and the electron transport between the two photosystems (see Krause and Weis, 1991). Incubation of leaf discs for 3 min at temperature up to 45 °C did not provoke considerable changes in F_v/F_m in all variants (Fig. 3). The data show the presence of acclimation effect on the relative variable fluorescence, the latter being strongly displayed only after additional stress (AS variant). The thermal tolerance of acclimated plants increases with the rise in acclimation temperature until a definite limit. For example, the F_v/F_m ratio in control plants, measured at 50 °C, was about 10±8% of that registered at 25 °C (Fig. 3). After the acclimation this ratio was preserved at higher level: about 40±15% for 38 °C (Fig. 3*A*), 46±14% for 43 °C (Fig. 3*B*), 72±16% for 45 °C (Fig. 3*C*), and 50±5% for 47.5 °C acclimation temperature (Fig. 3*D*).

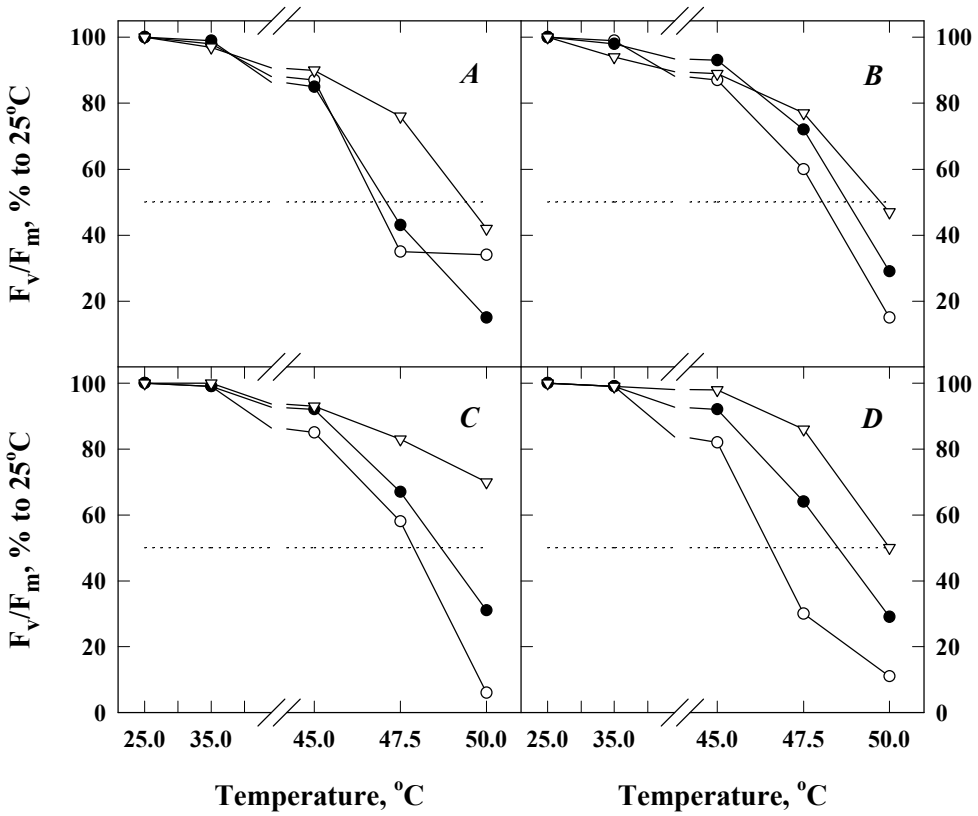


Fig. 3. Temperature dependence of relative variable fluorescence (F_v/F_m) in leaf discs from bean plants subjected to different acclimation procedures. The conditions of acclimation and measurement procedures as well as the curves symbols are as in Fig. 1. Data are presented in percent to F_v/F_m value measured at 25 °C

Influence of duration of acclimation

The acclimation effect on the photosynthetic apparatus developed according to the duration of plant treatment. The parameters of induction kinetics were not modified considerably immediately after applying different acclimation procedures (Table 2), but the latter could alter the thermal sensitivity of plants. As already mentioned, acclimation influenced the course of F_0 heat tolerance, the most noticeable effect being

Table 2. Effect of acclimation procedure duration on chlorophyll fluorescence parameters. Young been plants were grown for 8 days at 25 °C (Control variant), treated for a definite time (30 min–8 h) at temperature 45 °C and on the next day for 5 h at 55 °C (Acclimated variants) or exposed directly for 5 h at 55 °C (Control stressed variant). The data shown are mean values ± standard error (SE) from 4–5 experiments and are presented as a percent to control variant values

Variant	F_0±SE	F_v±SE	F_m±SE	F_v/F_m±SE
Control	100.0±4.0	100.0±1.0	100.0±1.0	100.0±0.6
0.5 h acclimation	97.8±4.5	87.5±2.6	89.8±2.4	97.5±0.7
1 h acclimation	88.2±3.1	87.0±3.3	87.0±3.0	100.0±0.6
2 h acclimation	103.0±4.1	97.2±2.4	98.1±2.1	98.3±0.8
4 h acclimation	109.1±3.1	99.3±3.4	101.1±2.3	98.1±0.8
8 h acclimation	89.9±1.3	94.1±3.6	93.4±2.9	100.7±0.8
Control stressed	95.7±4.2	86.0±2.6	87.7±2.4	98.0±0.9

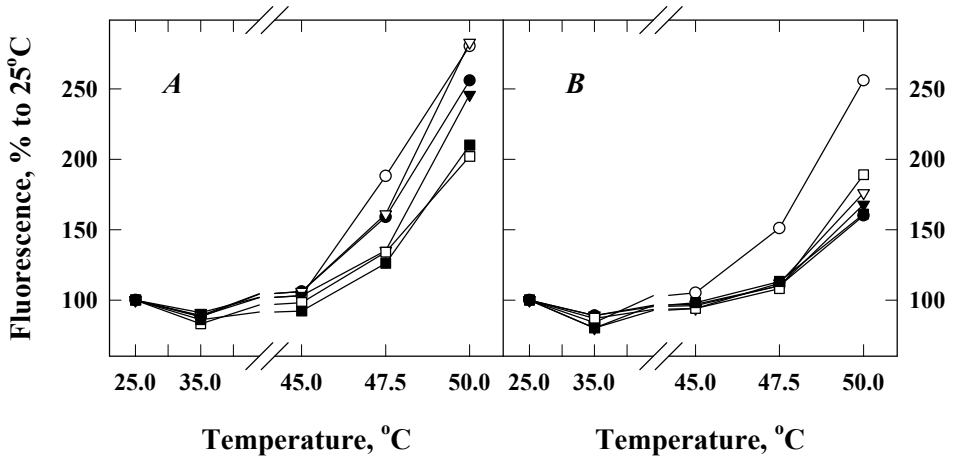


Fig. 4. Temperature dependence of initial chlorophyll fluorescence (F_0) in leaf discs from bean plants subjected to different acclimation procedures. Acclimation was carried out at 45°C different time: 0 h (Control, —○—), 0.5 h (—●—), 1 h (—▽—), 2 h (—▼—), 4 h (—□—) and 8 h (—■—). Acclimated plants were analyzed either directly (*A*) or after 5 h additional stress treatment at 55 °C (*B*). Leaf discs dark incubation (3 min) and fluorescence kinetics measurement were carried out at the same temperature. Exciting light intensity was 2250 μmol.m^{-2}.s^{-1}. Data are presented in percent to F_0 value measured at 25 °C

observed in the 45–50 °C interval (Fig. 4). The increase of F_0 (in all acclimated plants induced by 3 min preincubation of leaf discs at 47.5–50 °C) was weaker, which could be considered as a protective effect of acclimation. When the acclimation procedure was prolonged from 30 min up to 4 h, the effect increased. A certain stabilization occurred up to the 8 hour treatment (Fig. 4*A*). Leaving the plants for 5 h at 55 °C on the day after acclimation treatment, developed further the stabilizing effect (Fig. 4*B*). The heat-induced (3 min, 50 °C treatment) F_0 increase was 93±9% immediately after acclimation at 45 °C, and after the stress procedure it was only 56±4%. These values were respectively 56±2% and 53±3% in plants, acclimated for 8 h, which means that obviously a maximal degree of acclimation was almost attained and additional stay at 55 °C enhanced it only insignificantly. Even a short time of acclimation (30 min or 1 h) provoked a significant stabilizing effect in the variants with stress treatment, which was not so strongly dependent on the duration of acclimation.

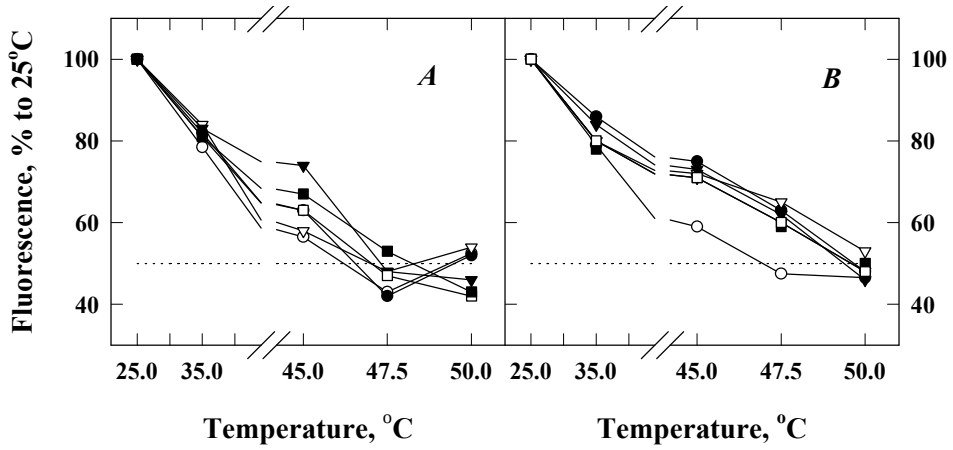


Fig. 5. Temperature dependence of maximal chlorophyll fluorescence (F_m) in leaf discs from bean plants subjected to different acclimation procedures. The conditions of acclimation and measurement procedures as well as the curves symbols are as in Fig. 4. Data are presented in percent to F_m value measured at 25 °C

The temperature sensitivity of F_m changed in the process of acclimation in a more complex way (Fig. 5). It was influenced by acclimation in a wider temperature interval (35–50 °C). The protective effect observed, was strongly dependend on the duration of acclimation, when fluorescence kinetics was measured immediately after heating the plants at 45 °C (30 min to 8 h, Fig. 5*A*). The additional stress on the next day almost unified the temperature dependence of F_m in all samples (besides control) (Fig. 5*B*). The thermal stabilization of F_m by stress treatment is well expressed in 45–47.5 °C interval, which led to inactivation curve shift toward higher temperatures.

An acclimation effect of high temperature treatment was also observed for F_v/F_m ratio reflecting the relative change of variable fluorescence (Fig. 6). Evidently this effect was revealed after incubation of leaf discs at temperature above 35 °C and no noticeable differences between the variants of this parameter, measured at room temperature, could be observed (Table 2). In the 35–45 °C interval, the acclimation was expressed as a weaker inhibition of F_v/F_m after 3 min incubation, and when heating up to 45–50 °C was applied – as T_{50} rise. An increase of thermotolerance was observed with elongation the term of hardening as was the case with F_m (Fig. 6*A*). The acclimation effect was best expressed at 47.5 °C and 50 °C measuring temperature.

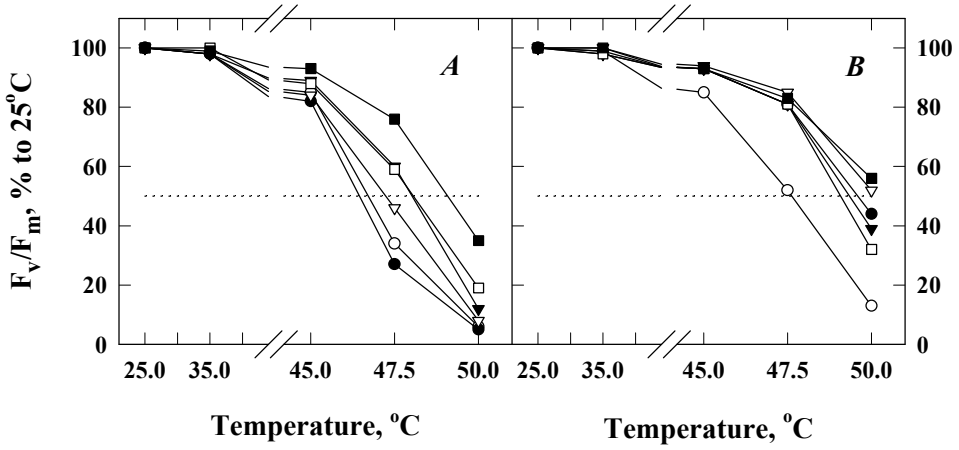


Fig. 6. Temperature dependence of relative variable fluorescence (F_v/F_m) in leaf discs from bean plants subjected to different acclimation procedures. The conditions of acclimation and measurement procedures as well as the curves symbols are as in Fig. 4. Data are presented in percent to F_v/F_m value measured at 25 °C

When the stress treatment was applied, differences from the control appeared already in plants subjected to 30 min acclimation procedure. The effect remained approximately at a constant level for all other periods of acclimation (1–8 h, Fig. 6*B*). The maximal protective effect after 8-hour-treatment, combined with the stress procedure, was close to the measured immediately after acclimation of plants.

Our finding, that even 30 min exposure at 45 °C is sufficient for acclimation of bean plants, is in agreement with Havaux's results (Havaux, 1993) that transfer of potato leaves from 25 °C to temperature slightly lower than the threshold one (T_c) for a short time (half-time ≈ 20 min) causes an upward shift of T_c value without any appreciable loss of PS2 activity. Unlike the results of Havaux, where the effect has been demonstrated immediately after short hardening, in our experiments it was displayed only after additional high temperature stress. This may be a result of different acclimation conditions used.

As a criterion for the thermal tolerance of the photosynthetic apparatus, the temperature, T_{50}, at which a 50% inhibition of the process occurs, was used (Goltsev et al., 1987; Yordanov et al., 1987). The changes of T_{50} of the relative variable fluorescence, registered immediately after the acclimation procedure or after additional stress treatment (5 hours, 55°C), versus duration of hardening are presented in Fig. 7. Evi-

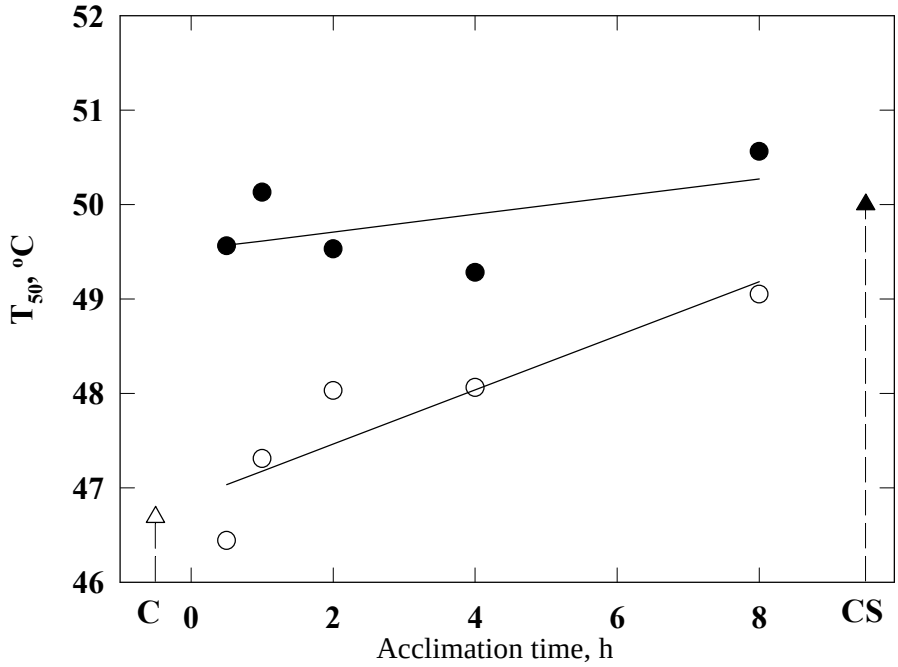


Fig. 7. Dependence of half-inactivation temperature (T_{50}) of the F_v/F_m ratio on the duration of acclimation at 45 °C immediately after treatment (—○—) or after additional 5 h, 55 °C stress procedure (—●—). With triangles the values of the parameter for Control (holed) and Control stressed plants (filled) are presented

dently, T_{50} increased proportionally to duration of acclimation (open circles). After stress treatment T_{50} increased with 2–3 °C (closed circles) and its values were not dependent on the duration of acclimation. The additional increase of F_v/F_m stability as a result of stress treatment was maximal for the short periods of acclimation (30 min–1 h) and insignificant for the longer ones (4–8 h).

Discussion

The heat stress in the interval from 30 min up to several hours can imitate a situation of naturally occurring short periods of overheat observed in areas with big temperature oscillations.

At conditions of temperature variation, including stress, acclimation depends on the capability of plants living at moderate climate conditions, to endure an occasional heat stress. In this case the plant adaptation includes a certain degree of physiological tolerance and mechanisms for an escape of heat stress (Kappen, 1981). In most cases the adaptive mechanisms to different heat regimes can be considered as compensatory, because they allow the plants to buffer the effect of temperature shift on their metabolic systems. The ability of easy recovery after heat stress is decisive, because the functional disorders as inhibition of photosynthetic or protein metabolism decreases the resistance power of the plant.

Thermal inhibition of photosynthesis occurs at temperatures that damage membrane connected processes. The observation that heat injury leads to significant changes in prompt and delayed chlorophyll fluorescence (Berry et al., 1975; Schreiber and Berry, 1977; Schreiber and Armond, 1978; Berry and Björkman, 1980; Yordanov et al., 1987; Goltsev et al., 1987; Bilger and Schreiber, 1990), as well as to abnormal behaviour of the pigment and protein components (Smillie and Nott, 1979; Sundby et al., 1986) shows that a fast disturbance of the integrity of chloroplast membrane occurs when plants are exposed to high temperature. Uncoupling of photophosphorylation and inhibition of the photoreductive ability of chloroplasts can be only symptoms of the more direct effect of high temperature on membrane structure (Mukohata et al., 1974).

The acclimation effects that we observed, regarding thermal inactivation of fluorescence parameters, show that the acclimation procedures applied, provoke an increase of heat tolerance of photosynthetic reactions.

In regard to F_0 the acclimation effect is expressed as a reduction of the heat-induced increase in the 47.5–50 °C interval. One probable reason for that is the heat-induced transition of $PS2_\alpha$ into $PS2_\beta$ centres with their following transformation in Q_B-nonreducing centres (Sundby et al., 1986; Cao and Govindjee, 1989; Guenther and Melis, 1990). The inactivated centres of PS2 sharply increase the effectivity of the emitted fluorescence so that even at very low light intensities (at which F_0 is measured) a great part of it may include its variable component. This is presumably due to the increase of the relative concentration of Q_A-states in Q_B-nonreducing reaction centers of PS2 that have a long life-time – about 3 seconds (Chylla and Whitmarsh, 1989; Ort and Whitmarsh, 1990). An other cause for F_0 rise could be the disturbance of excitation energy migration from LHCII to PS2 core complexes.

So, in frame of this concept, acclimation can influence the velocity of the heat-induced diffusion of PS2 by changing the microviscosity of the lipid bilayer of the thylakoid membrane (e.g. by raising the relative concentration of more saturated digalactosyldiacyl glycerides in the acclimated chloroplasts), as it has been shown by Süss and Yordanov (1986). On the other hand, the newly synthesized heat-shock proteins in acclimated plants are disposed on the outer surface of the unstacked thylakoid membrane building a protecting shield, thus keeping the proteins of $PS2_\beta$

from adverse conformational changes. Indirect confirmation of this proposition may be the observed increase of negative electric surface charge density in chloroplasts isolated from acclimated bean and pea plants (Goltsev et al., 1987; Georgieva et al., 1989). It may be the result of association of newly sinthesized heat-shock proteins with thylakoid membrane surface.

The variable component of fluorescence is sensitive to changes in excitation-energy transport rate within the pigment apparatus, as well as to photochemical and dark reactions in the thylakoid membranes. The drop of F_m in thermoinactivated leaf discs is most probably due to the electron transport inhibition in the donor side of PS2 (Katoh and San Petro, 1967; Yamashita and Butler, 1968; Goltsev and Yordanov, 1992). Another reason for it may be the acceleration of internal conversion at higher temperature.

The degree of reducing maximal fluorescence measured at 45 °C in acclimated plants is considerably smaller than that of the controls, which is evidently connected with the enhanced stability of O_2 evolving complex. It consists of about 25–30% in the variants with maximal expressed acclimation effect, which correlates to the fluorescence diminution, due only to the elevated internal conversion in excited chlorophyll molecules (Goltsev and Yordanov, unpublished). This shows that in contrast to control plants, the treatment of leaf discs of acclimated plants with 45 °C does not lead to inactivation of PS2 donor side.

Analyses of the acclimation effect's dependence of the fluorescent parameters on temperature and on duration of hardening show some peculiarities. The increased thermal tolerance acquired as a result of hardening depends to a considerable degree on quantitative characteristics of the provoking stress (time duration and temperature). A maximal acclimation effect is observed at temperatures close to lethal if the plant can endure it and is able to normalize its functions. In our experiments such effect was found after 5 h treatment of the plants with 53 °C or 55 °C (air temperature). The ability of plants to endure such high temperature loading is build up after their previous hardening at more moderate stress conditions. The acclimation procedure itself really leads to formation of increased thermal tolerance of fluorescence parameters, as well as of integral photosynthetic process (unpublished data). Moreover, the degree of acquired thermal stability is defined by the regime of acclimation. For all parameters investigated a monotonous increase of the stabilizing effect is observed with the increase of the duration of hardening at a given temperature (Fig. 4–7), as well as with the increase of the hardening temperature (Fig. 1–3).

Acquired increased thermostability of the fluorescent parameters after stress treatment (5 h at 53 °C or 55 °C) does not practically depend on duration and temperature, at which preliminary acclimation has taken place. This shows that all conditions of hardening that we applied induce time-developing processes, which determine the increased thermal tolerance. Moreover, when longer hardening is applied, the formation of heat tolerance ends with the process of hardening itself, thus after 5 hour's addi-

tional treatment with 53°C or 55°C it changes insignificantly. Approximately the same thermotolerance can be achieved by short acclimation combined with stress treatment.

Acclimation to a wide temperature range (38–47.5°C) develops high thermostability in young bean plants, which allows them to survive at stress conditions. The acclimation process develops and ends after approximately 5 hours hardening at 45°C is applied. Maximum thermal tolerance of the photosynthetic apparatus is achieved at conditions, close to lethal ones. In initial periods of hardenning the thermotolerance of fluorescence parameters is not enhanced, but the plants are able to endure a following stress, which allows the assumption that under this condition, acclimation effect is realized not on the level of primary photosynthetic reactions, but on the cellular level.

***Acknowledgements*:** This work was supported by Grants No. B-72/1991 and K-13/1991 from the National Science Fund, Bulgaria.

References

Alexandrov, V. Y., 1975. Cell, Macromolecules and Temperatures. Nauka, Moscow (In Russ.).

Armond, P., U. Schreiber, O. Björkman, 1978. Photosynthetic acclimation to temperature in desert shrub *Larrea divaricata*. II. Light harvesting efficiency and electron transport. Plant Physiology, 61, 411–415.

Berry, J., O. Björkman, 1980. Photosynthetic response and adaptation to temperature in higher plants. Annual Reviews of Plant Physiology, 31, 491–543.

Berry, J., D. Fork, S. Garrison, 1975. Mechanistic studies of thermal damage to leaves. Carnegie Institution Washington Yearbook, 74, 751–759.

Berry, J., J. Raison, 1981. Responses of macrophytes to temperature. In: Encyclopedia of Plant Physiology 12A. (Eds O. Lange et al.), Springer Verlag, Berlin-Heidelberg, 277–338.

Bilger, W., U. Schreiber, 1990. Chlorophyll luminescence as an indicator of stress-induced damage to the photosynthetic apparatus. Effect of heat-stress in isolated chloroplasts. Photosynthesis Research, 25, 161–171.

Björkman, O., 1975. Photosynthetic responses of plants from contrasting environments. Thermal stability of the photosynthetic apparatus in intact leaves. Carnegie Institution Washington Yearbook, 74, 748–751.

Cao, J., Govindjee, 1989. Chlorophyll *a* transient as an indicator of active and inactive photosystem II in thylakoid membranes. Biochimica et Biophysica Acta, 1015, 180–188.

Chylla, R., J. Whitmarsh, 1989. Inactive photosystem II complexes in leaves: turnover rate and quantitation. Plant Physiology, 90, 765–772.

Georgieva, K., V. Goltsev, I. Iordanov, 1989. Influence of high temperature on electrophoretic mobility of thylakoid membranes from two pea cultivars. Photosynthetica, 23, 399–402.

Goltsev, V., I. Yordanov, 1987. High temperature damage and acclimation of the photosynthetic apparatus. 3. Modification of the thylakoid membrane electrophoretic mobility. Photosynthetica, 21, 189–192.

Goltsev, V., I. Yordanov, 1992. Mathematical model of thermal inactivation of variable fluorescence. In: Proc. VI Nat. Conf. Biomed. Phys. and Engineering, Sofia, 155–161 (In Bulg.).

Goltsev, V., I. Yordanov, T. Stoyanova, O. Popov, 1987. High temperature damage and acclimation of the photosynthetic apparatus. II. The effect of mono- and divalent cations and pH on the temperature sensitivity of some functional characteristics of chloroplasts isolated from heat-acclimated and non-acclimated bean plants. Planta, 170, 478–488.

Guenther, J. A., A. Melis, 1990. The physiological significance of photosystem II heterogeneity in chloroplasts. Photosynthesis Research, 23, 105–109.

Havaux, M., 1993. Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. Plant, Cell and Environment, 16, 461–467.

Kappen, L., 1981. Ecological significance of resistance to high temperature. In: Encyclopedia of Plant Physiology 12A. (Eds O. Lange et al.), Springer Verlag, Berlin Heidelberg, 439–474.

Katoh, S., A. San Pietro, 1967. Ascorbate-supported NADP photoreduction by heated Euglena chloroplasts. Archives of Biochemistry and Biophysics, 122, 144–152.

Krause, G., E. Weis, 1991. Chlorophyll fluorescence and photosynthesis. The basis. Annual Review of Plant Physiology and Plant Molecular Biology, 42, 313–349.

Kritenko, S., A. Titov, 1990. Influence of abscissic acid and cytokinin on protein synthesis at low and high temperature adaptation of plants. Soviet Plant Physiology, 37, 126–132.

Mukohata, Y., T. Yagi, M. Higashida, A. Matsuno, Y. Sugiyama, 1974. Comparative studies on the properties of spinach chloroplasts prepared by media containing choline chloride or sucrose. Plant and Cell Physiology, 15, 619–628.

Neumann, D., L. Nover, B. Parthier, R. Rieger, K. Scharf, L. Wollgiehn, 1989. Heat shock and other stress response systems of plants. Biologische Zentrblat, 108, 2–78.

Ort, D., J. Whitmarsh, 1990. Inactive photosystem II centers: a resolution of discrepancies in photosystem II quantitation. Photosynthesis Research, 23, 101–104.

Ougham, H. J., J. L. Stoddart, 1986. Synthesis of heat shock protein and acquisition of thermotolerance in high temperature tolerant and high temperature succeptible lines of sorghum. Plant Science, 44, 163–168.

Raison, J., C. Pike, J. Berry, 1982. Growth temperature-induced alteration in thermotropic properties of *Nerium oleander* membrane lipids. Plant Physiology, 70, 215–218.

Santarius, K., M. Müller, 1979. Investigation on heat resistance of spinach leaves. Planta, 146, 529–538.

Schreiber, U., P. Armond, 1978. Heat-induced changes of chlorophyll fluorescence in isolated chloroplasts and related heat-damage at the pigment level. Biochimica et Biophysica Acta, 502, 138–151.

Schreiber, U., J. Berry, 1977. Heat-induced changes of chlorophyll fluorescence in intact leaves correlate with damage of the photosynthetic apparatus. Planta, 136, 233–238.

Smillie, R., R. Nott, 1979. Heat injury in leaves of alpine, temperate and tropical plants. Australian Journal of Plant Physiology, 6, 135–141.

Steponkus, P., 1981. Responds to extreme temperatures: cellular and sub-cellular bases. In: Encyclopedia of Plant Physiology, 12A. (Eds O. Lange et al.), Springer Verlag, Berlin-Heidelberg, 371–402.

Sundby, C., A. Melis, P. Maenpaa, B. Andersson, 1986. Temperature dependent changes in antennae size of PS II. Reversible conversion of $PS2_{\alpha}$ to $PS2_{\beta}$. Biochimica et Biophysica Acta, 851, 475–483.

Süss, K.-H., I. Yordanov, 1986. Biosynthetic cause of *in vivo* acquired thermotolerance of photosynthetic light reactions and metabolic responses of chloroplasts to heat stress. Plant Physiology, 81, 192–199.

Titov, A., S. Drozdova, S.P. Kritenko, V.V. Talanova, E.G. Sherudilo, 1986. Cytokinin influence on cold resistance of active developing plants. Physiology and Biochemistry of Crop Plants (Kiev), 18, 64–69 (In Russ.).

Weis, E., 1984. Temperature-induced changes in the distribution of excitation energy between photosystem I and photosystem II in spinach leaves. In: Advances in Photosynthesis Research (Ed. C. Sybesma), Vol. 3, M.Nijhoff / Dr.W. Junk Publs. The Hague, 291–294.

Yamasita, T., W. Butler, 1968. Inhibition of chloroplast by UV irradiation and heat treatment. Plant Physiology, 43, 2037–2040.

Yordanov, I., 1981. Photosynthetic characteristics of *Phaseolus vulgaris* plants adapted to high temperatures. In: Photosynthesis VI. Photosynthesis and Environment (Ed. G. Akoyunoglou), Balaban Intern. Sci. Services, Philadelphia, 379–388.

Yordanov, I., S. Dilova, R. Petkova, T. Pangelova, V. Goltsev, K.-H. Süss, 1986. Mechanisms of the temperature damage and acclimation of the photosynthetic apparatus. Photobiochemistry Photobiophysics, 12, 147–155.

Yordanov, I., V. Goltsev, T. Stoyanova, P. Venediktov, 1987. High temperature damage and acclimation of the photosynthetic apparatus. I. Temperature sensitivity of some photosynthetic parameters of chloroplasts isolated from acclimated and non-acclimated bean leaves. Planta, 170, 471–477.

Yordanov, I., E. Weis, 1984. The influence of leaf-aging on the heat-sensitivity and heat-hardening on the photosynthetic apparatus in *Phaseolus vulgaris* L. Zeitschrift für Pflanzenphysiologie, 113, 383–393.