PHOTOSYNTHETICA 44 (3): 404-410, 2006

Mechanisms of energy dissipation in peanut under water stress

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Abstract

Effect of drought on the mechanisms of energy dissipation was evaluated in two-month-old *Arachis hypogaea* cvs. 57-422, 73-30, and GC 8-35. Plants were submitted to three treatments: control (C), mild water stress (S1), and severe water stress (S2). Photosynthetic performance was evaluated as the Hill and Mehler reactions. These activities were correlated with the contents of the low and high potential forms of cytochrome (cyt) b_{559}, plastoquinone, cyt b_{563}, and cyt *f*. Additionally, the patterns of carotenoids and chlorophylls (Chls), as well as the alterations of Chl *a* fluorescence parameters were studied. Under mild water stress the regulatory mechanism at the antennae level was effective for 57-422 and GC 8-35, while in the cv. 73-30 an overcharge of photosynthetic apparatus occurred. Relative to this cv., under S1 the stability of carotene and the dissipative cycle around photosystem (PS) 2 became an important factor for the effective protection of the PS2 reaction centres. The cyclic electron flow around PS1 was important for energy dissipation under S1 only for the cvs. 57-422 and 73-30.

Additional key words: *Arachis*; chlorophyll fluorescence; cultivar differences; cytochromes; energy dissipation; Hill reaction; Mehler reaction; photosynthetic electron carriers; photosystems 1 and 2; plastoquinone; xanthophyll cycle.

Introduction

Photosynthetic metabolism is a main target to reactive forms of oxygen (Lidon and Henriques 1993, Foyer *et al.* 1994, Mishra *et al.* 1995). Even in the absence of stress, protection mechanisms against the deleterious effects of oxy-radicals are necessary, since excess energy can be transferred to chlorophyll (Chl) and O_2 (Asada 1994, Foyer *et al.* 1994, Sgherri *et al.* 1996, Hidég 1997).

Under drought, plants can reduce stomatal conductance to attenuate the rate of water loss, but a decrease in CO_2 uptake rate also occurs. Additionally, in this process the rate of photon absorption largely exceeds its consumption in the carboxylative reactions (Genty *et al.* 1989, Demmig-Adams 1990). Consequently, an energetic over-excitation of thylakoids and an exacerbate production of reactive molecules develops (Demmig-Adams 1990, Young and Britton 1990, Lawlor 1995). Linked to this production, under drought an overproduction of triplet state Chl ($^3Chl^*$) might occur, if the energy received by the complex protein-pigments becomes excessive (Takahama and Nishimura 1976, Oxborough and Horton 1988). From $^3Chl^*$ singlet oxygen ($^1O_2$) can be further produced. This highly reactive form may cause irreversible bleaching of pigments, namely of P_{680}, as well as the oxidation of several other molecules, including the D1 proteins and membrane phospholipids (Demmig-Adams 1990, Barber and de las Rivas 1993, Foyer *et al.* 1994). Under drought, photoinhibition can occur in the donor side, allowing the production of the highly oxidative form P_{680}^+ which becomes extremely reactive for carotenoids and Chl (either by direct action or implicating generation of active forms of oxygen). In the acceptor side, photoinhibition can additionally develop, leading to an over-

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Received 5 August 2005, *accepted* 16 January 2006.

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Abbreviations: C = control; Chl = chlorophyll; cyt = cytochrome; DCPIP = 2,6-dichlorophenolindophenol; $DCPIPH_2$ = reduced 2,6-dichlorophenolindophenol; DPC = 1,5-diphenyl-carbazide; F_m = maximum fluorescence; F_v = variable fluorescence; F_0 = initial, minimal, or basal fluorescence; MV = methyl viologen; N = neoxanthin; PQ = plastoquinone; PS = photosystem; q_p = photochemical quenching; RuBPCO = ribulose-1,5-bisphosphate carboxylase/oxygenase; RWC = relative water content; S1 = mild water stress; S2 = sever water stress; V = violaxanthin; Z = zeaxanthin; Φ_e = quantum yield of PS2 electron transport.

reduction of the quinone pool, whereas the radical pair recombination (P_{680} $^{+}Pheo^{-}$) favours the production of triplet state of P_{680} (Barber and de las Rivas 1993). The overproduction of reactive molecules may further occur at the photosystem (PS) 1 level, since an increasing leakage of electron toward oxygen increases, synthesising the superoxide anion, and therefore leading to the production of hydrogen peroxide and hydroxyl radicals.

Under drought, plant defences are limited to the development of mechanisms favouring the reduction of photon absorption rate (Matthews *et al.* 1983, Ludlow and Björkman 1984) or the activation of processes that allow the dissipation of excess energy (Björkman and Demmig-Adams 1995). Several mechanisms of excess energy dissipation have been referred at the PS1 (Krause 1988, Demmig-Adams 1990, Young and Britton 1990) and PS2 levels (Harbinson *et al.* 1989, Björkman and Demmig-Adams 1995).

In a previous study (Lauriano *et al.* 2000) we showed that the photosynthetic machinery of the cv. GC 8-35 is more sensitive to water deficit, while those of the cvs. 57-422 and 73-30 display a more effective control of excess energy as suggested by its stability. In this context, the peanut cv. GC 8-35 reveals a middle sensitivity to drought. Using the above mentioned cvs. as test systems, the aim of this work was to evaluate the model effects of water stress in dissipation of excess energy in the three peanut cvs. that have different adaptive characteristics.

Materials and methods

The effects of drought were evaluated in two-month-old peanut (*Arachis hypogaea*) plants (cvs. 57-422, 73-30, and GC 8-35). After germination in Petri dishes, the seedlings were chimed in 2 500 cm^3 pots (one plant per pot) filled with a mixture of vermiculite and *Trio-hum* substrate (4 : 5, v/v). Plants were grown in a glasshouse, from June to August, under natural irradiation. They were kept well watered and fed weekly with a Hoagland nutrient solution.

Drought was imposed by withholding irrigation and the measurements took place at relative water contents (RWC) of 95–90, 80–70, and 60–55 %. These RWC values were considered the control (C) or mild (S1) and severe (S2) water stress treatments, respectively. Relative water content was determined gravimetrically according to Čatský (1960), using leaf discs of 0.5 cm^2 each.

The determination of photosynthetic electron transport rates followed the method of Droppa *et al.* (1987). The Hill ($H_2O \rightarrow DCPIP$ and $DPC \rightarrow DCPIP$) and the Mehler ($DCPIPH_2$-MV) reaction rates were determined using a Clark-type oxygen electrode (*LW2*, *Hansatech*, Kings Lynn, UK) and adding chloroplasts to a final concentration equivalent to 30 µg Chl. Chl concentration was determined according to Arnon (1949). Leaves were homogenised in a medium containing 0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 2 mM EDTA, 1 mM $MnCl_2$, 2 mM ascorbate, 0.4 % bovine serum albumin (BSA), and 50 mM MES (pH 6.4). After filtration, the chloroplasts were washed twice in the isolation medium and resuspended in 0.33 M sorbitol, 10 mM NaCl, 1 mM $MgCl_2$, 2 mM EDTA, 1 mM $MnCl_2$, 0.4 % BSA, and 50 mM HEPES (pH 7.5). For determination of PS2 activity including the water oxidation complex, 2,6-dichlorophenolindophenol was used as electron acceptor from the quinone pool. For measurement of the PS2 without the water oxidation complex, 1,5-diphenyl-carbohydrazide was used. To measure the electron transport associated to PS1, reduced 2,6-dichlorophenolindophenol ($DCPIPH_2$) was used as electron donor to cytochrome (cyt) b_6/f, with methyl viologen as electron acceptor. Sodium azide and 3-(3,4-dichlorophenyl)-1,1-dimethylurea were also applied to inhibit peroxidase activities and the electron transport before the plastoquinone (PQ), respectively.

Chloroplasts for cyt determination were isolated according to Spiller and Terry (1980). The contents of cyt b_{559HP}, b_{559LP}, b_{563}, and f were measured according to Houchins and Hind (1984). Values were obtained at 545 nm, using the isosbestic wavelengths of 545 and 568 nm for cyt b_{559}. An extinction coefficient of 20 mM^{-1} cm^{-1} was assumed. The values of cyt f were obtained at 554 nm, considering an extinction coefficient of 19.7 mM^{-1} cm^{-1}. Sub-chloroplast fractions for PQ determination were obtained following Droppa *et al.* (1987), and the content of this molecule was determined according to Redfearn and Friend (1962). The absorption difference spectrum between the oxidised and the reduced form of PQ at 255 nm was used, considering the isosbestic wavelengths of 276 and 308 nm. An extinction coeficient of 14.8 mM^{-1} cm^{-1} was assumed. For measuring the photosynthetic electron carriers, a *Shimadzu CPS-240* UV-Visible spectrophotometer was used.

The extraction from isolated chloroplasts and quantitative determination of neoxanthin+violoxanthin (N+V), lutein (L), zeaxanthin (Z), and carotene were carried out according to Hager and Meyer-Bertenrarth (1966), under a constant nitrogen flow. The Chl content was measured using sub-samples of an intimate mixture following Arnon (1949).

Chl *a* fluorescence parameters were measured using the *PAM 2000* system (*H. Walz*, Effeltrich, Germany). Leaves were dark-adapted overnight before F_0 measurement, and the ratio F_v/F_m was determined. The q_p was calculated according to van Kooten and Snell (1990), and measured under steady-state photosynthetic conditions, at ambient CO_2, temperature of about 30 °C, 800 µmol m^{-2} s^{-1} of "actinic light", and saturating flashes of 4 300 µmol m^{-2} s^{-1}, with a duration of 1 s. The Φ_e was calculated according to Genty *et al.* (1989).

For statistical analyses, a two-way ANOVA was applied to the studied parameters, for a significance level of $p \leq 0.05$. Based on the ANOVA results, a Tukey test for mean comparison was performed for a 95 % confidence level. The letters *a*, *b*, and *c* represent differences between the degree of drought, while the letters *r*, *s*, and *t* represent differences between the cvs.

Results

Under S1 and S2 in the cv. 57-422 (Fig. 1*A,B*), the rate of reactions associated to PS2 ($H_2O \rightarrow DCPIP$, $DPC \rightarrow DCPIP$) and the contents of cyt b_{559HP} (Fig. 2*A*) and PQ (Fig. 3) decreased significantly. No significant differences were observed between the two water stresses. The reactions coupled to $DCPIPH_2 \rightarrow MV$ (Fig. 1*C*) and the content of cyt *f* (Fig. 2*A*) displayed non-significant changes under water stress, but the contents of cyt b_{559LP} and cyt b_{563} decreased under S2. Under S1, F_0, the ratio F_v/F_m, and q_p remained unchanged (Fig. 4). The content of carotene remained relatively stable, while the contents of L, V+N, and Z decreased significantly under S1 and S2 (Table 1). F_0 and q_p were stable, which was coupled to a significant decrease of the ratio F_v/F_m and Φ_e under S2 (Fig. 4).

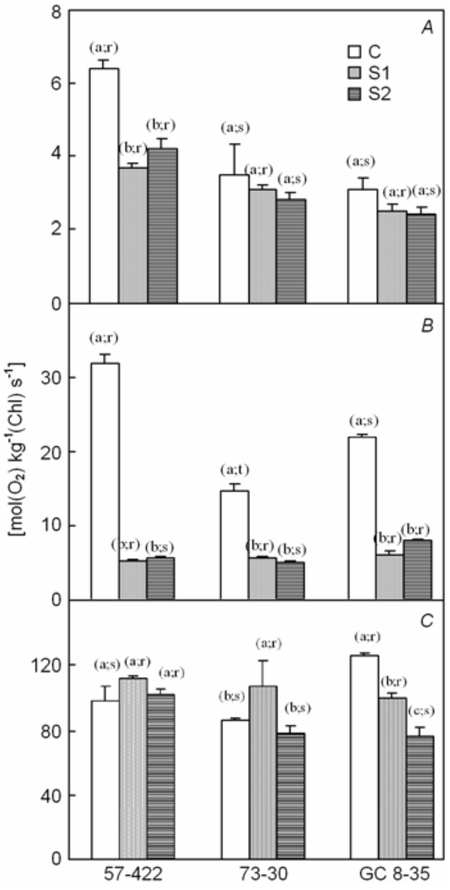

Fig. 1. Changes in (*A*) electron transport rates associated to photosystem (PS) 2 including the oxygen evolving complex, OEC ($H_2O \rightarrow DCPIP$), (*B*) electron transport rates associated to PS2 without OEC, $DPC \rightarrow DCPIP$, and (*C*) electron transport rates of PS1 ($DCPIH_2 \rightarrow MV$) in leaves of cvs. 57-422, 73-30, and GC 8-35 under different water conditions: control (C), middle water stress (S1), and severe water stress (S2). Means ± SE ($n = 3$).

Under water stress in cv. 73-30 the reaction $H_2O \rightarrow DCPIP$ was stable, which was coupled to a significant decrease of $DPC \rightarrow DCPIP$ (Fig. 1*B*). The contents of cyt b_{559} forms (Fig. 2*B*) and PQ (Fig. 3) decreased only under S2. The rate of reaction $DCPIPH_2 \rightarrow MV$ (Fig. 1*C*) and the content of cyt b_{563} (Fig. 2*B*) increased significantly under S1. Under S2 they reached values similar to those for C. The content of cyt *f* (Fig. 2*B*) decreased significantly only under S2. The q_p remained constant, F_0 increased significantly at S1, but an opposite trend was detected for F_v/F_m and Φ_e (Fig. 4). Contents of L, V+N, and Z also decreased significantly, but the carotene content remained constant (Table 1). Following severe water stress, F_0 increased, while F_v/F_m, q_p, and Φ_e

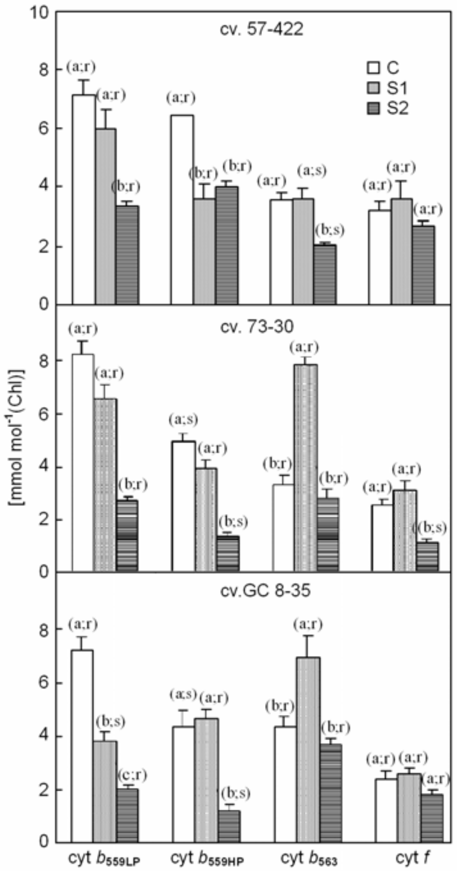

Fig. 2. Changes in the contents of cytochromes in cvs. 57-422, 73-30, and GC 8-35 submitted to different water conditions: control (C), middle water stress (S1), and severe water stress (S2). Means ± SE ($n = 3$).

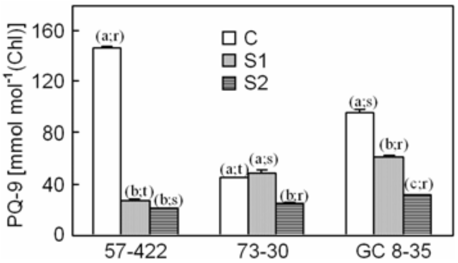

Fig. 3. Changes in the content of quinone (PQ-9) of the peanut cvs. 57-422, 73-30, and GC 8-35 submitted to different water conditions: control (C), middle water stress (S1), and severe water stress (S2). Means ± SE (n = 3).

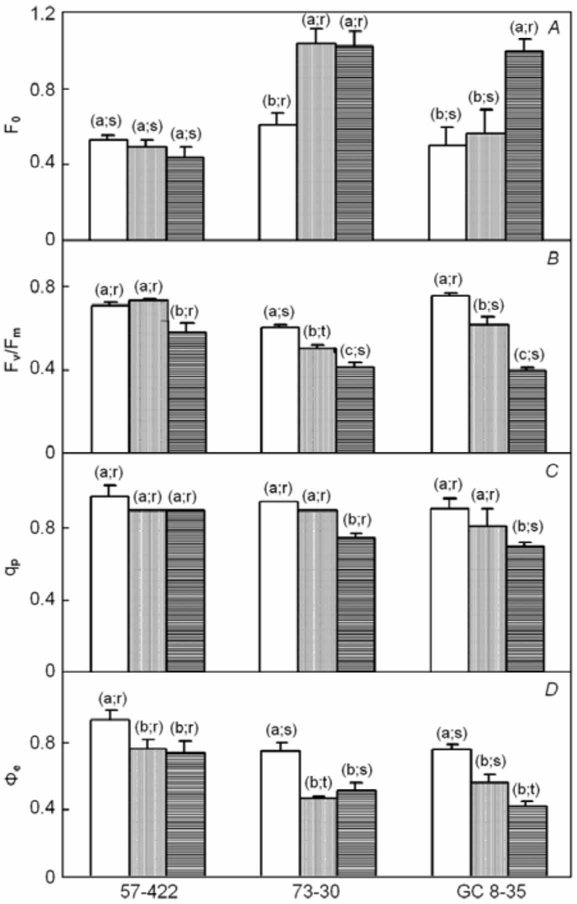

Fig. 4 Changes in the chlorophyll a fluorescence parameters of the peanut cvs. 57-422, 73-30, and GC 8-35 submitted to different water conditions: control (C), middle water stress (S1), and severe water stress (S2). Means ± SE (n = 3).

significantly decreased (Fig. 4). Plants recovered the contents of V+N and Z to those observed in C, but the carotene content remained constant (Table 1).

Table 1. Changes in the contents of lutein (L), zeaxanthin (Z), violaxanthin+neoxanthin (V+N), and carotene (Car) of the cvs. 57-422, 73-30, and GC 8-35 submitted to different water conditions: control (C), middle water stress (S1), and severe water stress (S2). Means ± SE (n = 3).

Cv.		C	S1	S2
57-422	L	5.2 ± 0.1 $^{(a;rs)}$	1.9 ± 0.1 $^{(b;st)}$	1.3 ± 0.1 $^{(b;s)}$
	Z	2.7 ± 0.1 $^{(a;r)}$	0.8 ± 0.0 $^{(b;s)}$	0.5 ± 0.0 $^{(c;s)}$
	V+N	1.5 ± 0.1 $^{(a;r)}$	0.8 ± 0.0 $^{(b;s)}$	0.5 ± 0.0 $^{(c;s)}$
	Car	1.5 ± 0.0 $^{(a;s)}$	1.1 ± 0.1 $^{(b;r)}$	1.2 ± 0.0 $^{(ab;r)}$
73-30	L	4.3 ± 0.2 $^{(a;s)}$	1.0 ± 0.0 $^{(c;t)}$	2.5 ± 0.1 $^{(b;r)}$
	Z	1.5 ± 0.1 $^{(a;s)}$	0.4 ± 0.0 $^{(b;s)}$	1.0 ± 0.1 $^{(a;r)}$
	V+N	0.9 ± 0.0 $^{(a;s)}$	0.4 ± 0.0 $^{(b;t)}$	1.0 ± 0.0 $^{(a;r)}$
	Car	1.7 ± 0.1 $^{(a;s)}$	1.1 ± 0.0 $^{(b;r)}$	1.8 ± 0.1 $^{(a;r)}$
GC 8-35	L	5.8 ± 0.5 $^{(a;r)}$	3.6 ± 0.4 $^{(b;r)}$	2.4 ± 0.1 $^{(c;r)}$
	Z	1.6 ± 0.2 $^{(a;s)}$	1.7 ± 0.1 $^{(a;r)}$	0.8 ± 0.2 $^{(b;r)}$
	V+N	1.1 ± 0.1 $^{(b;r)}$	1.7 ± 0.0 $^{(a;r)}$	0.8 ± 0.2 $^{(b;rs)}$
	Car	3.2 ± 0.2 $^{(a;r)}$	0.7 ± 0.0 $^{(c;r)}$	1.9 ± 0.2 $^{(b;r)}$

The rate of reaction $H_2O \rightarrow DCPIP$ remained unchanged in the cv. GC 8-35 (Fig. 1*A*). Moreover, the rate of reaction DPC→DCPIP (Fig. 1*B*) and the content of PQ (Fig. 3) significantly decreased under S1 and S2. The content of cyt b_{559LP} displayed an antagonistic pattern with increasing water deficit, but the content of cyt b_{559HP} decreased only under severe stress (Fig. 2*C*).

The rate of the reaction $DCPIPH_2 \rightarrow MV$ decreased more under S2 compared to S1 (Fig. 1*C*). The content of cyt b_{563} increased under mild water stress (Fig. 2*C*). After S2 it reached similar value to that observed for C plants. Under water stress, the content of cyt *f* revealed non-significant changes (Fig. 2*C*). Relative to the Chl *a* fluorescence parameters (Fig. 4) during S1 interaction, the stability of F_0 and q_p and a significant decrease of F_v/F_m and Φ_e was observed. The content of V+N rose, content of Z remained unchanged, while those of carotene and L decreased significantly (Table 1). After reaching the S2 conditions, F_0 increased sharply, while F_v/F_m (Fig. 4) and the contents of V+N and Z sharply decreased (Table 1). Carotene content, however, after a partial recuperation, displayed a significant decrease (Table 1).

Discussion

Under drought, due to the stomata closure, CO_2 becomes a limitation of the plant physiology. Thereafter, a full reduction of the available electron acceptors occurs, leading to a rapid photo-inactivation, and to a subsequent photodestruction. This process can be minimised at the PS1 level, through alternative metabolic pathways limiting the photosynthetic cycles of electron transport, modulation of the photorespiration rates, and specific transport of molecular oxygen (Harbinson and Foyer 1991, Havaux 1992, Heber and Walker 1992). Moreover, the dissipative cycles around PS2, in a mechanism that avoids photoinhibition at the donor and/or at the acceptor sides

(Thompson and Brudvig 1988, Buser *et al.* 1992, Prasil *et al.* 1996) can also attenuate the photoinhibitory mechanisms. In this context, carotenoid synthesis, which permits a diversion of massive amount of excitation energy from photochemical reaction centres (RCs) in a form of radiation-less process, involving all of them or at least Z in a specific mechanism (Krause and Weis 1988, Demmig-Adams 1990, Horton *et al.* 1996), can also shield against photo-destruction.

Under S1, the cv. 57-422, although showing a strong inhibition of the PQ content, presented unchanged F_0 and F_v/F_m. This pattern, which was also found in *Digitalis lanata* (Stuhlfauth *et al.* 1990), indicated that the photon absorption and the energy transfer capacity from antennae to PS2 RCs remained unaffected. Additionally, the quantum yield of PS2 was not inhibited. Nevertheless, V+N and Z contents significantly decreased, even under S1, which suggested that the xanthophyll cycle is not determinant in the maintenance of the integrity of the antennae and PS2 RCs. Indeed, a different pattern was found when compared with several findings in other plant species (Stuhlfauth *et al.* 1990, Schäfer and Schmidt 1991, Sharma and Hall 1993, Brestic *et al.* 1995).

The carotene molecules integrate the photosynthetic RCs, having protective functions (Telfer and Barber 1995), namely in the prevention of singlet oxygen production and/or in its quenching/deactivation. In the cv. 57-422, eventually, the unchanged content of carotene had an important role in the overall process of energy dissipation and in the protection of PS2 RCs.

The content of cyt b_{559LP} did not vary significantly, contrary to the two other components of the dissipative cycle around PS2. The overall conclusion was that a sharp loss of the efficiency of this cycle occurred. Moreover, the unchanged rates of the reactions between $DCPIPH_2$ and MV and the maintenance of the contents of the cyt b_{563} and *f* further support previous statements (Havaux 1992) about the higher sensibility of PS2. The general assumptions of these data suggest that the water stress increases the utilisation of new pathways for the consumption of photosynthetic electrons. Thus, during mild water stress PS1 eventually drives and dissipates the excess of energy, avoiding the over-reduction of the overall electron transport chain.

After reaching the severe water stress condition, the significant inhibition of the PQ content induced the photoinhibition in the acceptor side, which promoted the antennae damage and a loss of efficiency of PS2 open RCs. Yet this effect was not observed in cv. 57-422, since F_v/F_m decreased significantly, while F_0 remained unchanged. Such tendency is a reflex of increasing energy dissipation (as heat), thus of a protective thermal de-excitation of PS2 (Cornic *et at.* 1991, Epron *et al.* 1992). This mechanism probably triggered an adequate photon flow to PS2 RCs. The stability of F_0 associated to the decrease in F_v/F_m seemed further related to the regulatory mechanisms in the antennae level. Additionally, these mechanisms contributed to alleviate the energy overcharge on the photosynthetic apparatus, namely in the antennae and PS2 RCs.

L is involved in the antennae radiation absorption and energy transfer (Sieferman-Harms 1985, Stuhlfauth *et al.* 1990, Schindler *et at.* 1994). Similar to Stuhlfauth *et al.* (1990), our results showed a decrease in the content of this carotenoid, suggesting that L was not decisive for the observed F_0 stability. Furthermore, regulatory mechanisms at the antennae level, involving maximum antennae energy trapping, for the consumption of reducing equivalents by the xanthophyll cycle were also reduced due to the lower xanthophyll pool. Nevertheless, due to the decrease of these regulatory mechanisms at the antennae level, F_v/F_m decreased slightly but significantly, while q_p remained unchanged. This process can be interpreted following the previous suggestion of Krause (1988). He stated that under drought the proportion of centres that act as traps for excitation increases. The centres therefore convert excitation energy into heat (diminishing F_m). Thus, the remained unaltered centres appear to be capable of normal photochemistry and might justify the observed stability of q_p. Nevertheless, since the dissipative cycle around PS2 decreased its efficiency (as suggested by the strong affectation of its components, mainly cyt b_{559LP} and PQ), our results indicated that the relative stability of PS2 RCs might also be related to the maintenance of some energy dissipation. Similarly, the stability of PS1 played an important role in the overall energy dissipation process, because although the content of cyt b_{563} decreased, the rate of Mehler reaction and content of cyt *f* remained unchanged.

In cv. 73-30 under mild water stress, the rise of F_0 suggested an increase of excitation loss during the transfer of excitation energy from the antenna pigments to the RCs (Krause and Weis 1991). A decline of F_v/F_m associated with an increase in the F_0 further suggested a decrease of the maximum quantum efficiency of open PS2 centres, as well as an increase in the energy dissipation as heat (Krause 1988), and seemed to indicate a damage of the PS2 centres (Krause 1988, Franklin *et al.* 1992). Therefore, the decrease of F_v/F_m might result from a failure of the regulatory mechanisms at the antennae level, which led to the overcharge of photosynthetic apparatus, the damage of PS2 RCs, and to a loss in the efficiency of the PS2 energy dissipation as heat.

Although F_0 increased sharply, F_v/F_m decreased slightly. This indicated the existence of other mechanisms allowing the dissipation of excess energy and the protection of the PS2 RCs. Indeed, this is an indication of the well known primary site of damage (Franklin *et al.* 1992). The dissipative cycle around PS2 might be important in the overall dissipation of excess energy by maintaining the efficiency of PS2 RCs. Although Φ_e declined significantly, q_p remained unchanged. Considering that the damage of PS2 limits the energy dissipation throughout Φ_e, this stability suggested a reduced photo-production at the

PS2 level, or a control of photosynthesis through carbon fixation rather than by electron transport (Dietz *et al.* 1984, Sukenik *et al.* 1987). Thus only a strong decrease in the rate of electron transport has a reflex on photosynthesis. The reaction $DCPIPH_2 \rightarrow MV$ and the content of cyt b_{563} increased significantly between control and mild water stress, reaching values similar to these observed for C plants. The cyt *f* content only decreased significantly under severe water stress. Under S1, the stability of PS1 activity and the patterns of the cyt b_{563} and *f* suggested an important role of this photosystem for the control of dissipation of excess energy. After reaching S2, the cv. 73-30 was the only one that displayed significant recoveries in the contents of all studied pigments (L, V+N, Z, and Car). This recovery eventually limited the increase of F_0. In this way, if these photoprotective pigments shield the PS2 RCs, the F_v/F_m decrease under S2 could reflect loss of photochemical efficiency due to thermal energy dissipation. Under such water deficit conditions, the dissipative cycle around PS2 also showed a significant loss of efficiency, despite the probable photoprotection given by the carotenes that reach values similar to those observed in control conditions. Accordingly, the quantum yield of PS2 linear electron transport decreased, and a loss of efficiency in the proportion of available excitation energy used in photochemistry developed.

After reaching S1, the cv. GC 8-35 showed that the content of cyt b_{559HP} remained unchanged, while those of cyt b_{559LP} and PQ were already significantly affected. These parameters clearly indicated that the efficiency of the dissipative cycle around PS2 decreased. Yet, the significant decline of the carotene content suggests also a decrease in the dissipation energy status. The alteration of F_v/F_m resulted of the concurrent decrease of F_m. This supposes the existence of regulatory mechanisms at the antennae level that might be played by Z, that remained unchanged. This carotenoid acts as competitor for excitation energy at the antenna level (Sharma and Hall 1990). This increases radiation-less energy dissipation and allows the reduction of the state of over-energisation of the PS2 RCs. Consequently, a lesser damage of the PS2 RCs could occur. So, the observed stability of Z in S1 could be involved in maintainance of relatively stable values of F_0 and F_v/F_m. Under S2, Z content sharply decreased, F_0 increased, and F_v/F_m decreased, suggesting that the control of radiation-less dissipation involving Z has a lower efficiency. Yet, a significant decrease of all components of the PS2 dissipative cycle further suggests a decrease in its functioning. Considering that the rate of reaction $DCPIPH_2 \rightarrow MV$ decreased significantly, already under S1, the present results confirm the previous ones (Lauriano *et al.* 2000) that indicate a higher drought sensitivity of the photosynthetic apparatus of GC 8-35, when compared to 57-422 and 73-30 cvs.

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