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Leaf rolling and photosystem II efficiency in *Ctenanthe setosa* exposed to drought stress

H. NAR[*], A. SAGLAM[*], R. TERZI[*+], Z. VÁRKONYI[**], and A. KADIOGLU[*]

Department of Biology, Faculty of Art and Science, Karadeniz Technical University, 61080, Trabzon-Turkey[*]
Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, H-6701, Hungary[**]

Abstract

Photochemical efficiency of PSII of *Ctenanthe setosa* was investigated to understand the photosynthetic adaptation mechanism under drought stress causing leaf rolling. Stomatal conductance (g_s), the levels of photosynthetic pigments and chlorophyll (Chl) fluorescence parameters were determined in leaves that had four different visual leaf rolling scores from 1 to 4, opened after re-watering and mechanically opened at score 4. g_s value gradually decreased in adaxial and abaxial surfaces in relation to scores of leaf rolling. Pigment contents decreased until score 3 but approached score 1 level at score 4. No significant variations in effective quantum yield of PSII (Φ_{PSII}), and photochemical quenching (q_p) were found until score 3, while they significantly decreased at score 4. Non-photochemical quenching (NPQ) increased at score 2 but then decreased. After re-watering, the Chl fluorescence and other physiological parameters reached to approximately score 1 value, again. As for mechanically opened leaves, g_s decreased during drought period. The decrease in adaxial surface was higher than that of the rolled leaves. NPQ was higher than that of the rolled leaves. Φ_{PSII} and q_p significantly declined and the decreases were more than those of the rolled leaves. In conclusion, the results indicate that leaf rolling protects PSII functionality from damage induced by drought stress.

Additional key words: chlorophyll fluorescence, *Ctenanthe setos*a, drought, leaf rolling, protection, PSII functionality.

Introduction

Ctenanthe setosa Eichl., Marantaceae (Grey-maranta) is a tropical herbaceous perennial plant, and is cultivated as a greenhouse ornamental and houseplant for its attractive foliage. It prefers semi-shaded places and rolls its leaves in response to drought stress. *C. setosa* is a convenient model plant for leaf rolling studies because its leaves show gradual rolling and the duration of this process is long (30 to 40 days), therefore observation of leaf rolling is easy (Kadioglu and Terzi 2007).

Leaf rolling, a dehydration avoidance mechanism, protects some plants from the effects of high irradiance (photodamage) under water deficit stress (Kadioglu and Terzi 2007). Leaf rolling, most often interpreted as a response to water stress (Heckathorn and DeLucia 1991), represents a dynamic behavioral response in plants whereby by the normal sunlight orientation of the two leaf surfaces may be reversed (Smith 2008). Another advantage of leaf rolling is to minimize the potential damages caused by increased leaf temperature that results from high levels of solar radiation. Leaf rolling also decreases transpiration rate by reducing the effective leaf area exposed to sunrays. The rolling also increases drought resistance in cereal crops by relieving water stress (Townley-Smith and Hurd 1979, Omarova *et al*. 1995).

Photosynthesis is one of the main metabolic processes directly affected by drought stress. These effects are generally classified as stomatal and non-stomatal

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[+]Corresponding author; fax: 0090 (462) 325 31 95, e-mail: trabiye@yahoo.com

Abbreviations: Chl – chlorophyll; ETR – electron transport rate; g_s – stomatal conductance; F_v/F_m – maximum quantum yield of PSII photochemistry; F_m – maximum Chl fluorescence yield in the dark-adapted state; F_m' – maximum Chl fluorescence yield in the light-adapted state; F_0 – minimum Chl fluorescence yield in the dark-adapted state; F_s – steady-state Chl fluorescence yield in the light-adapted state; FM – fresh mass; DM – dry mass; TM – turgid mass; NPQ – non-photochemical quenching of Chl fluorescence; q_p – photochemical quenching of Chl fluorescence; Rfd – vitality index; RWC – relative water content; Φ_{PSII} – effective quantum yield of PSII photochemistry; Ψ_{leaf} – leaf water potential.

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limitations. Stomatal closure is often considered as an early physiological response to water deficit, which results in decreased photosynthesis, through limited CO_2 availability in the mesophyll (Cornic 2000). It has been reported that drought stress decreased photochemical efficiency of PSII, evidenced by a lower quantum yield of PSII, larger NPQ and decreased fraction of open PSII reaction centers under light (Giardi *et al.* 1996).

Stress effects on photosynthesis can be quantified in various ways. Chl fluorescence is one of these tools that provide rapid and accurate data to asses the situation of photosystem under stress. The approach for measuring photosynthetic traits such as Chl content and Chl fluorescence parameters might estimate influence of the environmental stress on growth and yield since these traits were closely correlated with the rate of carbon exchange (Fracheboud *et al.* 2004).

Stomata may be able to remain open (Matthews *et al.* 1990) or partially open (O'Toole and Cruz 1980) in rolled leaves. Stoma opening is an advantage for plants during leaf rolling, because it provides some level of unrestrictive CO_2 entry. Therefore, the rolling alters the microclimate surrounding the leaf surfaces. Its response to drought stress may be different in comparison with plants without rolling mechanism. Moreover, it is well known that many species are able to reduce the quantity of radiation by leaf folding and paraheliotropism, or leaf rolling. Besides the great progress in understanding the effects of drought stress on photosynthesis in plants without leaf rolling, there are limited works regarding the changes of photosynthesis during leaf rolling. For example, Corlett *et al.* (1994) reported interactions between leaf rolling and susceptibility to photoinhibition in sorghum subjected to mild water deficit. However, to date, there has been no study related to the photochemical efficiency of PSII regarding leaf rolling in detail.

Thus, in order to examine the role of leaf rolling in photosynthetic performance, we hypothesize that leaf rolling is a good mechanism to protect the photosynthetic apparatus and to allow a compromise with incident radiation, g_s and efficiency of PSII in order to continue photosynthesis in plants under drought stress. For these purposes, leaf water potential (Ψ_{leaf}), g_s and photosynthetic pigment contents and Chl fluorescence parameters of leaves were determined in leaves that had four different visual leaf rolling scores from 1 to 4 (score 1: not rolled, score 2: slightly rolled, score 3: strongly rolled, score 4: completely rolled), opened after re-watering and mechanically opened by clamping with plastic wires at score 4.

Materials and methods

Growth of plants and stress applications: *C. setosa* (Rosc.) Eichler (Marantaceae) plants were vegetatively propagated from their rhizomes and grown in plastic pots containing peat and sand (5:1) in a growth chamber with the following parameters: 16 h light (a fluorescent lamp, *OSRAM FLUORA 36W L77*) and 8 h darkness at 25 °C, relative humidity 70 %, photosynthetic photon flux density at the surface of the leaves 300 µmol m^{-2} s^{-1}. The plants at the same age (three years old) were chosen and their old and wilted leaves were trimmed. Some plants were well-watered (control) throughout the experiment while other plants were subjected to drought stress to achieve different visual leaf rolling scores from 1 to 4 by withholding water through 56 days (Fig. 1). Visual leaf rolling scores were also used in other studies (*e.g.* O'Toole and Moya 1978). The leaves of stressed plants showed pronounced rolling after 35 days of drought period. After score 4, some plants were re-watered to unroll their leaves. The rolled leaves of the other plants at score 4 were also mechanically opened by clamping with plastic wires so that we could demonstrate whether leaf rolling was effective in protecting the photosynthetic apparatus against drought. The reason of choosing the leaves at score 4 is that the chance of obtaining the obvious and definite results at maximum score is probably high. Following parameters were measured during leaf rolling, on the first and second days after re-watering and in mechanically opened leaves by clamping during 5 days of drought stress after score 4.

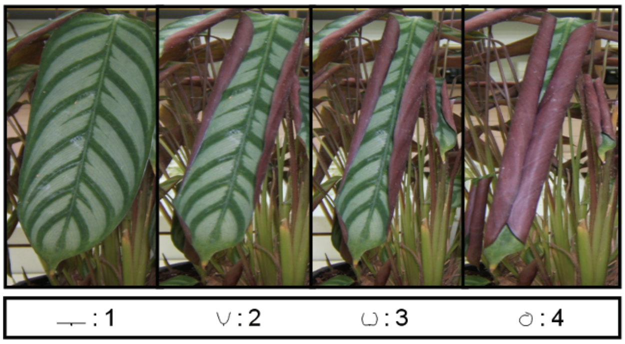

Fig. 1. Visual leaf rolling scores

Relative water content (RWC) and leaf water potential (Ψ_{leaf}): RWC of the leaves was estimated according to the method of Castillo (1996). Samples (0.5 g) were saturated in 100 ml of distilled water for 24 h at 4 °C in the dark and their turgid masses were recorded. Samples were then oven dried at 80 °C for 24 h and their dry masses were recorded. RWC was calculated as given below:

$$\text{RWC [\%]} = [(\text{FM} - \text{DM})/(\text{TM} - \text{DM})] \times 100,$$

where FM, DM, and TM are fresh, dry and turgid mass, respectively. All measurements were done with eighteen replicates.

Ψ_{leaf} was measured with a thermocouple psychrometer (*Wescor PSYPRO*, Logan, UT, USA). Discs about 6 mm in diameter were cut from the youngest fully expanded leaves of plants and sealed in the *C-52* psychrometer chamber. Samples were equilibrated for 90 minutes before the readings were recorded by a *Wescor PSYPRO* water potential datalogger in the psychrometric mode. Measurements were done three times from 6 leaves at the same age.

g_s: There are stomata on both sides of the leaf blade in *C. setosa* (Kutlu *et al.* 2009). g_s was monitored by using a dynamic diffusion porometer (*AP4, Delta-T Devices*, Burwell, Cambridge, UK) after calibrating with a standard calibration plate following manufacturer's instructions. The rolled leaves were opened and g_s was measured on abaxial and adaxial surfaces of the leaves. Thus measured part had a plane arrangement. Data were obtained from the leaves that rolled adaxial side inside. Measurements were conducted six times on three leaves from three plants at the same age.

Chl and carotenoid (Car) contents were determined by following the method of Arnon (1949) and Jaspars (1965), respectively. Fresh leaf samples (0.03 g) were selected randomly from three plants and homogenized in a mortar with a pestle in 80 % acetone. The extract was centrifuged at 5000 × *g* for 5 min. Aliquots of the extract (3 ml) were transferred to a cuvette and the absorbance was read at 645, 663, and 450 nm with a spectrophotometer (*Nicolet Evolution 100, Thermo Electron Corporation*, Altrincham, UK) against 80 % acetone as blank. Photosynthetic pigment contents were expressed as mg g^{-1}(DM).

Chl fluorescence measurements were performed by *OS1-FL*, a pulse modulated fluorometer (*OptiScience Corporation*, Tyngsboro, MA, USA) according to Zhang *et al.* (2005). The measurements were taken from the inner sides of the inwardly rolled leaves. A total of eighteen typical leaves were selected and dark-adapted for 20 min before Chl fluorescence was measured. During measurements, the rolled leaves were opened and the leaves had a plane arrangement. Minimum Chl fluorescence yield (F_0) was determined under weak modulated λ_{660}-irradiation (<0.1 µmol m^{-2} s^{-1}). Maximum Chl fluorescence yield (F_m) was reached by exposing PSII to saturating λ_{690} pulse (0.8 s) of white light (8000 µmol m^{-2} s^{-1}). After dark measurements, the leaves were exposed to an actinic light (5.5 W a halogen lamp, *ML S990, Micron*, Tokyo, Japan). Steady state Chl fluorescence (F_s) was achieved after exposure to the actinic light for 10 min. Intensity of the actinic light was 120 µmol m^{-2} s^{-1}. Saturating pulses (0.8 s) of white light (8000 µmol m^{-2} s^{-1}) were applied to determine maximum Chl fluorescence in the light (F_m'). Definitions of Chl fluorescence parameters (q_p, NPQ, F_v/F_m and Φ_{PSII}) were used as described by van Kooten and Snel (1990). F_v/F_m and Φ_{PSII} are indicators of the maximum and effective quantum yield of PSII, respectively. q_p and NPQ were calculated according to the equation, $(F_m' - F_s)/(F_m' - F_0)$ and $(F_m - F_m')/F_m'$ (Bilger and Björkman 1990), respectively. F_v/F_m and Φ_{PSII} were automatically calculated by fluorometer according to equations ($F_v/F_m = (F_m - F_0)/F_m$, $\Phi_{PSII} = (F_m' - F_s)/F_m'$) of Genty *et al* (1989). ETR was measured with special PAR clip and calculated as follows. ETR = (Φ_{PSII} × PAR × 0.5 × 0.84).Vitality index (Rfd) was also calculated by using an equation Rfd = $(F_m - F_s)/F_s$ (Lichtenthaler and Rinderle 1988). All parameters were quantified at module 4 of the *OS1-FL*.

Statistical analysis: Variance analysis of means (*ANOVA*) was performed with *Duncan Multiple Comparison* test using *SPSS* software (*Ver. 10.0, SPSS Inc.*, Chicago, USA) on eighteen replicates. Statistical significance was determined at the 5 % ($p<0.05$) level.

Results

Ψ_{leaf} and g_s: RWC decreased during leaf rolling under drought condition. It was determined that RWC was by 97 % at score 1 and 79 % at the highest score. Ψ_{leaf} gradually decreased during leaf rolling due to drought. While Ψ_{leaf} was –0.33 MPa at score 1, its value was –1.60 MPa at score 4. Also, after re-watering of the stressed plants, the rolled leaves opened and their values of RWC and Ψ_{leaf} approximately reached score 1 value again (Table 1). Significant negative correlations between leaf rolling and RWC ($r = -0.99$), and leaf rolling and Ψ_{leaf} ($r = -0.92$) were observed during the rolling period (Table 4).

g_s gradually decreased during the stress period. While abaxial g_s was 31.1 mmol m^{-2} s^{-1} at score 1, it was found to be 5.8 mmol m^{-2} s^{-1} at score 4. Exposure to drought stress resulted in decreases in g_s at the rates of 75 %, 79 % and 81 % at scores 2, 3 and 4, respectively (Table 1). However, adaxial g_s was 11.5 mmol m^{-2} s^{-1} at score 1 while it was 6.9 mmol m^{-2} s^{-1} at score 4. Decreases in adaxial g_s were at the rates of 30 %, 36 %, and 40 % at scores 2, 3, and 4, respectively. Two days after re-watering, adaxial g_s returned to score 1 value but abaxial g_s did not reach the level of score 1 (Table 1). As for mechanically opened leaves by clamping at score 4, g_s decreased in the leaves during 5 days after score 4 (Table 2). The decrease in adaxial surface was lower in the rolled leaves than those of the clamped leaves during 5 days of drought. In addition, g_s values were higher at adaxial surfaces of the rolled leaves than the clamped leaves. On the other hand, g_s values at abaxial surfaces of the clamped leaves were lower than those of the rolled leaves up to 3 days of drought after score 4 but later there was not any difference between the rolled and clamped leaves (Table 2).

Table 1. Changes of water potential (Ψ_{leaf}), relative water content (RWC), stomatal conductance (g_s), total chlorophyll (Chl), and total carotenoid (Car) levels during leaf rolling under drought stress and after re-watering (RW). Means±SD, $n = 18$. *Different letters in each line are significant at $p<0.05$ by *Duncan*'s multiple range test.

	Leaf rolling scores				Re-watering	
	1	2	3	4	RW1	RW2
Ψ_{leaf} [MPa]	–0.33±0.02 ^{f}	–1.22±0.03 ^{c}	–1.44±0.03 ^{b*}	–1.6±0.02 ^{a}	–0.63±0.04 ^{d}	–0.45±0.02 ^{e}
Leaf RWC [%]	97±1.0 ^{d}	92±2.0 ^{c}	84±1.0 ^{b}	79±3.0 ^{a}	95±3.0 ^{d}	99±1.0 ^{e}
Abaxial g_s [mmol m^{-2} s^{-1}]	31.1±2.6 ^{f}	7.8±0.7 ^{c}	6.6±0.4 ^{b}	5.8±0.3 ^{a}	11.3±0.2 ^{d}	23.4±0.4 ^{e}
Adaxial g_s [mmol m^{-2} s^{-1}]	11.5±0.5 ^{c}	8.1±0.5 ^{ab}	7.4±0.3 ^{ab}	6.9±0.7 ^{a}	9.2±0.6 ^{ab}	10.8±0.5 ^{c}
Total Chl [mg g^{-1}(DM)]	38.8±3.9 ^{c}	31.3±3.4 ^{b}	23.1±1.1 ^{a}	36.2±2.5 ^{bc}	24.3±1.8 ^{a}	24.4±0.7 ^{a}
Total Car [mg g^{-1}(DM)]	5.4± 0.9 ^{b}	3.8±0.5 ^{a}	3.8±0.2 ^{a}	5.8±0.5 ^{b}	3.6±0.2 ^{a}	3.8±0.6 ^{a}

Table 2. Changes in stomatal conductance (g_s) in the rolled, and the mechanically opened leaves by clamping during 5 days after score 4. Means±SD, $n = 18$. *Different letters in each line and column are significant at $p<0.05$ by *Duncan*'s multiple range test.

	g_s [mmol m^{-2} s^{-1}]				
Time [d]	1	2	3	4	5
Abaxial surface					
Rolled leaf	5.8±0.6 ^{l}	4.4±0.4 ^{jk}	3.9±0.2 ^{hij}	3.3±0.2 ^{efgh}	3.3±0.4 ^{def}
Clamped leaf	3.7±0.2 ^{fghi}	3.3±0.2 ^{defg}	3.2±0.4 ^{def}	3.1±0.3 ^{de}	2.7±0.3 ^{cd}
Adaxial surface					
Rolled leaf	6.9±0.7 ^{m}	5.9±0.7 ^{l}	4.8±0.3 ^{k}	4.0±0.4 ^{ij}	3.9±0.4 ^{ghi}
Clamped leaf	3.3±0.5 ^{def}	2.8±0.1 ^{cde}	2.3±0.2 ^{bc}	2.1±0.1 ^{ab}	1.6±0.1 ^{a}

Table 3. Changes in chlorophyll fluorescence parameters in the rolled leaves and mechanically opened leaves by clamping during 5 days after score 4. Means±SD, $n = 18$. *Different letters in each column are significant at $p<0.05$ by *Duncan*'s multiple range test.

Day	F_v/F_m	Φ_{PSII}	q_p	NPQ	ETR	Rfd
Rolled leaf						
1	0.80±0.01 ^{a}	0.76±0.01 ^{ef}	0.96±0.02 ^{c}	0.06±0.02 ^{a}	21.3±3.2 ^{c}	2.8±0.1 ^{a}
2	0.80±0.01 ^{a}	0.73±0.02 ^{cd}	0.94±0.02 ^{c}	0.11±0.04 ^{a}	23.5±2.4 ^{c}	2.9±0.3 ^{a}
3	0.80±0.01 ^{a}	0.75±0.01 ^{def}	0.95±0.03 ^{c}	0.11±0.01 ^{a}	22.7±0.8 ^{c}	2.8±0.3 ^{a}
4	0.81±0.01 ^{a}	0.73±0.02 ^{de}	0.92±0.05 ^{bc}	0.12±0.05 ^{a}	21.4±1.5 ^{c}	2.6±0.5 ^{a}
5	0.80±0.03 ^{a}	0.70±0.01 ^{bc}	0.89±0.02 ^{ab}	0.09±0.03 ^{a}	22.5±0.7 ^{c}	2.6±0.2 ^{a}
Clamped leaf						
1	0.81±0.03 ^{a}	0.76±0.01 ^{f}	0.96±0.04 ^{c}	0.21±0.06 ^{b}	20.6±1.6 ^{c}	2.8±0.2 ^{a}
2	0.80±0.01 ^{a}	0.70±0.03 ^{ab}	0.92±0.03 ^{bc}	0.23±0.07 ^{b}	20.1±0.6 ^{c}	2.8±0.2 ^{a}
3	0.81±0.01 ^{a}	0.69±0.03 ^{ab}	0.89±0.05 ^{ab}	0.38±0.13 ^{c}	17.8±1.2 ^{cb}	2.8±0.6 ^{a}
4	0.79±0.02 ^{a}	0.67±0.02 ^{a}	0.87±0.03 ^{a}	0.39±0.08 ^{c}	14.1±1.5 ^{ab}	2.3±0.6 ^{a}
5	0.81±0.01 ^{a}	0.67±0.01 ^{a}	0.86±0.02 ^{a}	0.37±0.06 ^{c}	11.3±1.9 ^{a}	2.4±0.3 ^{a}

There were significant correlations between adaxial g_s and leaf rolling ($r = –0.91$), abaxial g_s and Ψ_{leaf} ($r = 0.98$), and adaxial g_s and Ψ_{leaf} ($r = –0.99$) (Table 4).

Photosynthetic pigments: The total Chl and Car contents tended to decrease until score 3 under drought stress. Photosynthetic pigment contents were enhanced at score 4 compared to score 3, and reached to the level of score 1. While total Chl and Car contents were 36.7±2.0 and 5.6±0.6 mg g^{-1}(DM) in the rolled leaves, they were 28.1±2.1 and 4.6±0.8 mg g^{-1}(DM) at the clamped leaves on the 3rd day of drought period after score 4, respectively. After re-watering, the photosynthetic pigment contents unexpectedly decreased as compared to the values of score 1 (Table 1).

Chl fluorescence parameters: Water deficit did not significantly affect the ratio of variable to the maximum Chl fluorescence in the dark adapted state or maximum quantum yield of PSII (Fig. 2). At score 1 and 2, Rfd values were above 3.0. However, Rfd was 2.8 and 1.96 at score 3 and 4, respectively. But after re-watering, it reached the level of score 1 again. Drought stress did not significantly affect the Φ_{PSII} up to score 3 but it

Table 4. Linear correlations among the descriptive parameters of physiological state and some chlorophyll fluorescence parameters. The correlation coefficient (*r*) and significant differences are given. * $p<0.05$, ** $p<0.01$.

	Leaf rolling	Ψ_{leaf}	RWC	g_s (abaxial)	g_s (adaxial)	Rfd	q_P	Φ_{PSII}
Ψ_{leaf}	–0.92*							
RWC	–0.99**							
g_s (abaxial)	–0.83	0.98*	0.80					
g_s (adaxial)	–0.91*	0.99**	0.88	0.99**				
Rfd	–0.99**	0.95**	0.98**	0.84	0.91*			
q_p	–0.96*	0.89*	0.94**	0.52	0.64	0.91*		
Φ_{PSII}	–0.92*	0.83*	0.93**	0.62	0.73	0.95**	0.99**	
F_v/F_m	–0.63	0.87*	0.78	0.85	0.91*	0.75	0.62	0.53
NPQ	–0.40	0.05	0.40	–0.19	–0.01	0.28	0.36	0.44
ETR	–0.95*	0.98**	0.94**	0.92*	0.96*	0.98**	0.96**	0.91*

significantly decreased at score 4. q_p did not change up to score 3 and then decreased at score 4. NPQ increased up to score 2 and then decreased. ETR did not significantly change up to score 3 but declined at score 4. After the plants with rolled leaves were watered, the values of all Chl fluorescence parameters in the re-opened leaves rapidly returned to those of score 1 (Fig. 2).

Significant correlations were observed between Rfd and ETR ($r = 0.98$), Rfd and Φ_{PSII} ($r = 0.95$), Rfd and q_p ($r = 0.91$) (Table 4). Leaf rolling was significantly ($p<0.05$) related to values of Φ_{PSII} ($r = –0.92$), q_p ($r = –0.96$), ETR ($r = –0.95$) and Rfd ($r = –0.99$). Significant ($p<0.01$) positive correlations between ETR and RWC ($r = 0.94$), ETR and Ψ_{leaf} ($r = 0.98$) were observed during the rolling period (Table 4).

As for mechanically opened leaves by clamping, there was no difference in F_v/F_m ratio between the rolled and the clamped leaves during 5 days of drought period after score 4. NPQ was high at the clamped leaves compared to the rolled leaves. ETR did not change in the rolled leaves but decreased in the clamped leaves. Φ_{PSII} and q_p significantly declined and the decreases were more than those of the rolled leaves. However, the changes in Rfd were not significant between the rolled and the clamped leaves (Table 3).

Discussion

The Ψ_{leaf} range used to define drought stress levels (mild stress from 0 to –1 MPa, moderate stress from –1 to –2 MPa, and severe stress for Ψ_{leaf} greater than –2 MPa) usually depends on plant species and growth conditions (Lawlor 1983). Water deficit stress in *C. setosa* decreased Ψ_{leaf} and RWC during the rolling. In addition, these parameters were not downed to low values as observed in stressed plants in other studies (*e.g.* Sanchez-Rodriguez *et al.* 1997). Even at the highest score of leaf rolling in *Ctenanthe*, the range of Ψ_{leaf} indicated that the stress was moderate owing to leaf rolling mechanism.

The leaves of *C. setosa* roll inward during drought stress. The outer part of the leaf is exposed to full irradiance, but the inner part of the leaf is shaded by the rolled outer part of leaf. For this reason, we measured g_s in outer (abaxial) and inner (adaxial) surfaces of the leaves separately during the rolling period. g_s in abaxial surface of the leaves decreased faster than that of the adaxial surface due to different number of stomata in abaxial and adaxial surfaces (stomatal indexes , the ratio of the number of stomata in a given area, were 12.6±0.3 and 2.5±0.1, respectively) during the rolling (Table 1). Also when the rolling reached to score 4, the rolled leaves were mechanically opened in order to show the effect of leaf rolling. We observed that g_s values at abaxial surfaces of the clamped leaves were lower than those of the rolled leaves up to 3 days of drought after score 4 but later there was not any difference between the rolled and the clamped leaves (Table 2). Because of the effects of full irradiance on abaxial surfaces of the leaves, there was no difference between the rolled and the clamped leaves about the stomatal conductance of the abaxial surface. Furthermore, g_s on the adaxial surface of the rolled leaves were high compared to clamped leaves (Table 2). High g_s in adaxial surfaces of the rolled leaves may be derived from higher moisture at the inner surface due to different shade conditions as a result of leaf rolling. Results of g_s also showed that rolling was an effective process to control the stomatal closure to maintain CO_2 uptake under drought stress. Smith *et al.* (1997) reported that leaf orientation generates the well known sun vs. shade leaf structure that can function to increase the overlap of photosynthetic efficiency. Soares *et al.* (2008) showed that a C_4 grass species, during natural leaf rolling that reversed the orientation of the upper and lower leaf surfaces, had accompanying stomatal changes inside the leaf that enhanced its photosynthetic capability.

It has been reported that the degradation of Chl and Car was observed in drought-stressed plants, which was presumably associated with photo-oxidative process in chloroplast (Munné-Bosch and Peñuelas 2004). In the present experiment, both photosynthetic pigment contents

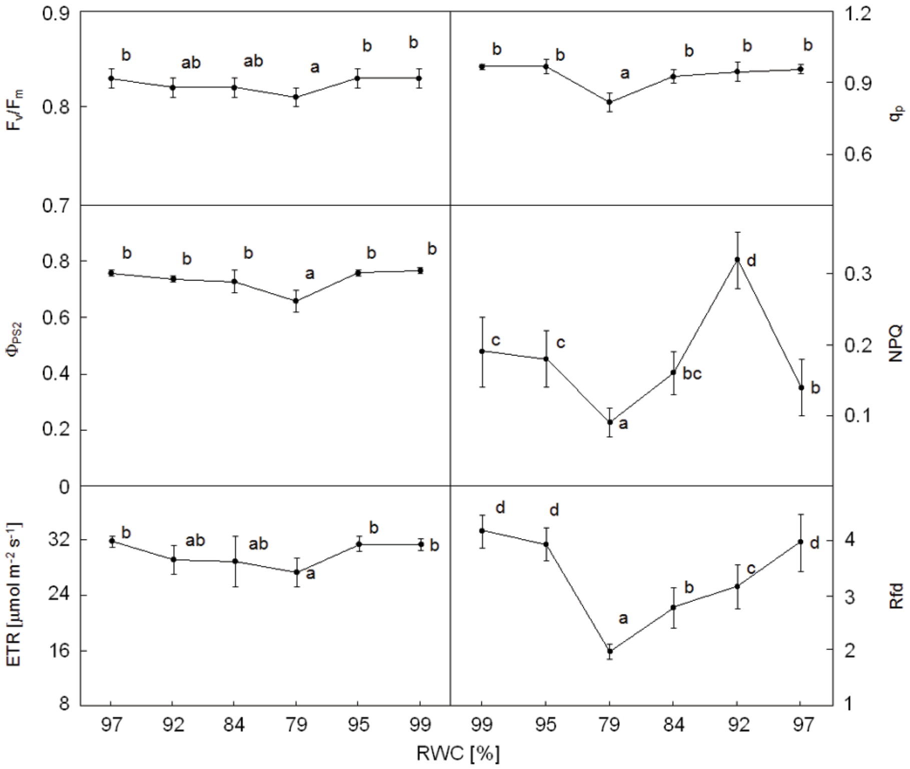

Fig. 2. Changes in Chl fluorescence parameters during leaf rolling and after re-watering. The vertical bars indicate standard deviation and different letters represent significant differences at $p<0.05$, $n = 18$. Values of relative water contents (RWC) (97, 92, 84, 79, 95, 99) indicate score 1, 2, 3, 4 and the first and second days after re-watering, respectively.

declined up to score 3 but attained to score 1 value at score 4. Decreased or unchanged Chl level during drought stress has been reported in other species, depending on the duration and severity of drought (Kyparissis *et al.* 1995). The changes in photosynthetic pigment contents at score 4 may be derived from microclimate parameters such as irradiance and temperature as a result of the rolling. For instance, there was no significant temperature difference between score 1 (24°C±0.2) and score 4 (24.4 °C±0.2) but temperature was high (25.1°C±0.2) at mechanically opened leaves. However, in spite of many repetitions in the experiment, we found unexpected results on contents of photosynthetic pigments at the opened leaves after re-watering. So, we suggest that plants may sense the incident irradiance as a new stress factor because of the fact that the inner surface of the leaves exposes to light after a long time of darkness. On the other hand, in the clamped leaves, total Chl and Car contents decreased more than those of the rolled leaves on the 3rd day after score 4. According to the results, it can be said that the increase in leaf rolling probably prevents degradation of photosynthetic pigments resulted from photo-oxidative damage. On the other hand, Fig. 1 clearly shows the presence of anthocyanins in the abaxial tissue of the leaf. Anthocyanins prevent chloroplasts from photooxidative stress by absorbing light/UV-radiation and dissipating their energy in a harmless way (Feild *et al.* 2001). Further studies are likely to allow us to understand effects of leaf rolling on Chl photooxidation.

A sustained decrease in F$_v$/F$_m$ indicates the occurrence of photoinhibitory damage in response to many environmental stresses (Maxwell and Johnson 2000). However, the invariance of the F$_v$/F$_m$ ratio during leaf rolling shows that maximum quantum yield of the primary PSII reaction remains high during drought stress. The fact that F$_v$/F$_m$ did not change upon desiccation indicates that photosynthetic electron transport chain was very resistant to dehydration (Cornic and Massacci 1996). In the present study, there was also not significant difference in the F$_v$/F$_m$ ratio between the rolled and the clamped leaves at score 4 under drought stress. On the other hand, there are several papers showing that the quantity of F$_v$/F$_m$ is not sensitive to drought stress up to 60 % RWC (*e.g.* Matouskova *et al.* 1999).

NPQ increased at score 2 but it decreased at the later scores. Furthermore, a clear increase in NPQ value was observed in the clamped leaf compared with the rolled leaf. Increased NPQ indicated that a part of energy load on leaves was thermally dissipated, so accounting for the apparent down-regulation of PSII (Tezara *et al.* 2005). It is known that drought may lead to an increase in NPQ (Sheuermann *et al.* 1991, Biehler *et al.* 1997). These data suggest that adaptation traits to cope with the excess radiation have been developed at score 2 in the direction of enhancing dissipation mechanism (Galmes *et al.* 2007). Thermal dissipation minimized the accumulation of excess excitation energy and the probability of photodamage. After score 2, lowering leaf area due to leaf rolling may decrease incident light and the leaf may not need effective mechanism of thermal dissipation. The increases in NPQ in opened leaves after re-watering and clamped leaves also supported the above mentioned idea.

The decline in Φ_{PSII} was a consequence of increase in the thermal dissipation of excitation energy from PSII (reviewed by Cornic *et al.* 1992, Demming-Adams and Adams 1992). In opposition to these studies, in present study, although thermal dissipation of excess energy load on leaf diminished, Φ_{PSII} also decreased at score 4. The reason of the decline in photochemical yield was probably the decrease in leaf area of the inner side of the rolled leaves that was exposed to ambient light and dark through increase in leaf rolling. We also observed a decrease in Φ_{PSII} of the clamped leaves in comparison with the rolled leaves after score 4. Thus, leaf rolling may protect the plant from photodamage.

q_p is a measure of the redox state of the primary quinone acceptor (Q_A). The results indicated that the oxidized Q_A amount did not change up to score 3, but it decreased slightly at score 4. In addition, in the clamped leaves, q_p decreased more than that of the rolled leaves. Electron transport was maintained well up to score 3 in *C. setosa*. ETR results were in accordance with q_p measurements. ETR did not change up to score 3 under drought stress, but declined at score 4 and then started to increase following re-watering. According to these results, the plant protected its PSII against drought up to score 3, but then the resistance to the stress decreased and some responses got also loosen. The notable decrease in ETR in the clamped leaves supports the idea that leaf rolling is an important protector for photosynthesis under drought stress.

Rfd is a good indicator of whole plant physiological state (Pukacki and Kamińska-Rożek 2005). Rfd values above 3.0 indicate very efficient photosynthesis and high photosynthetic rates per leaf area unit while at Rfd values below 1.0, the leaves no longer exhibit a net CO_2 assimilation (reviewed by Lichtenthaler and Rinderle 1988). In current study, Rfd values were above 3.0 at score 1 and 2. Then it started to decrease to 2.8 and then 1.96 at scores 3 and 4, respectively. Following re-watering, Rfd rose to score 1 level. The increase attests to a great plasticity and tolerance to drought stress of reactions on PSII and in dark pathway of photosynthesis. There was a decrease in Rfd, although it was not statistically significant in the clamped and the rolled leaves. The decrease was more in the clamped leaves than that of the rolled leaves. Rfd also provides information about the enzymatic reactions of Calvin cycle (Lichtenthaler and Rinderle 1988). The Calvin cycle enzymes were not probably affected up to score 4. So, we suggest that the enzymes of Calvin cycle should be investigated during leaf rolling in the future. Rapid intercellular communication linking the environment with leaf photosynthesis is an important area for future research (Yano and Terashima 2004).

Plants are protected by several mechanisms capable of preventing drought-induced photodamage, the most important of which is leaf rolling (Kadioglu and Terzi 2007). As a result of this study, we can conclude that leaf rolling can minimize adverse effects of the high level of solar radiation by reducing leaf area and maintains stomatal conductance under drought stress. By re-watering of rolled leaves, the Chl fluorescence values reached to approximately the control values, again. In accordance with the information above, our results related to the descriptive parameters of physiological state and Chl fluorescence have supported the idea that there was no important defect on photosynthesis machinery during leaf rolling of *C. setosa*. These results also confirmed that leaf rolling saved PSII complex in plants under drought stress.

In conclusion, these results generally showed that leaf rolling was an advantageous mechanism to allow a compromise among incident radiation, stomatal conductance and efficiency of PSII in plants under drought stress. In addition, it can be seen that leaf rolling mechanism protects photosynthetic apparatus in plants during drought periods.

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