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PHOTOSYSTEM II FUNCTIONALITY AND ANTIOXIDANT SYSTEM CHANGES DURING LEAF ROLLING IN POST-STRESS EMERGING *CTENANTHE SETOSA* EXPOSED TO DROUGHT

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We studied the changes in antioxidant system and chlorophyll fluorescence parameters in post-stress emerging *Ctenanthe setosa* (Rosc.) Eichler (Marantaceae) plants (PSE plants) having reduced leaf area under drought stress causing leaf rolling and re-watering. PSE plants were compared to primary stressed plants (PS) in previous studies. The parameters were measured at different visual leaf rolling scores from 1 to 4 (1 is unrolled, 4 is tightly rolled and the others is intermediate form). Water potentials and stomatal conductance of leaves were gradually decreased during leaf rolling. Similarly, maximum quantum efficiency of open PS II center and quantum yield of PS II decreased during the rolling period. Non-photochemical quenching of chlorophyll fluorescence decreased at score 2 then increased while photochemical quenching did not change during leaf rolling. Electron transport rate decreased only at score 4 but approximately reached to score 1 level after re-watering. Superoxide dismutase activity was not constant at all leaf rolling scores. Ascorbate peroxidase, catalase and glutathione reductase activities generally tended to increase during leaf rolling. Lipid peroxidation and H_2O_2 content increased at score 2 but decreased at the later scores. On the other hand, $O_2^{\bullet-}$ production increased during the rolling period. After re-watering of the plants having score 4 of leaf rolling, antioxidant enzyme activities were lower than those of score 1. Other physiological parameters also tended to reach the value of score 1. The results indicated that PSE plants gained drought tolerance by reducing leaf area effectively induced their antioxidant systems and protected the photosynthesis under drought stress similar to PS plants.

Keywords: Antioxidant enzymes – *Ctenanthe setosa* – chlorophyll fluorescence – leaf rolling – post-stress emerging plant

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Abbreviations: APX – Ascorbate peroxidase; Car – carotenoid; Chl – chlorophyll; CAT – catalase; F_m – maximum fluorescence yield; F_0 – minimum fluorescence yield; F_s – steady-state fluorescence yield; F_v/F_m – quantum efficiency of photosystem II; *NPQ* – non-photochemical quenching of chlorophyll fluorescence; PSII – photosystem II; *qP* – photochemical quenching; GR – glutathione reductase; RW – Re-watering; RWC – relative water content; g_s – stomatal conductance; SOD – superoxide dismutase; Ψ_{leaf} – leaf water potential; $\Phi_{PS\ II}$ – quantum yield of PSII

INTRODUCTION

Drought stress induces several physiological, biochemical and molecular responses which provide plants to adapt limited environmental conditions [7]. Leaf rolling is one of the avoidance mechanisms in plants [22]. Some economically important Gramineae plants such as wheat, maize, sorghum and rice have leaf rolling mechanism under drought stress [29]. *Ctenanthe setosa* (Grey-maranta), a species that shows a leaf rolling response to drought, is a member of rhizome-forming tropical herbaceous plants (Marantaceae), originated from Brazil and grown for its attractive foliage. It is a good model plant for leaf rolling studies because its leaves show gradual rolling and the duration of this process takes a long time (30 to 40 days), therefore the observation of leaf rolling is easy [22].

Stress avoiders are just able to survive during water deficit whereas stress tolerant plants are able to survive during more severe drought stress through their protective mechanism. Avoidance and tolerance are the two main defense mechanisms induced against drought stress in vegetative tissues. *Ctenanthe setosa* is an interesting plant that has both avoidance and tolerance mechanisms. When unstressed *C. setosa* plants were exposed to severe drought-stress and the leaves of drought-stressed plants were cut after drought, leaf areas of new growing plants from the rhizomes of stressed plants after stress were significantly reduced as compared to the unstressed plants. The plants, subjected to primary stress, were named as primary-stress plant (PS) while the new grown plants after stress were named as post-stress emerging plant (PSE) [32].

Plant stress physiologists are particularly interested in photosynthesis because it is a very good indicator of the overall fitness of the plant. In addition, chlorophyll fluorescence was studied for many years to obtain an understanding of functioning of the photosynthetic apparatus [15]. The advantage of this method is that the measured parameters are assumed to closely reflect the functioning of photosystem II.

Under water stress conditions, stomatal closing is a common response of plants, which cause declining CO_2 uptake and increasing accumulation of NADPH. In that case, oxygen is the final electron acceptor instead of limited NADP, which results in formation of superoxide [42]. Through a variety of reactions, superoxide leads to the formation of hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$) and other reactive oxygen species (ROS) all of which can cause damage in various ways [33]. Generation of ROS results in lipid peroxidation which is one of the most important damaging effects of oxidative stress [9, 14]. Plants have antioxidant defense mechanism including enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), guaiacol peroxidase (GPX, EC 1.11.1.7), glutathione reductase (GR, EC 1.6.4.2) and catalase (CAT, EC 1.11.1.6) to decrease the deleterious effects of ROS. Modulation in the activities of these enzymes may be an important factor in tolerance of various plants to environmental stress [31]. In addition, high activities of the enzymes during drought are related to lowered lipid peroxidation [8]. Unfortunately, little is known about changes of antioxidant enzymes in

plants having reduced leaf area after stress. There is only a report about morphological and biochemical adaptation of post-stress emerging plants under a second drought-stress cycle [32]. In addition, there is no available study about photosynthetic parameters during the rolling period in this plant.

We hypothesized that PSE plants which gained drought tolerance by reducing leaf area induce the antioxidant system and protect their photosynthetic apparatus during leaf rolling under drought stress. To test the hypothesis, we measured leaf water potential, stomatal conductance, lipid peroxidation, some antioxidant enzyme activities and chlorophyll fluorescence parameters of leaves during leaf rolling.

MATERIALS AND METHODS

Growth of the plants and stress applications

Ctenanthe setosa (Rosc.) Eichler (Marantaceae) plants were vegetatively propagated and grown in plastic pots (14 cm high, 16 cm top and 11 cm bottom diameter) containing peat and sand (5 : 1) in a growth chamber under the following treatment conditions: 16 h light and 8 h darkness at 25 °C, relative humidity 50%, photon flux density at the surface of the leaves 300 μmol m^{-2} s^{-1}. The plants were subjected to 70 days of drought stress. These plants subjected to primary stress (PS plant), were trimmed and then the pots were irrigated. New plants were produced from the rhizomes of these plants (PSE plants) and grown for 90 days. PSE plants were exposed to drought to achieve leaf rolling scores from 1 to 4 (—: 1, ∨ : 2, ⌣: 3, ○ : 4) by withholding water through 56 days. A score of 1 indicates no rolling (well-watered), while a score of 4 indicates complete rolling. Visual leaf rolling scores were also used in other studies [30]. PSE plants were re-watered to unroll their leaves at the end of drought period again. Following parameters were measured during leaf rolling scores and on the fourth day of re-watering.

Leaf water potential

Leaf water potential (Ψ_{leaf}) was measured with a C-52 thermocouple psychrometer (Wescor, Inc., 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 45 min before the readings were recorded by a Wescor PSYPRO water potential datalogger in the psychrometric mode.

Stomatal conductance

Stomatal conductance (g_s) was monitored by using a dynamic diffusion porometer (AP4, Delta-T Devices, Burwell, Cambridge, UK) after calibrated with a standard calibration plate following manufacturer's instructions.

Chlorophyll and carotenoid contents

Leaf samples were selected randomly from the plants and homogenized in 80% acetone. The extract was centrifuged at 5000 *g* for 5 min. Absorbance of the supernatant was spectrophotometrically recorded at 663, 645 and 450 nm (Thermo Nicolet Evolution 100, England). The amounts of chlorophyll and carotenoid were estimated according to Arnon [3] and Jaspars [21], respectively. Photosynthetic pigment contents were expressed as mg per g dry weight.

Lipid peroxidation

Lipid peroxidation was measured in the term of malondialdehyde content (MDA, $\varepsilon = 155$ mmol^{-1} cm^{-1}), a product of lipid peroxidation following the method of Heath and Packer [19]. Leaf samples (0.5 g) were homogenized in 10 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 *g* for 5 min. To 1 ml aliquot of supernatant 4 ml of 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 *g* for 10 min, the absorbance of the supernatant was recorded at 532 nm. The value for non-specific absorption at 600 nm was substracted. The MDA content was expressed as nmol MDA per g fresh weight.

Hydrogen peroxide assay

Leaves were ground in 5% TCA with activated charcoal at 0 °C [2]. The homogenate was filtered, centrifuged at 20,000 *g* for 20 min and the supernatant was adjusted to pH 3.5 with 4 N KOH. H_2O_2 content was measured according to the method of Capaldi and Taylor [10]. The sample (200 µl) was added to 100 µl of reagent solution containing 3.4 mM 3-methyl-2-benzothiazolinone hydrazone and 3.32 mM formaldehyde. The reaction was initiated by adding 500 µl of the solution of horseradish peroxidase (0.5 U) in 0.2 M sodium acetate buffer (pH 3.5), and after 2 min it was quenched with 1400 µl of 1N HCl. The absorbance at 630 nm was measured 15 min after quenching. H_2O_2 content was estimated from a standard curve prepared with known concentrations of H_2O_2, and was expressed as mmol per g fresh weight.

Determination of superoxide radical production

Superoxide release to the medium was determined spectrophotometrically, using the tetrazolium salt XTT {(2, 3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide} (Sigma-Aldrich Co., Israel) as described by Frahry and Schopfer [16] with minor modification. For quantitative

determination of superoxide radicals leaves (0.5 g) were cut into small pieces and the leaf segments were vacuum-infiltrated for 20 min with 5 ml of 10 mM Na-citrate buffer (pH 7.0) containing 500 μM XTT, with/without 3.5 Uml^{-1} superoxide dismutase in dark. Then, samples were totally incubated in the buffer for 2 h. The buffer containing $O_2^{\bullet-}$ which was released by the samples was used for $O_2^{\bullet-}$ determination. The increase in XTT reduction was read at 470 nm in a spectrophotometer. Specific absorbance due to the presence of $O_2^{\bullet-}$ was calculated as the difference in A_{470} between samples with and without SOD. It was transformed into molar concentration using an extinction coefficient of 2.16×10^4 M^{-1} cm^{-1}.

Extractions of antioxidant enzymes

For the enzymes extractions, leaf materials (0.5 g) were homogenized with 1% polyvinylpolypyrrolidone (PVPP) in 5 ml extraction buffer (50 mM potassium-phosphate buffer, 1 mM EDTA, pH 7.0). For APX activity, 5 mM ascorbate was added. The homogenate was centrifuged at 18,000 *g* for 20 min at 4 °C. Leaf extracts were stored at –20 °C until used for analysis.

Enzyme analysis

Superoxide dismutase activity was determined using the nitroblue tetrazolium (NBT) reduction method of Beauchamp and Fridovich [4]. For activity assay 50 mM potassium-phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 2 μM riboflavin, 13 mM L-methionine and 75 μM NBT was used. Initial absorbance at 560 nm was recorded then samples were exposed to 200 μmol photons m^{-2} s^{-1} for 10 min. Absorbance at 560 nm was then remeasured. The SOD activity was expressed in U; 1 unit corresponding to the amount needed to cause a 50% inhibition in the absorbance of controls performed for each individual assay.

Ascorbate peroxidase activity was determined from the decrease in absorbance at 290 nm, following ascorbate (ASC) consumption [26]. The assay contained 50 mM K_2HPO_4 (pH 7.0), 750 μM ASC, 5 mM H_2O_2. APX activity was calculated by using an extinction coefficient of 2.8 mM^{-1} cm^{-1} for ASC at 290 nm.

Catalase activity was determined from the decrease in absorbance at 240 nm, following the consumption of H_2O_2 [1]. The assay contained 50 mM K_2HPO_4 (pH 7.0), 30 mM H_2O_2 and 20 μl enzyme extract. CAT activity was calculated by using an extinction coefficient of 39.4 mM^{-1} cm^{-1} for H_2O_2 at 240 nm.

Glutathione reductase activity was determined following the decrease in absorbance at 340 nm associated with the oxidation of NADPH [13]. The assay contained 50 mM Tris-HCl (pH 7.8), 150 μM NADPH, 500 μM oxidised glutathione (GSSG) and 50 μl extract.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were performed by an OS1-FL fluorometer (OptiScience Corporation, Tyngsboro, MA) according to Zhang et al. [41]. A total of fifteen typical leaves were selected and dark-adapted for 20 min before the chlorophyll fluorescence was measured. The minimal fluorescence yield (F_0) were determined under weak red light. Maximal fluorescence yield (F_m) was reached by exposing PSII to saturating pulse (0.8 s) of white light. After dark measurements, the leaves were exposed to actinic light. The actinic light provided by a 5.5 W halogen lamp (Micron, Japan) to read F_s, which is the steady-state fluorescence immediately prior to saturating pulses. Intensity of the actinic light was 1500 μmol photons m^{-2} s^{-1}. Saturating pulses (0.8 s) of white light (8000 μmol photons m^{-2} s^{-1}) were applied to determine F'_m, maximum fluorescence in the light. Definitions of fluorescence parameters (*qP, NPQ,* F_v/F_m and $\Phi_{PS\,II}$) were used as described by van Kooten and Snel [40]. F_v/F_m and $\Phi_{PS\,II}$ are indicators of the maximum and effective quantum yield of the *PSII*, respectively. The photochemical quenching (*qP*) and non-photochemical quenching (*NPQ*) were calculated according to the equation,

$$\frac{(F'_m - F_s)}{(F'_m - F_0)} \quad [12] \quad \text{and} \quad \frac{(F_m - F'_m)}{F'_m} \quad [6],$$

respectively, where is the maximum value taken before exposure to actinic light. Maximum quantum yield of photosystem II photochemistry (F_v/F_m) and quantum yield of PSII photochemistry ($\Phi_{PS\,II}$) were read by machine. R_{fd}, proposed as vitality index, was also calculated as

$$R_{fd} = \frac{(F_m - F_s)}{F_s} \quad [24].$$

All parameters were quantified module 4 of the OS1-FL.

Statistical analysis

Variance analysis of means of ten replicates from three plants were performed with Duncan Multiple Comparison test by using SPSS software for Microsoft Windows (Ver. 13.0, SPSS Inc., USA) and significance was determined at the 5% ($P < 0.05$) level.

RESULTS

Water potential and stomatal conductance

Leaf water potential gradually decreased during the drought stress. While water potential of leaves was –0.15 MPa at score 1, it gradually decreased with the increase in leaf rolling and the value of Ψ_{leaf} was –0.65 MPa at the leaves of score 4. However, after re-watering of the stressed plants, rolled leaves opened and value of Ψ_{leaf} started to increase again. It was –0.41 MPa upon re-watering at the fourth day (Table 1).

Stomatal conductance gradually decreased with increased leaf rolling score. However, g_s started to increase again after re-watering. Upon irrigation of the plants, g_s slowly increased but did not reach to score 1 level (control). For example, g_s was found 28.6 and 1.0 mmol m^{-2} s^{-1} at score 1 and 4, respectively. Exposure to drought stress resulted in a decrease at the rates of 73, 89 and 97% in g_s at rolling scores of 2, 3 and 4, respectively. However, g_s started to increase again during the re-watering period and it was found that g_s was 4.26 mmol m^{-2} s^{-1} (Table 1).

Photosynthetic pigments

The total chlorophyll and carotenoid contents were not particularly affected during leaf rolling in post-stress emerging *C. setosa* under drought stress. Similarly after re-watering, the photosynthetic pigment contents did not change significantly as compared to the other values of leaf rolling scores (Table 1).

Lipid peroxidation

Lipid peroxidation measured by MDA-content increased at score 2 but decreased at the later scores. For example, the contents of MDA were found to be 96.5 nmol at score 1 but 130.3 nmol per g fresh weight at score 2. However, the contents of MDA at scores 3 and 4 were 103.8 nmol and 95.5 nmol per g fresh weight, respectively. After re-watering of the stressed plants, lipid peroxidation slightly decreased but the decrease was not significant compared to score 4 (Table 1).

ROS production

Hydrogen peroxide content significantly increased at score 2 and then decreased in comparison with score 1. These decreases were 2.2- and 3.4-fold at scores 3 and 4 compared to score 1, respectively. After re-watering, H_2O_2 content increased again compared to score 4 (Table 1).

$O_2^{\bullet-}$ production did not significantly change at score 2 but then increased at scores 3 and 4 compared to score 1. The increase of $O_2^{\bullet-}$ production was 2.7- and 3.8-fold

Table 1
Changes in relative water content (RWC), stomatal conductance (g_s), water potential (Ψ_{leaf}) total chlorophyll (Chl), total carotenoid (Car) levels, MDA content, level of H_2O_2 and $O_2^{\bullet -}$ during leaf rolling in leaves of post-stress emerging *Ctenanthe setosa* under drought stress condition

	Leaf rolling score				Re-watering
	1	2	3	4	
Ψ_{leaf} (– MPa)	0.15±0.02*[a]	0.35±0.02[b]	0.44±0.01[c]	0.65±0.02[d]	0.41±0.02[c]
g_s (mmol m^{-2} s^{-1})	28.6±4.6[e]	7.86±0.2[d]	3.06±0.1[b]	1.01±0.01[a]	4.26±0.04[c]
Total Chl (mg g^{-1} dw)	13.2±1.7[a]	12.0±2.0[a]	12.1±0.8[a]	14.1±2.5[a]	12.3±1.3[a]
Total Car (mg g^{-1} dw)	4.4±0.5[a]	4.4±1.4[a]	5.2±1.1[a]	4.9±0.1[a]	4.2±0.8[a]
MDA (mmol g^{-1} fw)	96.5±2.8[a]	130.3±5.4[c]	103.8±4.2[b]	95.5±0.2[a]	94.8±1.3[a]
H_2O_2 (μmol g^{-1} fw)	37.3±0.7[d]	46.1±0.5[e]	16.5±0.2[b]	10.7±0.6[a]	28.6±2.0[c]
$O_2^{\bullet -}$ (nmol min^{-1} g^{-1} fw)	0.92±0.01[a]	1.02±0.2[a]	2.5±0.4[b]	3.5±0.3[c]	2.2±0.6[b]

* Different letters in each line indicate significant differences ($P < 0.05$)

at scores 3 and 4 as compared to score 1, respectively. After re-watering, $O_2^{\bullet -}$ production declined again compared to score 4 (Table 1).

Antioxidant enzyme activities

CAT activity enhanced at score 3 but decreased at score 4 during rolling period. After re-watering of plants with rolled leaves, CAT activity was lower than that of score 1 (Fig. 1A).

APX activity increased at score 2, then decreased at score 3 in comparison with score 1, but increased at score 4 again. On the other hand, APX activity significantly decreased again after re-watering period in comparison with score 1 during rolling period (Fig. 1B).

SOD activity in PSE plants decreased at score 2 and score 4 in comparison with score 1 during rolling period. However, the activity of SOD did not change at score 3 compared to score 1. After re-watering, the activity decreased in comparison with score 1 (Fig. 1C).

GR activity significantly increased compared to score 1 during leaf rolling. However, the activity started to decrease after re-watering compared to score 1 (Fig. 1D).

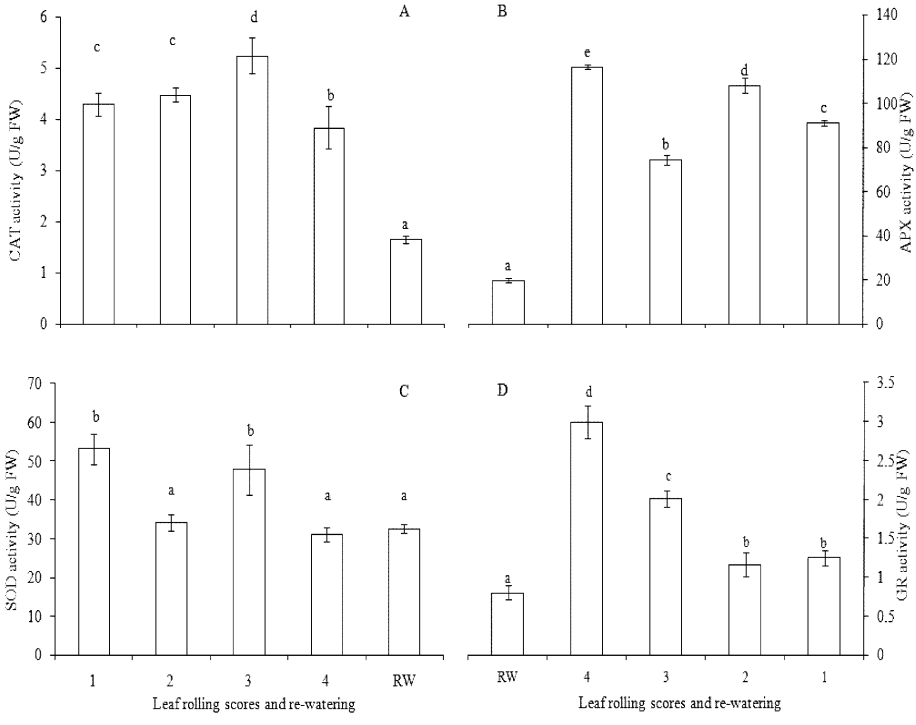

Fig. 1. Antioxidant enzyme activities during leaf rolling and after re-watering. A: Catalase, B: Ascorbate peroxidase, C: Superoxide dismutase, D: Glutathione reductase. Vertical bars indicate SD and different letters denote significant differences ($P < 0.05$)

Chlorophyll fluorescence parameters

The quantum efficiency of photosystem II and $\Phi_{PS\ II}$ decreased during the rolling period and after re-watering. *NPQ* decreased at score 2, but increased at score 4 and after re-watering. The photochemical quenching did not change significantly during leaf rolling and after re-watering (Fig. 2). R_{fd} declined at score 2 but later approximately reached to score 1 level (Fig. 3).

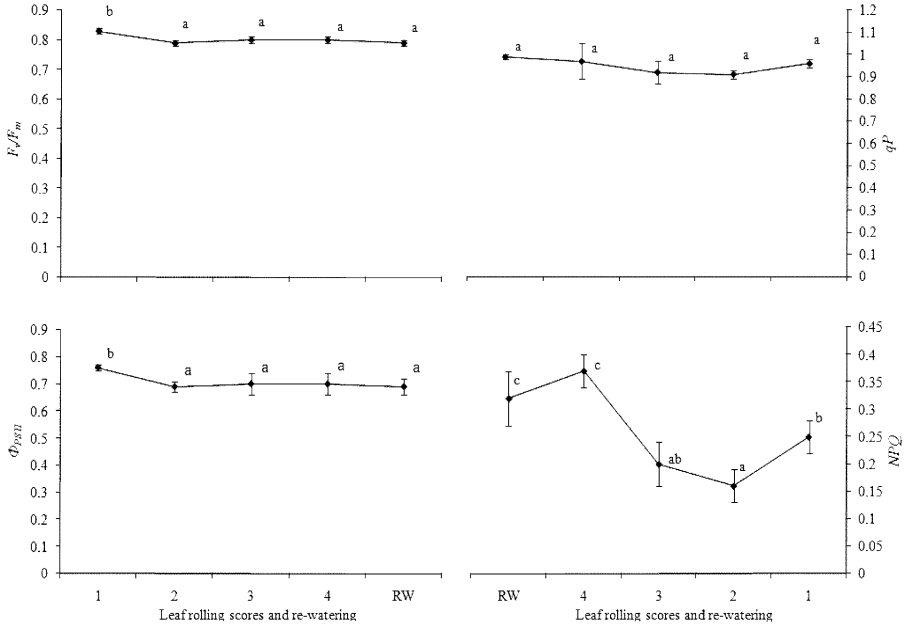

Fig. 2. Changes in F_v/F_m, $\Phi_{PS\,II}$, *qP* and *NPQ* during leaf rolling and after re-watering

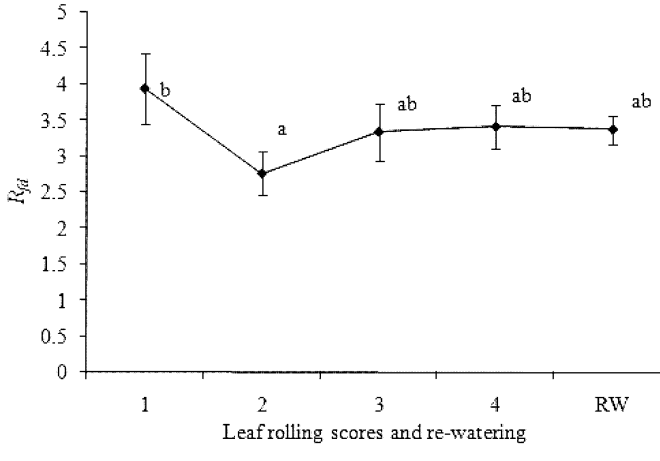

Fig. 3. Changes in R_{fd} during leaf rolling and after re-watering

DISCUSSION

Upon re-watering of PSE plants, we observed that opening response of the rolled leaves was slower than that of PS plants. For example, rolled leaf of PS plant was opened on the first day after re-watering, but rolled leaf of PSE plant was fully opened on the fourth day after re-watering. It was reported that the leaves of PSE plants were rolled earlier than those of PS plants [32]. In the previous study, the leaf area of PSE plants decreased by 69.3% as compared with those of unstressed plants. PSE plants with reduced leaf have more extended tolerance than PS plants. In addition, PSE plants are more cautious than PS plants to the stress changes.

In the present study, exposure to drought stress resulted in decreasing of leaf water potential during leaf rolling. Leaf water potential of the PSE plants was compared to the PS plants in a previous study [35]. Values of Ψ_{leaf} in the PSE plants were higher than those of the PS plants. For instance, at score 4, Ψ_{leaf} in PSE and PS plants was –0.65 and –1.18 MPa, respectively. After re-watering of the plant, Ψ_{leaf} started to increase. Similarly, Sağlam et al. [32] recorded that RWC in PSE plants was also higher than that of PS plants. Therefore, water loss of PSE plants may be at a lower level than PS plants due to reduction in the leaf area.

Drought stress increased stomatal closure in 4 to 28 times during leaf rolling period of PSE plants in the present experiment. These values were 4- to 10-folds in PS plants [35]. However, the change of g_s in PSE plants during leaf rolling had similar trend with that of PS plants. The result also showed that response of stomatal opening in PS plant after re-watering was quicker than that of PSE plant. This data also supports the above idea that PSE plant was more cautious to drought stress changes than PS plant.

The total chlorophyll and carotenoid contents did not significantly change during leaf rolling in PSE plants. Contrarily, in previously reported PS plants, the pigment contents decreased at the beginning of leaf rolling but later approximately reached to control (score 1) level [38]. Thus, the reduction of leaf area may contribute to keeping of photosynthetic pigments. On the other hand, in our study, lipid peroxidation increased at score 2 but decreased at the later scores. The result showed that changes of lipid peroxidation in PSE plants during rolling period were found similar to the previously reported in PS plants [38]. The data in the present study supported that the highest rolling decreased membrane damage because less radiation was intercepted by leaf tissue. The highest value was found at score 2 because the leaves are under drought stress and high daylight effect.

In the present study, to explain the involvement of antioxidant defence mechanisms in tolerance of PSE plants to drought, we also investigated the capacity of some enzymes (SOD, APX, GR and CAT) of the cellular antioxidative system. Our results showed that activities of CAT, APX and GR generally increased during leaf rolling. Similarly, it was reported that antioxidant enzyme activities increased under drought stress in other plant species [25, 34, 37, 39].

In this work, SOD activity was not constant at all leaf rolling scores. It decreased at score 2 and score 4 but did not change at score 3 compared to score 1. However,

it did not significantly change during leaf-rolling in PS plants [38]. The decline in SOD activity would favor accumulation of $O_2^{\bullet -}$. When SOD activity was low, ROS scavenging, especially superoxide radical, was not done properly and damage to membranes increased at score 2.

APX generally showed an increase related to the leaf rolling scores in PSE plants. A decrease in APX activity occurred only at score 3 compared to score 1. Conversely, in the previous study, it did not significantly change in drought-stressed leaves compared to score 1 in PS plants [35]. The results indicated that APX in PSE plants played more important role in protecting the plants from oxidative stress compared with PS plants.

In the present study, CAT activity statistically rose up to score 3 during rolling period in PSE plants but then decreased. Changes in CAT activity in PSE plants during rolling period were found similar to the previously reported in PS plants [35]. Our results demonstrated that CAT effectively scavenged H_2O_2 during the rolling. On the other hand, decrease in APX activity at score 3, and CAT activity at score 4 may be related to reducing in the light intensity on the surface of the leaves because of leaf rolling. Indeed, it is known that stress-induced changes in certain antioxidant enzyme activities depend on the light [20].

The results indicated that GR activity in PSE plants enhanced during leaf rolling but there was no significant difference ($P < 0.05$) between scores 1 and 2. Similar to previous study [36], GR activity increased with enhanced leaf rolling in PS plants. The elevated levels of GR activity in response to drought may be able to increase the ratio of $NADP^+$/NADPH. Also, under drought stress, GR seems to be crucial in the protection against oxidative stress. The relation between drought stress and antioxidant enzyme activities was studied in some plant species [34] and it was reported that GR enhances the resistance of plants to desiccation or drought [17].

The increased enzyme activity can reduce oxidative injury arising from ROS forming under drought stress. In our experiment, superoxide radical production increased during leaf rolling in PSE plants. Furthermore, production of superoxide anion in PSE plants during rolling period was found similar to the previously reported in PS plants [35]. Formation of superoxide anion has been reported to increase under drought stress in a number of plant species [18]. On the other hand, in our study, a decline in the concentration of H_2O_2 was observed in PSE plants under drought stress although it increased at score 2. Decrease in H_2O_2 content may be resulted from the increase in antioxidant enzyme activities. Indeed, increased activity of APX and CAT may be attributed to the efficient removal of H_2O_2 produced under drought stress. As similar to our results, Sharma and Dubey [37] reported that the sharp decline of H_2O_2 level attributed to the efficient removal of H_2O_2 by increased activity of antioxidant enzymes as well as certain non-enzymatic reactions working efficiently in the stressed plants.

There is much evidence that water stress does not cause reductions in primary events of photosynthesis, i.e. PSII efficiency [11]. Chlorophyll fluorescence data in our experiment showed that *NPQ* and R_{fd} decreased at score 2, but then increased. These results can be explained that at the beginning of drought stress, adaptation to

stress condition was probably weak because of low leaf rolling score. As increased leaf rolling score, the adaptation to drought stress condition enhanced. Each chlorophyll fluorescence parameter will be discussed in detail below.

In our study, there was a decrease in F_v/F_m ratio and quantum yield of PSII during the rolling and after re-watering. The decreases were not high compared to the other plant species under drought stress in the literature [23]. The results showed that PSE plant adapted to drought stress. However, upon irrigation of PS plant, F_v/F_m value turned back to score 1 level [27]. The decreasing ratio of $\Phi_{PS\,II}$ in PSE plant during the rolling process was lower than previously reported PS plant. Upon irrigation to plants, $\Phi_{PS\,II}$ changed as similar with F_v/F_m [27]. Thus, similar to PS plants, PSE plant was also not affected by adverse environmental condition.

Photochemical quenching did not change during leaf rolling and after re-watering. On the other hand, no significant variations were found up to score 3 in *qP* but declined at score 4 in PS plants [27]. This indicated that both PSE and PS plants could tolerate drought stress.

In the present study, non-photochemical quenching decreased at score 2, but then increased. These results were opposite to PS plants [27]. It is known that drought may lead to an increase in non-photochemical quenching [5]. The importance of the non-photochemical quenching is that the level of excitation energy in the PS II antenna can be regulated. *NPQ* prevents over-reduction of the electron transfer chain therefore provides the protection against photo-damage of plants [28].

In conclusion, our results pointed out post stressed plants having aquired drought tolerance by reducing leaf area displayed different responses in terms of photosynthetic apparatus and antioxidant system in comparison with primary stressed plants.

REFERENCES

1. Aebi, H. E. (1983) Catalase. In: Bergmeyer H.U. (ed.) *Methods of Enzymatic Analysis*. Verlag Chemie, Weinhern., pp. 273–286.
2. Aroca, R., Irigoyen, J. J., Sanchez-Diaz, M. (2003) Drought enhances maize chilling tolerance. II. Photosynthetic traits and protective mechanism against oxidative stress. *Physiol. Plant. 117,* 540–549.
3. Arnon, D. I. (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol. 24,* 1–15.
4. Beauchamp, C., Fridovich, I. (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem. 44,* 276–287.
5. Biehler, K., Haupt, S., Beckmann, J., Fock, H., Becker, T. W. (1997) Simultaneous CO_2 and $^{16}O_2$: $^{18}O_2$-gas exchange and fluorescence measurements indicate differences in light energy dissipation between the wild-type and the phytochrome-deficient aurea mutant of tomato during water stress. *J. Exp. Bot. 48,* 1439–1449.
6. Bilger, W., Björkman, O. (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynth. Res.* 25, 173–185.
7. Blum, A. (1996) Crop responses of drought and the interpretation of adaptation. *Plant Growth Regul. 20,* 135–148.

8. Bowler, C., van Montagu, M., Inzé, D. (1992) Superoxide dismutase and stress tolerance. *Annu. Rev. Plant Phys. 43,* 83–116.
9. Butow, B., Wynne, D., Sukenik, A., Hadas, O., Tel-Or, E. (1998) The synergistic effect of carbon concentration and high temperature on lipid peroxidation in *Peridinium gatunense. J. Plankton Res. 20,* 355–369.
10. Capaldi, D. J., Taylor, K. E. (1983) A new peroxidase color reaction: oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) with its formaldehyde azine. Application to glucose and choline oxidases. *Anal. Biochem. 129,* 329–336.
11. Cornic, G., le Gouallec, J. L., Briantais, J. M., Hodges, M. (1989) Effect of dehydration and high light on photosynthesis of two C3 plants (*Phaseolus vulgaris* L. and *Elatostema repens* (Lour) Hall f.). *Planta. 177,* 84–90.
12. Dall'Osto, L., Fiore, A., Cazzaniga, S., Giuliano, G., Bassi, R. (2007) Different roles of α- and β-branch xanthophylls in photosystem assembly and photoprotection. *J. Biol. Chem. 282,* 35056–36068.
13. Foyer, C. H., Halliwell, B. (1976) Presence of glutathione and glutathione reductase in chloroplast: a proposed role in ascorbic acid metabolism. *Planta 133,* 21–25.
14. Foyer, C. H., Descourvieres, P., Kunert, K. J. (1994) Protection against oxygen radicals: an importance defence mechanism studied in transgenic plants. *Plant Cell Environ. 17,* 507–523.
15. Fracheboud, Y., Leipner, J. (2003) The application of chlorophyll fluorescence to study light, temperature, and drought stress. In: DeEll, J. R., Toivonen, P. M. A. (eds) *Practical Applications of Chloropyll Fluorescence in Plant Biology*. Kluwer Acad. Pub., USA, pp. 126–147.
16. Frahry, G., Schopfer, P. (2001) NADH-stimulated, cyanide-resistant superoxide production in maize coleoptiles analyzed with a tetrazolium-based assay. *Planta 212,* 175–183.
17. Gamble, P. E., Burke, J. J. (1984) Effect of water stress on the chloroplast antioxidant system I. Alterations in glutathione reductase activity. *Plant Physiol. 76,* 615–621.
18. Halliwell, B., Gutteridge, J. N. C. (1999) *Free Radicals in Biology and Medicine.* 3rd ed. Oxford University Press, Oxford.
19. Heath, R. L., Packer, L. (1968) Photoperoxidation in isolated chloroplast. I. Kinetics and stochiometry of fatty acid peroxidation. *Arch. Biochem. Biophys. 125,* 189–198.
20. Janda, T., Szalai, G., Lesko, K., Yordanova, R., Apostol, S., Popova, L. P. (2007) Factors contributing to enhanced freezing tolerance in wheat during frost hardening in the light. *Phytochem. 68,* 1674–1682.
21. Jaspars, E. M. J. (1965) Pigmentation of tobacco crown-gall tissues cultured in vitro in dependence of the composition of the medium. *Physiol. Plant. 18,* 933–940.
22. Kadıoğlu, A., Terzi, R. (2007) A dehydration avoidance mechanism: leaf rolling. *The Bot. Rev. 73,* 290–302.
23. Li, Q. M., Liu, B. B., Wu, Y., Zou, Z. R. (2008) Interactive effects of drought stresses and elevated CO_2 concentration on photochemistry efficiency of cucumber seedlings. *J. Intgr. Plant Biol. 50,* 1–11.
24. Lichtenthaler, H. K., Rinderle, U. (1988) The role of chlorophyll fluorescence in detection of stress conditions in plants. *Crit. Rev. Anal. Chem. 19,* 529–585.
25. Mittler, R., Zilinskas, B. A. (1994) Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought and following recovery from drought. *Plant J. 5,* 397–405.
26. Nakano, Y., Asada, Y. (1987) Purification of ascorbate peroxidase from spinach chloroplasts: Its inactivation in ascorbat depleted medium and reactivation by monodehydroascorbate radical. *Plant Cell Physiol. 28,* 131–135.
27. Nar, H., Sağlam, A., Terzi, R., Várkonyi, Z., Kadıoğlu, A. (2009) Leaf rolling and photosystem II efficiency in *Ctenanthe setosa* exposed to drought stress. *Photosynthetika 47* (in press).
28. Niyogi, K. K. (1999) Photoprotection revisited: genetic and molecular approaches. *Ann. Rev. Plant Physiol. Plant Mol. Biol. 50,* 333–359.

29. Oppenheimer, H. R. (1960) *Plant Water Relationships in Arid and Semi-Arid Conditions*, Unesco, UK, pp. 105–138.
30. O'Toole, J. C., Moya, T. B. (1978) Genotypic variation in maintenance of leaf water potential in rice. *Crop Sci. 18,* 873–876.
31. Rensburg, L. V., Kruger, G. H. J. (1994) Evaluation of components of oxidative stress metabolism for use in selection of drought tolerant cultivars of *Nicotiana tabacum* L. *J. Plant Physiol. 143,* 730–737.
32. Sağlam, A., Kadıoğlu, A., Terzi, R., Saruhan, N. (2008) Physiological changes in them in post-stress emerging *Ctenanthe setosa* plants under drought conditions, *Russ. J. Plant Physiol. 55,* 48–53.
33. Sairam, R. K., Shukla, D. S., Saxena, D. C. (1997/1998) Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes. *Biol. Plantarum. 40,* 357–364.
34. Sairam, R. K., Chandrasekhar, V., Srivastava, G. C. (2001) Comparison of hexaploid and tetraploid wheat cultivars in their responses to water stress. *Biol. Plantarum. 44,* 89–94.
35. Saruhan, N., Terzi, R., Sağlam, A., Nar, H., Kadıoğlu, A. (2010) Scavenging of reactive oxygen species in apoplastic and symplastic areas of rolled leaves in *Ctenanthe setosa* under drought stress. *Acta Biol. Hung. 61* (in press).
36. Saruhan, N., Terzi, R., Sağlam, A., Kadıoğlu, A. (2009) The role of leaf rolling in regulation of ascorbate-glutathione cycle enzymes in apoplastic and symplastic areas of *Ctenanthe setosa*. *Biol. Res. 42,* 315–316.
37. Sharma, P., Dubey, S. (2005) Drought induces oxidative stres and enhances the activities of antioxidant enzymes in growing rice seedlings. *Plant Growth Regul. 46,* 209–221.
38. Terzi, R., Kadıoğlu, A. (2006) Drought stress tolerance and the antioxidant enzyme system in *Ctenanthe setosa*. *Acta Biol. Cracov. Bot. 48,* 89–96.
39. Türkan, İ., Bor, M., Özdemir, F., Koca, H. (2005) Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Sci. 168,* 223–231.
40. van Kooten, O., Snel, J. F. H. (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology, *Photosynth. Res. 25*, 147–150.
41. Zhang, Y. J., Zhao, C. J., Liu, L. Y., Wang, J. H., Wang, R. C. (2005) Chlorophyll fluorescence detected passively by difference reflectance spectra of wheat (*Triticum aestivum* L.) leaf. *J. Intgr. Plant Biol. 47,* 1228–1235.
42. Zlatev, Z. S., Lidon, F. C., Ramalho, J. C., Yordanov, I. T. (2006). Comparison of resistance to drought of three bean cultivars. *Biol. Plantarum. 50,* 389–394.