Leaf rolling reduces photosynthetic loss in maize under severe drought

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Abstract – Effects of leaf rolling (LR) on maize photosynthesis under severe drought stress were studied in two cultivars with opposite drought responses, Batem 56-55 (drought tolerant) and Batem 51-52 (drought sensitive). Drought stress and artificial prevention of leaf rolling (PLR) were applied at grain filling stage for 30 days. LR in Batem 56-55 occurred later than in Batem 51-52. Leaf water potential (Ψ_{leaf}) did not change in Batem 56-55 but decreased in Batem 51-52 at LR. Maximum quantum yield of photosystem II (F_v/F_m), effective quantum yield of photosystem II (Φ_{PSII}) and electron transport rate (ETR) of the cultivars decreased during LR more significantly in Batem 56-55 in comparison to Batem 51-52. The same was observed for the decrease in net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration (E) and intracellular level of CO_2 (C_i). Rubisco activity and content were reduced at LR, but were less affected in Batem 56-55 than in Batem 51-52. Ear and kernel weights also decreased at LR. All parameters at PLR were more reduced than those of LR. These results implied that LR was an important and necessary mechanism protecting photosynthesis and reducing yield loss under drought stress by maintaining the leaf hydration, preventing loss of the photosynthetic pigments, sustaining the activity of PSII, keeping the stomata open, and conserving the activity of Rubisco.

Keywords: drought, leaf rolling, maize, photosynthesis, yield

Abbreviations: Car – carotenoid, Chl – chlorophyll, C_i – intracellular CO_2, dw – dry weight, E – transpiration, ETR –electron transport rate, F_v/F_m – maximum quantum yield of PSII, Φ_{PSII} – effective quantum yield of PSII photochemistry, g_s – stomatal conductance, LR – leaf rolling, NPQ – nonphotochemical quenching, P_N – net photosynthetic rate, PSII – photosystem II, PLR – prevention of leaf rolling, Ψ_{leaf} – leaf water potential

Introduction

Maize is one of the key sources of human nutrition and is becoming an increasingly valuable crop due to the decrease in rice production and an enhanced demand for meat products

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and dairy (Salvi et al. 2007). Maize is grown mostly under rain-fed conditions and is more susceptible to drought than other cereals. In addition drought is unpredictable. Therefore drought tolerance is an important factor in maize breeding (Banziger and Araus 2007). Grain yield is reduced by drought but depends on plant species, developmental stages, variety of the plants, and intensity of the stress (Grzesiak et al. 2013). Maize is especially vulnerable to soil drought during grain-filling periods (Bai et al. 2006). The decrease of grain yield in maize under drought stress has been the subject of recent studies. For instance, a five-day drought stress during pollination has caused formation of aborted embryos and decrease in kernel numbers in maize (Zinselmeier et al. 1999). Drought stress and shading during pre and post pollination stages have decreased kernel set in the apical regions of maize ears (Setter et al. 2001).

The photosynthetic process is impaired mostly by drought stress (Chaves et al. 2009) due to both stomatal and non-stomatal limitations, which lead to a decrease in photosynthetic activity (Zlatev and Yordanov 2004). Stomatal closure is one of the earliest responses of the plants to drought, reducing transpiration, CO_2 uptake and photosynthetic activity (de Souza et al. 2013). The limitation of CO_2 uptake causes an imbalance between PSII activity and the Calvin cycle, increasing the excitation energy on PSII, and subsequent photodamage (Baker and Rosenqvist 2004), mainly to the PSII oxygen-evolving complex, and the disruption of D1 protein, resulting in inactivation of PSII reaction centers (Ashraf and Harris 2013).

A number of mechanisms are involved in plant defense strategies against drought stress such as reduction of leaf size, leaf movement or leaf rolling (LR) (Kadioglu et al. 2012). Although, there are many studies about the effects of drought on photosynthesis in plants, there are still few studies showing changes in photosynthesis during LR under drought stress. For example, a relation between LR and susceptibility to photoinhibition of sorghum photosynthesis under drought was reported (Corlett et al. 1994). In *Ctenanthe setosa*, LR was reported to protect PSII against damage under severe drought (Nar et al. 2009). Furthermore, moderate LR increased efficiency of photosynthesis in rice (Lang et al. 2004). However, the effects of LR on photosynthetic yield regarding agronomic parameters and cultivars have not been studied. Thus, in the present study, the effects of LR on photosynthesis yield and drought-response mechanisms are elucidated. We hypothesized that LR could protect photosynthesis from drought stress and preserve maize yield. In addition, delayed or late LR has been associated with drought tolerance in rice cultivars (Singh and Mackill 1990). On the other hand, how delayed LR affects photosynthesis and yield has not yet been fully elucidated. Therefore, the effects of artificial prevention of leaf rolling (PLR) on photosynthesis under drought stress was investigated as well as the way in which LR affects photosynthesis in cultivars with contrasting drought tolerance.

Material and methods

Growth of the plants and stress treatments

The experiment was carried out between May and August 2008, 2009 and 2010 at Karadeniz Technical University, Greenhouse Field (40°59′N, 39°46′E, 179 m). A rainout shelter (8 m width × 15 m length × 3 m height) was constructed as a tunnel open at both ends and kept 150 cm above ground to allow free airflow and avoid rises in temperature due to

the greenhouse effect on an open field and covered with polyethylene transmitting more than 90% of sunlight. The plants were grown in the rainout shelter under sunlight. Average temperature of the field was 21.8 ± 4.1 °C between May and August in 2008–2010. Seeds of the maize cultivars (Batem 51-52 and Batem 56-55) were obtained from Western Mediterranean Agricultural Research Institute, Antalya, Turkey; Batem 51-52 was reported as drought sensitive and Batem 56-55 as drought tolerant (Saruhan et al. 2012). Cultivars were determined as drought tolerant/sensitive based on their leaf water potentials, stomatal conductance, proline and malondialdehyde (MDA) contents after 30 days of drought stress, to which the plants were exposed at grain filling stage (R2). Eight seeds were sown in pots (30 cm diameter and 50 cm deep) filled to 47.5 cm height with 14 kg of fully water-saturated sandy soil. There were twelve pots for each cultivar. After full germination, seedlings were reduced to two in each pot. Plants were kept well irrigated and grown to stage R2 by adding one and half liters of water to each pot every other day. At stage R2, half of the plants were kept well watered (control), while the others were subjected to drought stress by withholding water for 30 days until maturity was reached. To investigate the effect of LR on photosynthesis during drought, LR was artificially inhibited (PLR) by clamping leaves with plastic wires in the beginning of drought application. Leaves (6th leaf from top) were harvested on the 30th day of drought. Leaf rolling degree, water status and temperature were measured, in addition to photosynthetic pigment content, chlorophyll *a* fluorescence, photosynthetic gas exchange parameters, Rubisco activity, Rubisco content and some agronomic characteristics.

Leaf rolling degree and water status

Leaf rolling degree was measured according to Premachandra et al. (1993) as a percentage reduction in the width of the leaf mid-portion. Leaf water potential (Ψ_{leaf}) was measured with a thermocouple psychrometer (C-52, Wescor) after it had been calibrated by using NaCl solutions of known water potentials. Discs about 6 mm in diameter were cut from the leaves of three plants and sealed in the C-52 sample chamber. Samples were equilibrated for 60 min before the readings were recorded by a water-potential data logger (PSYPRO, Wescor Inc., Logan, UT USA) in the psychrometric mode.

Lipid peroxidation

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content ($\varepsilon = 155$ mM^{-1} cm^{-1}), a product of lipid peroxidation following the method of Heath and Packer (1968). Leaf samples (0.5 g) were homogenized in 10 ml of 0.1% (w/v) trichloroacetic acid (TCA). As much as 4 ml of 0.5% (w/v) thiobarbituric acid containing 20% (w/v) of TCA was added to 1 ml aliquot of supernatant. The absorbance of the supernatant was recorded at 532 and 600 nm and MDA content was expressed as nmol MDA per g fresh weight (nmol MDA g^{-1} fw).

Photosynthetic pigments

Total chlorophyll (Chl) and carotenoid (Car) contents were determined following the method of Arnon (1949). The 6th leaf from the tops of different plants of the same age were selected randomly and homogenized in a mortar in 80% acetone. The extract was centri-

fuged at 5,000 g for 5 min. Absorbance of the supernatant was recorded at 663, 645, and 450 nm by spectrophotometer (Nicolet evolution 100, Thermo Scientific, USA). Content was expressed as mg of pigments per g dry weight (mg g^{-1} dw).

Thermal imaging

Thermal images were obtained using a Thermovision A20 infrared camera (FLIR Systems, USA) according to VOLLSNES et al. (2009). The infrared camera was mounted vertically ~10 cm above the leaf. Each leaf was positioned in the camera view field to focus the image and to maximize the image area covered by the leaf. The images of the 6th leaf from the tops of six different plants were captured. Images were analyzed for temperature determination using the ThermaCAM Researcher software (LIU et al. 2011).

Chlorophyll a fluorescence measurements

Chlorophyll *a* fluorescence measurements were performed by pulse modulated fluorometer (OS1-FL, OptiScience Corporation, Tyngsboro, MA, USA) according to NAR et al. (2009) between 10:00 and 14:00 h at the grain-filling stage in the rainout shelter under sunlight. For each treatment, the 6th leaf from six different plants was selected and clamped at the center point with the OS1-FL leaf clip holder. Leaves were dark-adapted for 20 min and the Chl fluorescence was then measured. 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 (8,000 μmol m^{-2} s^{-1}). After dark measurements, the leaves were exposed to the red actinic light of the fluorometer together with the sunlight functioning as actinic light source. Steady state Chl fluorescence (F_s) was achieved after exposure to the actinic light. A simultaneous saturating pulse (0.8 s) of white light (8,000 μmol m^{-2} s^{-1}) was applied to determine maximum Chl fluorescence in the light (F_m'). Maximum quantum yield of PSII photochemistry (F_v/F_m) and effective quantum yield of PSII photochemistry (Φ_{PSII}) were calculated according to the following equations ($F_v/F_m = (F_m - F_0)/F_m$ and $\Phi_{PSII} = (F_m' - F_s)/F_m'$) of GENTY et al. (1989). Nonphotochemical quenching (NPQ) was calculated according to the equation, NPQ = $(F_m - F_m')/F_m'$. Electron transport rate (ETR) was calculated according to following equation, ETR = $(\Phi_{PSII} \times PAR \times 0.5 \times 0.84)$. PAR was measured with a PAR clip. Value 0.5 indicates partitioning of energy between PSII and PSI, while value 0.84 presents proportional light absorbance by photosynthetic tissue.

Photosynthetic gas exchange measurements

Stomatal conductance (g_s) was monitored on the 6th leaf from six different plants by using a dynamic diffusion porometer (AP4, Delta-T Devices, Burwell, Cambridge, UK) after it had been calibrated with a standard calibration plate following the manufacturer's instructions. Other gas exchange parameters were measured using a portable photosynthesis system (TPS-2 Photosynthesis System, PP Systems, Amesbury, MA, USA) equipped with a 25 × 18 mm PLC6 automatic universal leaf cuvette on the 6th leaf from six different plants under natural sunlight. Middle parts of the selected leaves were measured from 10:00 to 14:00 h. The parameters measured were transpiration (E), net photosynthetic rate (P_N) and intracellular CO_2 (C_i).

Agronomic characteristics

Grain yield and yield components were evaluated through ear weight and 100-kernel weight in six plants.

Determination of Rubisco activity

About 0.25 g of frozen maize leaves were ground to a powder using a chilled mortar and pestle with liquid N_2, a small amount of quartz sand, and insoluble polyvinylpolypyrrolidone (PVP). Tissues were homogenized with cooled extraction buffer containing 50 mM of Tris–HCl (pH 7.5), 1 mM of ethylenediaminetetraacetic acid (EDTA), 10 mM of $MgCl_2$, 12% (v/v) of glycerol, 0.1% (v/v) of β-mercaptoethanol and 1% (w/v) of PVP-40 (soluble PVP) at 0–4 °C. The homogenate was centrifuged at 15,000 g for 15 min at 4 °C. Protein contents of the homogenates were determined as described by BRADFORD (1976) using bovine serum albumin as a standard. The Rubisco activity in the supernatant was assayed according to SAWADA et al. (2003) with minor modifications. The Rubisco activity was measured at 30 °C for 10 minutes by adding 100 μl of supernatant into 900 μl of assay buffer containing 50 mM of HEPES–KOH (pH 8.0), 1 mM of EDTA, 20 mM of $MgCl_2$, 25 mM of dithioerythritol, 10 mM of $NaHCO_3$, 5 mM of ATP, 0.15 mM of nicotinamide adenine dinucleotide (NADH), 5 mM of creatine phosphate, 0.6 mM of ribulose-1,5-bisphosphate (RuBP), 10 units of phosphocreatine kinase, 10 units of glyceraldehyde-3-phosphate dehydrogenase and 10 units of phosphoglycerate kinase. Total Rubisco activity was expressed in units per milligram protein.

Determination of Rubisco content

To determine Rubisco content, leaves were homogenized as described in the Rubisco activity assay. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) of leaf protein was carried out in a vertical slab gel discontinuous buffer system following the method of LAEMMLI (1970) using a 7% acrylamide gel concentration. A total volume of 20 μl protein extract solution including 30 μg of total protein was loaded into each well. In addition, to quantify the amount of Rubisco in the leaf homogenates, Rubisco recombinant protein standards ranging from 0.0625 to 0.25 pmol were also loaded. Protein molecular mass standard (Thermo Fisher Scientific, Waltham, MA) was used. The gels were run at approximately 80 mA for 30–40 min in electrophoresis buffer by using a Mini-PROTEAN Tetra Cell electrophoretic unit (Biorad, Germany). Proteins were then transferred to polyvinylidene fluoride (PVDF) transfer membrane (Whatman, Dassel, Germany) in McFarland's electroeluting buffer (12 mM Tris base, 96 mM glycine, and 20%, v/v, methanol) at < 25 V for 45 min. The blotted membrane was subjected to immunodetection of Rubisco according to MA et al. (2009). The PVDF blot was placed in 10 ml of blocking solution (TBST; 5%, w/v, nonfat dry milk in 24.8 mM Tris, pH 7.4, 150 mM NaCl, 2.7 mM KCl, and 0.5%, v/v, Tween 20) and rocked gently on a rotary shaker or platform rocker for 30 min then washed four times for 5 min each with TBST and incubated for 1 h on a shaker-rocker with polyclonal rabbit anti-rubisco antibody (anti-rubisco large subunit global antibody, diluted 1:6,000; Agrisera, Vännäs, Sweden). After four 2.5-min washes with TBST, the blot was incubated with goat anti-rabbit immunoglobulin G (IgG) polyclonal antibody conjugated to horseradish peroxidase (diluted 1:2,000; Agrisera, Vännäs, Sweden) for 30 min on the shaker. After four 2.5-min washes, chromogenic substrate solution for horseradish peroxidase

(0.015%, w/v, of 4-chloro-1-napthol and 0.05%, v/v, hydrogen peroxide in TBS containing 16.7%, v/v methanol) was added, and the blot was allowed to develop 15 min. The blot was then rinsed three times in water for 2 min each. The developed blot was allowed to dry on a clean piece of filter paper and stored in a ziplock bag at −20 °C. The Western blot image of protein bands was captured with a scanner (HP Scanjet 4850; Hewlett Packard, Palo Alto, CA). A comparison of the average intensity of each band from western blot analysis was quantified with the use of the ImageJ analysis program (ImageJ 1.34s; http://rsbweb.nih. gov/ij/).

Statistical analysis

Each measurement was conducted three times with six biological replicates. Variance analysis of mean values was performed with Duncan Multiple Comparison test (two-way ANOVA) using SPSS software for Microsoft Windows (Ver. 13.0, SPSS Inc., USA) and significance was determined at $P < 0.05$ level.

Results

Leaf rolling degree and water status

Leaves of Batem 56-55, drought tolerant cultivar, rolled two days later than Batem 51-52, drought sensitive cultivar, under drought. In addition, LR degree (%) in Batem 51-52 (77.0 ± 0.5) was higher than Batem 56-55 (65.0 ± 2.7).

The leaf water potential (Ψ_{leaf}) control values were -0.30 ± 0.01 and -0.50 ± 0.03 MPa in control groups of Batem 51-52 and Batem 56-55, respectively. The Ψ_{leaf} significantly decreased (6-fold) in Batem 51-52 at LR (-1.81 ± 0.03 MPa) compared to the control (Fig. 1). However, the Ψ_{leaf} did not change in Batem 56-55 (-0.51 ± 0.02 MPa). At PLR, decreases in Ψ_{leaf} were measured in both Batem 51-52 (-2.30 ± 0.02 MPa) and Batem 56-55 (-1.20 ± 0.03 MPa) compared to the controls (Fig. 1). Ψ_{leaf} in Batem 56-55 at PLR was 7.8 fold lower than its control, while Ψ_{leaf} in Batem 51-52 was 2.4 fold lower than its control.
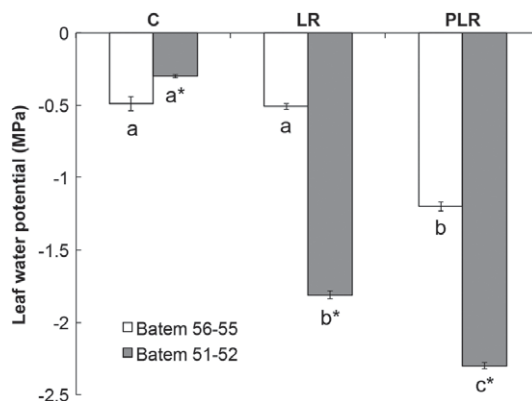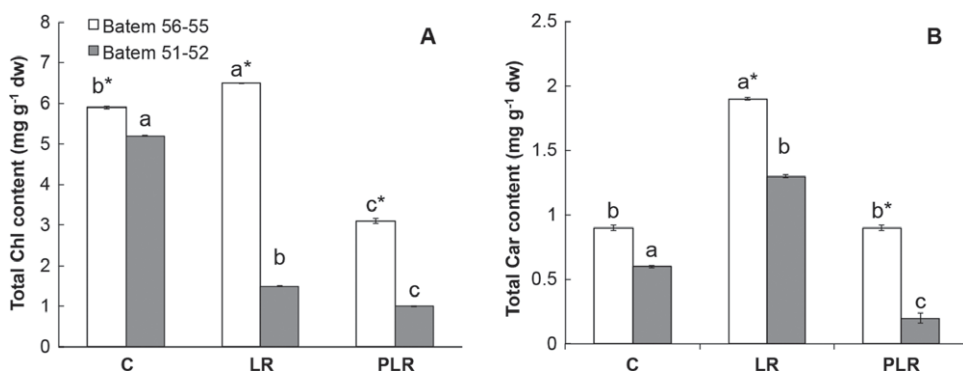

Fig. 1. Changes in leaf water potential of maize cultivars. C – control, LR – leaf rolling, PLR – prevention of leaf rolling. Different small letters show statistical differences at $P < 0.05$ among C, LR and PLR of same cultivar. Asterisk denotes significant differences between cultivars. Vertical bars are standard deviation of means.

Photosynthetic pigments

The total Chl content in Batem 51-52 decreased from 5.20 ± 0.01 mg g^{-1} dw (control) to 1.50 ± 0.01 mg g^{-1} dw at LR (Fig. 2A) while the total Chl content increased from 5.9 ± 0.03 mg g^{-1} dw (control) to 6.50 ± 0.01 mg g^{-1} dw in Batem 56-55 at LR. Total Chl content in Batem 51-52 was 3.5 fold lower at LR than in the control. On the other hand, the Chl content in Batem 56-55 was 1.1 fold higher at LR than in the control. In addition, the total Chl content at PLR was also lower than that of the control. The Chl content in Batem 51-52 was 1.00 ± 0.01 mg g^{-1} dw at PLR, a 5.2-fold decrease as compared to control. It was 3.10 ± 0.06 mg g^{-1} dw in Batem 56-55 at PLR, a 1.9 fold decrease as compared to control.

Total carotenoid (Car) content increased in Batem 51-52 and Batem 56-55 at LR compared to the controls. Total Car contents in rolled leaves of Batem 51-52 (1.30 ± 0.01 mg g^{-1} dw) and Batem 56-55 (1.9 ± 0.01 mg g^{-1} dw) were 2.2 and 2.1-fold higher than the controls (0.6 ± 0.01 mg g^{-1} dw and 0.9 ± 0.02 mg g^{-1} dw) respectively. In addition, a significant decrease in the Car contents was determined in Batem 51-52 at PLR. The Car content in Batem 51-52 (0.2 ± 0.04 mg g^{-1} dw) at PLR was 7.5-fold lower than the control (0.60 ± 0.01 mg g^{-1} dw) (Fig. 2B).

Fig. 2. Changes in photosynthetic pigment contents of maize cultivars. A) total Chl content and B) total Car content; C – control, LR – leaf rolling, PLR – prevention of leaf rolling. Different small letters show statistical differences at P < 0.05 among C, LR and PLR of same cultivar. Asterisk denotes significant differences between cultivars. Vertical bars are standard deviation of means.

Thermal imaging

Leaf temperature increased in both tolerant and sensitive cultivars at LR compared to the controls. Leaf temperature was 21.3 ± 0.2 °C in the control group of Batem 51-52 and 20.7 ± 0.1 °C in the control group of Batem 56-55. It increased to 21.7 ± 0.05 °C in Batem 51-52, and to 21.4 ± 0.2 °C in Batem 56-55, at LR. In addition, the leaf temperature at PLR was determined to be higher than that of LR in Batem 56-55. There was no significant difference between LR and PLR in Batem 51-52. The leaf temperature was 21.7 ± 0.06 °C in Batem 51-52 and 21.9 ± 0.1 °C in Batem 56-55 at PLR (Fig. 3).
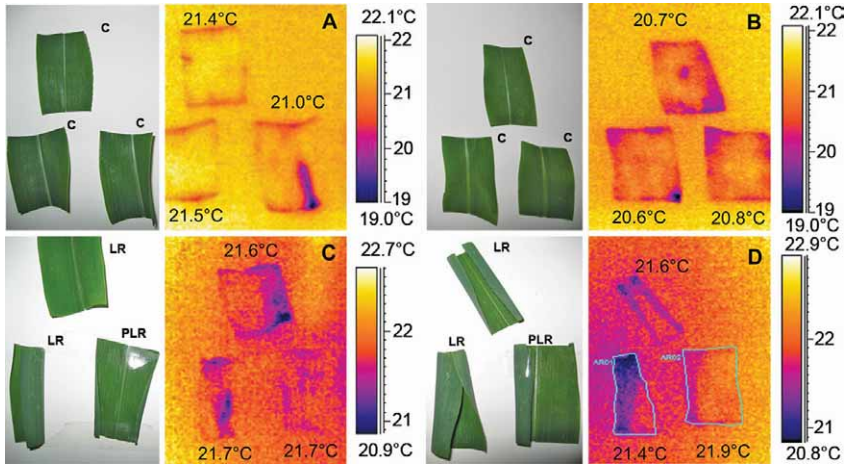
Fig. 3. Thermal images of maize leaves. A) Batem 51-52, sensitive control, B) Batem 56-55, tolerant control, C) Batem 51-52, upon drought stress and D) Batem 56-55, upon drought stress. C – control, LR – leaf rolling, PLR – prevention of leaf rolling.
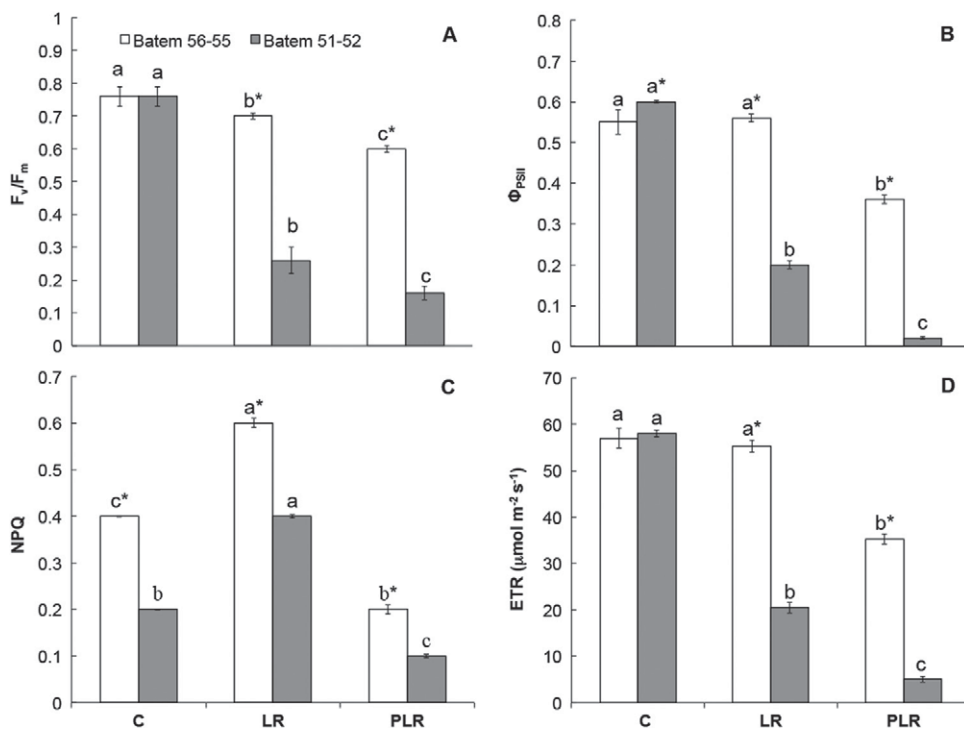
Chlorophyll fluorescence parameters

Maximum quantum yield of PSII photochemistry (F_v/F_m) decreased in both cultivars at LR (Fig. 4A). The decrease in the F_v/F_m was higher in Batem 51-52 than in Batem 56-55. F_v/F_m in the rolled leaves of the Batem 51-52 (0.26 ± 0.04) was 2.9-fold lower than in the control (0.76 ± 0.03) while F_v/F_m in Batem 56-55 (0.7 ± 0.01) was 1.1-fold lower at LR than in the control (0.76 ± 0.03). Moreover, significant decreases in F_v/F_m were determined in both cultivars at PLR compared to the controls. F_v/F_m values decreased from 0.76 (control) to 0.16 and 0.6 in Batem 51-52 and Batem 56-55 at PLR, respectively. F_v/F_m values in Batem 51-52 and Batem 56-55 were 4.8 and 1.3 fold lower than in their controls (Fig. 4A).

Effective quantum yield of PSII photochemistry (Φ_{PSII}) decreased in Batem 51-52 at LR compared to the control while it did not change in Batem 56-55 (Fig. 4B). As compared to control (0.6 ± 0.004), a 3-fold decrease in Φ_{PII2} (0.2 ± 0.01) was determined in Batem 51-52 at LR. A significant decrease in Φ_{PSII} was also determined at PLR. The Φ_{PSII} in Batem 51-52 and Batem 56-55 diminished to 0.02 ± 0.003 and 0.36 ± 0.01 at PLR, respectively. The values of Φ_{PSII} in Batem 51-52 and Batem 56-55 were 30.0 and 1.5 fold lower than in the control groups, respectively (Fig. 4B).

Non-photochemical quenching (NPQ) increased in rolled leaves of both cultivars compared to the controls (Fig. 4C). Furthermore, the increase in NPQ was higher in Batem 56-55 than that of Batem 51-52 at LR. NPQ in Batem 51-52 increased from 0.200 ± 0.001 to 0.400 ± 0.004 (a 2-fold increase). NPQ in Batem 56-55 also increased (from 0.400 ± 0.001 to 0.60 ± 0.01) compared to control, a 1.3 fold increase. In the case of PLR, NPQ decreased in both cultivars significantly compared to the controls. NPQ in Batem 51-52 was 0.100 ± 0.004 at PLR. It was 0.2 ± 0.01 in Batem 56-55. The rates of the decreases in NPQ were 2.0 fold in both Batem 51-52 and Batem 56-55 compared to the controls (Fig. 4C).

Electron transport rate (ETR) decreased in Batem 51-52 at LR (Fig. 4D). ETR decreased from 58.0 ± 0.8 μmol m^{-2} s^{-1} (control) to 20.5 ± 1.2 μmol m^{-2} s^{-1} at LR, which was 2.8 fold lower than in the control. On the other hand, ETR did not change in Batem 56-55 during LR.

Fig 4. Changes in Chl fluorescence parameters of maize cultivars. A) Fv/Fm – maximum quantum yield of PSII photochemistry B) Φ_{PSII} – effective quantum yield of PSII photochemistry, C) NPQ – non-photochemical quenching and D) ETR – electron transport rate; C – control, LR – leaf rolling, PLR – prevention of leaf rolling. Different small letters show statistical differences at $P < 0.05$ among C, LR and PLR of same cultivar. Asterisk denotes significant differences between cultivars. Vertical bars are standard deviation of means.

In the case of PLR, ETR decreased in both cultivars compared to the controls. ETRs in Batem 51-52 and Batem 56-55 were 5 ± 0.6 μmol m^{-2} s^{-1} and 35.3 ± 1.1 μmol m^{-2} s^{-1}. There were 12.0 and 1.6-fold decreases in Batem 51-52 and Batem 56-55 as compared controls, respectively (Fig. 4D).
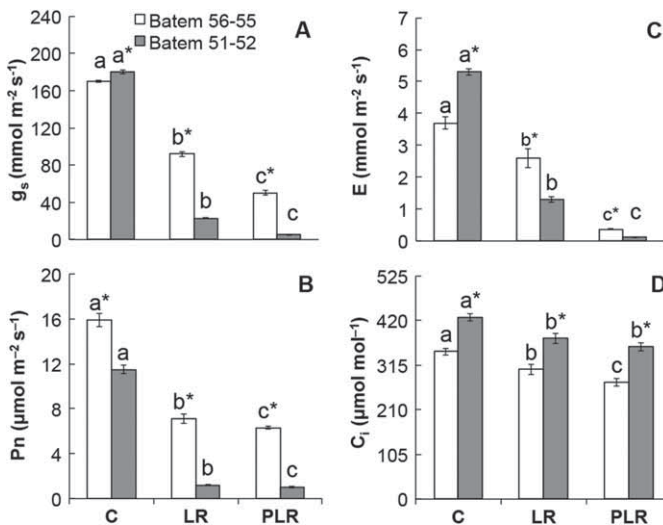
Photosynthetic gas exchange measurements

Stomatal conductance (g_s) diminished in both cultivars at LR. g_s in Batem 51-52 decreased from 180 ± 2.2 mmol m^{-2} s^{-1} (control) to 23 ± 0.5 mmol m^{-2} s^{-1} (a 7.8-fold decrease). The decrease in g_s was greater in Batem 51-52 than in Batem 56-55 at LR (Fig. 5A). g_s in Batem 56-55 diminished from 170 ± 1.3 mmol m^{-2} s^{-1} to 92 ± 2.7 mmol m^{-2} s^{-1}, which was a 1.9-fold decrease. Remarkable decreases in g_s were measured in both cultivars at PLR compared to the controls. g_s values in Batem 51-52 and Batem 56-55 were 5 ± 0.4 mmol m^{-2} s^{-1} and 50 ± 2.5 mmol m^{-2} s^{-1}, respectively at PLR. g_s in Batem 51-52 and Batem 56-55 were 36 and 3.4-fold lower than the controls, respectively.

Transpiration rate (E) decreased in both cultivars at LR compared to the controls (Fig. 5B). E in Batem 51-52 decreased from 5.3 ± 0.1 mmol m^{-2} s^{-1} (control) to 1.3 ± 0.1 mmol

m^{-2} s^{-1}. E in Batem 56-55 was reduced from 3.7 ± 0.2 mmol m^{-2} s^{-1} (control) to 2.6 ± 0.3 mmol m^{-2} s^{-1}. Decrease in E was higher in Batem 51-52 than in Batem 56-55 at LR. The rates of decreases were 1.4 and 4.1 in Batem 56-55 and Batem 51-52 compared to control, respectively. The decreases in E were more at PLR than those of LR as compared to controls. E values were 0.110 ± 0.007 mmol m^{-2} s^{-1} and 0.37 ± 0.02 mmol m^{-2} s^{-1} in Batem 51-52 and Batem 56-55 at PLR, respectively (Fig. 5B). E values in Batem 51-52 and Batem 56-55 were 48 and 10-fold lower than the control groups, respectively.

Photosynthetic rate (P_N) decreased in both cultivars at LR compared to the controls (Fig. 5C). P_N values were reduced from 11.5 ± 0.4 µmol m^{-2} s^{-1} (control) to 1.2 ± 0.04 µmol m^{-2} s^{-1} in Batem 51-52. It was reduced from 15.9 ± 0.6 µmol m^{-2} s^{-1} (control) to 7.1 ± 0.4 µmol m^{-2} s^{-1} in Batem 56-55 at LR. P_N values were reduced at the rates of 9.6 and 2.3 in Batem 51-52 and Batem 56-55 at LR compared to their controls, respectively. P_N decreased more in both cultivars at PLR than LR compared to control. P_N values were 1.03 ± 0.06 µmol m^{-2} s^{-1} and 6.3 ± 0.14 µmol m^{-2} s^{-1} in Batem 51-52 and Batem 56-55 at PLR, respectively (Fig. 5C). The rates of decrease in P_N were 11.1 and 2.5 in Batem 51-52 and Batem 56-55 at PLR compared to the controls, respectively.

Intracellular CO_2 concentration (C_i) was reduced by drought at LR (Fig. 5D). C_i in Batem 51-52 decreased from 428.0 ± 8.6 µmol mol^{-1} (control) to 378.0 ± 11.6 µmol mol^{-1}. It was reduced from 347.0 ± 4.9 µmol mol^{-1} to 305.0 ± 16.3 µmol mol^{-1} in Batem 56-55. The rates of the decrease in C_i values were 1.1 and 1.1 in Batem 51-52 and Batem 56-55 at LR compared to their controls. The reductions in C_i at PLR were higher than those of LR compared to control. C_i values were 358.0 ± 9.0 µmol mol^{-1} and 275.0 ± 2.1 µmol mol^{-1} in Batem 51-52 and Batem 56-55 at PLR, respectively. The rate of the decrease in C_i was 1.2 in Batem 51-52 compared to the control while it was 1.3 in Batem 56-55 at PLR.


Fig. 5. Changes in photosynthetic gas exchange parameters of maize cultivars. A) g_s – stomatal conductance, B) P_n – net photosynthetic rate, C) E – transpiration and D) C_i – intracellular CO_2; C – control, LR – leaf rolling, PLR – prevention of leaf rolling. Different small letters show statistical differences at $P < 0.05$ among C, LR and PLR of same cultivar. Asterisk denotes significant differences between cultivars. Vertical bars are standard deviation of means.

Rubisco activity

Rubisco activity in Batem 51-52 was higher than Batem 56-55. Rubisco activity decreased in both cultivars at LR compared to the controls. Rubisco activity in Batem 51-52 decreased from 1286.0 ± 15.0 U mg^{-1} protein (control) to 869.0 ± 24.0 U mg^{-1} protein. It was reduced from 464.0 ± 13.0 U mg^{-1} protein (control) to 303 ± 11.0 U mg^{-1} protein for Batem 56-55 (Fig. 6A). There was a 1.5-fold reduction in the Rubisco activity in both cultivars at LR compared to their controls. Rubisco activities determined at LR were higher than PLR for both cultivars. Rubisco activities were 557 ± 19.0 U mg^{-1} protein and 128.0 ± 9.0 U mg^{-1} protein in Batem 51-52 and Batem 56-55 at PLR, respectively. The reductions in the activities were higher at PLR than LR compared to the controls (Fig. 6). The reductions in the activities were 2.3-fold and 3.6-fold in Batem 51-52 and Batem 56-55 at PLR compared to the controls, respectively.

Rubisco content

Rubisco content in Batem 51-52 was higher than Batem 56-55 in control plants (Fig. 6B). It was 0.9 ± 0.05 pmol µg^{-1} protein in Batem 51-52 and 0.4 ± 0.01 pmol µg^{-1} protein in Batem 56-55. Rubisco contents of both cultivars decreased at LR as compared to control. They were 0.2 ± 0.008 pmol µg^{-1} protein in Batem 51-52 (4.5-fold lower than control) and 0.2 ± 0.002 pmol µg^{-1} protein in Batem 56-55 (2-fold lower than control). Rubisco contents of the cultivars at PLR were lower than those of LR plants. There was 0.002 ± 0.0005 pmol µg^{-1} protein in Batem, 450-fold lower than control and 0.002 ± 0.0009 pmol µg^{-1} protein in Batem 56-55, 200-fold lower than control.
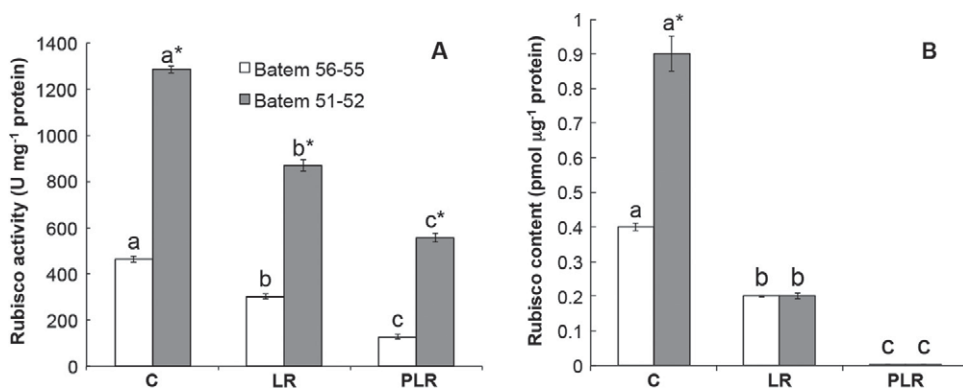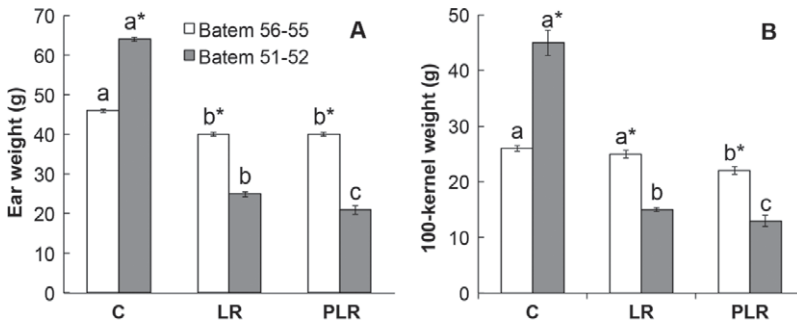

Fig. 6. Changes in Rubisco activity (A) and content (B) of maize cultivars; C – control, LR – leaf rolling, PLR – prevention of leaf rolling. Different small letters show statistical differences at $P < 0.05$ among C, LR and PLR of same cultivar. Asterisk denotes significant differences between cultivars. Vertical bars are standard deviation of means.

Agronomic characteristics

As compared to control, ear weights were reduced in both cultivars at LR. Ear weights decreased from and 64.0 ± 0.5 g to 40.0 ± 0.5 g in Batem 51-52 and 46.0 ± 0.4 g to 25.0 ± 0.7 g in Batem 56-55 at LR, respectively. The reduction in the ear weight was higher in

Batem 51-52 (2.6 fold) than Batem 56-55 (1.2 fold) at LR (Fig. 7A). Ear weights decreased more at PLR than LR compared to the controls. Ear weights in Batem 51-52 and Batem 56-55 were 21 ± 1.1 g and 40 ± 0.5 g at PLR, respectively. The decreases in ear weight were 3.0 and 1.5 fold in Batem 51-52 and Batem 56-55 as compared to their controls, respectively. One hundred-kernel weight also declined at LR. The kernel weight in Batem 51-52 diminished from 45.0 ± 2.3 (control) to 15 ± 0.4 g at LR. It was not changed in Batem 56-55. The reduction in 100-kernel weight in Batem 51-52 was 3.0 fold compared to the control (Fig. 7B). The decreases were more pronounced in both cultivars at PLR compared to the controls. Decreases in 100-kernel weights of Batem 51-52 and Batem 56-55 were 3.5 and 1.2 fold at PLR compared to the controls, respectively (Fig. 7B).

Fig. 7. Changes in agronomic characteristics of maize cultivars. A) ear weight and B) 100-kernel weight; C – control, LR – leaf rolling, PLR – prevention of leaf rolling. Different small letters show statistical differences at P < 0.05 among C, LR and PLR of same cultivar. Asterisk denotes significant differences between cultivars. Vertical bars are standard deviation of means.

Discussion

In the present study, leaf water potential in rolled leaves of drought sensitive cultivar was reduced by drought. Confirming our study, decreases in leaf water potential during LR were also reported in maize and *C. setosa* plants subjected to drought stress (Terzi et al. 2009, Saruhan et al. 2012). On the other hand, plants having LR mechanism were reported to exhibit a resistance to drought and high temperature and had higher water use efficiency (Kadioglu et al. 2012). That the decrease in leaf water content in our study was enhanced by PLR compared to LR suggested that LR might be a water-saving regulatory mechanism and may protect maize photosynthesis from drought stress.

To evaluate effects of environmental stress on growth and yield, Chl content and Chl fluorescence parameters can be measured because these traits are related with carbon exchange rate (Fracheboud et al. 2004). The effect of LR on photosynthetic pigment content was studied in maize under drought stress. Chl content decreased under drought in the sensitive cultivar at LR in our study while it increased in the tolerant one. Similarly, a high decrease in pigment content was reported in a sensitive maize cultivar under drought stress (Chugh et al. 2013). The increase in pigment concentration could be related to decreased leaf surface and assembling chlorophylls on less area of leaf (Tourian et al. 2013). Relation

between LR and photosynthetic pigment content was shown under drought stress in *C. setosa* (NAR et al. 2009). During early LR, a decrease in photosynthetic pigment content was observed. However, it approached control values in the late LR situation under severe drought stress. In our study, significant decreases in pigment content were observed at PLR compared to LR. These significant reductions in the pigment content could be related to changes in microclimate parameters such as irradiance and temperature differences, which might be created by the LR. However, the PLR may disrupt humid environment formed in rolled leaves, which have low temperature inside. Thermal images of maize leaves indicated that rolled leaves were cooler than artificially opened leaves. Leaf temperature of rolled maize leaves was 21.4 ± 0.2 °C while the temperature of artificially opened leaves was 21.9 ± 0.1 °C. In addition, reduced irradiance upon the leaf surfaces (PAR) due to LR was reversed by PLR in our study. PAR in control (1081 ± 0.2) decreased to 295 ± 42 during LR then increased to 1092 ± 20 during PLR. Therefore we concluded that LR might prevent light damage on PSII by reducing leaf exposure to light.

Chlorophyll *a* fluorescence provides information about the effects of LR on photosynthetic apparatus under drought stress (NAR et al. 2009). Thus, changes in fluorescence parameters were investigated in this study. One of those parameters, F_v/F_m has been widely used to detect changes in the photosynthetic apparatus due to stress (BAKER and ROSENQVIST 2004). In other words, changes in the fluorescence yield reflect changes in photochemical efficiency. Low F_v/F_m values in plants under stress indicate damage to the PSII reaction center (NAR et al. 2009). In this study, the decrease of F_v/F_m in the sensitive cultivar was more pronounced than in the tolerant cultivar during LR, indicating a higher drought tolerance in the latter cultivar. In addition, higher decreases in F_v/F_m at PLR indicated that LR might have a protective role on photosynthetic apparatus. However, no change in F_v/F_m was found in *C. setosa*, a drought tolerant plant (TERZI and KADIOGLU 2006), during LR (NAR et al. 2009).

Increase in NPQ resulting from drought stress is a well-known phenomenon (EFEOĞLU et al. 2009, HUANG et al. 2013). In the present study, NPQ increase during LR indicated the thermal dissipation of energy load on the leaves, perhaps preventing photo damage. Maize genotypes were found to conserve their photosystems from adverse effects of drought by increasing NPQ (DE SOUZA et al. 2013). Carotenoids are known as accessory photosynthetic pigments protecting the chlorophylls from oxidative stress damage during photosynthesis, dissipating energy through non-photochemical quenching and having an important role in the xanthophyll cycle (DE SOUZA et al. 2013). The increases in NPQ and Car content on rolled leaves of maize indicated thermal dissipation of excess energy in our study, which supported this hypothesis. Decreases in Car content and NPQ at PLR also supported the protective role of LR on the photosynthetic system.

Reduction in effective quantum yield of PSII photochemistry was observed in sensitive cultivar at LR in this study. Down-regulation of electron transport can be estimated by decreased in Φ_{PSII}. This decrease can also be related with increases in excitation energy quenching in the PSII antennae (HORTON et al. 1996). Consequently, the increase of NPQ in the sensitive cultivar was lower than in the tolerant, resulting in more energy on PSII and also more damage under drought. The decreases in Φ_{PSII} showed increased excitation energy quenching process in the PSII antennae and the regulation of electron transport. Therefore, the increase in NPQ during LR may cause a decrease in Φ_{PSII}. On the other hand, high NPQ in the tolerant cultivar did not affect Φ_{PSII} at LR. Increased Chl content in the tolerant cultivar

at LR might compensate for energy loss through NPQ in the tolerant cultivar. In addition, Φ_{PSII} at PLR was reduced more than LR in our study. The decrease in Φ_{PSII} at PLR could also be a proof for a protective role of LR on PSII.

A decline in electron transport rate was determined in the present study. This decrease in ETR may be associated with the increased trans-thylakoid ΔpH. This change in ΔpH causes the xanthophyll de-epoxidation that follows increasing thermal dissipation of energy when photosynthesis is reduced (Lu and Zhang 1999). In addition, photochemical down-regulation under stress conditions could cause reductions in ETR. Drought stress was recorded to limit photosynthetic machinery of six maize genotypes (Cruz de Carvalho et al. 2011). On the other hand, higher decreases in ETR during PLR supported the hypothesis that LR could be a protective mechanism for photosystems under drought.

Stomatal conductance is one of the parameters that affect photosynthesis under drought stress (Siddique et al. 1999, Jiang et al. 2006). Because g_s restricts CO_2 uptake, it is considered to be one of the main reasons for reduced photosynthesis (Ashraf and Harris 2013). In this respect, reduction in photosynthesis probably depends more on CO_2 in the chloroplast than on leaf water potential (Medrano et al. 2002). In our study, g_s was reduced by drought in rolled leaves of maize plants probably due to reduction in leaf water potential. A decrease in g_s and Ψ_{leaf} and a positive correlation between them were reported in maize plants under water stress (Cruz de Carvalho et al. 2011; Zia et al. 2011). On the other hand, sharp decreases in g_s and increases in leaf temperature following PLR in our study suggest that LR may maintain CO_2 uptake by creating a cool and humid environment inside the rolled leaf thus allowing some of stomata to remain open. Similarly, g_s values were found to be higher at adaxial (inner) surfaces of the rolled leaves than those of PLR during drought stress (Nar et al. 2009)

In the present study, the net photosynthetic rate decreased in rolled leaves of both maize cultivars under drought. However, the reduction in P_N of the tolerant cultivar was less than in the sensitive cultivar. Similarly, photosynthesis of drought-tolerant and sensitive maize cultivars responded to drought in different ways (Cruz de Carvalho et al. 2011). In addition to P_N and transpiration rate, intracellular CO_2 concentration also decreased in rolled leaves of maize cultivars in the present study. In addition, similar results were reported for drought tolerant and sensitive maize cultivars subjected to drought stress (Zhang and Liu 2009). Stomatal and non-stomatal factors cause reduction in photosynthesis. In the present study, decreases in P_N, g_s, C_i and E indicated that reduction in photosynthesis was due to stomatal factors. However, as compared to LR, notable reductions in all gas exchange parameters occurred following PLR. These differences may indicate that LR may minimize photosynthetic losses due to drought stress, functioning as a protective mechanism.

The effect of LR on the activity of the CO_2-fixing enzyme Rubisco was investigated in the current study. Reductions in the Rubisco activity were observed in rolled leaves of both cultivars. Furthermore, Rubisco activity was higher in the sensitive than in the tolerant cultivar. This might be related to the high amount of Rubisco in the drought sensitive cultivar. On the other hand, high content of inactive Rubisco in tolerant cultivar at LR might be a reason for low activity even if tolerant and sensitive cultivar has same amount of Rubisco. In addition the decrease in Rubisco activity in our study may be a result of stomatal closure due to long-term drought stress. Stomata closure reduces photoassimilates and concentration of C_i (Baczek-Kwinta et al. 2010) leading to Rubisco deactivation (Chaves and Oliveira 2004). Decrease in C_i in our study supports the hypothesis that the reduction in Rubisco

activity may be a result of stomatal closure. As compared to LR, higher decreases in Rubisco activity after PLR indicate that the LR mechanism may preserve activity of CO_2 fixation enzymes.

Drought stress causes reductions in maize development and growth (SHIRAZI et al. 2011). XIAO et al. (2005) and TI-DA et al. (2006) reported that cob characters especially number of kernels of corncob, ear weight and 100-kernel weight were decisive factors for maize yield loss under drought stress. Decreases in kernel weight of corn cultivars subjected to drought were also observed previously (KHALILY et al. 2010). Decreases in ear weight and 100-kernel weights at PLR indicated that LR could be a mechanism to reduce yield loss in our study.

In conclusion, leaves were rolled in drought tolerant maize cultivars later than in sensitive cultivars under drought stress. The results showed that monitoring of LR would be useful for the early detection of drought stress. Significant decreases in Chl fluorescence parameters of PLR plants compared to LR implied that LR might protect the photosynthesis under severe drought. Also, the study showed that LR allowed photosynthesis to occur by protecting PSII efficiency and Rubisco activity under severe drought by maintaining leaf water content. 100-kernel and ear weights at LR were less affected by drought as compared to PLR. LR might also be important for maize yield under drought stress. Therefore, we suggest that maize cultivars with late LR may be planted in order to avoid yield losses resulting from drought.

Acknowledgements

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