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Physiological response of maize (*Zea mays* L.) to high temperature stress

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Abstract

This study was conducted to investigate physiological response of maize to high temperature stress. Field trial was carried out in a randomized block design with three replications during the second crop season of the year 2008 in Sanliurfa, Turkey. RI, chlorophyll content, and *Chla/b* were measured in laboratory. LT, DF, and GY were measured in field trial. The relations among the investigated characteristics were evaluated by correlation analysis. We found that early mature varieties had higher values with regard to chlorophyll content and GY compared to late mature varieties. However, early mature varieties had lower values with regard to RI, LT, and DF than late mature varieties. Correlation analysis revealed significant relations among the characteristics measured. It was concluded that early mature varieties having lower LT and RI levels compared to late mature varieties might be recommended as second crop for the region with extremely high temperatures during the second crop season.

Keywords: *Zea mays*; chlorophyll content; high temperature stress; cell membrane stability; grain yield.

Abbreviations; GAP-Southeastern Anatolian Project; CMS-cell membrane stability; LT-leaf temperature; RI-relative injury; *Chla*-chlorophyll a; *Chlb*-chlorophyll b; GY-grain yield; DF-days to flowering; *Chla/b*-chlorophyll a to chlorophyll b ratio; EC-electrical conductivity.

Introduction

Turkey had 4.25 million tons of grain maize production in the year 2009 and 15 % of which comes from Southeastern Anatolia Region and 8 % of which comes from Sanliurfa province of the region. Silage maize production of Turkey was 11 million tons and 4 % of which comes from Southeastern Anatolia Region and 2 % of which comes from Sanliurfa province of the region (Oktem and Oktem, 2009). Harran Plain has the most fertile lands of the region and has an irrigated land area of 142 000 hectares. Maize should definitely be placed within crop rotation as the second crop over the irrigated lands of GAP area. Maize has a high yield capacity in the region and is suitable as the second crop after cereals and lentil (Kun, 1992). Exposure of plants to high temperatures causes irreversible changes in membrane structure of the plants (Nguyen and Joshi, 1992) and cell membrane instability (Yildiz and Terzi, 2007). The cell membranes are thought as the first structures physiologically damaged by high temperatures (Blum, 1988). Active oxygen species (singlet oxygen, super oxide, hydrogen peroxide and hydroxyl radical) formed as high temperature response of the plant get into reaction with membranes and cause changes in functions of these structures (Francisco et al., 2002; Diego et al., 2003). Under normal growth conditions, active oxygen species are produced by membrane-related oxidase (Desikan et al., 1996) and electron transport chain (Shewfelt and Purvis, 1995). Cell membrane stability was decreased by cell wall stretch (Khadem et al., 2010). High temperature stress impairs the flow of carbon assimilation processes in cereals and causes distinctive yield loses (Stone, 2001). Cell membrane stability test is a convenient test to trace the high temperature tolerance of the plants. It measures electrolyte leakage from high temperature-damaged leaves (Sullivan, 1972). Chlorophyll biosynthesis is affected by exposure to high temperatures (Havaux, 1993, 1998). Photosynthetic activity is decreased due to slow down of chlorophyll biosynthesis with high temperatures (Hodgins and van Huystee, 1986). It was stated that chlorophyll accumulation capacity of wheat decreases at temperatures over 35°C (O'Mahony et al., 2000). It was also indicated that differences among net photosynthesis ratios of wheat varieties exposed to high temperatures were related to changes in chlorophyll a to chlorophyll b ratio due to low chlorophyll concentrations and rapid leaf senescence (Harding et al., 1990; Yildiz and Terzi, 2007). Net photosynthesis is inhibited in C_4 plants when the leaf temperature exceeds 38°C (Berry and Bjorkman, 1980; Edwards and Walker, 1983). Increase in cell membrane damage may inhibit the mobility of water, ions and soluble organic solids within plant cell membranes, and consequently, carbon production, transport and accumulation may be effected from these conditions (Christiansen, 1978). Cereals generally respond to high temperatures with an increase in grain growth ratios due to decrease in dry matter accumulation period (Zakaria et al., 2002). Decrease in dry matter accumulation may be caused by production of grains not fully filled and smaller than normal ones (Yoshida, 1981; Tashiro and Wardlaw, 1991). Although high temperatures affect nitrogen and carbon flow to grain, carbon flow is more sensitive against high temperatures than nitrogen flow (Triboi and Triboi-Blondel, 2002). A high correlation coefficient (r=0.926) between yield and photosynthetic rate in sweet maize under Mediterranean conditions was reported by Efthimiadou et al. (2010). This study was conducted to investigate physiological responses in early development stage of maize to high temperature stress. Maize can't be produced as a main crop fails due to high temperature and low air humidity at the flowering stage in Harran Plain. In addition, high temperature and low air humidity cause low grain yield with missing kernels in maize (Oktem et al. 2004). Average maximum temperature is 38.56°C in July based on a 24-years meteorological data (Karipcin, 2009). Effects of high temperature to maize are very complex. Some parameters such as chlorophyll content, *Chla/b*, LT, RI, DF and their correlation with GY were investigated in ten hybrid dent maize varieties with different maturation groups in this study. These parameters were used largely in abiotic stress studies such as salt stress, drought stress. There are little studies for high temperature stress in maize with such parameters.

Results and discussion

Chlorophyll content

While there were not significant differences ($p \geq 0.05$) among varieties with regard to *Chla/b*, significant differences ($p<0.05$) were observed among varieties with regard to *Chla*, *Chlb*. Experimental results showed that *Chla* varied from 1.361 mg g^{-1} to 1.839 mg g^{-1}. *Chlb* varied from 0.325 mg g^{-1} to 0.449 mg g^{-1}. *Chla/b* varied from 3.981 to 4.519 (Fig.1). Heat shock reduces the amount of photosynthetic pigments (Todorov et al. 2003). Differences among chlorophyll content of varieties may be due to different response of varieties to high temperature stress. Hodgins and van Huystee (1986), Havaux (1993, 1998), and O'Mahony et al. (2000) reported that chlorophyll biosynthesis of plants is affected by exposure to high temperatures. *Chla/b* may be an important parameter for final grain yield of cereals. Thus differences among net photosynthesis ratios of wheat varieties exposed to high temperatures were related to changes in *Chla/b* due to low chlorophyll concentrations and rapid leaf senescence (Harding et al., 1990; Yildiz and Terzi, 2007). As reported by Wang et al. (2008), increasing the chlorophyll content in crops may be an effective way to increase biomass production and grain yield.

Relative injury and leaf temperature

Significant differences ($p<0.05$) were observed among the varieties with regard to RI and LT. RI varied from 61.38 % to 69.78 %. LT varied from 36.60 °C to 39.15 °C (Fig.2). The varieties with high LT and RI might produce lower grain yields due to effects of high temperature exposure on chlorophyll biosynthesis (Havaux, 1993, 1998) and inhibition of photosynthesis in C_4 plants at temperatures over 38°C (Berry and Bjorkman, 1980; Edwards and Walker, 1983). In maize the net photosynthesis reduced at leaf temperatures above 38 ◦C (Wahid et al. 2007). Sheikh et al. (2010) reported that differences among RI of varieties possibly caused by better maintaining of cellular membrane integrity of some varieties then others under high temperature stress. Salvucci and Crafts-Brandner (2004) reported that the progressive decrease in Rubisco activation that accompanies increasing leaf temperatures closely correlates with the extent of photosynthetic inhibition in different plant species (cotton, tobacco, spinach, jojoba, Antarctic hairgrass, and creosote bush). Ajaz and Warsi (2009) reported that leaf temperature was significantly negative correlated (-0.296) with grain yield of maize. CMS is an indicator of plant resistance to environmental stresses (Saneoka et al., 2004). CMS appears to predict reasonably well the relative heat tolerance of wheat genotypes in terms of their yield level in hot environments (Fokar et al., 1998). Therefore, RI may be used as a marker to high temperature tolerance.

Days to flowering and grain yield

Significant differences ($p<0.05$) were observed among the varieties with regard to days to flowering and grain yield. Days to flowering varied from 52.67 days to 56.67 days. Grain yield varied from 1021 kg da^{-1} to 1296 kg da^{-1} (Fig.2). Similar results for grain yield for maize under the similar environmental conditions were found by Oktem and Oktem (2003, 2009) in Turkey, Sharief et al. (2009) in Egypt, Khadem et al. (2010) in Iran. Differences among grain yield of varieties may be due to a decrease in carbon accumulation (Christiansen, 1978; Triboi and Triboi-Blondel, 2002) or a decrease in dry matter accumulation period (Zakaria et al., 2002; Yoshida, 1981; Tashiro and Wardlaw, 1991) as a response to high temperatures. Modarresi et al. (2010) reported that high temperature significantly decreased wheat grain yield (46.63%). Early mature varieties have generally higher grain yields than late mature varieties. It may be concluded that early mature varieties provided a tolerance against high temperature stress by keeping their leaf temperatures low. Although the early mature varieties has lower yield capacities than late mature varieties (Kirtok, 1998), higher grain yields of early mature varieties bring into mind the significant impacts of high temperature stress on the grain yield.

Correlation analysis

Correlation analysis revealed positive relationships between RI and LT, DF, and *Chla/b*; between LT and DF; between *Chla* and *Chlb*; between DF and *Chla/b*. Negative correlations between GY and DF, RI, LT, and *Chla/b* were observed (Table 2). Although there was not any relationship between grain yield and *Chla* or *Chlb*, negative correlations were observed between grain yield and *Chla/b*. These results are supported by the findings of Harding et al. (1990) indicating that differences among net photosynthesis ratios of wheat varieties exposed to high temperature stress was related to changes in *Chla/b* due to low chlorophyll concentration and rapid leaf senescence. Ajaz and Warsi (2009) reported that leaf temperature was significantly negative correlated (-0.296) with grain yield of maize. Ahsan et al. (2008) determined that CMS had negative direct effect on grain yield of maize. Positive correlations were also reported between membrane thermo stability and grain yield in seedling stage (Reynolds et al. 1994, Yildirim et al. 2009) and at flowering-early milk stage (Shanahan et al. 1990, Dhanda and Munjal 2006, Yildirim et al. 2009) in wheat under heat stress. It was also indicated that differences among net photosynthesis ratios of wheat varieties exposed to high temperatures were related to changes in *Chla/b* due to low chlorophyll concentrations and rapid leaf senescence (Harding et al., 1990; Yildiz and Terzi, 2007). A high correlation coefficient ($r=0.926$, $p<0.001$) between yield and photosynthetic rate in sweet maize under Mediterranean conditions was reported by Efthimiadou et al. (2010). Therefore, our results provide negative correlation between grain yield and *Chla/b*. In temperate regions, heat stress has been reported as one of the most important causes of reduction in yield and dry matter production in many crops, including maize (Giaveno and Ferrero 2003). High temperatures caused significant declines in shoot dry mass, relative growth rate and net assimilation rate in maize, though leaf expansion was minimally affected (Ashraf and Hafeez 2004, Wahid 2007). In conclusion, based on the findings, DF, RI, LT, and *Chla/b* may be used as physiological markers for early yield estimation in maize grown under

Table 1. Some characteristics of maize varieties used as the plant materials in the study.

Commercial name	Hybrid form	Grain form	Days to grain maturation
Cadiz	Single hybrid	Dent	130-135
TTM 815	Single hybrid	Dent	130-135
Ada 523	Single hybrid	Dent	130-135
Rx 9292	Single hybrid	Dent	125-130
Özgem	Single hybrid	Dent	120-130
Truva	Single hybrid	Dent	115-120
Mataro	Single hybrid	Dent	110-115
DK - 626	Single hybrid	Dent	105-110
Ada 8924	Single hybrid	Dent	100-105
P. 3394	Single hybrid	Dent	100-105

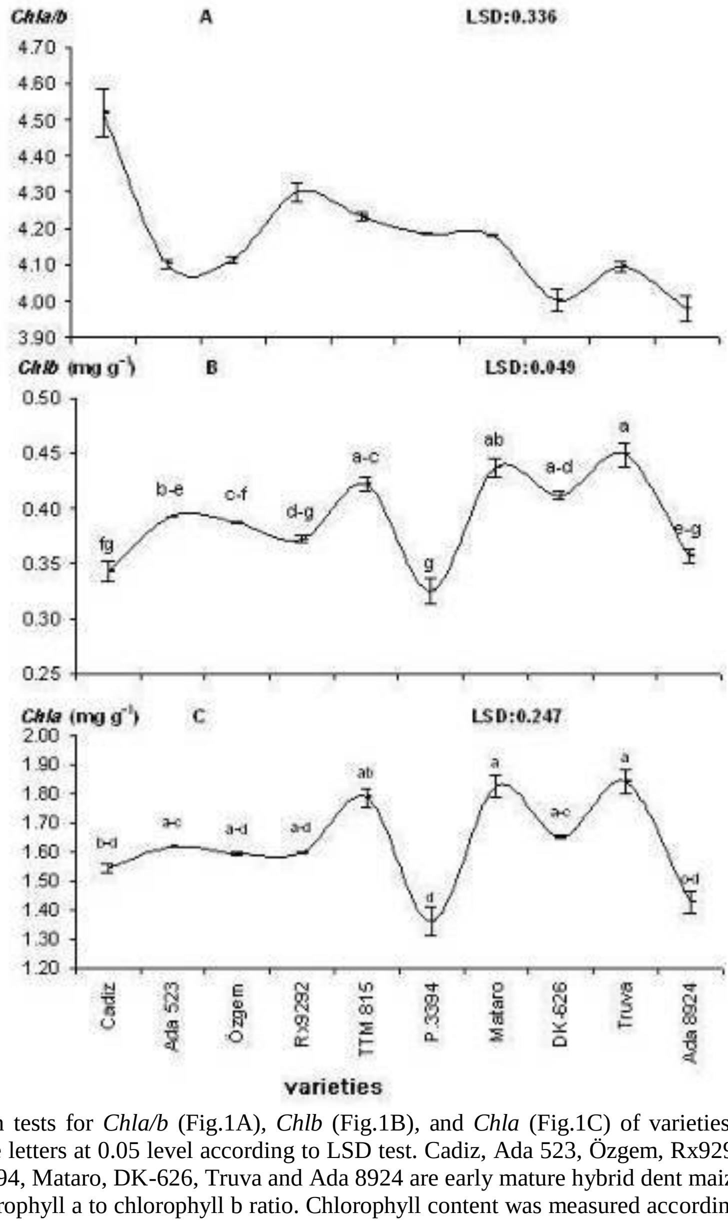

Fig 1. LSD multiple comparison tests for *Chla/b* (Fig.1A), *Chlb* (Fig.1B), and *Chla* (Fig.1C) of varieties used in the study. There is no statistical difference among same letters at 0.05 level according to LSD test. Cadiz, Ada 523, Özgem, Rx9292 and TTM 815 are late mature hybrid dent maize varieties. P.3394, Mataro, DK-626, Truva and Ada 8924 are early mature hybrid dent maize varieties. *Chla*-Chlorophyll a; *Chlb*-Chlorophyll b; *Chla/b*-chlorophyll a to chlorophyll b ratio. Chlorophyll content was measured according to method of Arnon (1949) by using young leaf samples (6^{th} leaf) were taken from maize plants in the morning when average air temperature was measured as 28 ^{o}C.

Table 2. Correlation coefficients for characteristics studied.

	LT	*Chla*	*Chlb*	GY	DF	*Chla/b*
RI	0.751**	0.058^{NS}	-0.129^{NS}	-0.605**	0.653**	0.456*
LT		0.035^{NS}	-0.155^{NS}	-0.545**	0.747**	0.276^{NS}
Chla			0.922**	-0.014^{NS}	-0.003^{NS}	0.346^{NS}
Chlb				0.136^{NS}	-0.160^{NS}	-0.145^{NS}
GY					-0.734**	-0.369*
DF						0.393*

^{NS}-non-significant; *-significant at 0.05 probability level; **-significant at 0.01 probability level; RI-Relative injury; LT-Leaf temperature; *Chla*-Chlorophyll a; *Chlb*-Chlorophyll b; GY-Grain yield; DF-Days to flowering; *Chla/b*-chlorophyll a to chlorophyll b ratio

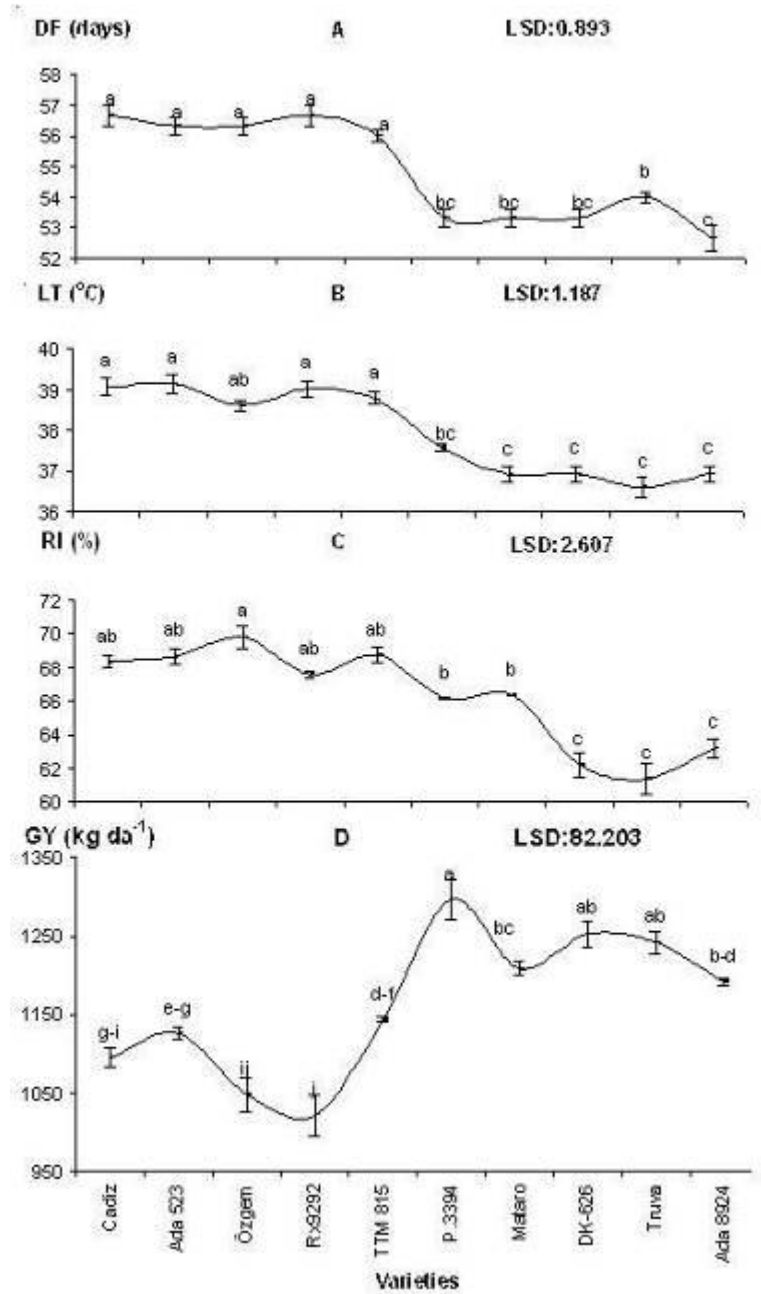

Fig 2. LSD multiple comparison tests for DF, LT, RI, and GY according to varieties. There is no statistical difference among the same letters at 0.05 level according to LSD test. Early varieties have lower DF (Fig.2A), LT (Fig.2B), RI (Fig.2C) values and higher GY (Fig.2D) values than late varieties. Earliness may be one way of escape from high temperature stress for maize. RI-relative injury; LT-leaf temperature; GY-grain yield; DF-days to flowering. RI was measured according to method of Sullivan (1972) by the using young leaf samples (6th leaf) were taken from maize plants in the morning when average air temperature was measured as 28 ^{o}C. LT of three plant samples from each plot (three plots) were measured Fluke 62 mini IR Thermometer (Fluke Co.; China) with a 30^{o} angle from the upper surface of sixth leaf of plant during noon hours under the field conditions when average air temperature was measured as 43 ^{o}C.

high temperature conditions. Also early mature varieties might be considered as second crop season because they had lower LT and RI levels than late mature varieties for the region with extremely high temperatures. Further investigations may be necessary for the different developmental stages.

Materials and methods

Plant material and experimental area

Some characteristics of maize varieties used as the plant materials in the study were taken in Table 1. Field trial was carried out in Harran Plain of Turkey in 2008. The research area has alluvial, deep, almost smooth Harran serial soils. Typical reddish profiles have clay texture. Entire profile contains lime, pH varies between 7.3-7.8, organic material content is low and cation exchange capacity is high. Annual total precipitation is 365.2 mm, average annual temperature is 17.2 ^{o}C, the highest temperature is 46.8 ^{o}C, and the lowest temperature is -16.8 ^{o}C based on a 24-years meteorological data. Average temperatures during the experiment (July, August, September, October) were 30.0, 29.5, 23.1, 18.0 ^{o}C, average maximum temperatures were 39.8, 38.7, 37.6, 34.8 ^{o}C, and average minimum temperatures were 21.1, 21.4, 19.9, 17.7 ^{o}C, respectively (Karipcin, 2009).

Experimental design and agronomic applications

Randomized block design with three replications was used for field experiments. Plot sizes were arranged as 5 m × 0.7 m × 4 rows = 14 m^2 (on-row spacing is 20 cm and inter-row spacing is 70 cm) during plantation and as 5 m × 0.7 m × 2 rows = 7 m^2 during harvest season. Seeds were planted on 7th July 2008. Fertilization was carried out a day before and entire phosphorous and partial nitrogen (based on 10 kg da^{-1} pure nitrogen and 10 kg da^{-1} pure phosphorous) were applied as composed fertilizer form (20 % pure N + 20 % P_2O_5) by over spraying and mixed into the soil with proper machinery. Rest of nitrogen (15 kg da^{-1} pure nitrogen) was applied in NH_4NO_3 form (33 % pure N) before neck filling. Border strip irrigation was applied eight times based on plant water requirements. Chemicals (Foramsulfuron 22.5 g l^{-1} and Carbofuran5%) were applied for weeds and insects. Harvest was performed on 17th November 2008 for grain yield.

Description of relative injury

Young leaf samples (6th leaf) were taken from top of the maize plants for the determination of relative injury in the morning when average air temperature was measured as 28 ^{o}C (average of 5 measurements with 5 minutes intervals). Samples were taken from three plants from each plot (three plots). Samples were rapidly transferred to laboratory in 4 °C ice bags for protect to leaf natural moisture. Relative injury was determined according to the method of Sullivan (1972). For this purpose, a total of ten leaf discs (flakes) with a diameter of 10 mm were taken from both sides of center line of leaf blade as to form two sets of sample. One set (five samples) was used for heat treatments and the other was used for control treatments. Leaf discs were washed 2-3 times with deionized water and strained samples were placed into glass tubes with 2 ml deionized water. Tubes were closed to prevent water loss. Tubes containing high temperature-treatments were placed into hot water bath adjusted ahead to 50 ^{o}C for 1 hour and control treatment samples were kept at room temperature (24-25 °C). Then, 10 ml deionized water was added to each tube and samples were kept at 10 ^{o}C for 24 hours to provide electrolyte leakage. After that, the samples were shaken on a shaker until they reach to room temperatures (24-25 °C). Electrical Conductivity (EC) of samples was measured by using the “HI 98311 EC/TDS/Temperature Tester” (Hanna Inst., USA). Following the first measurements, samples were autoclaved under 0.1 Mpa pressure for 15 minutes. They were brought to room temperatures again and second EC measurements were performed. Relative injury was calculated in accordance with (Sullivan, 1972) by using Equation 1 and 2;

$$CMS\% = \left[1 - \frac{1 - \left(\frac{T_1}{T_2} \right)}{1 - \left(\frac{C_1}{C_2} \right)} \right] \times 100 \qquad (1)$$

$$RI\% = 100 - CMS \qquad (2)$$

Where T_1 and C_1 represent the EC measured after incubating at 10 ^{o}C for 24 h, and T_2 and C_2 represent the total EC measured after autoclaving the leaf tissues.

Measurements of chlorophyll content and leaf temperature

Young leaf samples (6th leaf) were taken from maize plants for the determination of chlorophyll content in the morning when average air temperature was measured as 28 ^{o}C (average of 5 measurements with 5 minutes intervals). Leaf samples were taken from three plants from each plot (three plots). Chlorophyll content of samples was observed by using the method specified by Arnon (1949). Leaf temperatures of three plants from each plot (three plots) were measured by the infrared thermometer (Fluke 62 mini IR Thermometer by Fluke Co.; China) with a 30^{o} angle from the upper surface of sixth leaf of plant during noon hours under the field conditions. Average air temperature during leaf temperature measurements was measured as 43 ^{o}C (average of 5 measurements with 10 minutes intervals).

Statistical analysis

Least significant difference (LSD) multiple comparison test at the 0.05 probability level and correlation analysis were performed by using JMP 5 statistical software (JMP, 2002).

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