Association of Some Photosynthetic Characteristics with Canopy Temperature in Three Cereal Species under Soil Water Deficit Condition

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ABSTRACT

Canopy temperature (CT) is used as a selection tool to improve crop adaptation to drought. The aim of this work was to investigate association of some photosynthetic characters with CT during grain filling in three cereal species. For this objective, a two-year study (2009-2011) was carried out in Kurdistan Province in western Iran. Four genotypes of triticale, three cultivars of bread wheat, and a new variety of barley were compared under well watered (WW, \( \Psi_{\text{soil water}} = -3 \text{ bar} \)) and deficit water (DW, \( \Psi_{\text{soil water}} = -12 \text{ bar} \)) conditions in a randomized complete block design (RCBD) with a split-plot arrangement. Compared with well-watered plants, water stressed plants displayed 1.44°C higher canopy temperature irrespective of plant species. Under water stress condition, the high yielding genotypes had lower canopy temperature than low yielding ones by 1.23°C. Results showed that under water deficit condition triticale relative to commercial cultivar of wheat and barley had a higher adaptability to drought as indicated by its higher yield (59.5 g m\(^{-2}\) more than average) and lower CT (0.28°C lower than average). The main physiological traits correlated with canopy temperature under DW treatment were stomatal conductance \((g_s)\) \((r = -0.73^*)\), photosynthetic rate \((P_n)\) \((r = -0.76^*)\), the maximal quantum yield of primary photochemistry \(\left( \frac{F_v}{F_m} \right)\) \((r = -0.71^*)\), chlorophyll content at grain filling \((r = -0.72^*)\) and leaf temperature \((L_T)\) \((r = 0.82^{**})\). Thus, canopy temperature seems to play an important role in the physiological basis of grain yield in different cereal species, and can be used as a selection tool in breeding programs under water deficit condition in Iran.

Keywords: Barley, Chlorophyll fluorescence, Drought stress, Net photosynthetic rate, Triticale.

INTRODUCTION

Canopy temperature (CT) is a non-destructive monitoring parameter of whole-plant used to evaluate plant response to environmental stresses including drought (Rashid et al., 1999; Royo et al., 2002). Blum et al. (1989) and Balota et al. (2007) have proposed low CT as a selection tool for drought tolerance. In contrast to leaf stomatal conductance \((g_s)\), canopy temperature has higher potential value as it can be used at the whole plant or canopy level (Munns et al., 2010).

In general, CT is an indicator of plant water status because it involves many physiological responses to drought and heat (Amani et al., 1996). Depending on crop species and environment, several mechanisms have been attributed to cooler canopy. Under favourable environments, the main physiological and morphological traits that contribute to low CT

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are stomatal conductance ($g_s$), crop water use (Amani et al., 1996; Fischer et al., 1998; Hura et al., 2007; Reynolds et al., 1994), assimilate translocation (Reynolds et al., 1994) and leaf area index (Ayeneh et al., 2002). In drought conditions, smaller leaf sizes with higher thickness (Balota et al., 2008), photosynthetic enzymes activity (Burke et al., 1988; Wanjura et al., 1995) and root traits (Lopes and Reynolds, 2010; O’Toole et al., 1998) have major roles in keeping lower canopy temperature. As explained by Reynolds (2002), high canopy temperature depression (CTD) or low CTD may be indicative of a high demand for photo-assimilation caused by many rapidly filling kernels (i.e. sink strength) in physiologically well-adapted lines, higher metabolic capacity, and a good vascular system capable of meeting evaporative demand.

Since physiological traits related to low CT under water-limited environments of Iran are not clearly known, understanding of these traits may help breeders for indirect selection of high or low yielding genotypes by using CT and its related traits among large number of germplasms. Therefore, this study was carried out to find out the main physiological traits contributing to cool canopy, and to evaluate triticale as a new species in comparison with wheat and barley in this regard.

**MATERIALS AND METHODS**

**Plant Materials and Plant Growth Conditions**

The experiment was conducted at the experimental fields of Grizheh Station in Kurdistan Province, west of Iran, during two consecutive growing seasons (2009-2011). The experimental design was a randomized complete block design (RCBD), with a split-plot arrangement of treatments. Two water regimes were allocated to the main plots and eight genotypes of triticale, bread wheat, and barley were in the subplots. The water regimes were a well-watered (WW) treatment in which irrigation water was applied when soil water potential was -3 bar ($\Psi_{\text{soil water}} = -3$ bar), and a deficit water (DW) treatment that was irrigated when soil water potential was -12 bar ($\Psi_{\text{soil water}} = -12$ bar). Soil water potential was set based on a soil moisture retention curve (pF).

Plots were replicated three times. During rainy days, a mobile rain shelter for each block (10x6x2.5 m) was used in the DW treatment to prevent the infiltration of the rain. Thus, the DW soil moisture was approximately kept at –12 bar. To limit the lateral movement of water into DW plots, a drainage ditch dug around the rain shelter. A drip irrigation system with drip lines between the rows was used. Irrigation was applied from the developmental stage of stem elongation onwards.

Four hexaploid genotypes of triticale including ARDI-1/TOPO14//ERIZO-9CTY87352, Juanillo92, RONDO/BANT_5//ANOAS_2/3/VICUNA_4 and SRIER-29/FARS-1//MANATI-1 (originated from CIMMYT and labelled as T1, T2, T3 and T4, respectively), three commercial bread wheat cultivars (Alvand, Pishgam, and Zarrin), and a new variety of barley (Bahman) were used for monitoring physiological responses to different soil moisture regimes. Standard cultural practices were implemented, including the usual sowing rate, fertilizer application and post-emergence herbicide application, followed by hand-hoeing where necessary to control weeds. Each plot consisted of 6 rows, 20 cm apart, with 450 seeds per square meter.

**Grain Yield**

In each treatment, grain yield (GY), thousand kernel weight (TKW), grain per spike (Grain Spike$^{-1}$) and spike per square meter (Spike m$^{-2}$) were measured.

**Canopy Temperature (CT)**

A handheld infrared thermometer (Model AZ 8866, Taiwan) was used to measure CT ($^\circ$C) around grain filling stage (Zadoks 71-
TraitsRelatedtoCanopyTemperatureinCereals

75). The data was taken from the same side of each plot at 1-m distance from the edge and approximately 50 cm above the canopy at an angle of 30° to the horizontal (Bilge et al., 2008). Readings were made between 12:00 and 14:00 hours on five sunny days. Data obtained are the means of five measurements taken during grain filling on cloudless days with no or low wind.

LeafGasExchanges

Leaf gas exchange including leaf temperature (LT), stomatal conductance (g_s) and net photosynthetic rate (P_n), were measured on three labelled, fully expanded flag leaves around grain filling (at 5- to 15-day after grain filling) using a portable gas exchange system (IRGA. ADC, LCA4. UK).

ChlorophyllFluorescence

The maximal quantum yield of primary photochemistry or the ratio of variable to the maximal fluorescence of dark-adapted leaves (F_v/F_m) as the main parameter of chlorophyll fluorescence was measured using a Plant Stress Meter (Handy PEA V1.3, UK) for 2009-10 and a fluorometer (PAM 2000, Walz, Germany) for 2010-2011 as described by Grzesiak et al. (2003) and Subrahmanyam et al. (2006). Before measuring, the leaves were dark adapted for 25 min to relax all energy-dependent fluorescence quenching.

ChlorophyllContent

A hand-held meter (SPAD 502, Konica, Japan) was used to measure leaf greenness (SPAD unit) at three growth stages including heading, anthesis, and grain filling. This instrument provides a convenient means of assessing relative leaf chlorophyll concentration or leaf greenness. Five flag leaves were used to take chlorophyll meter readings from each plot at three growth stages (heading, anthesis, and grain filling) and the data presented are the means of the five readings.

LeafTraits

A leaf area meter (APEX-CJ589, Henan, China) was used to determine flag leaf area (FLA). After measuring leaf dry weight, specific leaf area (SLA) was calculated (cm² g⁻¹).

StatisticalAnalysis

The analysis of variance (ANOVA) was performed using SAS (version 9.1, 2005 for windows) software. Least significant differences (LSD) at probability levels of P = 0.01 and 0.05 were calculated. Correlation analysis was conducted to relate grain yield and CT to each of the traits.

RESULTS

The total rainfall in the 2009-2010 and 2010-2011 crop growing seasons was 455 and 351 mm, respectively. The rainfall pattern showed a historically normal variability during the two growth seasons (Figure 1). With rainfall uniformly distributed, there was no increase in competition for limiting resources (mainly water) during the vegetative and early reproductive growth stages, except in DW treatment, in which the plants were shielded from rainfall by rain shelter. Average temperature during grain filling (early June) was the same for both years as shown in Figure 1.

GrainYieldandItsComponents

The combined ANOVA showed significant main effects of year, water treatment, genotype and genotypetreatment (GxT) interactions for GY, TKW, Grain Spike¹, and Spike m⁻² (Table 1). Average
Figure 1. Monthly rainfall and mean temperature during two growing seasons (2009-2011) in Grizah research station.

Grain yield of the two years under imposed water deficit and well-watered treatments were 399.4 and 713.2 g m$^{-2}$, respectively (Table 1). Triticale genotypes produced more grain yield than wheat and barley by 18 and 13% in WW and 24 and 36% in DW treatments, respectively. However, based on grain yield in both treatments, the genotypes were grouped into high yielding (mean grain yield more than the average) and low yielding (mean grain yield lower than average). On this basis, the genotypes T2, T3, T4 and Pishgam produced more grain and were designated as the high yielding group, while the genotypes Alvand, Zarrin and Bahman with lower grain yield than average were in the low yielding group.

Components contributing to the final yield in our study varied with genotype and treatment. On average, reduction in TKW and Grain Spike$^{-1}$ was higher in low yielding genotypes than the high yielding ones, by 20 and 4%, respectively, while for Spike m$^{-2}$, this superiority was negligible.

**Canopy Temperature**

Average $CT$ in WW and DW treatments was 17.74 and 16.33°C, respectively. Therefore, Compared with well-watered, water stressed plants displayed warmer canopies by 1.44°C (Table 1). The difference between the coolest genotype i.e. Pishgam, and the warmest genotype, Zarrin, was 2.1°C under DW treatment. This difference decreased to 1.33°C in WW treatment. Increasing $CT$ in high yielding genotypes was nearly 1.23°C fewer degrees than the low yielding ones. Under DW condition, the low yielding genotypes, Alvand, Zarrin and Bahman, showed warmer canopies by 0.68°C over the average, while the high yielding genotypes had cooler canopy by 0.41°C below the average. However, $CT$ showed negative relationship with GY ($r = 0.77$, $P<0.05$), TKW ($r = 0.79$, $P<0.05$) and Grain Spike$^{-1}$ ($r = 0.74$, $P<0.05$) under DW conditions (Table 1). On average, triticale had lower $CT$ in comparison with wheat and barley.

**Leaf Gas Exchanges**

Water deficit decreased flag leaf stomatal conductance ($g_s$), in all genotypes by 41% (Table 1). The greatest decrease was for Bahman and Zarrin (54% in both) and the lowest for T1 (22%). T1 had the lowest $g_s$ (135 µmol m$^{-2}$ s$^{-1}$) in WW and intermediate in DW conditions (105 µmol m$^{-2}$ s$^{-1}$). Even though the high yielding genotypes showed low $g_s$ under WW, they achieved more $g_s$ by 32 µmol m$^{-2}$ s$^{-1}$ under DW conditions. The coolest genotype, Pishgam had the highest $g_s$ (155 µmol m$^{-2}$ s$^{-1}$), while the warmest genotype, Bahman, had the lowest (65 µmol m$^{-2}$ s$^{-1}$) under DW conditions. Negative
Table 1. Average values and analysis variance of two growing seasons for grain yield (GY), thousand kernel weight (TKW), grain per spike (Grain Spike⁻¹), canopy temperature (CT), stomatal conductance ($g_s$), net photosynthetic rate ($P_n$) and leaf temperature (LT) in four genotypes of triticale (T1, T2, T3 and T4), three wheat cultivars (Alvand, Pishgam and Zarrin), and one barley cultivar (Bahman) under two water regimes (well watered, WW and deficit water, DW).

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<th>Spike $\text{m}^{-2}$</th>
<th>CT $\text{C}^\circ$</th>
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$^a$ ARDI-1/TOPO1419/ERIZO-9CTY787352; $^b$ Juaanile92; $^c$ RONDO/BANT_5//ANOAS_2/3/VICUNA_4; $^d$ SRIER-29/FARS-1//MANATI-1; $^e$ Correlation with Canopy Temperature; $^f$ Correlation with Grain Yield. *: Significant at 5% level of probability; **: Significant at 1% level of probability; ns: Not significant
relationships were found between CT and gn in both WW and DW conditions (Table 1). Water deficit reduced Pn in all genotypes in both years. This reduction ranged from 11% for T1 to 55% for T2. However, the rate of reduction in triticale was lower than wheat, with the exception of T2. Although high yielding genotypes showed higher values of Pn under WW condition, excluding T2, they had lower reduction by DW. We could not find a significant correlation between Pn and grain yield under any WW and DW regimes (Table 1) Nonetheless, under DW conditions, the data revealed negative relationship between Pn and CT.

In both years, the Fv/Fm ratio sensitivity to DW was less than Pn and gn (Table 2), as indicated by its lower reduction by DW. However, under both WW and DW conditions a significant variation was observed among genotype, moisture treatment, and their interaction (Table 2). Overall, under DW condition Pishgam and Bahman had the maximum and minimum Fv/Fm values, respectively. A significant relationship was found between Fv/Fm and Pn under DW treatment (Figure 2-a). Moreover, negative relationships were observed among Fv/Fm, CT (Table 2) and LT (Figure 2-b)

Chlorophyll Content

For all genotypes SPAD unit increased slowly from heading to anthesis, but it sharply decreased after anthesis and during the grain filling period (Table 2). On average, the rate of reduction in the DW treatment was twofold (15.1% against 7.2% in WW). Under DW treatment, Pishgam had the highest chlorophyll content (47 SPAD units) at the grain filling stage. The destruction of chlorophyll content by stress regime was more in Bahman than T2 and Zarrin at the same stages of development. On average, the high yielding genotypes had more chlorophyll content at all three growth stages. Under DW significant correlations between chlorophyll content at grain filling stage with Fv/Fm and Pn were observed (Figures 2-c and -d), but these relations were not observed in the other two stages. A negative relationship between CT and chlorophyll content was found at grain filling stage (Table 2).

Flag Leaf Area and Specific Leaf area

On average, water deficit decreased flag leaf area (FLA) and specific leaf area (SLA) by 5.44 cm² and 15.32 cm² g⁻¹, respectively. Compared with other germplasms, Pishgam and Zarrin showed significantly (P< 0.05) higher FLA (Table 2) under WW and DW conditions, respectively, while Bahman had significantly (P< 0.01) smaller FLA under both WW and DW treatments (16.1 and 13.5 cm², respectively). Under WW treatment, by increasing FLA, CT decreased as shown in Table 2.

SLA varied from 150 to 195 cm² g⁻¹ under WW and 131 to 175 cm² g⁻¹ under DW, with the minimum and maximum belonging to T2 and Bahman, respectively.

DISCUSSION

The genotypes with low CT, including T2, T3, T4 and Pishgam, produced 112 g m⁻² more grain yield, suggesting that the cooler canopy resulted in better adaptation to water stress. Lopes and Reynolds (2010) reported that the wheat lines with low CT in grain filling produced more grain yield by 80 g m⁻². As reported by Olivares-Villegas et al (2007), cooler canopy temperature explained over 60% of yield variation in wheat populations. The negative correlation between CT and grain yield under DW condition clearly indicated that increase in CT was accompanied with yield reduction because plants could not keep adequate transpiration rate (Rashid et al., 1999). In fact, low CT induced avoidance of drought stress by sustaining turgor and physiological activity (Olivares-Villegas et al., 2007).
Table 2. Average values and analysis variance of two growing seasons for the maximal quantum yield of primary photochemistry ($F_v/F_m$), Chlorophyll content at heading, anthesis and grain filling stages, flag leaf area (FLA) and specific leaf area (SLA) in four genotypes of triticale (T1, T2, T3 and T4), three wheat cultivars (Alvand, Pishgam and Zarrin), and one barley cultivar (Bahman) under two water regimes (well watered, WW, and deficit water, DW).

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Year    | **  | **  | **  | **  | **  | **  | **  | **  |
Treatment (T) | **  | **  | **  | **  | **  | **  | **  | **  |
Genotype (G)  | **  | **  | **  | **  | **  | **  | **  | **  |
GxT       | ns  | ns  | ns  | ns  | ns  | ns  | ns  | ns  |

*a* ARDI-1/TOPO1419/ERIZO-9CTY87352; *b* Juanille92; *c* RONDO/BANT_5//ANOAS_2/3/VICUNA_4; *d* SRIER-29/FARS-1//MANATI-1; ‘Correlation with Canopy Temperature;’Correlation with Grain Yield. *a*, **: Significant at 5 and 1% level of probability, ns: Not significant.
Figure 2. Simple correlation between photosynthetic rate ($P_n$), and the maximal quantum yield of primary photochemistry: $F_v/F_m$ (a); leaf temperature, $LT$ and $F_v/F_m$ (b); $F_v/F_m$ and chlorophyll content at grain filling (c); $P_n$ and chlorophyll content at grain filling (d); $LT$ and $g_s$ (e) and, $g_s$ and $P_n$ (f) under water deficit ($Ψ_{soil\,water} = -12$ bars) condition. Significant correlations are indicated by *: $P<0.05$, and **: $P<0.01$.

Many reports have demonstrated the correlation of CT and grain yield in wheat (Winter et al., 1988; Royo et al., 2002). Further, studies in other crops, including cotton (Hatfield et al., 1987) and durum (Giunta et al., 2008) have indicated that genotypes with warmer CT, implying more-closed stomata, produced the highest yields. However, based on the number of genotypes, soil moisture status, and time and
number of $CT$ measurements, some discrepancies for association of $CT$ and grain yield (from negative to no or positive association) have been reported (Rebetzke et al., 2013).

Leaf stomatal conductivity ($g_v$) declined in response to DW treatment resulting in an increase in $LT$ and $CT$. Transpiration results from evaporation of water from within the leaf interior. Variation in $g_v$ contributes to differences in transpiration and subsequent changes in leaf cooling (Rebetzke et al., 2013). Genotypic variation in $g_v$ has potential in selection for improved adaptation to a broad range of growing conditions. A strong negative correlation was found between $LT$ and $g_v$ (Figure 2e) under both WW and DW conditions that is in agreement with the findings of others (Baker et al., 2007, Izanloo et al., 2008, Pasban Eslam, 2009). In addition, a positive correlation was observed between $CT$ and $LT$ ($r= 0.84, P< 0.01$) which was in agreement with the results of Nippert et al. (2009). A significant correlation between $CT$ and $g_v$ under DW condition ($r= -0.75, P< 0.05$) suggests that the genotypes can extract more water from the soil and have the ability to open their stomata, resulting in higher transpiration and lower $CT$. This is mainly due to high soil moisture uptake by deep root systems (Blum, 2009). Lopes and Reynolds (2010) found a significant negative correlation between $CT$ and root dry weight at grain filling stage, a sign of higher capacity of root to absorb soil water for maintenance of transpiration (Blum, 2009). Rebetzke et al. (2013) reported a large additive genetic correlation between $CT$ and leaf porosity. They concluded that the more rapid $CT$ assessment may be of greater value for indirect screening of high or low $g_v$ among large numbers of early-generation breeding lines. There are different reports for associations of $CT$ and $g_v$. For example, Amani et al. (1996), Pinter et al. (1990), and Fischer et al. (1998) reported phenotypic correlations of 0.60-0.76, 0.92, and 0.85, respectively, for $CTD$ and $g_v$. These estimates contrast with other reports (e.g. Araghi and Assad, 1998; Giunta et al., 2008) where the association of $CT$ or $CTD$ with $g_v$ was small and not statistically significant.

Strong positive correlation between $P_n$ and $g_v$ under DW (Figure 2-f) suggests that the stomatal limitation is a major factor in limitation of photosynthesis under mild drought stress conditions. In fact, $g_v$ affects the rates of diffusion of CO$_2$ into the leaf for photosynthesis and transpiration of water vapour out of the leaf (Rebetzke et al., 2013).

In this study, the reduction of $g_v$ by DW in triticale was less than wheat and barley (33.5%, 47.5% and 54.3%, respectively) indicating the better acclimation of triticale to stomatal limitation caused by water stress (Roohi et al., 2013). $F_{v}/F_{m}$ had lower reduction under DW than $g_v$ (7.26 % against 41%, respectively). Many reports demonstrated on lower susceptibility of $F_{v}/F_{m}$ than both $P_n$ and $g_v$ under drought conditions (Nippert et al., 2009; Resco et al., 2008). However, chlorophyll fluorescence under water limiting condition acts as a non-stomatal factor in limiting photosynthesis apparatus (Hura et al., 2007). The negative relationships among $F_{v}/F_{m}$, $CT$ ($r= 0.85, P< 0.01$) and $LT$ (Figure 2-b) suggest that any increase in leaf temperature by water stress leads to warming the canopy and reducing the $F_{v}/F_{m}$ and photosynthesis activity.

The results of chlorophyll content measurements during plant growth indicate that a cultivar with high chlorophyll content seems to stay green longer. More reduction of chlorophyll content (SPAD unit) under DW is a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Anjum et al., 2011). The reduction of chlorophyll content by drought reported in many species (Guerfel et al., 2009; Manivannan et al., 2007). The significant correlation between chlorophyll content at grain filling and $P_n$ (Figure 2-d) suggest that loss of chlorophyll content under water stress can be considered as a main cause of inactivation of photosynthesis (Anjum et al., 2011). Data also showed reduction of chlorophyll content at grain filling stage under water stress, indicating that increase in $CT$ because of chlorophyll loss is a symptom of inability to access water, which induces stomata closure and increases leaf temperature. Similar result is reported by Feng et al. (2009). However, a longer duration of leaf photosynthetic activity indicated by maintaining leaf greenness has contributed to yield in most major crops (Evans, 1993).

A negative correlation was found between $FLA$ and $CT$ under WW treatment ($r= -0.84, P<
0.01) suggesting that, under favourable environments, the leaves with higher area transpired more and showed lower CT (Ayeneh et al., 2002). Under DW, this relationship was not observed, while for SLA, excluding T2, genotypes with more SLA had warmer canopies. This result was strongly validated by low FLA and high SLA in Bahman, one of the warmest genotypes under DW treatment. Moreover, in the other two low yielding genotypes (Alvand and Zarrin) with warmer canopy, SLA was more than the average. Balota et al. (2008) reported that the genotypes with low SLA had cooler canopies under dry land conditions.

CONCLUSIONS

These results illustrated that genotypes with low CT could maintain superiority in many physiological traits. Also, our data reveal that CT is a powerful physiological tool that involves many physiological traits. Under DW condition, high $g_\text{c}$, $P_\infty$, $F/F_\infty$ and chlorophyll content at grain filling are some physiological traits that are attributed to cooler canopy. Results also showed that, under this condition, triticale related to the commercial wheat cultivars and barley had a higher adaptability to drought, as indicated by higher yield ($59.5 \text{ g m}^2$ more than the average of grain yield) and lower CT ($0.28^\circ \text{C}$ lower than the average of CT).

REFERENCES


صفات مورفولوژی و فیزیولوژی موثر در دماى سایه انداز سه گونه غلات تحت دو رژیم رطوبتی مختلف خاک


