Leaf Growth, Gas Exchange and Chlorophyll Fluorescence Parameters in Response to Different Water Deficits in Wheat Cultivars

Xiaoli Wu1,2 and Weikai Bao1

1Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041 China; 2Graduate University of Chinese Academy of Sciences, Beijing 100039, China

Abstract: We investigated responses of wet climate (CY17) and dry climate (XN889) Triticum aestivum L cultivars under 85, 55 and 25% field water capacity (FC). Less decrease in grain yield, relative water content, growth, gas exchange and chlorophyll fluorescence parameters indicated that XN889 was more drought-tolerant than CY17. At 55% FC, CY17 showed a lower net photosynthetic rate (Pn) than XN889 mainly due to stomatal closure. Stomatal closure was also observed in XN889, but its Pn was higher at 55% FC than at 85% FC. The higher Pn in XN889 may be associated with a higher chlorophyll content and resulting increase in photochemical quenching (qP), apparent electron transport rate, and effective quantum yield of photosystem II (PSII). Both cultivars showed photodamage at 25% FC, but XN889 showed less photodamage in terms of maximal PSII photochemical efficiency. XN889 showed higher qP and non-photochemical quenching than CY17, further demonstrating its superior drought tolerance.

Key words: Chlorophyll fluorescence, Gas exchange, Leaf growth, Water deficit, Wheat cultivar.

Water deficiency is one of the most common factor limiting crop production in many regions of the world (Sayed, 2001), often reducing growth of leaves and roots, stomatal conductance, photosynthesis, and dry matter accumulation (Blum, 1996). Reduced photosynthesis due to water deficit can be caused by a reduction in light interception due to smaller leaf area and specific leaf area, by a reduction in carbon fixation per unit leaf area, or by damage to the photosynthetic apparatus (Castrillo et al., 2001; Bruce et al., 2002). The effects of water deficit on leaf photosynthetic parameters have been a subject of research for over three decades (Santosi et al., 2009). Some authors reported that reduction in photosynthetic activity due to water deficit is primarily the result of stomatal closure (Shangguan et al., 1999). However, Subrahmanyam et al. (2006) concluded that water deficit inhibits photosynthesis through impaired photochemical reactions. In addition, Tang et al. (2002) argued that stomatal and non-stomatal effects on photosynthesis acted in combination, depending on the degree of drought, and even in well-hydrated plants (Yu et al., 2009). There has been considerable effort to elucidate the degree and nature of the genetic control of water use efficiency (WUE) in wheat. WUE is defined as the ratio of net photosynthesis to transpiration over a period of seconds or minutes (Wang et al., 2007). Therefore, studies on the response to water deficit of wheat cultivars of different genotypes are necessary.

In this study, we examined the leaf traits of wheat cultivars from different climatic regions in response to different water regimes, and discussed the possible differences between the two cultivars in the photosynthetic responses triggered by water deficiency. The differences between cultivars in water deficit may be used as criteria for genotype selection in different management regimes.

Materials and Methods

Two cultivars of wheat XN889 and CY17 from contrasting habitats were used. XN889 was from the dry climate region Shanmxii, 34°21’ N, 108°10’ E, with an...
annual rainfall of 573 mm. It is one of the major wheat cultivars in Shaanxi province and was bred by Northwest A&F University and released in 2005. CY17 was from the wet climate region Sichuan, 30°85’N, 104°12’E, with an annual rainfall of 1250 mm, and was bred by Chengdu Institute of Biology, Chinese Academy of Sciences and released in 2002. These wheat cultivars exhibited a large genetic variation. Experiments were performed twice in a period of two consecutive winter months. Wheat seeds were pre-soaked at 4°C for vernalization and five seeds per pot were sown at a depth of 3 cm in small plastic pots (25 cm diameters ×20 cm height). A peat-sand mixture (containing 80% peat and 20% sand) was used as growth medium in the pots. Shortly after emergence, seedlings were thinned to one plant per pot, and then grown for about three months before the start of experimental watering treatments. The cultivars were grown to full maturity in a greenhouse under a semi-controlled environment with a day temperature range of 12−35°C, a night temperature range of 9−20°C, and a relative humidity range of 30−81%. A total of 4 g slow-release fertilizer (7% N, 5% P and 26% K) was added to each pot at the beginning of the experiment.

The two wheat cultivars and three watering regimes [85, 55 and 25% field water capacity (FC)] were tested in a completely randomized design. Thirty individual plants of each cultivar were subjected to each watering regime, by supplying an amount of water equal to transpiration losses every other day. Evaporation from the soil surface was minimized by covering with a 3 cm layer of quartz gravel (Liu and Stützel, 2004). Transpiration was measured gravimetrically by weighing all pots every other day.

Ten replicate flag leaves from each cultivar / treatment combination were tested for leaf gas exchange during the anthesis period. Net photosynthetic rate, transpiration rate, intercellular CO2 concentration and stomatal conductance were assessed using an open gas exchange system with a 6 cm clamp-on leaf cuvette (LI-6400, LORIC Inc., Lincoln, NE, USA). Instantaneous water-use efficiency was calculated as the ratio of net photosynthetic rate to transpiration rate (Condon et al., 2002). Photosynthetic photon flux density (PPFD) was fixed at 1200 μmol m−2 s−1, using a red-blue LED light source built into the leaf cuvette, but other environmental factors were not controlled. Air was collected outside the greenhouse and passed through a buffering gallon and then pumped into the system, with mean CO2 concentration of 380 μmol mol−1. During the measurement, the air relative humidity was about 75% and the leaf temperature 28°C. All measurements were made in the morning (0900−1100) to avoid high afternoon temperature and low humidity.

Chlorophyll fluorescence and leaf gas exchange were measured simultaneously. Chlorophyll fluorescence was measured using a PAM-2100 fluorometer (Heinz Wälchli, Effeltrich, Germany). Initial fluorescence (Fo) and maximal fluorescence were measured after adaptation to darkness for 30 min. The intensity of saturation pulses used to determine the maximal fluorescence emission in the presence (Fm’) or absence (Fm) of quenching was 4000 μmol (photon) m−2 s−1, 0.8 s. The “actinic light” was set at 1200 μmol (photon) m−2 s−1. Steady-state fluorescence (Fs), basic fluorescence after light induction (F0’), and the variable fluorescence in both dark (Fv=Fm−F0) and light-adapted (ΔF=Fm’−Fs) were also determined.

Several photochemical variables were calculated based on the chlorophyll fluorescence parameters measured: potential activity of PSII (Fv/Fo), maximal PSII photochemical efficiency (Fv/Fm), effective quantum yield of PSII (ΦPSII=AF/Fm’), apparent electron transport rate (ETR=AF/Fm’×PPFD×0.5×0.84), photochemical [qP=(Fm−Fs)/(Fm−F0)] and non-photochemical [NPQ=(Fm−Fm’)/Fm’] fluorescence quenching (Maxwell and Johnson, 2000). For ETR estimation, 0.5 was used as the fraction of excitation energy distributed to PSII, and 0.84 as the fraction of light absorption.

Chlorophyll was extracted from the discs for at least 72 h in the dark using N, N-dimethylformamide. Absorbencies at 647 and 664 nm were determined with a UV-VIS spectrophotometer (UV2401PC, Shimadzu, Columbia, MD, USA). Chlorophyll a (Chla) and chlorophyll b (Chlb) were calculated using the equations of Inskipe and Bloom (1985).

Relative water content (RWC) was determined gravimetrically pre-dawn. Ten fully expanded flag leaves were collected to determine fresh weight (FW). Turgid weight (TW) was determined after the leaves were floated on distilled water in a closed container at 4°C in the dark for 24 h. Dry weight (DW) was determined for the same leaves after oven drying for 48 h at 70°C. RWC was calculated as: RWC (%) = [(FW−DW)/(TW−DW)]×100 (Jeon et al., 2006).

Plants were harvested the day after the leaf gas exchange measurements to assess total leaf area and above-ground dry matter. Each plant was divided into root, stem and leaf. Images of leaves were recorded with a scanner (Model F6800, Founder Electronics Co., Ltd, Beijing), and images were digitized by the Arcview 3.2a [Environmental Systems Research Institute (ESRI), Inc., New York] software in order to determine total leaf area. Then the roots, stems and leaves were dried in an oven for 48 h at 70°C for above-ground dry matter determination. Specific leaf area was calculated as the total leaf area divided by the dry mass of leaves. Grain yield was determined after the remaining plants had reached full maturity.

Experimental data were subjected to analyses of variance (ANOVA) for testing the cultivar and treatment differences. Statistical analyses were done with the Statistical Analysis
System (SAS version 8.0 for Windows, SAS Inc., IL, USA) software package.

**Results**

Water deficit reduced grain yield and above-ground dry matter in both cultivars. At 85%FC, CY17 had significantly higher grain yield and above-ground dry matter values than XN889, but at 25%FC, the grain yield and above-ground dry matter of CY17 and those of XN889 were decreased by about 60% and 35%, respectively, compared with that at 85%FC (Fig. 1A, B), indicating that XN889 was less sensitive to water deficit than CY17. Water deficit treatments also significantly decreased total leaf area, specific leaf area and relative water content in both cultivars (Fig. 1C, D, E). At 85%FC, XN889 had significantly lower total leaf area, specific leaf area and relative water content compared to CY17.
relative water content values as compared with CY17. At 25%FC, XN889 showed less change in relative water content and total leaf area than did CY17 (Fig. 1C, E).

CY17 showed decreases in net photosynthetic rate, stomatal conductance, intracellular CO₂ concentration and transpiration rate and increases in instantaneous water use efficiency under water deficit conditions (Fig. 1F, G, H, I, J). However, XN889, showed its highest net photosynthetic rate at 55%FC although the values of stomatal conductance, intracellular CO₂ concentration and transpiration rate were less at 55%FC than at 85%FC (Fig. 1F, G, H, I). Furthermore, a similar pattern was observed in stomatal conductance and transpiration rate in both wheat cultivars (Fig. 1G, I), but the reduction of stomatal conductance was smaller in XN889 than in CY17 under water deficit conditions. In XN889, instantaneous water use efficiency also increased under water deficit conditions, and was higher than that in CY17 (Fig. 1J).

Water deficits affected neither Chla nor Chlb content in CY17 at 55%FC, but Chla and Chlb in XN889 increased at 55%FC, and decreased at 25%FC, compared with the values at 85%FC (Fig. 2A, B). In both cultivars, ETR, Fv/Fo, ΦPSII and qN declined significantly at 25%FC, but not at 55%FC, relative to 85%FC (Fig. 2C, E, F, G). Furthermore, the non-photochemical quenching in both cultivars increased during water deficits, the increase in CY17 being less than that in XN889 (Fig. 2H).
Discussion

At 85% FC, CY17 had a higher grain yield, above-ground dry matter, total leaf area, specific leaf area, relative water content, net photosynthetic rate, stomatal conductance, chlorophyll content, ETR, Fv/Fo and ΦPSII than XN889 (Fig. 1, 2), perhaps because CY17, as a wet climate cultivar bred in regions with a high rainfall during growing season, is considered to utilize abundant soil water and grow rapidly. On the contrary, XN889, a dry climate cultivar cultivated in regions with a low rainfall during growing season, would be considered to have a high drought tolerance. Similar results were also reported for other plants (Zhang et al., 2005).

At 55% FC, water deficit inhibited growth (grain yield, above-ground dry matter, total leaf area, specific leaf area and relative water content) and photosynthetic parameters (net photosynthetic rate, stomatal conductance, intracellular CO2 concentration, and transpiration rate) in CY17. The decrease in growth parameters was mainly due to the depression of photosynthesis under water deficit conditions (Cui et al., 2006). In our study, intracellular CO2 concentration decreased in parallel to stomatal conductance and net photosynthetic rate at 55% FC (Fig. 1F, G, H), indicating that stomatal closure is the primary limiting factor of photosynthesis (Chaves and Oliveira, 2004; Flexas et al., 2006). However, net photosynthetic rate increased under the same condition in XN889 (Fig. 1F), although stomatal closure was also observed. Since an increase in Chl content was observed (Fig. 2A, B), 55% FC water deficit might promote chlorophyll synthesis and enhance net photosynthetic rate. Our experiments also showed the greater reduction in stomatal conductance under water deficits resulted in high instantaneous water use efficiency (Fig. 1). This was mainly due to the rapid adjustment of water loss through transpiration and absorption of CO2 through stomatal regulation (Sharkey et al., 2007; Wu et al., 2008). The increase in instantaneous water use efficiency under water deficit conditions was less in CY17 than in XN889, further indicating that CY17 was more drought-sensitive.

Furthermore, the 55% FC water deficit had no significant effect on Fv/Fm (Fig. 2E), implying that a moderate water deficit had no effect on the primary photochemistry of PSII (Subrahmanyan et al., 2006; Santosoi et al., 2009). ETR, ΦPSII and qP under 55% FC tended to be higher than those under 85% FC in XN889 (Fig. 2C, F, G), indicating that the proportion of reaction centers remaining open was larger (Lu and Zhang, 1999) and the electron transport capacity was improved (Zhao et al., 2007). Higher net photosynthetic rate in XN889 may be one explanation for the increase in ETR, ΦPSII and qP. Because an increase in photosynthetic capability could improve the “opening” of the PSII reaction centers (measured as qP), it may promote light photosynthetic electron transport, and thereby enhance photosynthetic electron transport capacity (measured as ETR and ΦPSII) (Zhao et al., 2007).

Under 25% FC, the decreased photosynthetic performance in both cultivars may be associated with the decrease in total leaf area, specific leaf area and chlorophyll contents. In both cultivars, the chlorophyll content was decreased showing that the light-harvesting complex was seriously damaged due to its relatively high Chlb content (Jeon et al., 2006; Wu et al., 2008). Net photosynthetic rate and stomatal conductance were lowered but intracellular CO2 concentration was not decreased from that at 55% FC (Fig. 1F, G, H). This suggested that stomatal closure was not the principal cause of decreased assimilation under the severe stress condition. The dark reaction may be another factor of decreased net photosynthetic rate in both cultivars.

Moreover, damage to PSII was also observed, while the effect was less marked in XN889 than in CY17 (Fig. 1, 2). Reduction of Fv/Fm and Fv/Fo (Fig. 2D, E) indicated damage to an important portion of the PSII reaction center (Zhang et al., 2010). ΦPSII and ETR decreased under water deficit conditions (Fig. 2C, F) mainly because water deficit decreased the efficiency of excitation energy capture of open PSII reaction centers (Roháček and Barták, 1999). The decrease in qP suggest that water deficit might harm PSII reaction centers and promote closure of PS reaction centers (Fig. 2G) (Roháček and Barták, 1999).

We also found that non-photochemical quenching was significantly increased by water deficit (Fig. 2H), indicating an increase in thermal dissipation compensating the reduced photochemical dissipation (Zhang et al., 2010). Higher non-photochemical quenching at 25% FC in XN889 than in CY17 may be associated with its larger Fv/ Fm, which indicated that the damage to PSII in XN889 was less than that in CY17.

Therefore, our results indicated that CY17 have greater productivity than XN889 under the relatively sufficient water condition at 85% FC, but presented greater decline under different water deficit condition at 55% FC and 25% FC (Fig. 1) because the former have stronger sensitivity in photosynthetic process and water use under water deficit than the latter (Fig. 1, 2), suggesting that cultivar from dry climate have stronger drought tolerance than cultivar from wet climate. We conclude that inbreeding by selecting cultivars from dry climate and wet climate would be an alternative to obtain high drought tolerance and productivity cultivar.

Acknowledgments

The authors want to thank Dr. Baoping Yang from Northwest A&F University and Professor Wu Yu from Chengdu Institute of Biology of the Chinese Academy of Sciences for the seed supply of two wheat cultivars. This
work was supported by the National science and technology programs of China (No.2008BAD98B03).

References
Bruce, W.B. et al. 2002. *J. Exp. Bot.* 53: 13-25.
Blum, A. 1996. *Plant Growth Regul.* 20: 123-138.
Castillo, M. et al. 2001. *Photosynthetica* 39: 221-226.
Chaves, M.M. and Oliveira, M.M. 2004. *J. Exp. Bot.* 55: 2595-2594.
Condon, A.G. et al. 2002. *Ann. Bot.* 82: 122-131.
Cai, L.J. et al. 2006. *Bot. Stud.* 47: 1-69.
Flexas, J. et al. 2006. *New Phytol.* 172: 79-82.
Inskeep, W.P. and Bloom, P.R. 1980. *Plant Physiol.* 66: 86-90.
Jean, M.W. et al. 2006. *Environ. Exp. Bot.* 55: 181-194.
Liu, F. and Strelitz, H. 2004. *Sci. Hort.* 102: 15-27.
Lu, C. and Zhang, J. 1999. *J. Exp. Bot.* 50: 1199-1206.
Maxwell, K. and Johnson, G.N. 2000. *J. Exp. Bot.* 51: 659-668.
Roháček, K. and Barták, M. 1999. *Photosynthetica* 37: 393-363.
Santosi, M.G. et al. 2009. *Biol. Plant.* (2): 229-236.
Sayed, O.H. 2001. In I. Prakash ed., *Ecology of Desert Environments*. Scientific Publishers, Jodhpur: 87-103.
Shangguan, Z. et al. 1999. *J. Plant Physiol.* 154: 753-758.
Sharkey, T.D. et al. 2007. *Plant Cell Environ.* 30: 1035-1040.
Subrahmanyam, D. et al. 2006. *Photosynthetica* 44(1): 125-129.
Tang, A.C. et al. 2002. *Ann. Bot.* 89: 861-870.
Wang, T. et al. 2007. *Biol. Plant.* 51(1): 181-184.
Wu, F.Z. et al. 2009. *Photosynthetica* 46(1): 40-48.
Yu, D.J. et al. 2009. *Biol. Plant.* 53: 133-137.
Zhang, X. et al. 2005. *J. Arid Environ.* 60: 567-579.
Zhang, X.H. et al. 2019. *Biol. Plant.* 54(1): 164-168.
Zhao, L.Y. et al. 2007. *Chinese J. Eco-Agr.* 15: 63-66*.

* In Chinese.