

Characteristics of flag leaf photosynthesis and root respiration of four historical winter wheat varieties released over recent decades in semi-arid Northwest China

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Abstract

A pot-culture experiment was conducted to characterize the flag leaf photosynthesis (P_n) and root respiration in four historical winter wheat cultivars on the Loess Plateau in Northwest China. We compared 4 varieties of winter wheat (*Triticum aestivum* L.) that were released over the recent decades. These are Bima 1 (released in the 1950s) and Changwu 7125 (released in the 1970s) that represent the old varieties, and Changwu 131 (released in the 1980s) and Changwu135 (released in the 2000s) that represent the new varieties. At anthesis, Flag leaf P_n was significantly higher ($p \leq 0.05$) for the new varieties than for the old ones during the day (between 11:00h and 15:00h) except in the early morning (at 11:00h) and the late afternoon (at 17:00h). By contrast, root respiration in the old varieties was higher than in the new ones in the morning (07:00 – 09:00 h) and evening (19:00 – 23:00 h), whereas it exhibited the opposite pattern between 11:00 h and 17:00 h. The new varieties also maintained significantly higher values of effective PSII quantum yield (Φ_{PSII}), apparent rate of photosynthetic electron transport (ETR), coefficient of photochemical quenching (qP) and coefficient of non-photochemical quenching (NPQ) than the old varieties at low soil-water content. The differences in chlorophyll fluorescence performance suggested that the new varieties might be more drought tolerant than the old ones, in consistence with that the former produced higher grain yields under drought conditions than the latter. Our study indicates that, over the past 6 decades, photosynthetic activities and drought tolerance had improved, while root respiration has fallen resulting in yield increases in the new winter wheat varieties compared with old varieties on the semi-arid Loess Plateau of China.

Keywords: winter wheat, Loess Plateau, photosynthesis, root respiration, grain yield.

Abbreviations: P_n : photosynthesis, Φ_{PSII} : the effective PSII quantum yield, ETR: apparent rate of photosynthetic electron transport, qP : coefficient of photochemical quenching, NPQ: coefficient of non-photochemical quenching, FWC: field water capacity, GYS: The grain yield stability.

Introduction

Plant breeders generally regard improvement in crop yield per unit area as a high-priority over other objectives (Evans, 1993). The increase in grain yield of winter wheat resulting from the use of new varieties over the several decades was primarily attributable to the improvement in canopy structure, including increase in leaf area index and increased harvest index. Thus, in order to achieve further increases in yield, total biomass production must be improved (Reynolds et al., 2000; Richards, 2000). Although there are many factors that affect biomass production, the efficiency with which solar radiation is used in biomass production is the most important among many factors (Russell et al., 1989). In general, it has been recognized that the photosynthetic performance of agricultural crops should be improved in order to increase the rate of biomass production and the yield potential (Horton, 2000; Reynolds et al., 2000; Richards, 2000). Several studies have shown a positive correlation between yield and net photosynthetic rate in wheat (e.g. Reynolds et al., 1994; Fischer et al., 1998; Wang et al., 2008). However, other researchers considered that the correlations among photosynthetic rate, dry matter production and yield were either weak or negative (Lawlor, 1995). Exactly, the relationship between photosynthesis (P_n) per unit leaf area and yield is complicated. This may be because that P_n is very

sensitive to changes in environmental conditions and also change with plant phenology (Liu et al., 2002). Also not all improvement in grain yield is necessarily attributable to improvement in net photosynthetic rate, but may be due to a number of other factors, such as improved root physiological traits (Liu et al., 2004). The root is a major consumer of photosynthates for growth and maintenance respiration (McCree, 1986; Liu et al., 2004). For example, more than 50% of the daily accumulated assimilates is transported to the root and around 60% of the carbon allocated to roots is respired in wheat (Liu et al., 2004). Thus, quantitative information about root respiration could improve understanding of the interaction between the carbon budget and grain yield (Lohila et al., 2003). Differences in root respiration amongst wheat varieties and their influence on grain yield are not well understood. Further knowledge of these interactions may guide variety selection and improvement especially for semiarid environment. The aim of this study was to explore the relationships among leaf P_n , root respiration and grain yield. We did this by measuring leaf photosynthesis and root respiration in four major wheat varieties released in the past 6 decades on the Loess Plateau in Northwest China.

Results

Diurnal changes in P_n in flag leaves of wheat varieties

At anthesis, there was no significant difference in P_n of the flag leaf between old (Bima 1 and Changwu 7125) and new wheat varieties (Changwu 131 and Changwu 135) at 09:00 h and 17:00 h (Fig. 1). However, the new varieties had higher P_n than old varieties between 11:00 h and 15:00 h. P_n in all varieties peaked at 11:00 h, when P_n in Changwu 131 was the highest and that in Bima 1 was the lowest among the four varieties. Changwu 135 had higher P_n compared with Changwu 131 at 13:00 h and had similar P_n to Changwu 131 at 15:00 h. During midday hours, the P_n of all varieties was decreased, although the midday decrease of P_n in the new varieties was less compared with the old varieties (Fig. 1).

Comparison of specific root respiration among wheat varieties

At anthesis, the diurnal specific root respiration rate exhibited a single-peak pattern, with the highest values observed around midday. The old varieties (Bima 1 and Changwu 7125) had higher specific root respiration rates compared with the new varieties (Changwu 131 and Changwu 135) in the morning (07:00 h–09:00 h) and the evening (19:00 h–23:00 h). There was no significant difference in the specific root respiration rate amongst the four varieties at 11:00 h and 17:00 h, but the new varieties had higher specific root respiration rates compared with the old varieties from 13:00 h to 15:00 h (Fig. 2).

Changes in Chlorophyll fluorescence parameters during progressive soil drying

At anthesis, chlorophyll fluorescence parameters (Φ_{PSII} , ETR and qP) decreased significantly with soil drying, but the new wheat varieties (Changwu 131 and Changwu 135) generally maintained higher Φ_{PSII} , ETR and qP than the old varieties (Bima 1 and Changwu 7125) during this period (Fig.3). NPQ increased initially with soil drying until soil water content dropped to less than 50% FWC but then declined with soil drying. With further soil drying, the values of NPQ were significantly higher in the new wheat varieties compared with the old ones.

Yield traits and grain yield stability of different wheat varieties

Under well-watered and dried conditions, there were no significant differences in spike number per pot between the old and new wheat varieties, although the latter had higher aboveground biomass and grain yields than did the old varieties. The new varieties (Changwu 131 and Changwu 135) also had higher harvest index compared with the old varieties (Bima 1 and Changwu 7125). The grain yield stability (GYS) of different varieties was calculated as the percentage of grain yield in dried pots relative to that of pots at 70% FWC. New varieties were less affected in having GYS of 64.12% and 67.21%, respectively, which were higher than for the old varieties (Table 1).

Discussion

P_n is the source of biomass production. However, the relationship between net photosynthetic rate and crop yield has been found to be inconsistent and confusion (Liu et al., 2002). It is well known that photosynthetic products in late growing stage account for at least 90% of the grain yield (Zelitch, 1982)

and that there is a genetic gain in yield associated with flag-leaf photosynthetic rate in F_5 sister lines, resulting in a positive relationship between photosynthetic rate and yield and biomass production (Gutierrez-Rodríguez et al., 2000). In the current study we only found strong relationship between flag leaf P_n near midday with grain yield at harvest. For instance, Changwu 135 that had higher flag leaf P_n of $23.1 \mu\text{mol g}^{-1} \text{s}^{-1}$ at 11:00h produced 27.4 g pot^{-1} of grain compared to Bima 1 that had flag leaf P_n of $20.6 \mu\text{mol g}^{-1} \text{s}^{-1}$ producing 19.4 g.pot^{-1} of grain. This suggests that measurement of P_n around midday can be a useful selection tool for high yields for wheat varieties. Root respiration rate is frequently affected by various abiotic factors, such as temperature (Maestre and Cortina, 2003), atmospheric and soil CO_2 concentration (Ham et al., 1995), and soil water content (Bouma and Bryla, 2000; Davidson et al., 2000). However, these factors always change with the time of day. In the current study, new varieties exhibited lower root respiration compared to the old varieties in the morning (07:00 – 09:00 h) and evening (19:00 – 23:00 h). The lower root respiration in new varieties might have spared more photosynthate for grain-filling. Wheat production in northern China is often affected by terminal drought (usually from late May to early June) and photosynthetic performance is often affected, especially during midday hours. The terminal drought coincides with the grain-filling process, resulting in a significant penalty on final yield. Levitt (1980) argues that high root activity can increase the ability of roots to absorb water and to maintain constant tissue moisture under drought stress conditions. Specific root respiration (respiration rate per unit root biomass) represents the root activity of plants. In the current study, the new varieties which exhibited less depression of photosynthesis had higher specific root respiration compared to old varieties during the midday period, which was possibly associated with enhanced water uptake. Therefore, a combination of superior leaf photosynthetic capacity and high root activity is potentially advantageous wheat production. It is well known that plant growth and crop productivity can be limited by insufficient photosynthates during drought stress. Of several strategies, selection of species with drought resistance is an economic and efficient means of alleviating such problems (Ashraf et al., 1992). Understanding the mechanism of adaptation to drought stress provides opportunities to improve crop breeding process. Photochemical properties associated with drought tolerance are important for the drought resistance mechanisms of plants. In the current study, chlorophyll fluorescence parameters were used in assessing plant drought tolerance ability. Chlorophyll fluorescence represents the primary reactions of P_n and the intricate relationships between fluorescence kinetics and P_n help current understanding of photosynthetic biophysical processes. Chlorophyll fluorescence quenching analysis has been shown to be a non-invasive tool in ecophysiological studies, and has been used extensively in assessing plant responses to environmental stress (Sayed, 2003). Our current study showed that water stress at anthesis damaged the PSII reaction centers in leaves and the new varieties performed better chlorophyll fluorescence compared with the old varieties indicating they could maintain photosynthetic capability much better when suffering water deficit, is in consistence with the grain yield stability. High NPQ indicated reduced risk of damage to the PSII reaction centers from excess radiation, which is then dissipated as heat in the PSII antenna complexes (Rohacek 2002). However, continued drought must have damaged the PSII reaction centers leading to NPQ declining with further soil drying. However, the new varieties maintained higher NPQ than the old varieties, suggesting that the former had a superior capacity to protect PSII reaction centers and hence were better

Table 1. Spike number per pot, aboveground biomass, grain yield, harvest index and grain yield stability (GYS) of different wheat varieties.

Varieties	Treatments	Spike number	Aboveground biomass (g pot ⁻¹)	Yield (g pot ⁻¹)	Harvest index	GYS (%)
Bima 1	Well-watered	20.0±1.15a	58.02±1.4b	19.4±0.4d	0.34±0.01c	51.23
	Dried	19.3±1.21a	48.2±1.1c	9.94±0.6d	0.21±0.03c	
Changwu 7125	Well-watered	21.3±0.88a	60.84±2.1a	22.2±0.95c	0.36±0.01c	58.12
	Dried	20.7±1.13a	52.75±1.7b	13.49±0.49c	0.26±0.03b	
Changwu 131	Well-watered	21.7±0.67a	62.44±1.8a	25.6±0.44b	0.41±0.02a	64.18
	Dried	20.3±1.14a	55.21±2.3a	16.43±0.52b	0.30±0.04a	
Changwu 135	Well-watered	22.3±0.33a	62.94±1.4a	27.4±0.32a	0.44±0.02a	67.21
	Dried	21.3±0.54a	55.97±2.1a	18.42±0.53a	0.32±0.02a	

Values are means ± standard errors ($n = 3$). In the same column, different letters under the same water condition imply that there is a significant difference between different wheat cultivars at $p \leq 0.05$.

Table 2. The main important characteristics of four historical winter wheat varieties.

Items	Bima 1	Changwu 7125	Changwu 131	Changwu 135
Year of release	1950	1971	1984	1998
Years of planting	1960-1970	1972-1984	1986-1998	2000-
Long term mean yield (kg hm ⁻¹)	1200	1550	3500	6200
Growth period (d)	247	242	239	238
Day of anthesis	May 2	April 30	April 27	April 26
Plant height (cm)	110.25	86.58	73.94	72.38
Grian weight of ear (g)	0.87	1.04	1.18	1.23
Stress-resistance characteristics	Poor resistance to plant diseases, drought and stem lodging	Strong resistance to leaf rust, freezing and drought	Strong resistance to stem lodging and drought	Strong resistance to leaf rust resistance, freezing, stem lodging and drought

at tolerating drought than the old ones. These differences in the values of chlorophyll fluorescence parameters show that new wheat varieties have higher drought tolerance compared with old wheat varieties. The grain yield stability (GYS) of a variety was also closely related to its drought tolerance. In our study, new varieties had higher GYS under drought stress condition compared with old varieties (Table 1). A provisional conclusion from this study is that recent gains in yield could be associated with improvements in the photosynthetic capacity, reduced root respiration (from 19:00 h p.m. to 09:00 h a.m. the next day) and drought tolerance in the new varieties compared to the old varieties. And the drought tolerance in the new varieties was partly due to their improved root respiration during the midday period.

Materials and methods

Plant materials

The pot experiment was conducted at the Institute of Soil and Water Conservation, Yangling, China (34°12'N; 108°7'E). We compared 4 varieties of winter wheat (*Triticum aestivum* L.) that were released over the last 6 decades. These are Bima 1 (released in the 1950s) and Changwu 7125 (released in the 1970s) that represent the old varieties, and Changwu 131 (released in the 1980s) and Changwu 135 (released in the 2000s) that represent the new varieties. The detail of these varieties was given in Table 2.

Experimental procedure

Seeds of winter wheat were grown in plastic pots (350 mm diameter × 500 mm height). The pots were filled with 10 kg of sieved topsoil [from farmland in the region with a field water capacity (FWC) of 26%, total N 0.62 g kg⁻¹, total P 1.45 g kg⁻¹, soil organic matter 14.7 g kg⁻¹, available N 54.6 mg kg⁻¹, available P 8.9 mg kg⁻¹]. Chemical fertilizers (N, P and K) were applied to the pots at 1.18, 0.54 and 0.43 g pot⁻¹, respectively,

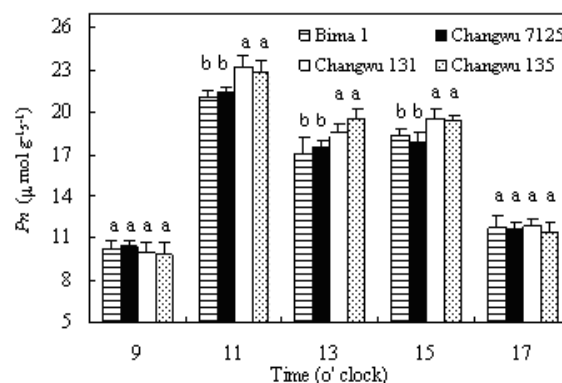


Fig 1. Diurnal change in P_n of flag leaves of different wheat varieties. Vertical bars represent the standard errors ($n = 9$).

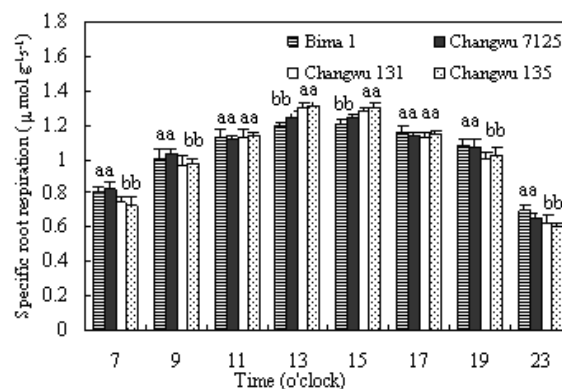


Fig 2. Diurnal change of specific root respiration rate in different wheat varieties. Vertical bars represent the standard errors ($n = 3$).

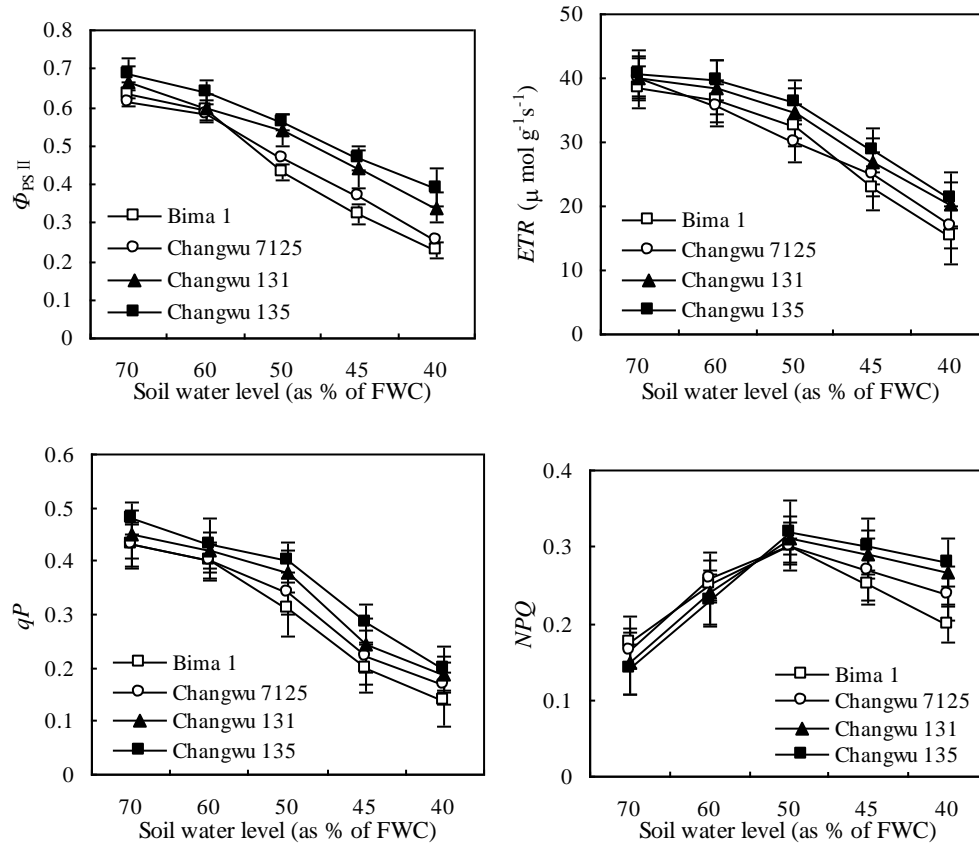


Fig 3. Comparison of chlorophyll fluorescence parameters among different wheat cultivars during the progressive soil drying. Vertical bars represent the standard errors ($n = 9$). Φ_{PSII} : the effective PSII quantum yield; ETR : apparent rate of photosynthetic electron transport; qP : coefficient of photochemical quenching; NPQ : coefficient of non-photochemical quenching.

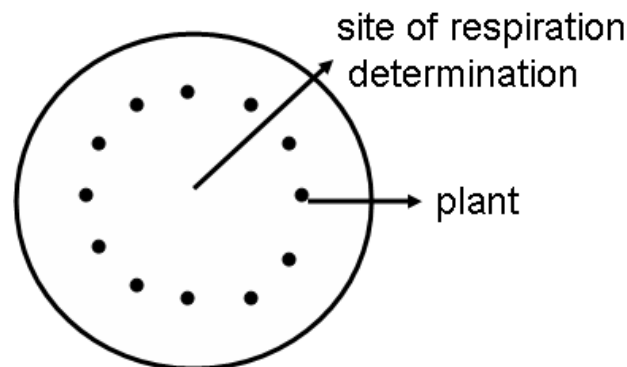


Fig 4. Schematic diagram of the sowing method and respiration measurement locations.

to ensure sufficient nutrition. The seeds were sown at a depth of 15-20 mm and a density of 12 seedlings per pot. In order to facilitate determination of root respiration *in situ*, seeds were distributed in the pot as shown in Fig. 4. All the 12 plants were retained in each pot throughout the experiment. The pots were maintained at 80% FWC before jointing and 70% FWC after jointing. All pots were arranged randomly under a movable transparent rain-out shelter. The pots were weighed and irrigated at 08:00 h and 16:00 h daily throughout the experimental period. At maturity (about 240 d after sowing), the shoot dry weight, grain yield and number of spike were measured.

Analytical methods

P_n and root respiration rate measurements

At anthesis (about 200 d after sowing), the instantaneous net photosynthetic rate (P_n) of the flag leaf was measured using a LI-6400 Portable Photosynthesis System (LI-Cor, Inc., Lincoln, Nebraska, USA) between 09:00 h and 17:00 h. Three flag leaves in each pot were measured. After P_n was measured, root respiration was assessed by using a closed system chamber (SRC-1 with EGM-4, PP-Systems, Hitchin, UK).

The chamber was held in the air to flush out before inserted into the soil to a depth of 30 mm. Measurement were taken 5 s after the chamber was inserted into the soil and the value was taken to represent instantaneous total soil respiration. Three pots per treatment were measured. Respiration from bare soil was measured in 3 pots that were not sown. Root respiration was estimated by subtracting the bare soil respiration from the total soil respiration. Root samples were then collected after the root respiration was measured and the samples were washed free of soil using a 0.5-mm mesh sieve. New roots (light brown in color) were then separated by hand from older roots from the filed (dark brown), soil particles and debris. The root samples were then dried in a forced draft oven at 75°C. The root weight was used to calculate specific root respiration (root respiration per root weight).

Chlorophyll fluorescence measurements

At anthesis, 8 pots selected randomly from each treatment were divided into two groups: one was maintained at 70% of FWC; the other was dried progressively by controlling water supply to achieve the following soil water level: 70% FWC (on the 1st day after the drought beginning), 60% FWC (2-3 day after the drought beginning), 50% FWC (4-5 day after the drought beginning), 45% FWC (6-7 day after the drought beginning), 40% FWC (8-9 day after the drought beginning). Soil water levels were determined gravimetrically by weighing pots and irrigating every 2 h in the daytime. Chlorophyll fluorescence parameters i.e., the effective PSII quantum yield (Φ_{PSII}), apparent rate of photosynthetic electron transport (*ETR*), coefficient of photochemical quenching (*qP*) and coefficient of non-photochemical quenching (*NPQ*), of the flag leaf were measured on randomly chosen plants in each pot using the Imaging-PAM (Walz Company, Effeltrich, Germany). The measurements were made from 09:00 h to 11:00 h on alternate days until the 9th day following imposition of drought. On nine replicate samples (3 leaves \times 3 pots) of each treatment were measured. Φ_{PSII} measures the proportion of light absorbed by chlorophyll associated with the Photosystem II (*PS II*) reaction centers of leaf that is used in photochemistry. *ETR* is the measurement of the apparent rate of photosynthetic electron transport. *qP* is the coefficient of photochemical quenching that gives an indication of the proportion of *PS II* reaction centers that are open. *NPQ* is the coefficient of non-photochemical quenching that reflects a change in the efficiency of heat dissipation. After chlorophyll fluorescence parameters were measured, the pots were maintained at 50% FWC. The shoot biomass and grain yields in these pots were measured at maturity.

Statistical analysis

All the statistical analyses were made using the one way ANOVA in SAS procedure. Mean values for treatments were compared using least significant difference (LSD) at $P \leq 0.05$.

Conclusion

In the present investigation, flag leaf photosynthesis (*P_n*) and root respiration in the four historical winter wheat cultivars indicated that, over the past 6 decades, the yield increases appeared to be associated with improved photosynthetic capability and reductions in root respiration. These new varieties maintained significantly higher photosynthetic capacity compared to the old ones when the soil was allowed to dry, suggesting the new varieties are more drought tolerant than the old ones.

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References

- Ashraf M, Bokhari H, Cristiti SN (1992) Variation in osmotic adjustment of lentil (*Lens culinaris* Medic.) in response to drought. *Acta Bot Neerland* 41:51-62
- Bouma TJ, Bryla DR (2000) On the assessment of root and soil respiration for soils of different textures: interactions with soil moisture contents and soil CO₂ concentrations. *Plant Soil* 227:215-221
- Davidson EA, Verchot LV, Cattanio JH, Ackerman IL, Carvalho HM (2000) Effects of soil water content on soil respiration in forests and cattle pastures of eastern Amazonia. *Biogeochem* 48:53-69
- Evans LT (1993) *Crop Evolution, Adaptation and Yield*. Cambridge University Press, Cambridge
- Fischer RA, Rees D, Sayre KD, Lu ZM, Condon AG, Saavedra AL (1998) Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. *Crop Sci* 38:1467-1475
- Gutierrez-Rodriguez M, Reynolds MP, Larque-Saavedra A (2000) Photosynthesis of wheat in a warm, irrigated environment. II. Traits associated with genetic gains in yield. *Field Crop Res* 66:51-62
- Ham BE, Owensby CE, Coyne PI, Bremer DJ (1995) Fluxes of CO₂ and water vapor from a prairie ecosystem exposed to ambient and elevated atmospheric CO₂. *Agric Forest Meteorol* 77:73-93
- Horton P (2000) Prospects for crop improvement through the genetic manipulation of photosynthesis: morphological and biochemical aspects of light capture. *J Exp Bot* 51:475-485
- Lawlor DW (1995) Photosynthesis, production and environment. *J Exp Bot* 46:1389-1396
- Levitt J (1980) *Response of Plants to Environmental Stress*, Vol. 2. Academic Press, New York
- Liu HQ, Jiang GM, Zhang QD, Sun JZ, Qu CM, Guo RJ, Gao LM, Bai KZ, Kuang TY (2002) Changes of gas exchanges in leaves of different cultivars of winter wheat released in different years. *Acta Bot Sin* 44:913-919
- Liu, H S, Li, FM, Xu, H (2004) Deficiency of water can enhance root respiration rate of drought-sensitive but not drought-tolerant spring wheat. *Agric Water Manage* 64:41-48
- Lohila A, Aurela M, Regina K, Laurila T (2003) Soil and total ecosystem respiration in agricultural fields: effect of soil and crop type. *Plant Soil* 251:303-317
- Maestre FT, Cortina J (2003) Small-scale spatial variation in soil CO₂ efflux in a Mediterranean semiarid steppe. *Appl Soil Ecol* 23:199-209
- McCree KJ (1986) Measuring the whole-plant daily carbon balance. *Photosynthetica* 2: 82 - 93.
- Reynolds MP, Balota M, Delgado MIB, Amani I, Fischer RA (1994) Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. *Aust J Plant Physiol* 21:717-730
- Reynolds MP, van Ginkel M, Ribaut JM (2000) Avenues for genetics modification of radiation use efficiency in wheat. *J Exp Bot* 51:459-473
- Richards RA (2000) Selectable traits to increase crop photosynthesis and yield of grain crops. *J Exp Bot* 51:447-458

- Rohacek K (2002) Chlorophyll fluorescence parameters: the definitions photosynthetic meaning, and mutual relationships. *Photosynthetica* 40:13-29
- Russell G, Jarvis PG, Monteith JL (1989) Absorption of radiation by canopies and stand growth. In: Russell G, Marshall B, Jarvis PG (Eds) *Plant canopies: their growth, form and function*. Cambridge University Press, Cambridge
- Sayed OH (2003) Chlorophyll fluorescence as a tool in cereal crop research. *Photosynthetica* 41:321-330
- Wang SH, Jing Q, Dai TB, Jiang D, Cao WX (2008) Evolution characteristics of flag leaf photosynthesis and grain yield of wheat cultivars bred in different years. *Chinese J Appl Ecol* 19:1255-1260
- Zelitch I (1982) The close relationship between net photosynthesis and crop yield. *Bioscience* 32:796-802