Universitat de Barcelona

Physiological traits associated with recent advances in yield of Chinese wheat

Bangwei Zhou

UNIVERSITAT DE BARCELONA

Physiological traits associated with recent advances in yield of Chinese wheat

(Rasgos fisológicos asociados con los recientes avances en el rendimiento del trigo chino)

Memoria presentada por **Bangwei Zhou** para optar al título de Doctor por la Universitat de Barcelona. Este trabajo se enmarca dentro del programa de doctorado de Biología Vegetal de la Facultad de Biología de la Universitat de Barcelona. Este trabajo se ha realizado en el Departamento de Biología Vegetal de la Facultad de Biología de la Universitat de Barcelona bajo la dirección del Dr. **Josep Lluís Araus Ortega** y la Dra. **M. Dolors Serret Molins**.

Doctorando	Directores de Tesis
Bangwei Zhou	Dr. José Luis Araus Ortega and Dra. M. Dolors Serret Molins

Barcelona, October, 2014

CHAPTER 6

General discussion

This last chapter of the thesis aims to:

- Recapitulate succinctly the main findings of the thesis, integrating the achievements across different experiments, and giving an overview of the agronomical and physiological traits contributing to the increase in grain yield and stress adaptation for recent winter wheats from Henan Province, China.

- Build a conceptual platform combining agronomical physiological and metabolic characteristics (i.e. an ideotype), together with visual criteria and high throughput phenotyping approaches, which may be deployed in future breeding programs with the aim of increasing the yield and stability of wheats from the Henan region.

1. Summary of major traits contributing to increasing the yield

Grain yield is a complex trait characterized by a low heritability and a high genotype × environment (G × E) interaction (Araus et al. 2003). During recent decades breeding for higher and more stable yields has been mostly based on a multitrial scheme, where grain yield, evaluated across years and environment, is the main trait for selection and eventually some secondary (i.e. indirect) traits are also considered. Usually these secondary traits are scored visually and refer to plant phenology, tolerance to pests and diseases, and morphological traits such as plant height or susceptibility to lodging. However, except for a few exceptions (e.g. carbon isotope discrimination in wheat, anthesis-to-silking-interval in maize, feed and food quality traits) few true physiological traits have been used systematically in breeding (Araus et al. 2008). The same may be said of the implementation of high throughput phenotyping at the field level (Araus and Cairns 2014). This is in spite of the fact of the low heritability inherent to grain yield and the cost of deploying large-scale yield trials. Thus, building up a conceptual model that integrates diverse secondary traits together with the proper high-throughput approaches to phenotype them are potentially key to ensuring that Chinese winter wheat breeding programs continue to deliver improved cultivars.

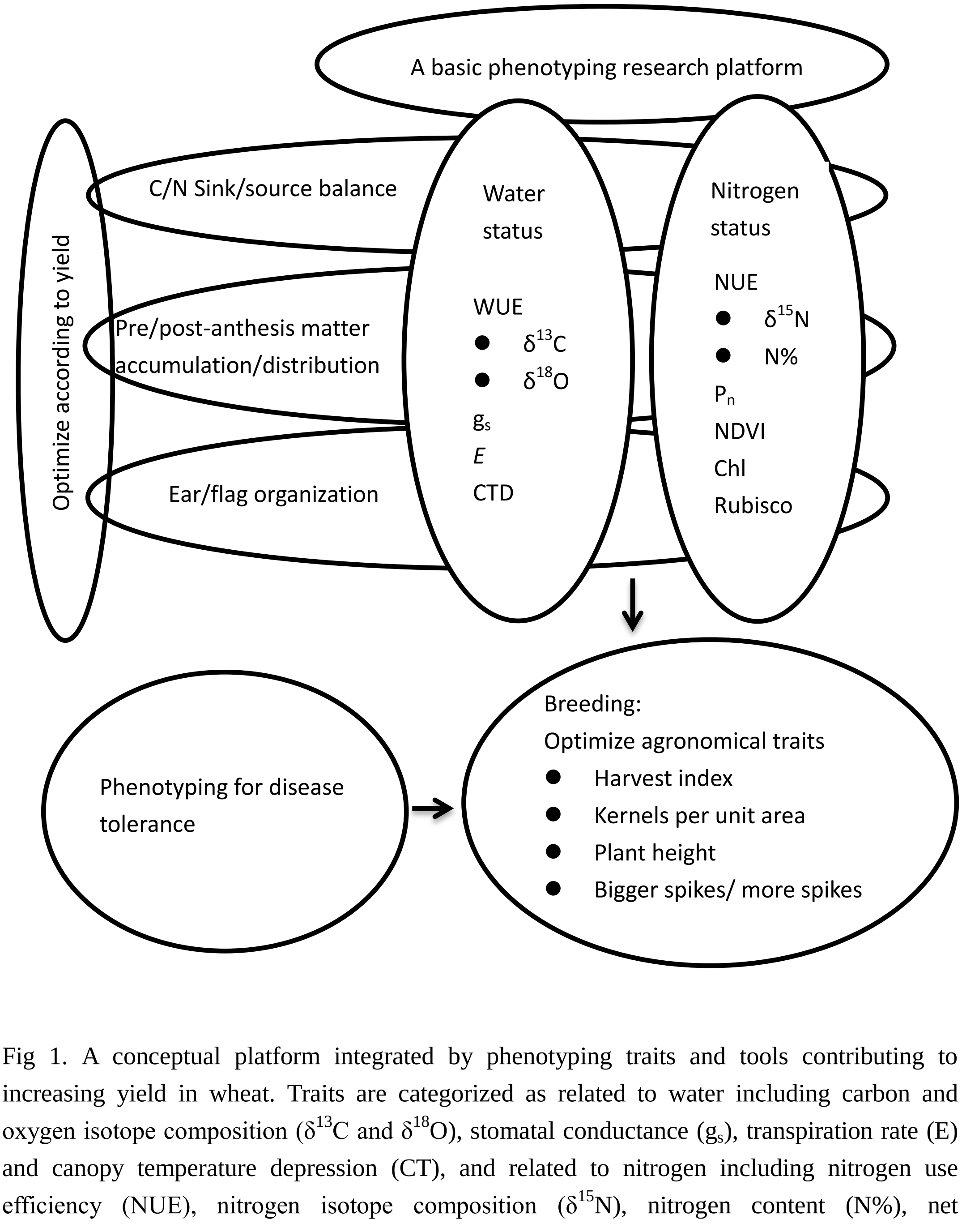


Fig 1. A conceptual platform integrated by phenotyping traits and tools contributing to increasing yield in wheat. Traits are categorized as related to water including carbon and oxygen isotope composition ($\delta^{13}C$ and $\delta^{18}O$), stomatal conductance (g_s), transpiration rate (E) and canopy temperature depression (CT), and related to nitrogen including nitrogen use efficiency (NUE), nitrogen isotope composition ($\delta^{15}N$), nitrogen content (N%), net photosynthesis (P_n), green biomass (evaluated remotely using for example a spectroradiometrical or RGB image-derived Vegetation Index), chlorophyll content (Chl) and Ribulose biphosphate carboxylase (Rubisco) content.

Trait selection is the cornerstone of modern wheat breeding and has made continual progress through incorporating the following types of traits: simply inherited agronomic characteristics; resistance to a spectrum of prevalent diseases; quality parameters determined by end use; and yield based on multiplication trials (Reynolds et al. 2011). In this thesis, a research platform was set up based on a set of physiological, agronomic, and phenotypic traits associated with yield improvements (Fig 1).

The genotypes studied come from one of the wheat baskets of Chinese agriculture, Henan province in the YHVWWZ, and include the most widely distributed cultivars released in recent decades in this province together with advanced lines. This thesis illustrates the wide range of yield exhibited by Chinese wheats in response to stress conditions, either abiotic such as moderate water stress (ranging between 5.7 to 7.5 tons ha^{-1}, which was lower than local Spanish genotypes), or biotic stresses, represented in this thesis by yellow rust (with yield ranging between 1-7 t ha^{-1}). Such a wide range in yield highlights that Chinese genotypes, especially for the more recently released varieties, exhibit a low acclimation capacity to maintain yield potential in stress conditions, even if they are characterized by a high yield potential. This may be just the consequence of neglecting stress adaptation in the modern breeding programs (Yang et al. 2006).

From 1950s, which is considered the beginning of modern breeding in YHVWWZ, three consecutive generations of improved cultivars have been released. For the generation bred in the 1980s, most of the cultivars released were derived from parent material with the 1B/1R gene, which aimed to reduce plant height and increase HI (Zhou et al. 2007). In this thesis, this generation is represented by the genotypes “Yumai 35”, “Lankao aizao 6” (aizao, in Chinese means short and early) and “Yumai 66”, with all three released during the 1990s, and they are characterized by short stature and a higher HI under optimal agronomical conditions than the first generation. Reducing plant height by the introgression of Rht genes aimed at chasing higher HIs has been a remarkable success, resulting in increased yield potential (He et al. 2011). However, these genotypes from the second breeding generation, which are characterized by a plant height that is even lower than other more recently released genotypes, still exhibit a HI that is lower than current genotypes (i.e. from the third generation) (see Chapters 2 and 3). Moreover, HI is strongly affected by stress conditions. On the other hand, the high linear correlations between grain number and grain yield found in this thesis under both optimal and stress conditions clearly indicate that grain number plays a key role in determining yield potential and stability, which agrees with previous studies (Zheng et al. 2011; Xiao et al. 2012). Furthermore, maintaining a high tiller capacity was the key subcomponent that satisfied sufficient grain numbers and thus grain yield. In a broad sense, maintaining an adequate tiller capacity and a high grain number was critically determined by the resource allocation prior to anthesis (Fischer, 2008; Sinclair and Jamieson, 2008). By contrast, thousand kernel weight (TKW) was not associated with grain yield improvement under any growing conditions, which agrees with the literature available on Chinese wheats (Wu et al. 2012; Xiao et al. 2012). This gives room for us to consider that the current Chinese wheats are to some degree sink limited (Slafer and Savin 1994; Madani et al. 2010).

Yield is by nature a very integrative trait, both in time and at the level of organization. Being integrative in time means that different environmental factors may affect yield throughout the plant cycle, and that grain yield is just the final result of such interaction between the crop and the environment. On the other hand, the crop is also the integrated result of different levels of organization, from the molecular and metabolic (e. g. Rubisco activity) to the canopy (e. g. canopy gas exchange) (Araus et al. 2003; Abbad et al. 2004; Araus et al. 2008). The relative importance of yield determinants depends strongly on the environment where the plant grows (e.g. optimal or stress-exposed), and is also related to the phenological stage when phenotyping takes place (e.g. pre/post anthesis). In this thesis, the physiological traits studied were associated with carbon (C) and nitrogen (N) metabolism and included both instantaneous (e.g. gas exchange measurements) as well as time-integrated traits (e.g. stable isotopes analysed in dry matter) and were mainly measured during the second part (i.e. reproductive stage) of the crop, when all agronomical yield components are defined (Kichey et al. 2007). Among the key organs, the ear actively contributes C and N towards grain filling, and should be considered in parallel with other organs (e.g. flag leaf) to further improve grain yield through a higher HI and TKW. Coincidently, the “ideotype model” established for wheat at YHVWWZ includes a big spike, strong culm, and a small flag leaf, and in fact the current breeding programs in this agroecological zone are giving increased importance to the ear (Guo et al. 2004; Ma et al. 2007). Concerning N, the ^{15}N labelling study of our thesis has also shown an increased role of the ear as a source of N to grains in the high yielding genotypes (Chapter 4). Overall this thesis provides further evidence of the key role of the ear, providing C and N assimilates, when defining an ideotype for high yielding conditions. (Araus et al. 1993; Tambussi et al. 2007; Aranjuelo et al. 2011; Sánchez-Bragado et al. 2014).

In wheat, N accumulated in the kernels is largely influenced by the amount of N accumulated in the biomass at anthesis (Martre et al. 2003), rather than by yield components as is the case for carbon assimilates. Modern cultivars require more N than older cultivars and respond more to N, which translates into higher economic values and higher returns when N fertilizer is available (Ortiz-Monasterio R. et al. 1997; Diaz et al. 2008; Gaju et al. 2014a). Improving NUE through optimizing the source/sink balance among the key organs (e.g. ear, flag leaf) has been proved to increase the N accumulation in the kernels and maximize productivity (Chapters 2, 3 and 4). Throughout this thesis, several approaches have been followed to test the differences in N metabolism and accumulation in grains. These methods include (i) the direct measurement of the N content in key organs to assess N storage capacity; (ii) the use of ^{15}N labelling as a tracer to estimate N uptake and remobilization; (iii) an indirect approach based on the use of stable N isotopes in their natural abundance, to assess N metabolism; or (iv) the use of stable N isotopes in their natural abundance to evaluate the role of N in determining the strength of the photosynthetic source (i.e. net photosynthesis rate, leaf duration, total green biomass). In the study under optimal conditions (Chapter 2), grain yield was negatively correlated with the N concentration of the ear two weeks post-anthesis, while such a correlation was not detected in the mild water stress conditions of the field study (Chapter 3). By contrast, grain yield was positively correlated with the N concentration of flag leaves under both fully irrigated (Chapter 2) and mild water stress (Chapter 3) conditions. As mentioned above, because the ear became an important organ to balance N storage during grain filling, the N accumulated in ears had more effect on grain filling when sink strength was the highest possible (i.e. reached its potential), which is the case under optimal growing conditions (Chapter 2). By contrast, the flag leaf was probably adequate as a source of N for grains under mild stress conditions (Chapter 3). Instead of N content, the natural abundance of $\delta^{15}N$ gave an indirect indication of the N status; specifically the NUE, N uptake efficiency and N accumulation in a variety of planting conditions (Schiltz et al. 2005; Tcherkez 2010). Under stressed field conditions, the $\delta^{15}N$ of the flag leaf, the ear or the mature kernels was negatively correlated with grain yield (Chapter 3). Negative correlations between $\delta^{15}N$ and grain yield have been reported before in durum wheat under Mediterranean conditions (Araus et al. 2013). In moderate stress conditions, the high negative correlations between grain yield and the total organic matter $\delta^{15}N$ of the flag, ear and grain showed that $\delta^{15}N$ was a powerful tool for genotypic screening (Yousfi et al. 2012; Yousfi et al. 2013).

On the other hand, a higher yield potential may involve a larger photosynthetic capacity of the whole canopy over the entire crop cycle, which also depends on N accumulation at the canopy level and its implication in a higher biomass (Parry et al. 2011). However, the ear may intercept nearly 50% of the solar irradiation after heading (Sánchez-Bragado et al. 2014). In spite of the high level of dark respiration, the positive correlation between grain yield and ear Pn (on area basis) (Chapter 2), together with the results of ^{15}N labelling (Chapter 4) further support the key role of the ear in providing photoassimilates during grain filling. Extending the ear duration (earlier heading date and late senescence) and the amount of light captured by improving the rate of leaf growth throughout the crop cycle may contribute to higher crop photosynthesis and thus yield (Parry et al. 2011). Besides this, a larger period for ear formation (which starts at the beginning of stem elongation) is postulated to also have a positive role in increasing yield potential through an increase in the number and potential size of kernels in the ear (Slafer and Savin 1994; Gaju et al. 2014b). Therefore, the high yielding genotypes combine a large green biomass and high canopy photosynthesis during the reproductive stage, together with a stay-green pattern and an efficient partitioning of N to the growing grains. Although the flag Pn (per unit area) did not correlate with grain yield under optimal (Chapter 2) and mild stress conditions (Chapter 3), other studies with Chinese wheats have reported that the positive relationships between the Pn of flag leaves and grain contributed to improvement in yield potential (Zheng et al. 2011; Xiao et al. 2012). However, methodological problems seem to exist with these papers that may weaken the conclusions attained by these studies (Hawkesford et al. 2013).

Improving drought resistance and seeking high agronomical water use efficiency (WUE) have also been critical in modern breeding programs. However, Chinese Breeders have mostly disregarded these traits in comparison to a higher yield potential (Kang et al. 2002). In this thesis, substantial efforts have been devoted to identify physiological traits associated with WUE in a variety conditions. The approaches applied included (i) the direct measurement of instantaneous stomatal conductance (gs) and the rate of transpiration (*E*) of the flag leaf, as well as the canopy temperature depression (CTD), and (ii) long-term indicators consisting of the $\delta^{13}C$ and $\delta^{18}O$ of ears, flag leaves and kernels. Moreover, high $\delta^{13}C$ has been proposed in commercial breeding as a criterion to select wheat with high WUE (Farquhar and Richards 1984; Araus et al. 2003; Monneveux et al. 2006). In our study, under optimal water conditions (but using bags as containers) positive correlations between $\delta^{13}C$ and grain yield were found (Chapter 2). However, the negative linear correlation between $\delta^{13}C$ and grain yield found under mild water stress conditions in the field (Chapter 3) suggests that the best performing genotypes are those able to maintain greater stomatal opening, and therefore greater rates of transpiration. In agreement with this hypothesis, the gs of the flag leaf was positively correlated with grain yield in the same field trials. A positive correlation between gs and grain yield has also been reported in studies on Chinese wheats under field conditions (Zheng et al. 2011). In field environments, where some degree of mild water stress is even present under “optimal” agronomical conditions, water restrictions may limit yield. Under such conditions, genotypes possessing higher stomatal conductance (gs) due to a better water status (e.g. due to a better access to soil water or some other reason) will grow faster and yield more while accumulating less ^{13}C in the organic dry matter (Araus et al. 2013). In contrast, for plants growing in absolutely optimal conditions, higher δ^{13}C may be the consequence of miscellaneous causes such as thicker or more compact leaves (Araus et al. 1997), and/or a higher transpirative cooling (Araus et al. 2003), or a larger intrinsic photosynthetic rate (e. g. caused by Rubisco activity) (Richards 2000).

Regarding biotic stresses, and specifically yellow rust (the case-study addressed in the thesis) fast, affordable and high-throughput methods to monitor the impact of disease may represent an effective way to prevent grain yield loss either through precision agriculture or phenotyping in breeding (Kuckenberg et al. 2008). Since yellow rust affects green area and photosynthetic capacity, any system that is able to assess the total amount of green area from the canopy in a reliable manner should be able to predict the impact of yellow rust on grain yield. In that sense, the use of RGB images to estimate green vegetation indices as a predictor of yield and tolerance to biotic stresses was proved reliable and affordable (Chapter 5). The colour components of Hue, Green Fraction, and Greener Fraction, combined with colour bands *a* and *u* were the most effective indicators to estimate he absolute grain yield and grain yield loss due to rust-infection. They performed much better than more conventional (albeit costly and time-consuming) approaches such as chlorophyll content of individual leaves, P_n, g_s, E or canopy temperature depression (Chapter 5). Whereas the use of

RGB images has been proposed in wheat and other cereals to assess the impact of abiotic stresses such as drought (Casadesús et al. 2007; Fiorani et al. 2012), to the best of our knowledge this is the first report on the use of RGB images from canopies to assess the impact of a fungal disease.

2. A conceptual plant model / ideotype for high yielding genotypes

Satisfying future Chinese demand for wheat will imply increasing yield and adaptation to stresses; all this in a context of climate change and growing scarcity of resources such as water or fertilizers. In fact, genetic gains in winter wheat resulting from breeding have decreased in recent decades in China (Zheng et al. 2011; Xiao et al. 2012). Therefore, future breeding strategies to increase yield potential and stability should be taken in a number of directions, including defining proper ideotypes and potential relevant secondary traits rather than only depending on just the innovative use of both germplasm and crossing strategies, followed by empirical selection for grain yield at multiple locations (Araus et al. 2008; Reynolds et al. 2011). Reynolds et al. (2009a; 2011) built a conceptual ideotype combining many traits to raise yield potential and stability. Ideotypes should be selected using a combination of visual criteria, precision phenotyping, and molecular marker-assisted approaches. Based on the physiological and agronomical traits investigated in this thesis, the conceptual model of Reynolds (2009a, 2011) has been redrawn to aid the design of crosses for raising the yield potential and stability of winter wheats for Henan Province (Fig 2). To date, increasing yield potential has been mainly achieved through a reduction in plant height, larger spike size and greater grain number per unit area, together with an increase in C and N accumulation in key organs (e.g. ears and leaves). As discussed above (Chapter 2), the lower yielding genotypes in this thesis were sink-limited under favourable conditions, with grain growth limited by the capacity of the grains to store assimilate during the grain filling period, which is a common problem in wheat (Gaju et al. 2014a). In fact, grain yield in wheat is either sink-limited or co-limited by both the source and sink (Slafer and Savin 1994), which indicated that enhanced sink capacity could be critical to increase yield potential. Improvement in crop yield is also associated with higher canopy photosynthesis throughout the crop cycle (Parry et al. 2011). However, the ear plays a very important photosynthetic role during grain filling. In another aspect, it should be emphasized that since Rubisco, a key enzyme from the Calvin cycle involved in CO_2 assimilation, is the most abundant protein in plants, its role as a pool of nitrogen storage was also important. In this sense, Rubisco seems to play an important role in contributing to N accumulation in grains, with the N originating not only from the flag leaf but also from the ear (Chapter 4). On the other hand, yield stability is also a target for future breeding, particularly in the context of increased climate unpredictability and growing environmental and economic concern about the indiscriminate use of fertilizers,. Yield stability may be achieved through a better water status, together with increased water uptake and NUE during grain filling (Chapter 3) as well as greater resistance to biotic stresses (such as. yellow rust) that are relevant to the target agroecological areas (Chapter 5).

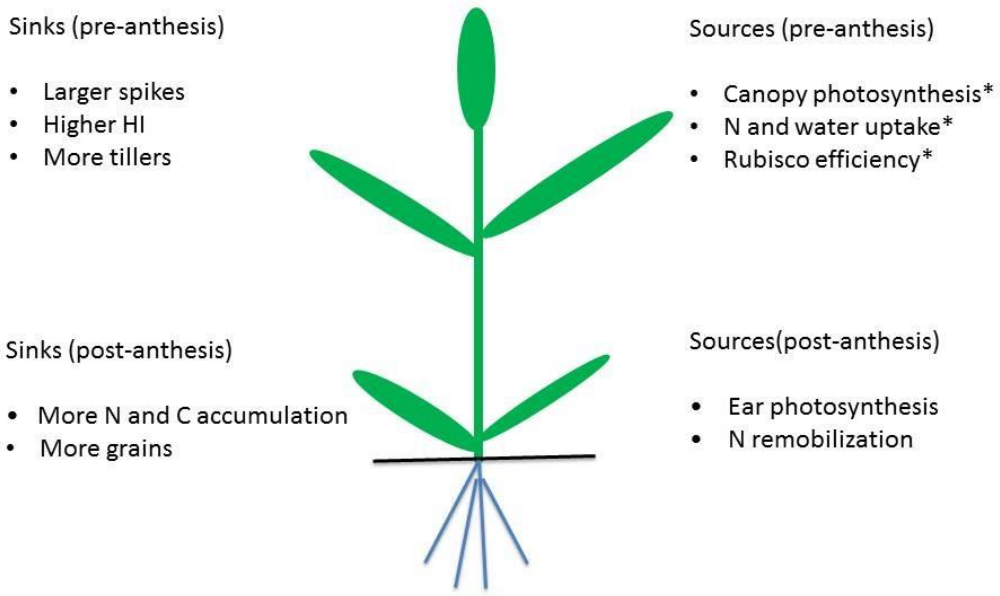


Fig. 2. A conceptual ideotype for designing crosses aimed at increasing yield potential and stability of winter wheats in Henan. All the traits were categorized as either belonging to sources or sinks, and either preferably assessed at pre-anthesis or post-anthesis, or both (*). Figure redrawn from Reynolds et al. (2009a; 2011).

3. General conclusions

- The increased grain yield potential of recent Chinese bread wheats from Henan Province appears to be the result of increases in HI, kernel number per unit land area and above-ground biomass

- Performance under mild water stress of the same genotypes seems related to a higher tillering capacity in exchange for smaller spikes and combined with moderate plant height as well as a high HI and TKW.

- Under mild stress conditions the best genotypes were those able to maintain a better water status in terms of high stomatal conductance and transpiration of the flag leaf together with a more negative $\delta^{13}C$ in the flag leaf and especially the kernels.

- Overall, the Chinese genotypes possess a low capacity for acclimation to less favourable conditions, probably as a consequence of a low tillering capacity and short stature, which make them prone to yield penalties even under moderate water stress conditions and/or with lower N fertilization levels.

- The higher yield potential of the most recent genotypes seems related to a higher $\delta^{13}C$ in plant matter and thus a higher WUE. However, this constitutive high WUE does not appear to be the consequence of a lower stomatal conductance in the most recent genotypes.

- The genetic advance in yield potential does not appear to be related to changes in photosynthesis rates on an area basis when measured in the flag leaf or the spike, but only to higher whole-spike photosynthesis. These results also highlight the key role of the spike as a photosynthetic organ during grain filling.

- Overall the genotypes with the highest yield under both stressed and well watered conditions were characterized by the highest uptake efficiency of N fertilizer together with the highest N utilization efficiency of leaves and the capacity to sustain a larger canopy during the reproductive stage.

- Under optimal conditions N remobilized from the shoot represented the most important N source to the kernel, while the $\delta^{15}N$ of flag leaves and glumes revealed different patterns in high and low grain-yielding genotypes. Thus, although N derived from flag leaf Rubisco represented a major N source in low yielding genotypes, N derived from ear Rubisco was more relevant in the high yielding ones. Moreover a high capacity for N accumulation in the ears and further translocation to the kernels represented higher yield potential

- Most of Chinese cultivars were susceptible to yellow rust, which caused a grain yield loss associated with a reduction in the green leaf area index together with a lower P_n on an area basis.

- The RGB imagery was an effective and low-cost method for yellow rust phenotyping under field conditions. The colour components of Hue, Green Fraction, and Greener Fraction, combined with colour bands *a* and *u* were the most effective indicators for estimation of the absolute grain yield and grain yield loss due to rust stress.

References (general introduction and general discussion):

Abbad H, Jaafari SE, Bort J, Araus JL (2004) Comparison of flag leaf and ear photosynthesis with biomass and grain yield of durum wheat under various water conditions and genotypes. **Agronomie** 24: 19-28

Aranjuelo I, Cabrera-Bosquet L, Morcuende R, Avice JC, Nogues S, Araus JL, Martinez-Carrasco R, Perez P (2011) Does ear C sink strength contribute to overcoming photosynthetic acclimation of wheat plants exposed to elevated CO_2? **Journal of Experimental Botany** 62: 3957-3969

Araus JL, Amaro T, Zuhair Y, Nachit MM (1997) Effect of leaf structure and water status on carbon isotope discrimination in field-grown durum wheat. **Plant, Cell & Environment** 20: 1484-1494

Araus JL, Bort J, Steduto P, Villegas D, Royo C (2003) Breeding cereals for Mediterranean conditions: ecophysiological clues for biotechnology application. **Annals of Applied Biology** 142: 129-141

Araus JL, Brown HR, Febrero A, Bort J, Serret MD (1993) Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory CO_2 to differences in grain mass in durum wheat. **Plant, Cell & Environment** 16: 383-392

Araus JL, Brown HR, Febrero A, Bort J, Serret MD (1993) Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory CO_2 to differences in grain mass in durum wheat. **Plant, Cell & Environment** 16: 383-392

Araus JL, Cabrera-Bosquet L, Serret MD, Bort J, Nieto-Taladriz MT (2013) Comparative performance of $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ for phenotyping durum wheat adaptation to a dryland environment. **Functional Plant Biology** 40: 595-608

Araus JL, Cairns JE (2014) Field high-throughput phenotyping: the new crop breeding frontier. **Trends in Plant Science** 19: 52-61

Araus JL, Slafer GA, Royo C, Serret MD (2008) Breeding for Yield Potential and Stress Adaptation in Cereals. **Critical Reviews in Plant Sciences** 27: 377-412

Araus JL, Villegas D, Aparicio N, del Moral LFG, El Hani S, Rharrabti Y, Ferrio JP, Royo C (2003) Environmental factors determining carbon isotope discrimination and yield in durum wheat under mediterranean conditions. **Crop Sciences** 43: 170-180

Barbour MM, Farquhar GD (2000) Relative humidity- and ABA-induced variation in carbon and oxygen isotope ratios of cotton leaves. **Plant, Cell & Environment** 23: 473-485

Berger B, Parent B, Tester M (2010) High-throughput shoot imaging to study drought responses. **Journal of Experimental Botany** 61: 1-13

Cabrera-Bosquet L, Albrizio R, Nogues S, Araus JL (2011) Dual $^{13}C/^{18}O$ response to water and nitrogen availability and its relationship with yield in field-grown durum wheat. **Plant, Cell & Environment** 34: 418-433

Cabrera-Bosquet L, Crossa J, von Zitzewitz J, Serret MD, Araus JL (2012) High-throughput phenotyping and genomic selection: the frontiers of crop breeding converge. **Journal of Integrative Plant Biology** 54: 312-320

Casadesús J, Kaya Y, Bort J, Nachit MM, Araus JL, Amor S, Ferrazzano G, Maalouf F, Maccaferri M, Martos V, Ouabbou H, Villegas D (2007) Using vegetation indices derived from conventional digital cameras as selection criteria for wheat breeding in water-limited environments. **Annals of Applied Biology** 150: 227-236

Cui Z, Chen X, Miao Y, Li F, Zhang F, Li J, Ye Y, Yang Z, Zhang Q, Liu C (2008) On-farm evaluation of winter wheat yield response to residual soil nitrate-N in North China Plain. **Agronomy Journal** 100: 1527

Diaz C, Lemaitre T, Christ A, Azzopardi M, Kato Y, Sato F, Morot-Gaudry JF, Le Dily F, Masclaux-Daubresse C (2008) Nitrogen recycling and remobilization are differentially controlled by leaf senescence and development stage in Arabidopsis under low nitrogen nutrition. **Plant Physiology** 147: 1437-1449

Farquhar G, Richards R (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. **Functional Plant Biology** 11: 539-552

Fiorani F, Rascher U, Jahnke S, Schurr U (2012) Imaging plants dynamics in heterogenic environments. **Current Opinion in Biotechnology** 23: 227-235

Foulkes MJ, Slafer GA, Davies WJ, Berry PM, Sylvester-Bradley R, Martre P, Calderini DF, Griffiths S, Reynolds MP (2011) Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. **Journal of Experimental Botany** 62: 469-486

Gaju O, Allard V, Martre P, Le Gouis J, Moreau D, Bogard M, Hubbart S, Foulkes MJ (2014a) Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. **Field Crops Research** 155: 213-223

Gaju O, Reynolds MP, Sparkes DL, Mayes S, Ribas-Vargas G, Crossa J, Foulkes MJ (2014b) Relationships between physiological traits, grain number and yield potential in a wheat DH population of large spike phenotype. **Field Crops Research** 164: 126-135

Hawkesford MJ, Araus J-L, Park R, Calderini D, Miralles D, Shen T, Zhang J, Parry MAJ (2013) Prospects of doubling global wheat yields. **Food and Energy Security** 2: 34-48

He ZH, Xia XC, Chen XM, and Zuang QS. 2011. Progress of wheat breeding in china and the future perspective. **Acta Agronomica Sinica**, 37: 202-215

He ZH, Xia XC, Chen XM, and Zuang QS. 2011. Progress of wheat breeding in China and the future perspective. **Acta Agronomica Sinica**, 37: 202-215

Huang W, Lamb DW, Niu Z, Zhang Y, Liu L, Wang J (2007) Identification of yellow rust in wheat using in-situ spectral reflectance measurements and airborne hyperspectral imaging. **Precision Agriculture** 8: 187-197

Jiang GM, Sun JZ, Liu HQ, Qu CM, Wang KJ, Guo RJ, Bai KZ, Gao LM, Kuang TY (2003) Changes in the rate of photosynthesis accompanying the yield increase in wheat cultivars released in the past 50 years. **Journal of Plant Research** 116: 347-354

Kang S, Zhang L, Liang Y, Hu X, Cai H, Gu B (2002) Effects of limited irrigation on yield and water use efficiency of winter wheat in the Loess Plateau of China. **Agricultural Water Management** 55: 203-216

Kichey T, Hirel B, Heumez E, Dubois F, Le Gouis J (2007) In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers. **Field Crops Research** 102: 22-32

Kuckenberg J, Tartachnyk I, Noga G (2008) Temporal and spatial changes of chlorophyll fluorescence as a basis for early and precise detection of leaf rust and powdery mildew infections in wheat leaves. **Precision Agriculture** 10: 34-44

Lenthe JH, Oerke EC, Dehne HW (2007) Digital infrared thermography for monitoring canopy health of wheat. **Precision Agriculture** 8: 15-26

Li GQ, Li ZF, Yang WY, Zhang Y, He ZH, Xu SC, Singh RP, Qu YY, Xia XC (2006a) Molecular mapping of stripe rust resistance gene YrCH42 in Chinese wheat cultivar Chuanmai 42 and its allelism with Yr24 and Yr26. **Theor Appl Genet** 112: 1434-1440

Li ZF, Zheng TC, He ZH, Li GQ, Xu SC, Li XP, Yang GY, Singh RP, Xia XC (2006b) Molecular tagging of stripe rust resistance gene YrZH84 in Chinese wheat line Zhou 8425B. **Theor Appl Genet** 112: 1098-1103

Liu X, Ju X, Zhang F, Pan J, Christie P (2003) Nitrogen dynamics and budgets in a winter wheat–maize cropping system in the North China Plain. **Field Crops Research** 83: 111-124

Ma W, Li J, Ma L, Wang F, Sisák I, Cushman G, Zhang F (2008) Nitrogen flow and use efficiency in production and utilization of wheat, rice, and maize in China. **Agricultural Systems** 99: 53-63

Madani A, Rad AS, Pazoki A, Nourmohammadi G, Zarghami R (2010) Wheat (*Triticum aestivum* L.) grain filling and dry matter partitioning responses to source:sink modifications under postanthesis water and nitrogen deficiency. **Acta Scientiarum Agronomy** 32: 145-151

Martre P, Porter JR, Jamieson PD, Triboi E (2003) Modeling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen remobilization for wheat. **Plant Physiology** 133: 1959-1967

Masclaux-Daubresse C, Reisdorf-Cren M, Orsel M (2008) Leaf nitrogen remobilisation for plant development and grain filling. **Plant Biology** 10 Suppl 1: 23-36

Maydup ML, Antonietta M, Guiamet JJ, Graciano C, López JR, Tambussi EA (2010) The contribution of ear photosynthesis to grain filling in bread wheat (*Triticum aestivum* L.). **Field Crops Research** 119: 48-58

Mirik M, Michels GJ, Kassymzhanova-Mirik S, Elliott NC, Catana V, Jones DB, Bowling R (2006) Using digital image analysis and spectral reflectance data to quantify damage by greenbug (Hemitera: Aphididae) in winter wheat. **Computers and Electronics in Agriculture** 51: 86-98

Moller M, Alchanatis V, Cohen Y, Meron M, Tsipris J, Naor A, Ostrovsky V, Sprintsin M, Cohen S (2007) Use of thermal and visible imagery for estimating crop water status of irrigated grapevine. **Journal of Experimental Botany** 58: 827-838

Monneveux P, Rekika D, Acevedo E, Merah O (2006) Effect of drought on leaf gas exchange, carbon isotope discrimination, transpiration efficiency and productivity in field grown durum wheat genotypes. **Plant Science** 170: 867-872

Ortiz-Monasterio R. JI, Sayre KD, Rajaram S, McMahon M (1997) Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. **Crop Sciences** 37: 898-904

Parry MA, Reynolds M, Salvucci ME, Raines C, Andralojc PJ, Zhu XG, Price GD, Condon AG, Furbank RT (2011) Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. **Journal of Experimental Botany** 62: 453-467

Reynolds M, Bonnett D, Chapman SC, Furbank RT, Manes Y, Mather DE, Parry MA (2011) Raising yield potential of wheat. I. Overview of a consortium approach and breeding strategies. **Journal of Experimental Botany** 62: 439-452

Reynolds M, Foulkes MJ, Slafer GA, Berry P, Parry MA, Snape JW, Angus WJ (2009) Raising yield potential in wheat. **Journal of Experimental Botany** 60: 1899-1918

Reynolds MP, Rajaram S, Sayre KD (1999) Physiological and genetic changes of irrigated wheat in the post–green revolution period and approaches for meeting projected global demand. **Crop Science** 39: 1611-1621

Reynolds MP, van Ginkel M, Ribaut JM (2000) Avenues for genetic modification of radiation use efficiency in wheat. **Journal of Experimental Botany** 51: 459-473

Richards RA (2000) Selectable traits to increase crop photosynthesis and yield of grain crops. **Journal of Experimental Botany** 51: 447-458

Richards RA (2000) Selectable traits to increase crop photosynthesis and yield of grain crops. **Journal of Experimental Botany** 51: 447-458

Richards RA (2006) Physiological traits used in the breeding of new cultivars for water-scarce environments. **Agricultural Water Management** 80: 197-211

Robinson D, Handley LL, Scrimgeour CM, Gordon DC, Forster BP, Ellis RP (2000) Using stable isotope natural abundances (δ^{15}N and δ^{13}C) to integrate the stress responses of wild barley (Hordeum spontaneum C. Koch.) genotypes. **Journal of Experimental Botany** 51: 41-50

Sánchez-Bragado R, Elazab A, Zhou B, Serret MD, Bort J, Nieto-Taladriz MT, Araus JL (2014b) Contribution of the ear and the flag leaf to grain filling in durum wheat inferred from the carbon isotope signature: Genotypic and growing conditions effects. **Journal of Integrative Plant Biology** 56: 444-454

Sánchez-Bragado R, Molero G, Reynolds MP, Araus JL (2014a) Relative contribution of shoot and ear photosynthesis to grain filling in wheat under good agronomical conditions assessed by differential organ $\delta^{13}C$. **Journal of Experimental Botany** 65: 5401-5413

Schiltz S, Munier-Jolain N, Jeudy C, Burstin J, Salon C (2005) Dynamics of exogenous nitrogen partitioning and nitrogen remobilization from vegetative organs in pea revealed by ^{15}N *in vivo* labeling throughout seed filling. **Plant Physiology** 137: 1463-1473

Slafer GA, Savin R (1994) Source-sink relationships and grain mass at different positions within the spike in wheat. **Field Crops Research** 37: 39-49

Sun ZJ, Livingston NJ, Guy RD, Ethier GJ (1996) Stable carbon isotopes as indicators of increased water use efficiency and productivity in white spruce (Picea glauca (Moench) Voss) seedlings. **Plant, Cell & Environment** 19: 887-894

Tambussi EA, Bort J, Guiamet JJ, Nogués S, Araus JL (2007) The photosynthetic role of ears in C_3 cereals: metabolism, water use efficiency and contribution to grain yield. **Critical Reviews in Plant Sciences** 26: 1-16

Tambussi EA, Bort J, Guiamet JJ, Nogués S, Araus JL (2007) The Photosynthetic Role of Ears in C3Cereals: Metabolism, Water Use Efficiency and Contribution to Grain Yield. **Critical Reviews in Plant Sciences** 26: 1-16

Tcherkez G (2010) Natural $^{15}N/^{14}N$ isotope composition in C_3 leaves: are enzymatic isotope effects informative for predicting the ^{15}N-abundance in key metabolites? **Functional Plant Biology** 38: 1-12

Tcherkez G (2010) Natural $^{15}N/^{14}N$ isotope composition in C_3 leaves: are enzymatic isotope effects informative for predicting the ^{15}N-abundance in key metabolites? **Functional Plant Biology** 38: 1-12

Tcherkez G, Hodges M (2008) How stable isotopes may help to elucidate primary nitrogen metabolism and its interaction with (photo)respiration in C_3 leaves. **Journal of Experimental Botany** 59: 1685-1693

Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. **Science** 327: 818-822

Vadivambal R, Jayas D (2011) Applications of thermal imaging in agriculture and food industry—A review. **Food and Bioprocess Technology** 4: 186-199

Wu X, Chang X, Jing R (2012) Genetic insight into yield-associated traits of wheat grown in multiple rain-fed environments. **PLoS ONE** 7: e31249

Xiao YG, Qian ZG, Wu K, Liu JJ, Xia XC, Ji WQ, He ZH (2012) Genetic gains in grain yield and physiological traits of winter wheat in shandong province, China, from 1969 to 2006. **Crop Science** 52: 44

Xu WG, Hu L, Wu Z, Ge JY. 2000. Genetic improvement of yield and yield components of wheat cultivars in the Guanzhong area. **Crops Journal**. 3: 25-33

Xu X, Yuan H, Li S, Monneveux P (2007) Relationship between carbon isotope discrimination and grain yield in spring wheat under different water regimes and under saline conditions in the Ningxia Province (North-west China). **Journal of Agronomy and Crop Science** 193: 422-434

Yang X, Chen X, Ge Q, Li B, Tong Y, Zhang A, Li Z, Kuang T, Lu C (2006) Tolerance of photosynthesis to photoinhibition, high temperature and drought stress in flag leaves of wheat: A comparison between a hybridization line and its parents grown under field conditions. **Plant Science** 171: 389-397

Yin GH, Wang JW, Wen WE, He ZH, Li ZF, Wang H, Xia XC (2009) Mapping of wheat stripe rust resistance gene <YrZH84> with RGAP Markers and its application. **Acta Agronomica Sinica** 35: 1274-1281

Yousfi S, Serret MD, Araus JL (2013) Comparative response of $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ in durum wheat exposed to salinity at the vegetative and reproductive stages. **Plant, Cell & Environment** 36: 1214-1227

Yousfi S, Serret MD, Marquez AJ, Voltas J, Araus JL (2012) Combined use of $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ tracks nitrogen metabolism and genotypic adaptation of durum wheat to salinity and water deficit. **The New Phytologist** 194: 230-244

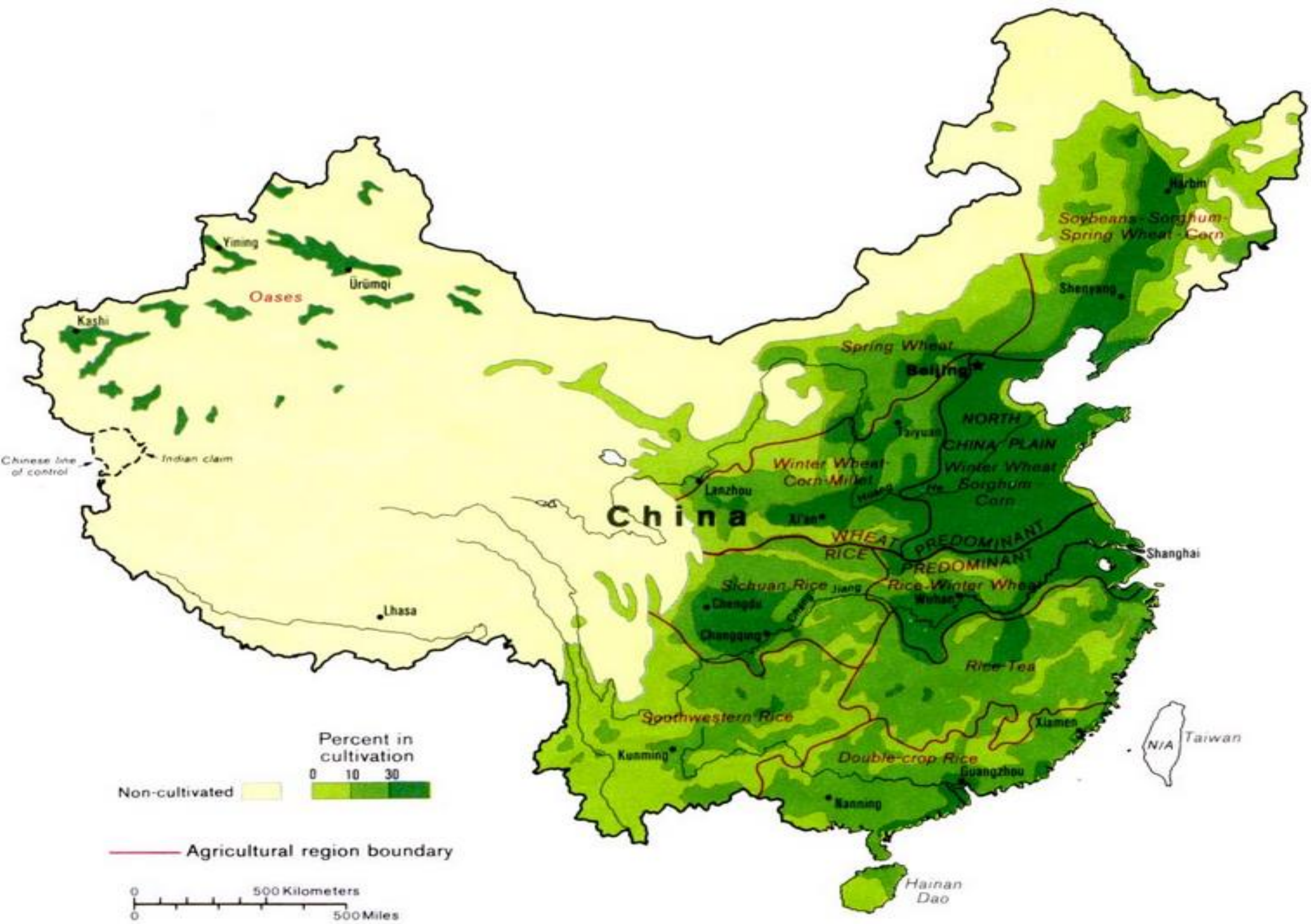
Zhao XL, Zheng TC, Xia XC, He ZH, Liu DQ, Yang WX, Yin GH, Li ZF (2008) Molecular mapping of leaf rust resistance gene LrZH84 in Chinese wheat line Zhou 8425B. **Theoretical and Applied Genetics** 117: 1069-1075

Zheng TC, Zhang XK, Yin GH, Wang LN, Han YL, Chen L, Huang F, Tang JW, Xia XC, He ZH (2011) Genetic gains in grain yield, net photosynthesis and stomatal conductance achieved in Henan Province of China between 1981 and 2008. **Field Crops Research** 122: 225-233

Zhou Y, He ZH, Sui XX, Xia XC, Zhang XK, Zhang GS (2007) Genetic improvement of grain yield and associated traits in the Northern China Winter Wheat Region from 1960 to 2000. **Crop Science** 47: 245-253

Zhu L, Li SH, Liang ZS, Xu X, Li Y (2010a) Relationship between carbon isotope discrimination, mineral content and gas exchange parameters in vegetative organs of wheat grown under three different water regimes. **Journal of Agronomy and Crop Science** 196: 175-184

Zhu XG, Long SP, Ort DR (2010b) Improving photosynthetic efficiency for greater yield. **Annual review of Plant Biology** 61: 235-261



Physiological traits associated with recent advances in yield of Chinese wheat