

# Proceedings

## of the 3<sup>rd</sup> International Workshop of the Wheat Yield Consortium



CENEB, CIMMYT, Cd. Obregón, Sonora, Mexico  
5-7<sup>th</sup> March 2013

Matthew Reynolds and Hans Braun  
(Editors)



Proceedings of the 3<sup>rd</sup> International Workshop  
of the  
**Wheat Yield Consortium**

CENEB, CIMMYT, Cd. Obregón, Sonora, Mexico

(5-7<sup>th</sup> March, 2013)

Matthew Reynolds and Hans Braun  
(Editors)

**Sponsored by**

SAGARPA through MasAgro, Mexico; and GRDC, Australia

**The International Maize and Wheat Improvement Center**, known by its Spanish acronym, CIMMYT® ([www.cimmyt.org](http://www.cimmyt.org)), is a not-for-profit research and training organization with partners in over 100 countries. The center works to sustainably increase the productivity of maize and wheat systems and thus ensure global food security and reduce poverty. The center's outputs and services include improved maize and wheat varieties and cropping systems, the conservation of maize and wheat genetic resources, and capacity building.

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**Abstract:** The abstracts herein are of presentations by crop experts for the "3<sup>rd</sup> International Workshop of the Wheat Yield Consortium". Sponsored by SAGARPA's international strategic component for increasing wheat performance, under the *Sustainable Modernization of Traditional Agriculture Program* (MasAgro); and GRDC, Australia.

The event covers innovative methods to significantly raise wheat yield potential, including making photosynthesis more efficient, improving adaptation of flowering to diverse environments, addressing the physical processes involved in lodging, and physiological and molecular breeding. The workshop represents the current research of the International Wheat Yield Consortium that involves scientists working on all continents to strategically integrate research components in a common breeding platform, thereby speeding the delivery to farmers of new wheat genotypes.

Thanks to Laura Martinez for her help in organizing the workshop and coordinating logistics, with the able assistance of Viridiana Silva and Eloisa Carrillo.

# Table of Contents

Program .....	v
List of participants .....	vii
<b>Background to Yield Potential Research</b>	
Achieving yield gains in wheat: Overview .....	ix
<i>Reynolds and Braun</i>	
A comparison of research outputs in rice and wheat .....	xii
<i>Vallaru et al.</i>	
<b>Improving Crop Biomass</b>	
Increasing photosynthetic capacity and efficiency of wheat .....	1
<i>Furbank et al.</i>	
Phenotypic selection for photosynthetic capacity and efficiency .....	4
<i>Furbank et al.</i>	
Phenotypic selection for spike photosynthesis .....	9
<i>Molero et al.</i>	
Optimizing and modeling canopy photosynthesis and photosynthetic duration .....	12
<i>Condon et al.</i>	
Genetic variation in radiation interception at different canopy levels and in radiation use efficiency .....	16
<i>López-Castañeda et al.</i>	
Optimizing RuBP regeneration to increase photosynthetic capacity .....	20
<i>Parry et al.</i>	
Improving the thermal stability of Rubisco activase .....	22
<i>Carmo-Silva et al.</i>	
Replacing the large subunit of Rubisco .....	23
<i>Martin Parry et al.</i>	
<b>Optimizing Partitioning to Maximize Agronomic Yield</b>	
Optimizing partitioning to grain while maintaining physiological and structural integrity .....	24
<i>Reynolds et al.</i>	
Optimizing harvest index through increasing partitioning to spike growth and maximizing grain number .....	26
<i>Foulkes et al.</i>	
Dynamics of floret development determining differences in spike fertility within the CIMCOG population .....	32
<i>Gonzalez et al.</i>	
Improving spike fertility by understanding and modifying its sensitivity to environmental cues .....	36
<i>Davies et al.</i>	
Improving grain filling and potential grain size .....	39
<i>Calderini et al.</i>	
Identifying traits and developing genetic sources for lodging resistance .....	45
<i>Berry et al.</i>	

<b>Breeding for Yield Potential and Research Support Platforms</b>	
An integrated approach to breeding for yield potential .....	49
<i>Griffiths et al.</i>	
Pre-breeding for yield potential.....	52
<i>Reynolds et al.</i>	
Expanding the genetic basis of wheat.....	56
<i>King and King</i>	
Genomic selection to increase breeding efficiency.....	58
<i>Bonnett et al.</i>	
<b>China Update</b>	
Wheat Yield Consortium update from China.....	61
<i>He et al.</i>	
<b>Germplasm Evaluation and Delivery</b>	
CIMCOG: Mexico and International data summary .....	63
<i>Chavez et al.</i>	
The CSISA wheat phenotyping network .....	70
<i>Pask et al.</i>	
<b>Gene Discovery Platforms</b>	
Advances in QTL discovery using using two wheat populations designed to minimize confounding agronomic effects .....	73
<i>Lopes and Reynolds</i>	
Phenotyping and genotyping of an elite wheat double haploid population: grain yield strategies in contrasting environments and QTL analysis .....	74
<i>Garcia et al.</i>	
Wheat mapping populations available at CIMMYT for yield potential research .....	78
<i>Sukumaran et al.</i>	

# Program: 3<sup>rd</sup> Wheat Yield Consortium Workshop (5-7<sup>th</sup> March, 2013)

## Cd. Obregon, Mexico

### Tuesday 5<sup>th</sup> March

**7:30-8:30am** *Registration, Quality Inn lobby*

**8:30-10:00** *Welcome and Background to Yield Potential Research (Chair Victor Kommerell)*

- Welcome Address (John Snape)
- The Wheat Yield Network (Vicky Jackson)
- Achieving Yield Gains in Wheat: Overview (Matthew Reynolds)
- A Comparison of Research Outputs in Rice and Wheat (Ravi Valluru)

**10:30-12:30** *Improving Crop Biomass (Chair Bill Davies)* (10 mins + 5min discussion)

- Overview: Increasing photosynthetic capacity and efficiency of wheat (Martin Parry)
- Optimizing leaf and canopy photosynthesis (John Evans)
- Phenotypic selection for spike photosynthesis (Gemma Molero)
- Optimizing RuBP regeneration to increase photosynthetic capacity (Elizabete Carmo Silva)
- Carbon concentrating mechanisms (Murray Badger)
- Improving the thermal stability of Rubisco activase (Elizabete Carmo Silva)
- Replacing the large subunit of Rubisco (Martin Parry)
- Group discussion led by Chair

**1:30-3:15** *Optimizing partitioning to maximize agronomic yield (Chair Martin Parry)* (10+5min discussion)

- Overview: Optimizing partitioning to grain while maintaining physiological and structural integrity (Jacques LeGouis)
- Optimizing harvest index through increasing partitioning to spike growth (John Foulkes)
- Optimizing developmental pattern to maximize spike fertility (Gustavo Slafer)
- Improving spike fertility by modifying its sensitivity to environmental cues (Bill Davies)
- Improving grain filling and potential grain size (Daniel Calderini)
- Identifying traits and developing genetic sources for lodging resistance (Pete Berry)
- Group discussion led by Chair

**3:45-5:30** *Breeding for Yield Potential and Research Support Platforms (Chair Daniel Calderini)* (10+ 5min)

- Overview: An integrated approach to breeding for yield potential (Simon Griffiths)
- Pre-breeding for Yield Potential (Matthew Reynolds)
- Genomic selection to increase breeding efficiency (David Bonnett)
- Germplasm evaluation and delivery
- The CSISA Wheat Phenotyping Network (Sindhu Sareen)
- CIMCOG: Mexico and International data summary (Perla Chavez)
- Gene Discovery
- Advances in QTL discovery using two wheat populations designed to minimize confounding agronomic (Marta da Silva)
- Phenotyping and genotyping of an elite wheat double haploid population (Daniel Miralles)
- Wheat mapping populations available at CIMMYT for yield potential research (Marc Ellis)
- Group discussion led by Chair

**7:00-9:00** *Dinner at La Catrina restaurant (in front of Quality Inn)*

**Wednesday, 6<sup>th</sup> March**

**Field Day, CENEB Field Station, Cd Obregon**

**8:00** Bus departs from Quality Inn

**9:00-12:00** **Wheat Yield Potential; Scientists working at MEXPLAT** (15+5 min)

- Light interception, radiation use efficiency and yield potential in wheat (Candido López Castañeda)
- High-throughput screening for photosynthetic efficiency and capacity in wheat (Viridiana Silva & John Evans)
- Use of stable isotopes to study spike photosynthesis contribution to grain filling (Rut Sanchez & Jose Luis Araus)
- Physiological and molecular traits associated with the determination of potential grain weight of wheat (Alejandro Quintero & Daniel Calderini)
- Phenotyping methods for measuring lodging resistance (Francisco Piñera & Pete Berry)
- Improving spike fertility in wheat by modifying its sensitivity to environmental cues (Arnauld Thiry & Bill Davies)
- Optimizing harvest index through increasing partitioning to spike growth and maximizing grain number (Carolina Rivera & John Foulkes)
- Increasing yield potential in wheat through enhanced allocation of dry matter to grain and optimized post-anthesis source-sink balance (Eliseo Trujillo & John Foulkes)

**12:00-1:30** Lunch at CENEB Station

**1:30-4:30** **Wheat Yield and Stress Adaptation, Physiology group and collaborators** (8+4min)

- Current genetic progress in wheat yield potential (Julio Huerta)
- Overview of the role of crop physiology in breeding and genetics (Matthew Reynolds)
- Studying spike photosynthesis (Gemma Molero)
- Adaptation to agronomic planting densities (Perla Chavez)
- Adapting phenology to different environments (Sivakumar Sukumaran)
- Improving heat tolerance (Mariano Cossani)
- Quantifying respiration at high temperature (Suzuky Pinto)
- Improving the modeling of high temperature response (Phillip Alderman)
- QTL mapping for drought (Bruno Bouffier and Shiferaw Gicaw)
- Airborne remote sensing platform for high-throughput phenotyping (Maria Tattaris)
- Root phenotyping with ground penetrating radar (Sean Thompson)
- The role of ethylene in abiotic stress response (Ravi Valluru)
- Pre-breeding activities for heat and drought adaptation (Marc Ellis)
- Hybrid Wheat (David Bonnett)

**5:00-9:00** **Carne Asada and Mariachis at CENEB Station**

**Thursday 7<sup>th</sup> March**

**Discussions, CENEB Field Station, Cd Obregon**

- 9:00-12:00 Follow Up among scientists and PhD students
- 12:00-1:30 Lunch at Station
- 1:30-5:00 Discussion on funding strategies
- 6:00 Departure for Airport or Quality Inn
- 7:00 Dinner at *Las Parillas*

# List of participants

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CIMMYT – Cd. Obregón, Son.

March 5-7<sup>th</sup>, 2013

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# Background to Yield Potential Research

## Achieving yield gains in wheat: Overview

Matthew Reynolds and Hans Braun  
CIMMYT Mexico

### Wheat and Food Security

Wheat provides 20% of the calories to the world's population and a similar proportion of daily protein for about 2.5 billion people in less developed countries (Braun et al. 2010). Although production has increased steadily, the price of wheat continues to increase at a considerably faster rate (Fig. 1). The future productivity of wheat will be of utmost importance for global food security since it is the most widely grown crop, being adapted to a broad range of latitudes, temperatures, water regimes, and nutritional levels. In spite of predicted increases in demand for wheat at a rate of around 1.6% p.a. until 2050 (Rosegrant and Agcaoili 2010), productivity in farmers' fields is increasing globally at only 1.1% p.a. (Dixon et al. 2009), and stagnating in some regions (Brisson et al. 2010). Factors ranging from climate change, diminishing natural resources, reduced input, and competition for most fertile land by higher value crops threaten to further reduce potential productivity. While theoretically more land could be brought into production, this is not desirable in terms of the long term sustainability of the global ecosystem. The most direct solution to these problems will be to increase productivity on currently cultivated land through adoption of cultivars with improved genetic yield potential which is related to farm level yields (Fischer and Edmeades 2010) and expressed under a broad range of conditions (Reynolds et al. 2009).

### A strategy for the genetic improvement of wheat

In spite of some studies suggesting that genetic gains of wheat are stagnating (Graybosch and Peterson 2010), a recent assessment of the impact of CIMMYT's Global Wheat Program reported genetic gains of between 0.6% and 1.0% p.a. for germplasm released between 1994 and 2010, based on data from ~1400 yield testing sites worldwide (Lopes et al. 2012; Manes et al. 2012; Sharma et al. 2012). In spite of this achievement, the numbers still fall short of meeting the predicted demands by 2050 (Rosegrant et al. 2010), a mismatch that represents a serious challenge for future food security. Given concerns about the ability of our agricultural system to meet future demand (Godfray et al. 2010) and the central role of wheat in maintaining food security, CIMMYT began consulting with crop experts worldwide in 2008 to establish a research network that would focus specifically on raising the genetic yield potential of wheat to its theoretical limit. Ongoing research efforts are being brought together to promote synergies between respective disciplines, including photosynthesis (Parry et al. 2011), partitioning to grain yield and lodging resistance (Foulkes et al. 2011), and breeding to accumulate yield potential traits (Reynolds et al. 2011).

There is a scientific rationale for focusing on these processes. At one end of the crop research spectrum (in terms of level of integration) sits conventional

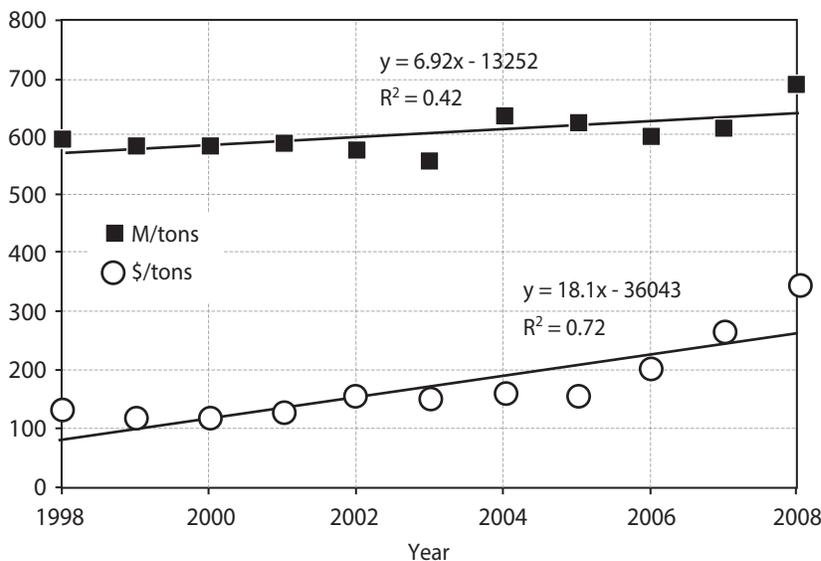


Figure 1. Comparison of global wheat production with wheat price, 1998-2008 (FAOSTAT 2010).

breeding for yield. Notwithstanding the fact that genes of major effect have been established for disease resistance and flowering time, when it comes to improvement of yield per se, crossing and selection is still based mainly on empirical approaches because genetic bases of yield are complex and in the majority of cases unknown. At the other end of the spectrum, molecular approaches have great potential to dissect yield into its genetic components. However, translation into routine breeding, even in model species like rice, has not occurred. This is because yield cannot be genetically dissected until its physiological components are themselves better defined. Growth analysis has helped better understand the integration of processes such as photosynthesis, partitioning, and phenology, but substantial knowledge gaps still exist in terms of what are the current bottlenecks to yield improvement, and until these are better defined, genetic dissection will remain elusive. For this reason, phenotyping approaches that sit somewhere in the middle of this research spectrum can play a central role in breeding for yield per se, as well as the eventual adoption of molecular breeding, as outlined below:

- Identify yield-limiting steps. By considering processes like photosynthesis and partitioning, with the hierarchy of physiological traits (PTs) falling under them, analysis can be performed using expression data from realistic field environments to determine which component PTs are rate-limiting. This information can be applied directly in hybridization strategies (below) as well as providing an efficient focus for more intensive research involving the advanced toolkit of modern biotechnology, reducing the empiricism of the latter.
- Design strategic crosses. Since the numbers of potential parental lines for crossing for a given target environment is typically relatively low (in the 100s), it is feasible to perform detailed phenotypic characterization at this scale to develop a catalogue of information that can be used to design strategic crosses aimed at combing complementary PTs through hybridization.
- Shift gene frequencies in breeding populations. Favorable expression of a PT represents the de facto favorable combination of unspecified alleles in a given genetic background; thus PTs can be used as proxy markers to shift gene frequencies in favorable directions in progeny selection where high throughput phenotyping platforms are available.

- Exploration of genetic resources. Analysis of yield limiting traits, combined with phenotyping of conventional gene pools, can help identify specific targets for exploration of genetic resources, thereby enabling isolated gene pools (winter wheat, landraces, primitive wheats, wild species) to be mined for traits and used in pre-breeding or wide crossing to augment current levels of expression. The same genetic resources can be used experimentally to determine molecular bases of yield-limiting traits.
- Gene discovery. Refinement and development of new high throughput phenotyping platforms hinge on the correct application of physiological tools and principles to ensure that data generated is scientifically meaningful. As such, these tools are very much a prerequisite to gene discovery of agronomically relevant traits. As genotyping tools become cheaper and increasingly sophisticated, the value of phenotypic data increases.

Theory suggests that the RUE of wheat could be increased ~50% through a combination of transgenic and non-transgenic interventions. While increasing photosynthetic potential will require considerable research focused at cellular and sub-cellular processes, this must go hand in hand with genetic improvement of structural and reproductive aspects of growth, since these will determine the net agronomic benefit of increased RUE. Partitioning to reproductive structures cannot be at the cost of physiological and structural integrity and crop development must be tailored to different latitudes, for example. To bring these traits together through hybridization will require a multifaceted breeding effort including modeling of optimal trait combinations, crossing among genetic resources to introduce necessary levels of trait expression, and the application of genomic and phenotyping tools to increase the efficiency of progeny screening and genetic resource exploration.

### **Product Delivery**

The target is a 50% increase in genetic yield potential of wheat targeted to relatively optimal growing regions (optimal environments currently represent 40% of the area in developing countries and 70% of grain production) within 20-25 years. The impact of research and breeding will depend on effective delivery of products. This will be achieved by distribution of germplasm with improved yield and yield potential characteristics through

the International Wheat Improvement Network (IWIN). CIMMYT has coordinated the IWIN for almost 50 years; this international wheat breeding effort, through its international nursery system, delivers ~1,000 new genotypes per year, targeted to the varying needs of national wheat programs in less developed countries (Braun et al. 2010). Impacts at the farm level are well documented (Lipton and Longhurst 1989; Evenson and Gollin 2003). Many of the traits conferring improved yield potential are also expected to have benefits in more marginal environments, albeit not necessarily as large. The spillover of genetic yield potential to more marginal wheat growing regions, that experience heat and drought stress for example, is well documented (Lantican et al. 2003).

### **Crop Management**

Improved methods of crop management are also expected to play a crucial role in increasing wheat productivity, for two main reasons: (i) improvements in genetic yield potential are more fully expressed where crop husbandry is optimized; and (ii) the practice of conservation agriculture and other resource-conserving technologies help stabilize soils and can lead to improvements in the fertility and productivity of cropping systems, especially those that are degraded from a history of intensive cultivation (Cook 2006; Montgomery 2007; Hobbs et al. 2008). These issues will be addressed by other research initiatives including CRP WHEAT, while genetic gains in yield potential will be measured under the appropriate range of cropping systems.

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# A comparison of research outputs in rice and wheat

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Rice and wheat account for more than 40% of global food production. In the past, both conventional and non-conventional breeding approaches, together with molecular and physiological efforts, have boosted yield potential of both crops. Genetic diversity for both crops has been maintained or slightly increased as genetic resources including landraces and wild relatives were used to accumulate yield-associated traits and to increase resistance to biotic and abiotic stresses. Here, we summarize these concepts and mark similarities and differences between the two species.

## **Breeding approaches largely explored (a)biotic stress resistance, quality traits but less to yield potential traits**

The breeding application of genomic technology for improving yield traits has been limited despite advances made in sequencing and functional genomic research in both crops. The application of a modified pedigree breeding scheme (Morais et al. 2006) and a recurrent selection philosophy (Breseghello et al. 2009) has been associated with yield gains in rice (Breseghello et al. 2011), while in wheat, the former has been complimented with wide crossing (Trethowan and Mujeeb Kazi 2008). However, as conventional approaches have yielded unsatisfactory results in bringing further genetic improvement in both crops, alternative approaches such as marker-assisted selection (MAS) have become the common approach employed for strategic crosses, which have indeed revealed a rich genetic architecture of complex traits (Zhao et al. 2011). However, the majority of such crosses were confined to disease resistance and quality traits, while less focussed on agronomic traits in both crops (Collard et al. 2008; Gupta et al. 2009; Akhtar et al. 2010; Liu et al. 2012). At CIMMYT, about 89,000 marker data points were performed in 2010 using markers associated with genes controlling disease, quality, and agronomy traits across all breeding programs (Dreisigacker 2011), in which, only 25% makers were used for Rht, Ppd, and Vrn genes for designing crosses aimed at transfer/stacking of genes (William et al. 2007; Gupta et al. 2010). At the global scale, genes conferring

dwarfism (Rht), photoperiod (Ppd), growth habit and heading date, vernalization (Vrn in wheat), pairing homolog (ph1 in wheat), and root traits (length and deep) for drought tolerance were commonly used in MAS programs in both crops (Collard et al. 2008; Gupta et al. 2009; Akhtar et al. 2010; Liu et al. 2012). While these new breeding and selection strategies rely on the availability of cheap and reliable marker systems, these systems theoretically provide a base for adding additional agronomic and yield traits to the elite material (Tester and Langridge 2010).

In addition, expanding the genetic bases of both crops has become a key resource for increasing yield potential. In rice, hundreds of diverse wild relatives of *Oryza sativa*, *O. rufipogon*/*O. nivara*, *O. glaberrima*, *O. barthii*, and *O. grandiglumis* were used to introgress QTLs for yield (Xiao et al. 1998; Brondani et al. 2002; Liang et al. 2004; Deng et al. 2007). For example, QTLs, yld1.1 and yld2.1, from *O. rufipogon* were introgressed into *O. sativa* and increased yield by 18% and 17% (Deng et al. 2007). In wheat, the 7Ag.7DL chromosome translocation has been widely used in breeding programs (Singh et al. 1998). Similarly, functional markers based on the rye secalin gene on 1RS were successfully applied in breeding (Liu et al. 2007).

## **Improving crop biomass through increasing photosynthetic efficiency**

In the past, improvements in wheat yield were strongly associated with improvements in harvest index (HI), while rice hybrids have significantly higher above ground biomass than inbred cultivars. In wheat, biomass has a lower genetic gain (0.3) as compared to HI (0.6; Donmez 2001; Zheng et al. 2011; Xiao et al. 2012), suggesting that biomass production may be the main way forward to further increase grain yields in wheat (Sylvester-Bradley et al. 2012). However, the potential of manipulating leaf anatomy to gain increased light interception, radiation use efficiency, and photosynthetic properties has so far received less attention in both crops (Tholen et al. 2012). In this respect, improving light interception could be further useful, which can

be achieved by introduction of a QTL influencing chlorophyll D as proposed in rice (Zhang et al. 2006). A phenotypic screening in rice identified higher vein density mutants (<http://www.slideshare.net/CIAT/engineering-c4-rice>; 25.10.2012), which would be useful to identify genes controlling leaf development. Interestingly, some vein density changes are closely associated with mesophyll cell expansion in rice mutants (Smillie et al. 2012) and plant height in sorghum (Paul 2011), emphasizing the importance of these genes in exploiting leaf anatomy variation in both species.

The use of conventional plant hybridization between sorghum and rice, oat and maize to transfer  $C_4$  traits to  $C_3$  plant rice seems unlikely due to several constraints (Rizal et al. 2012). In this respect, molecular engineering approaches looks attractive; however, manipulating cell biochemistry has yielded partial results in transgenic rice (Taniguchi et al. 2008). Nevertheless, the overexpression of genes encoding photosystem II subunits (PsbS; Hubbart et al. 2012), sedoheptulose-1,7-bisphosphate (SBPase), and fructose-1,6-bisphosphate (FBPase) have increased net photosynthesis by 6-12% in transgenic rice (Tamoi et al. 2006; Feng et al. 2009). These studies suggest that improving photosynthesis efficiency might be limited to specific set of biochemical changes, which would help wheat researchers to be focussed on specific, rather than on whole set of, biochemical changes through MAS approach, as a maize *ZmC4Ppc* gene via MAS increased phosphoenolpyruvate carboxylase, net photosynthetic rate, and grain yield of rice (Xiang et al. 2007). In addition, some important points to be considered for enhancing the photosynthetic efficiency include:

- Rubisco nucleotide diversity has not been explored in both species. At position 328 of Rubisco protein, many  $C_3$  species exhibit  $C_4$  dominant serine residue and mutation at this site could alter  $CO_2/O_2$  specificity of the Rubisco (Christin et al. 2008).
- The catalytic switches of faster carboxylation rate are yet to be identified in both crops while isoleucine 309 was identified as a catalytic switch in  $C_4$  species (Christin et al. 2008).
- The exact mechanism of interaction between Rubisco activase and Rubisco need to be understood although models predict that amino acids at 89-94 of the L-subunits are important targets of interaction with Rubisco activase (Portis et al. 2008).

### **Higher harvest index through optimizing the assimilates partitioning**

Crop HI is a complex key trait as it represents the efficiency of assimilates partitioning to grains that is highly influenced by environmental factors in both crops (Shrotria 1988). Therefore identifying the novel genetic loci with major effects on HI should be a key initiative in breeding programs as was proposed in rice using association mapping (Li et al. 2012). Increasing non-structural carbohydrates (NSCs) remobilization efficiency might be thus important to reduce the floret abortion as stem NSCs accumulation enhances the maximum endosperm cell number and thus high sink strength as was shown in inferior spikelets of rice (Fu et al. 2011). However, bottlenecks in the biochemical conversion of sucrose into starch can result in cessation of endosperm growth, which may be linked to low activity of carbohydrate metabolic enzymes and hormonal balance in the grains. Therefore, points needing to be considered include:

- Many of the carbon metabolic enzymes are temperature sensitive (Farooq et al. 2011). However, a single-nucleotide polymorphism was shown to control temperature sensitive of granule-bound starch synthase in rice (Larkin and Park 1999), which should be the target for genetic improvement in both crops.
- Grain hormonal contents largely regulate spikelet development, which is fairly understood in rice (Mohapatra et al. 2011) and this should be explored in wheat as well. Exploring the genotypic variation for hormonal contents in both crops should make sense, as hormonal contents are strongly cultivar and ploidy dependent (Eastmond and Jones 2005).
- Exploring the parental genome imbalance on the endosperm development might further help to improve grain size in both crops, as was shown in *Arabidopsis* (Lu et al. 2012).

Many of these genes could be introgressed into new elite lines via MAS. For example, in rice, grain number (GN1), grain weight (GW2), and grain size (GS3) (Ashikari et al. 2005; Xing and Zhang 2010); (Zong et al. 2012) genes were introgressed via MAS to increase grain yields. Recently, a novel pyramid-breeding scheme based on marker assisted and phenotype selection (MAPS) was proposed to pyramiding of as many as 24 QTLs at a single hybridization for grain-yield related traits in rice (Zong et al. 2012). In wheat, genes of sucrose synthase 2 (*Sus2*) and grain width (*GW2*) and cell-wall invertase (*CWI*) that are linked to

thousand kernel weight (TKW), were recently cloned and tested in Chinese wheat cultivars, which significantly improved TKW (Ma et al. 2010; Su et al. 2010). Further, association analysis highlighted that TaGW2-6A, Tacwi-A1b, and Tacwi-A1a loci not only increased TKW but also are associated with earlier flowering and maturity in these lines (Ma et al. 2010; Su et al. 2010).

### Lodging resistance

Sufficient assimilate partitioning to other organs (e.g. roots and stems) is important for improving lodging resistance as lodged wheat plants exhibited a strong source-limitation during grain-filling period (Acreche and Slafer 2011). Stem strength can be increased by the selection of thicker leaf sheath wrapping, as practiced in rice (had a lodging resistance of 30-60%; Setter et al. 1994). In addition, increasing the wheat stem diameter (~4.9 mm) from the current value that of rice (~5.5 mm; Ishimaru et al. 2005), which is also supported by the wheat lodging model (5.8 mm; Berry et al. 2007), might improve stem-lodging resistance in wheat. Since the genetic loci controlling several lodging resistant traits were commonly mapped on chr. 5 and 6 in both crops (Kashiwagi et al. 2008; Ookawa et al. 2010), these genomic regions should be explored further for trait deployment associated with lodging resistance in both crops.

### Conclusions

Both rice and wheat have been explored in multidirectional ways to improve the respective yield potential of these crops. Therefore, knowledge sharing between two crops would mutually benefit each other and may improve the current understanding of the constraints of yield potential. Based on the above literature survey, wheat can benefit from rice knowledge in the aspects of photosynthesis and grain growth and development, while rice can benefit from wheat knowledge for the potential of phenological phase linkages to yield potential.

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# Improving Crop Biomass

## Increasing photosynthetic capacity and efficiency of wheat

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The primary determinant of crop biomass is the cumulative rate of photosynthesis over the growing season. Although yields of wheat have increased over time, comparatively little of this increase can be attributed to increased biomass. Instead, improvements in agronomic practice and harvest index (the proportion of biomass that is grain) are largely responsible for the increased yield (see Austin et al. 1989; Fischer et al. 1998). The harvest index of two major food crops - rice and wheat - is now approaching a plateau and further increases in yield will necessitate an increase in productive biomass and therefore, an increase in photosynthesis. Provided that other constraints do not become limiting, and that partitioning of biomass to yield is optimized (see Partitioning overview, these proceedings), increasing photosynthesis will increase crop yields, as demonstrated by the effects on yield of CO<sub>2</sub> enrichment experiments (Ainsworth and Long 2005). Furthermore, experiments on historic wheat genotypes suggest that improvements in photosynthesis per unit leaf area may have already occurred in concert with improvements in harvest index and grain number (Fischer et al. 1998).

Total crop photosynthesis is dependent on: 1) the ability of the canopy to intercept and capture light; 2) the duration of light capture; and 3) photosynthetic capacity and efficiency. Once canopy architecture, light interception, and photosynthetic duration have been optimized, total photosynthesis can only be increased by increasing the photosynthetic rate per unit leaf area (Long et al. 2006; Parry et al. 2011). Recent experimental and theoretical analyses suggest there is substantial room to increase photosynthetic energy conversion efficiency (Zhu et al. 2008). The theoretical photosynthetic energy conversion efficiency of C<sub>3</sub> plants is about 4.6% (Zhu et al. 2008), while the recorded energy conversion efficiency in the field is usually less than 1/3 of this value. The projects in this section of the proceedings aim to improve leaf and canopy level photosynthetic capacity and efficiency; either by “mining” existing genetic variation in wheat photosynthetic performance

using a Phenomics screening approach across diverse germplasm sets, or via a candidate gene approach where targeted transgenic wheat germplasm is produced to alleviate metabolic constraints.

### Strategies to overcome photosynthetic limitation by Rubisco

Experiments using transgenic plants and modeling show that, under moderate to high light intensities and air CO<sub>2</sub> levels, leaf photosynthetic flux is largely limited by Rubisco, the primary enzyme of CO<sub>2</sub> fixation in C<sub>3</sub> plants (reviewed in Parry et al. 2011). Rubisco is an inefficient catalyst that discriminates poorly between CO<sub>2</sub> and oxygen as substrates in air; resulting in up to 30% of the energy harvested in photosynthesis being wasted through photorespiratory losses (Parry et al. 2011). To support high rates of photosynthesis, more than 25% of leaf N is invested in this enzyme. There is some evidence for genetic variation and plasticity in Rubisco content per unit leaf area in cereals, and modeling and nutritional experiments suggest that selecting for genotypes with high Rubisco content at a given N nutritional level would improve photosynthetic performance. However, given the likely nutritional limitations in the future, selecting for genotypes with improved Rubisco kinetic properties (Furbank et al. these proceedings) or engineering new varieties with improved kinetic properties (Parry et al. these proceedings) offer the largest potential benefits, although plastid transformation (presently intractable in cereals) is likely to be required for the latter approach (Parry et al. 2011; Reynolds et al. 2012). WYC research to date has established that there is considerable variation in photosynthetic performance in wheat, potentially due to both variation in the amount and properties of Rubisco, even across elite material bred in three countries. This work will allow the establishment of biparental populations to examine markers, genes, and mechanisms underpinning these phenotypes. There is also the opportunity to use a resequencing approach to examine allelic variation in the

Rubisco large and small subunit sequences in this material. Germplasm identified as having superior performance can be immediately incorporated into crosses for breeding improved material.

### **Exploiting photosynthesis in organs other than leaves**

In wheat and other cereals, spikes contain considerable chlorophyll, are highly photosynthetically competent and, for a large period of crop development both before and after flowering, intercept a considerable amount of incoming irradiation (see Parry et al. 2011). In wheat, there is evidence for spike photosynthesis contributing up to 25% of final grain carbon (Parry et al 2011). Many different parts of the reproductive structure are photosynthetically active and potentially under different genetic control to leaf functions. There is evidence for genetic variation in spike photosynthesis in durum wheat, though the difficulty of conducting high throughput measuring of spike photosynthesis limited breeding for this trait. Genetic variation in wheat spike photosynthesis has been established in a range of CIMMYT genotypes in the field (Molero et al. these proceedings), and a mapping population is under analysis to establish markers and candidate genes for breeding.

### **Photosynthesis at the canopy level**

For improvements in leaf level photosynthetic performance to be realized at the canopy level, light interception of the crop and light use efficiency of the canopy as a whole must be understood, modeled and integrated with the leaf and organ level physiology underpinning this theme. Leaf angle, thickness, width, tillering, and distribution of photosynthetic machinery between leaves in the canopy all affect realization of increased yields via photosynthetic improvements at the leaf level (Reynolds et al. 2012). Furthermore, truly high throughput remote sensing of photosynthesis is best carried out at the canopy level, and interpretation of data is highly dependent on understanding canopy architecture and associated radiative transfer modeling (see Munns et al. 2010). Scaling of leaf level biochemistry and physiology to the crop, apart from simple black box modeling, is a major deficiency in canopy level photosynthesis research. Combining digitization of canopies with modeling and leaf level measurements (Condon et al. these proceedings) offers the opportunity to inform not

only phenotypic selection in breeding but also candidate gene identification in transgenic breeding, by evaluating traits in silico. Advances have been made by using Lidar to extract canopy geometries and metrics, algorithms to allow substantive “value add” on remote sensing measurements, and in modeling canopy light interception and light use efficiency. These can be translated directly to breeding through improved screens in the field and in silico trait evaluation.

### **Chloroplast CO<sub>2</sub> pumps**

An alternative to improving wheat Rubisco to reduce photorespiratory energy losses, improve catalytic rates, and hence increase leaf level radiation use efficiency, is to elevate CO<sub>2</sub> at the site of Rubisco by installing a CO<sub>2</sub> concentrating mechanism (Price et al. 2013; Zarzycki et al. 2013). The efficacy of this approach is evidenced by the efficiency of the C<sub>4</sub> photosynthetic mechanism that confers C<sub>4</sub> crops with considerably enhanced radiation use efficiency, biomass, and yield, compared to C<sub>3</sub> crops (Reynolds et al. 2012). Projects within the WYC aim to install a CO<sub>2</sub>-concentrating mechanism in wheat, by mimicking the carbon concentrating mechanism present in cyanobacteria. Theoretically, this approach requires only a small set of transgenes, whereas C<sub>4</sub> photosynthesis requires many genes encoding enzymes, transporters, and morphological regulators to be cloned and transferred. This work is currently unfunded in the WYC, but has progressed in two laboratories via third party funding agencies and has made progress in expression of bicarbonate transporters in model plant species.

### **Improved RuBP regeneration**

While Rubisco exerts the majority of control over photosynthetic rate in air at high light levels, in a canopy environment, many leaves are operating at sub-saturating light for much of the day, particularly in conditions of direct rather than diffuse radiation. Under these conditions, the light-dependent regeneration of the substrate for Rubisco, RuBP, becomes limiting for photosynthesis. From experiments with transgenic plants (reviewed in Parry et al. 2011), the enzyme SBPase has been shown to be a major limiting step in the regeneration of RuBP. Over-expression of this enzyme in dicots results in improved photosynthesis and growth. Work described in these proceedings seeks to introduce additional

copies of the SBPase gene to increase expression levels and relieve this bottleneck. Investigations will also use high throughput sequencing at the RNA level to determine optimal relative expression levels of enzymes involved in regenerating RuBP under limiting N nutrition (Zhu et al. 2007). Considerable progress has been made in producing these transgenic wheat plants, which will be crossed into appropriate elite lines for testing.

### **Thermostability of Rubisco activase**

High temperatures (one of the consequences of climate change) cause inactivation of Rubisco and decreased photosynthetic rate, thereby compromising yield. The heat stability of Rubisco activase, the protein required to restore and maintain Rubisco activity, is an important determinant of the heat stability of photosynthesis (Salvucci and Crafts-Brandner 2004). There is natural diversity in the optimal temperature of Rubisco activase from different species (Carmo-Silva and Salvucci 2011), and enhanced thermal tolerance of Rubisco activase has been created by directed evolution (Kurek et al. 2007). Rubisco activase from cotton has a high thermal tolerance and can, *in vitro*, interact with and activate wheat Rubisco. Thus, the dual cotton Rubisco activase and wheat Rubisco is expected to function *in vivo* and at a higher temperature range than with wheat Rubisco activase. Research is underway to test the hypothesis that transgenic wheat expressing cotton Rubisco activase will have increased thermal tolerance of photosynthesis. Putative transgenic lines are under selection.

### **Engineering Rubisco kinetic properties**

The characteristics of Rubisco make it surprisingly inefficient and compromise photosynthetic productivity at today's CO<sub>2</sub> and O<sub>2</sub> concentrations. Over 30 years of directed mutagenesis have failed to improve Rubisco in crops. However, a wide range of natural diversity in the catalytic properties in Rubisco in diverse photosynthetic organisms has become apparent (Whitney et al. 2011; Parry et al. 2012). This suggests that changes in turnover rate,

affinity, or specificity for CO<sub>2</sub> can be introduced to improve Rubisco performance in specific crops and environments (Parry et al. 2011; 2012). The challenges we endeavor to address are to identify the 'best' naturally occurring enzymes and then to express both the small and large subunits into functional protein in wheat plastids. Reliable plastid transformation is currently limited to tobacco and a few horticultural crops (Hanson et al. 2013); thus, transplanting better Rubisco requires the development of robust protocols for wheat plastid transformation. The development of plastid transformation protocols will also facilitate the optimization of cyanobacterial CO<sub>2</sub>-concentrating-mechanisms in wheat (Price et al. 2013; Zarzycki et al. 2013).

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# Phenotypic selection for photosynthetic capacity and efficiency

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## Summary

This work has focused on: 1) Developing new, higher throughput tools for leaf level and canopy level screening, and 2) Screening germplasm sets from CIMMYT (Mexico), UK, and Australia using existing physiological tools such as infrared gas analysis as proxies for variation in Rubisco kinetics and amounts in wheat leaf material in the glasshouse and the field.

## Major Objectives

1. Identification of wheat germplasm with variation in photosynthetic capacity and efficiency under optimal and heat stress conditions.
2. Elucidation of mechanisms underlying improved photosynthesis in candidate lines.
3. Validation of increased photosynthetic performance and yield potential in the field.

## Results and Discussion

### Identification of wheat germplasm with variation in photosynthetic capacity and efficiency: New tools and techniques

A glasshouse pot grown trial was carried out in Canberra in 2012 on 16 genotypes from a panel previously selected for variation in vigour and transpiration efficiency. These genotypes were

grown at limiting and non-limiting nitrogen nutrition to generate a large range of photosynthetic capacity with 3-fold replication. Photosynthetic rates in air and at a range of CO<sub>2</sub> concentrations were measured to allow extraction of Rubisco kinetic parameters by modelling (von Caemmerer and Farquhar 1981), along with specific leaf area, leaf greenness index (SPAD, using 650 and 940nm) and leaf reflectance from 350 to 2500nm using a leaf spectrometer and a modified leaf clip (Fig. 1). A subset of lines was sampled for chlorophyll content and biochemical analysis. Photosynthetic rates in air (A) varied from 6 to 26  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , providing a wide range of photosynthetic performance for validation of new methods.

From reflectance spectra collected from leaves of all genotypes and the other physiological measurements, a partial least squares statistical approach was used to produce a model weighting individual reflectance bands to test the ability of this model to predict photosynthetic parameters in this germplasm set (see Serbin et al. 2012). While the published model for woody species (Serbin et al. 2012) was not suitable for wheat, strong correlations were observed between reflectance features at 380, 540, 706, and 1900 nm, and a robust predictive algorithm for assimilation rate was derived (Fig. 1).

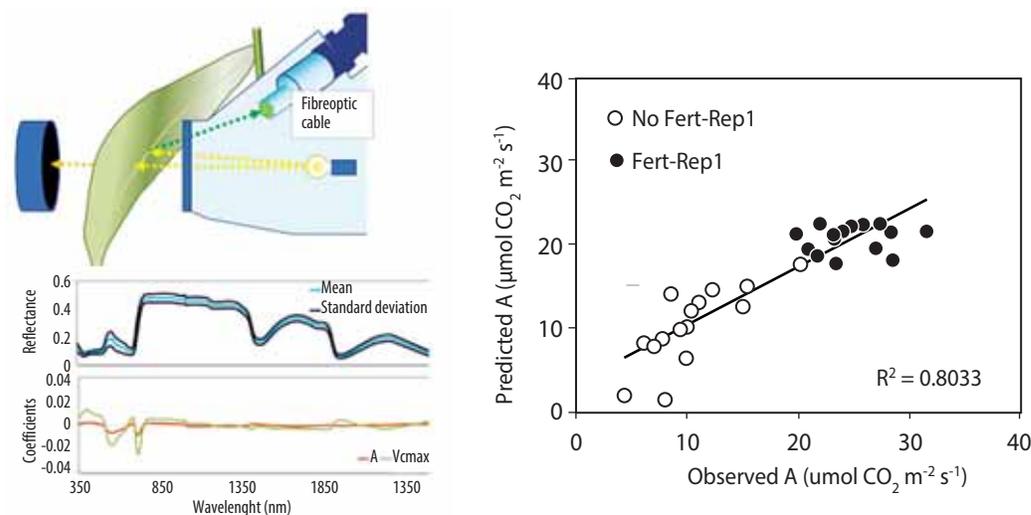


Figure 1. PLS modelling of leaf spectral reflectance for predicting photosynthetic parameters.

SPAD readings correlated with A ( $R^2 = 0.71$ ) and  $V_{cmax}$  ( $R^2 = 0.65$ ). A set of 25 commonly used reflectance indices were calculated from the leaf reflectance data and plotted against photosynthetic parameters. In general, predictive power was poor and NDVI, a reflectance measurement commonly used for crop N estimates, was very poorly correlated with A and  $V_{cmax}$  ( $R^2 < 0.1$ ). Under high fertiliser levels, the predictive capacity of pigment based indices declines due to the saturation of the reflectance signal at high chlorophyll content.

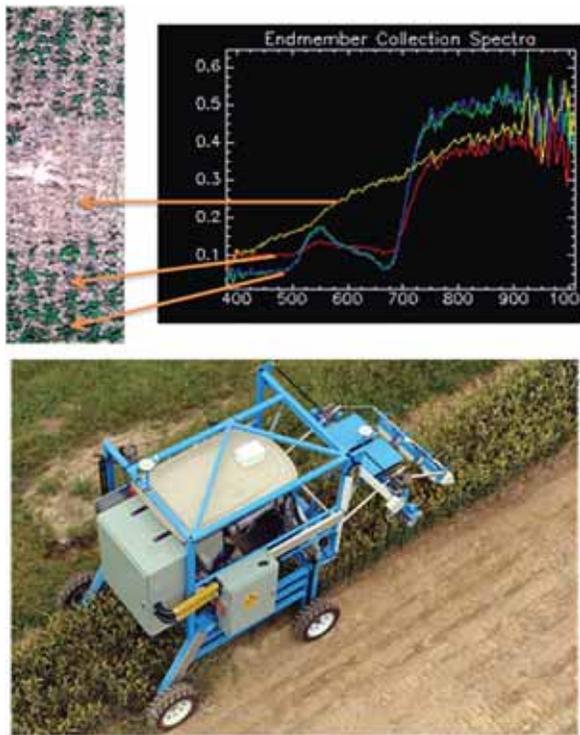


Figure 2. Hyperspectral imaging of a wheat trial pre-canopy closure

A set of 72 diverse genotypes (the **Best Unreleased Yield Potential** or **BUNYIP** set), including Triticale and durum wheat and members of the CIMCOG set, were also grown in the field in August 2012 and similar experiments were performed to test the predictive power of spectral reflectance models at field levels of N nutrition. A subset of 30 of these lines was also grown in single pots in the glasshouse for comparison.

The BUNYIP trial was also measured with a hyperspectral imaging system based on a buggy (the Phenomobile) to test whether these measurements could be scaled to a canopy level (Fig 2).

### Establishing genetic variation in elite wheat germplasm

Photosynthetic characteristics of the CIMCOG population composed by CIMMYT Elite lines were studied in Mexico under controlled conditions (2011, El Batan) and a subset was studied at MEXPLAT (2011-2012, Ciudad Obregon; Fig 3).

Gas exchange measurements were performed at 4 leaf stage under greenhouse conditions and at booting and nine days after anthesis in the field. Genetic variation and genetic range in  $A_{sat}$  and  $g_s$  under both experimental conditions at different growth stages, modeled Rubisco kinetics (maximum carboxylation capacity,  $V_{cmax}$ ) and electron transport rate (ETR) were established for CIMCOG population (Table 1). Genetic variation for chlorophyll content (SPAD), biomass (except seven days after anthesis), radiation use efficiency, and yield were also observed under field conditions (Table 1). Analysis of the data at different growth stages shows 39-50% variation in photosynthetic  $CO_2$



Figure 3. Photosynthesis measurements were performed under greenhouse conditions at 4 leaf stage (A), at booting stage (B) and nine days after anthesis (C) under field conditions.

assimilation rate at ambient CO<sub>2</sub> (Table 1).  $A_{\text{sat}}$  was positively correlated with  $V_{\text{cmax}}$  ( $r=0.72$ ) indicating that  $A_{\text{sat}}$  could be a useful tool to predict Rubisco carboxylation capacity in early leaf stage. ETR was also positively correlated with  $A_{\text{sat}}$  under controlled conditions ( $r=0.68$ ) and could be used as a surrogate to predict photosynthetic carbon assimilation rate in the screening for high photosynthetic rate in large populations (Table 1).

The results shown here indicate that there is genetic variation in the rate of leaf photosynthesis during different growth stages, as previously reported by Evans (1993). The correlation between the rate of leaf photosynthesis and grain yield and biomass at maturity was not observed at initiation of booting ( $r=0.193$  and  $r=0.108$ , respectively), but was observed in the rate measured nine days after anthesis (Figure 4). These results indicate the importance of grain filling photosynthesis to final grain yield and biomass. It is important to consider whether selection for high rates of leaf photosynthesis could

be effective due to spot measurements varying with development stage and other environmental factors (Richards 2000). In this sense it is important to corroborate the present results with additional measurements during 2012-2013 growth cycle, and to complement with additional measurements latter during grain filling.

At Rothamsted, UK, under the BBSRC-funded projects 20:20 Wheat® and CIRC BB/I017372/1, photosynthetic capacity and efficiency was measured for 64 lines in the ERYCC (Earliness & Resilience for Yield in a Changed Climate) panel. A replicated field experiment was set up with the ERYCC panel; phenology was monitored and recorded. At key growth stages, leaf chlorophyll (SPAD) and green canopy cover (spectral reflectance ratios) were measured and leaf area index was estimated. Using fully-emerged flag leaves prior to ear emergence (Zadoks 4.0 – 5.0), the response of photosynthesis to the intercellular CO<sub>2</sub> concentration ( $A/C_i$  curves) and Rubisco

**Table 1. Photosynthetic rate measured at saturating light ( $A_{\text{sat}}$ ), stomatal conductance ( $g_s$ ), maximum carboxylation capacity of Rubisco ( $V_{\text{cmax}}$ ) and electron transport rate (ETR) measured under greenhouse conditions at 4 leaf stage (n=60).  $A_{\text{sat}}$ ,  $g_s$ , SPAD (all measured in flag leaf) and aboveground biomass measured at booting and nine days after anthesis in a selected subset of CIMCOG population (n=30). Biomass was measured seven days after anthesis. Yield and radiation use efficiency (RUE) were measured at maturity also in the same subset of the CIMCOG population (n=30).**

CIMCOG-Green House (2011) and Field Experiment (2011-2012)				
		Mean	P-value (ENT)	Genetic range (LSD 5%)
4 leaf stage	$A_{\text{sat}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	21.4	***	15.1 — 30.4 (5.0)
	$g_s$ ( $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ )	0.455	***	0.193 — 0.693 (0.153)
	$V_{\text{cmax}}$ ( $\mu\text{molCO}_2\text{m}^{-2} \text{s}^{-1}$ )	122.5	**	94.0 — 155.0 (27.2)
	ETR ( $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ )	126.4	***	80.8 — 157.2 (24.9)
Booting	$A_{\text{sat}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	29.9	***	22 – 36 (2.9)
	$g_s$ ( $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ )	0.459	*	0.263 – 0.624 (0.180)
	SPAD units	47.4	***	40.8 – 55.3 (1.5)
	Biomass ( $\text{g m}^{-2}$ )	667	***	553 – 865 (108)
Anthesis +7/9	$A_{\text{sat}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	23.3	***	15.7 – 27.8 (3.5)
	$g_s$ ( $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ )	0.349	***	0.183 – 0.610 (0.128)
	SPAD units	52.3	***	46.0 – 57.8 (0.9)
	Biomass ( $\text{g m}^{-2}$ )	1110	ns	970 – 1249 (193)
Maturity	Yield ( $\text{g m}^{-2}$ )	677	***	594 – 797 (44)
	Biomass ( $\text{g m}^{-2}$ )	1464	***	1310 – 1623 (111)
	RUE ( $\text{g MJ}^{-1}$ )	1.69	**	1.33 – 2.10 (0.34)

\* Significant at the 0.05 probability level; \*\* significant at the 0.01 probability level; \*\*\*Significant at the 0.001 probability level.

content were determined for all the genotypes. Preliminary analysis of the data showed a 33% variation in photosynthetic CO<sub>2</sub> assimilation rate at ambient CO<sub>2</sub> (Fig 4) and 25% at saturating CO<sub>2</sub> concentrations (Fig 5). Further data analysis is underway and a second trial has been sown with the same lines.

### Next Steps

Hyperspectral reflectance models will be tested on CIMCOG material at Obregon this 2013 field season. BUNYIP data at the leaf level will be modeled and compared to canopy level measurements. Glasshouse and field trials will be carried out on the CIMCOG set introduced to Australia after passage

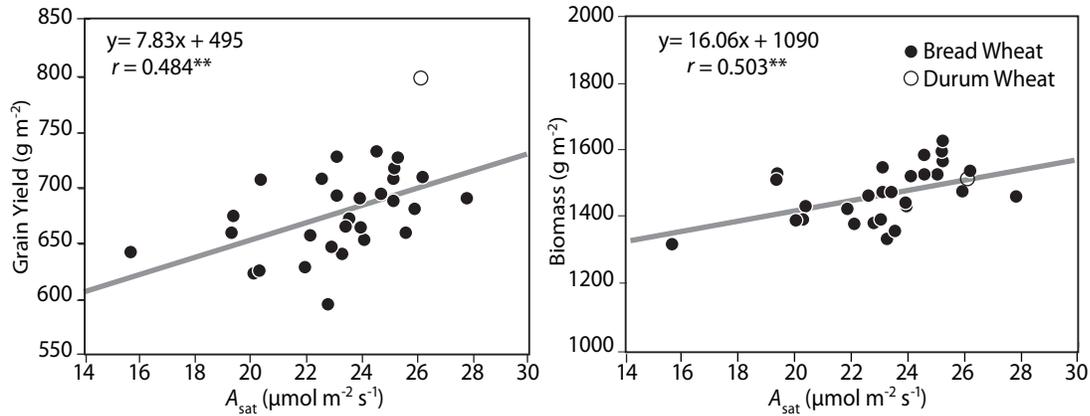


Figure 4. Correlation between grain yield and biomass (at maturity), with photosynthetic rate measured at saturating light ( $A_{sat}$ ) nine days after anthesis in a selected subset of the CIMCOG population (n=30) during the growth cycle 2011-2012.

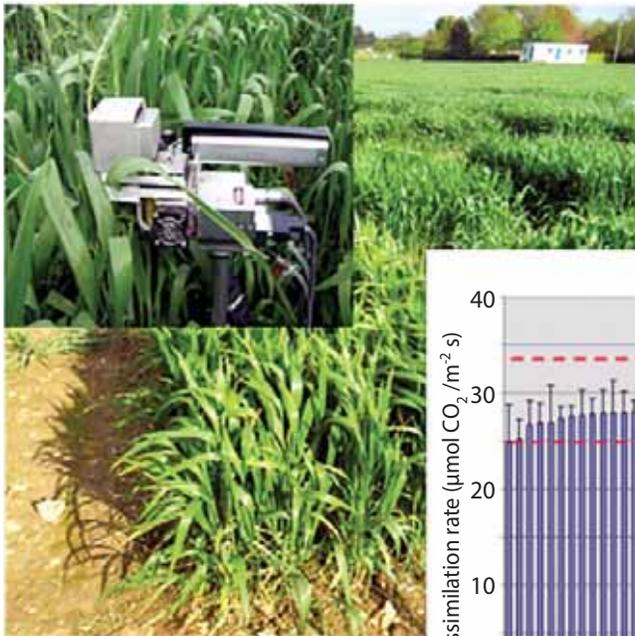
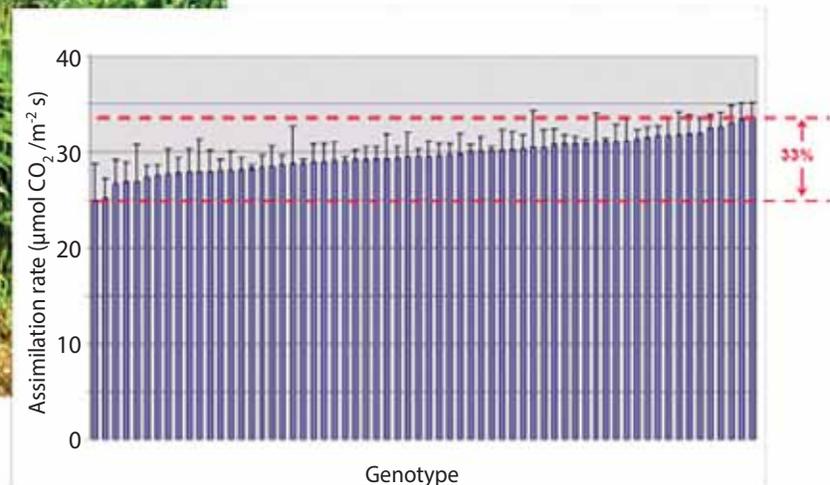


Figure 5. Photosynthetic screening of wheat germplasm at Rothamsted using infrared gas analysis



through quarantine and BUNYIP experiments replicated. Correlations between Rubisco amount and kinetic properties from destructive analysis of leaf material used in the physiological screening in Canberra and UK will be compared with gas exchange, high throughput hyperspectral reflectance, chlorophyll fluorescence, and canopy temperature measurements. Relationships between biomass, harvest index, yield, and photosynthetic characteristics will be collated for all three trial sites.

Measurements of the rate of leaf photosynthesis and at different growth stages together with  $A/C_i$  curves and hyperspectral reflectance measurements will be performed in the subset of CIMCOG population in MEXPLAT during the 2013 cycle in order to (i) determine consistency of genetic variation in photosynthetic related parameters, (ii) determine maximum carboxylation capacity under field conditions, and (iii) establish a model to predict photosynthetic parameters based on hyperspectral reflectance as described above.

Additional to these measurements, leaf photosynthetic rate and  $A/C_i$  curves will be measured in a selected subset of lines from a landrace population selected from the germplasm bank. These lines were selected under field conditions during 2012 for their high Biomass. The objective will be to identify candidate lines with high photosynthetic rate during grain filling and high carboxylation capacity of Rubisco to incorporate these candidate lines in strategic crosses with selected CIMCOG lines.

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# Phenotypic selection for spike photosynthesis

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## Summary

In wheat and other C<sub>3</sub> cereals, reproductive structures are sources of photosynthates that can substantially contribute to grain filling (Tambussi et al. 2007). In our previous studies of the CIMMYT Core Germplasm (CIMCOG) population, spike photosynthesis contributed 20-50 % of mature grain dry mass (see Reynolds et al. 2012), which is consistent with previous studies (e.g. Maydup et al. 2010). The importance of spike photosynthesis has been recognized for 50 years (Thorne 1963), but as yet, breeding programs have made no systematic attempts to improve this trait. Despite the fact that awns contribute significantly to total ear surface area (Blum 1985) and affect canopy light interception, their contribution to grain filling still remains unclear and it is also not addressed in breeding programs. In this sense, it is important to conduct studies in populations with contrasting spike characteristics to identify wheat germplasm with genetic variation in spike photosynthesis.

The main limitation in studying spike photosynthesis in crops is that it is not easy to measure the photosynthetic contribution of the ear to grain filling. The development of surrogates to estimate its contribution to grain filling is therefore of great importance. In our present work we developed a chamber to directly measure spike photosynthesis in the field and we have tested the usefulness of surrogates (inhibition of

photosynthesis treatments and the use of stable isotopes) to estimate the contribution of spike photosynthesis to grain filling. The latter techniques have been validated in Mexico for identifying significant genetic effects for integrated contribution over time of spike photosynthesis to grain filling. These tools can also be extremely valuable in gene discovery, so that phenotypic screening of spike photosynthesis can be complemented by molecular marker assisted selection.

This work has focused on two major objectives: 1) development of screening tools for photosynthesis and 2) identification of wheat germplasm with genetic variation in spike photosynthesis. The next step will be the development of molecular markers for spike photosynthesis, which is already underway.

## Results and Discussion

### Development of screening tools for spike photosynthesis

An attempt at high throughput screening for spike photosynthesis used three different treatments to estimate the contribution of ear photosynthesis to grain weight: 1) ear photosynthesis was reduced with an aluminum shading treatment, 2) ear photosynthesis was reduced with a textile shading treatment, and 3) ear photosynthesis was reduced with DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), a specific inhibitor of photosystem II (Figure 1).

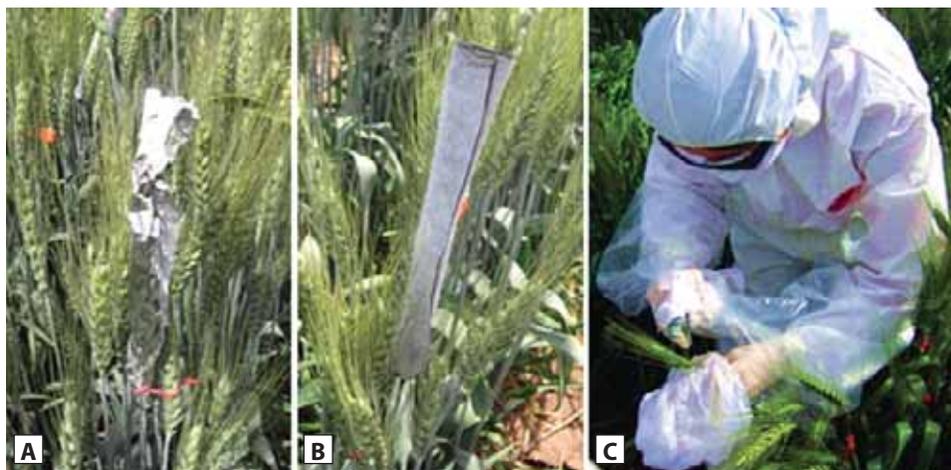


Figure 1. Screening for spike photosynthesis contribution to grain yield using three different treatments: (A) ear photosynthesis reduced with an aluminum shading treatment, (B) ear photosynthesis reduced with a textile shading treatment, and (C) ear photosynthesis reduced with the application of DCMU.

The spike grain weights of control and treated spikes were compared in order to estimate spike contribution to grain yield (Maydup et al. 2010). Although there were differences in the apparent contribution of spike photosynthesis among different treatments (data not shown), the inhibition of ear photosynthesis had comparable effects on grain weight and was consistent across all three treatments (Table 1). In order to validate the use of these treatments to estimate ear contribution to grain yield, they were tested under a range of conditions on a panel of lines used previously to generate mapping populations (PADS POP; Table 1).

The use of stable isotopes such as  $\delta^{13}\text{C}$  can contribute to understanding source-sink interactions. Usually the isotopic composition of spikes and the flag leaf are different. This difference could be used to predict the sources of photosynthates contributing to grain filling. The analysis of isotopic composition

**Table 1. Correlation coefficients (r) between grain weight per spike of different treatments and coefficient of variation (CV) of each treatment in a population composed of parental lines (PADS POP, n=12).** The same treatments were tested in two environments (irrigated and heat stressed environment).

Treatments	Irrigation	Heat
	R	
Aluminum vs. Textile	0.604**	0.872***
Aluminum vs. DCMU	0.702***	0.876***
Textile vs. DCMU	0.721***	0.873***
	CV (%)	
Aluminum	8.5	11.8
Textile	5.3	3.9
DCMU	8.1	7.1

**Table 2. Isotopic composition of carbon ( $\delta^{13}\text{C}$ ) from dry matter (DM) and water soluble fraction (WSF) of different plant organs in a subset CIMCOG genotypes (n=6).** Plant tissues were harvested during grain filling and the grains at physiological maturity.

Plant tissue	$\delta^{13}\text{C}$ (‰) DM	$\delta^{13}\text{C}$ (‰) WSF
Flag leaf	-28,1 <sup>a</sup>	-29,7 <sup>a</sup>
Peduncle	-25,9 <sup>c</sup>	-26,9 <sup>b</sup>
Glumes	-26,2 <sup>b</sup>	-26,5 <sup>c</sup>
Awns	-25,5 <sup>d</sup>	-25,4 <sup>d</sup>
Mature Grains	-26,3 <sup>b</sup>	-26,3 <sup>c</sup>
Level of significance		
Genotype	,003	ns
Tissue	,000	,000
GxT	ns	ns

of total dry matter (DM) and the water soluble fraction (WSF) were performed in a subset of six lines of the CIMCOG population (Table 2). Data confirmed genetic variability among the lines for  $\delta^{13}\text{C}$  of DM, and differing strategies of carbon assimilation between spikes and leaves and among spike organs (Table 2). Complementary experiments will be performed during 2012-2013 to complete these results.

### Identification of wheat germplasm with genetic variation in spike photosynthesis

The wheat mapping population (RILs ATIL C 2000/T. DICOCCON PI94628) was previously identified for having parents that contrasted in the relative contribution of spike photosynthesis to grain filling (Table 3). Spike photosynthesis inhibition treatments were applied to 100 recombinant inbred lines (RILs) from the Atil/Dicocum cross, in order to estimate spike contribution to grain weight and undertake further QTL analyses. These treatments were also applied to elite CIMCOG lines obtaining 22-47% of spike photosynthesis contribution to grain yield as previously reported (Reynolds et al. 2012). These results indicate a highly significant genetic variation of spike photosynthesis contribution to yield among

**Table 3. Combined analysis of Spike contribution to grain weight (%) during 2010-2011 and 2011-2012 growing cycles on MEXPLAT in parental lines population (PADS POP, n=12) and RILs ATIL/DICOCCON mapping population (n=100).**

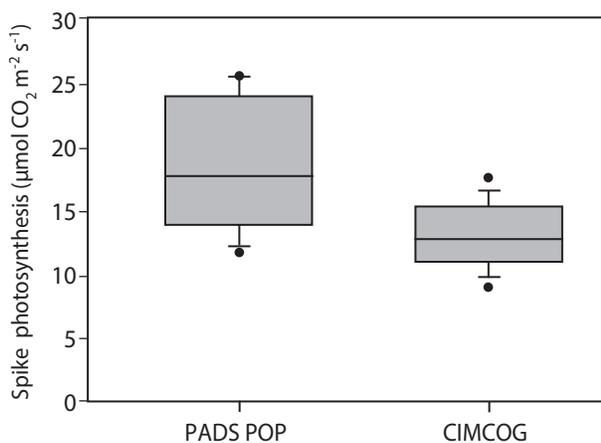
Cross Name	Spike contribution (%)	
	Y10-11 & Y11-12	
<i>PADS POP</i>		
RAC875	33.9	
KUKRI	48.4	
T.DICOCCON PI94628	14.6	
ATIL C 2000	40.1	
SERI M 82	25.7	
BAVIACORA M 92	33.7	
GLADIUS	35.0	
DRYSDALE	39.0	
PASTOR//HXL7573/2*BAU	38.1	
WEEBILL1	26.3	
SERI/BAV92	33.2	
SOKOLL	42.9	
Mean	34.3	
Genetic Range (LSD 5%)	14.6 - 48.4 (11.5)	
P-value(ENT)	0.064	
RILs ATIL/DICOCCON n = 100		
Mean	26.3	
Genetic Range (LSD 5%)	9.5 - 44.6 (14.4)	
P-value(ENT)	0.0001	

cultivars (Table 3) and highlight the importance of spike photosynthesis contribution to grain yield under irrigated conditions.

Direct measurements of spike photosynthesis were conducted on PADS POP and a subset of the CIMCOG population using a hand-made chamber connected to an infra-red gas analyzer (IRGA). Genotypic differences were observed for spike photosynthetic rate in both populations (Figure 2). These genotypic differences in spike photosynthesis were also observed in genotypes with contrasting spike architecture (Reynolds et al. 2012). One important and differential trait associated with spike morphology is awn length. Awns make a significant contribution to spike photosynthesis, and they could be easily incorporated into breeding programs. We therefore analyzed the possible correlation between awn length and grain weight in the CIMCOG population. Highly significant correlations were found for awn length and thousand grain weight in the subset (n=30) evaluated under yield potential ( $r=0.636$ ) and heat conditions ( $r=0.639$ ), which is consistent with the idea that larger awns have a positive effect on grain filling.

### Next Steps

From these results and previous data, it is clear that spike photosynthesis has an important role in grain filling, even without stress. Evidence indicates that source limitation (or at least source-sink co-limitation) is emerging in modern cultivars of wheat. Further increases of grain number per



**Figure 2. Box Plot of spike photosynthesis rate measured after anthesis at saturating light with a handmade chamber connected to an IRGA (LI-6400 XT) in parent lines (PADS POP, n=12) and a subset of the CIMCOG population (n=18).**

ear by breeding, with the concomitant increase of 'sink' demand, will require an increased assimilate availability to fill the grains. For this reason, and since spike photosynthesis has been confirmed as a target for genetic improvement in wheat, it is necessary to continue research on spike photosynthesis with a special focus on:

- Development of alternative high throughput phenotyping methodologies suited to large populations, for routine screening of spike photosynthesis in wheat based on i.e. i) thermal imaging and ii) spectral reflectance indices. As an example, in order to study genotypic variability in ear temperature, thermal imaging can be used to monitor the transpiration rate of different plant organs of elite cultivars during grain filling (Leinonen and Jones 2004). Such approaches have value in both identifying genetic variation and in gene discovery.
- Up-scaling data measured on individuals considering spike density so that genetic differences in spike photosynthesis at the canopy level can be estimated (i.e. spike photosynthesis  $m^{-2}$ ). Modeling approaches (see Zhu et al. 2010) that consider the response of both leaf and spike photosynthesis to light intensity can also help to establish thresholds of efficient light distribution within the whole canopy including spikes. The ability to accurately measure these effects and model them at the canopy level is a prerequisite to being able to combine their favorable expression through breeding, as well as allowing potentially unfavorable genetic linkages to be broken.
- Identification of QTL associated with the spike contribution to grain filling in RILs/Atil Dicocon as a first step towards the development of fine markers for spike photosynthesis, eventually facilitating the combination of spike photosynthesis with other RUE-related traits in breeding.

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# Optimizing and modeling canopy photosynthesis and photosynthetic duration

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## Summary

Our major objective is optimize seasonal radiation interception (RI) and radiation use efficiency (RUE) by: 1) evaluating effects on RI, RUE, biomass production, and yield of genetic variation in leaf area duration, within-canopy leaf area and N distribution and stomatal conductance, and 2) using the outputs from part 1, modeling the impacts on RI and RUE of changes of within-canopy and within-season distributions of leaf area and N.

Research in 2012 focused on: 1) testing effects of canopy architecture on growth and yield, 2) developing and testing new tools for providing user-friendly data on canopy level features, and 3) developing and testing mathematical descriptions of canopy-structure to evaluate, in silico, the likely effects of targeted changes in canopy characteristics.

## Results and Discussion

### Measuring the effects on canopy performance of variation in canopy structure

The CIMCOG set was grown on raised beds at MEXPLAT, Ciudad Obregon, Mexico, in 2012 and several studies were initiated. In one of these, some effects of canopy structure on leaf photosynthetic parameters were studied by Gemma Molero. Four genotypes that were similar in phenology were chosen for the study, based on visual contrasts in

canopy structure, viz: ‘open’ vs. ‘closed’ and ‘erect’ vs. ‘floppy’ leaf display. The pair of genotypes with erect-leaved canopies out-yielded the pair with floppy-leaved canopies, but consistent differences in photosynthetic traits between open and closed canopies or between erect-leaved and floppy-leaved canopies were not apparent (Table 1).

### Developing and testing tools for measuring canopy structure and function

Traditional methods for capturing information on the within-canopy distribution and temporal dynamics of N and other indicators of photosynthetic performance are prohibitively time-consuming. Remote sensing of spatial and temporal indicators of variation in photosynthetic and non-photosynthetic pigment distribution and of chlorophyll fluorescence should be much less laborious and cheaper than traditional techniques. Within the WYC, both aerial- and ground-based remote-sensing platforms are being developed and tested for these purposes. In 2012 at MEXPLAT, Maria Tattaris used a multispectral camera mounted on a light, remote-controlled UAV (Astec Falcon 8-rotor Unmanned Aerial Vehicle) to take aerial measurements of spectral reflectance in late-sown trials, which were then used to calculate canopy values of NDVI (Normalised Difference Vegetation Index; approximating to a measure

**Table 1. Comparison of yield and leaf photosynthetic traits among four CIMCOG genotypes selected for similar phenology but visually contrasting canopy architecture under irrigated conditions.** Measurements were performed during grain filling (18-20 days after anthesis) at different canopy strata (FL: flag leaf; L2: penultimate leaf; L3: third leaf). Data are means of four plants, two from each of two plots. YLD: yield (g m<sup>-2</sup>); A<sub>sat</sub>: photosynthetic rate measured under light-saturated conditions (μmol CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>); V<sub>cmx</sub>: maximum carboxylation rate allowed by Rubisco; J: rate of photosynthetic electron transport based on NADPH requirement; Chl: total chlorophyll content (a + b) (g m<sup>-2</sup>).

	ERECT LEAVES						FLOPPY LEAVES					
	YLD	Leaf	A <sub>sat</sub>	V <sub>cmx</sub>	J	Chl	YLD	Leaf	A <sub>sat</sub>	V <sub>cmx</sub>	J	Chl
OPEN CANOPY	685	FL	18.6	115.0	214.9	0.281	555	FL	15.6	90.8	199.8	0.314
		L2	15.2	94.4	172.3	0.314		L2	15.5	104.9	202.9	0.292
		L3	9.2	55.7	104.3	0.174		L3	6.7	67.3	114.7	0.294
CLOSED CANOPY	646	FL	16.2	87.4	187.5	0.343	590	FL	16.9	104.4	227.8	0.38
		L2	12.2	68.9	139.4	0.279		L2	13.3	87.3	169.4	0.28
		L3	11.9	57.8	112.6	0.189		L3	6.6	66.7	126.2	0.23

of “standing chlorophyll”). These NDVI values were subsequently related to yield (Fig. 1). Similar data have been captured by the ground-based “Phenomobile” at HRPPC, Canberra, which has a suite of sensing systems.

### Developing and applying tools for modeling canopy structure and function

Crop models indicate considerable scope to optimize leaf-area growth and distribution and further boost crop RUE via more effective spatial and temporal distributions of canopy N and/or different enzymes involved in photosynthetic carbon capture. In order to study the influence of canopy architectural and physiological parameters, even of agronomic practices, on canopy photosynthesis rates, a fully mechanistic model of canopy photosynthesis, including both detailed canopy architecture and light distribution and also canopy photosynthetic properties, is required. At the University of Nottingham, a three-year project funded by BBSRC began in May 2012, with the aim of identifying the processes that limit leaf photosynthesis for a given canopy structure/environment interaction. Bringing together crop scientists, image analysts, and mathematical modelers, the objective is to first capture the 3D nature of entire cereal crop canopies grown in field conditions, and then to model light distribution in order to predict the processes that limit photosynthesis. An example of this process is presented for rice in Song et al. (2013). This information for wheat will be used to direct the breeding for optimal canopy structure.

The reconstruction of entire canopies in the field rather than single plants in the lab presents unique problems. In collaboration with Qinfeng Song and

Xinguang Zhu at CAS, Shanghai, we are modeling the penetration of individual rays of light entering a crop canopy. To assist this process, several methods of reconstructing 3D plant canopies are being developed and assessed. Progress to date consists of:

1. Laser Scanning: Hardware-based approaches using laser scanning are capable of capturing plant canopies at a variety of scales and have been used with some success in the field. However, they are expensive, and very sensitive to lighting conditions and noise.
2. Kinect Fusion: Microsoft’s motion capture peripheral can be used to reconstruct 3D point clouds of a variety of objects. Each frame of a Kinect scan produces a depth map of the scene and multiple frames can be stitched together to reconstruct a plant or a canopy. This technique has so far been shown to work well on broader-leaved plants such as maize. Additional graphical modeling software can be used to “re-mesh” the data and fill in missing areas. The advantage of this technique is that it can be developed cheaply and is extremely rapid and quite precise. However, techniques are needed for outdoor use where the Kinect sensor cannot operate.
3. Stereo Reconstruction: Stereo vision uses two or more cameras placed in a static arrangement. While Kinect’s limited resolution prohibits accurate capture of thin leaved grasses such as wheat, stereo reconstruction now benefits from the millions of pixels available in modern DSLR cameras. This technique will also work effectively on field crop canopies as well as on individual plants. Further algorithmic development is required to combine multiple views of a plant or canopy into a mesh that can be used in ray tracing.

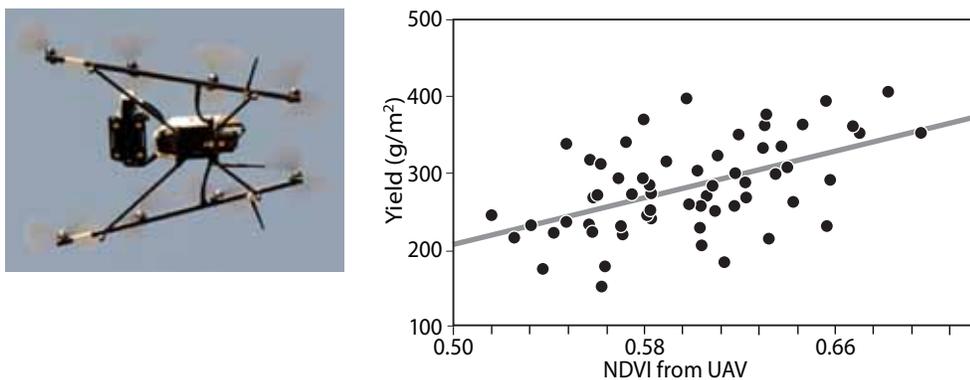


Figure 1. Left panel: Astec Falcon 8-rotor UAV carrying Tetracam ADC-lite multispectral camera. Right panel: Yield vs. NDVI for the late-sown CIMCOG Subset trial, taken from the UAV ( $r^2=0.52$ . Sixty entries, two reps, May 11 2012).

At HRPPC Canberra, an alternative system based on LIDAR (LIght Detection And Ranging) is being developed and tested. LIDAR is an optical remote sensing technology that can measure the distance to targets, or other optical properties of the targets, by illuminating the target with laser light and analyzing the backscattered light. LIDARs, mounted on the Phenomobile, are being used to quantify several aspects of canopy structure such as canopy height (an important covariate that influences canopy temperature independent of stomatal conductance), crop establishment, ground cover, leaf angle, stem and spike number. Some of these features can be accomplished using RGB cameras, but this very dependent on uniform, stable light conditions. LIDAR is an “active” system that functions independently of incident light and, for this reason, it has greater flexibility.

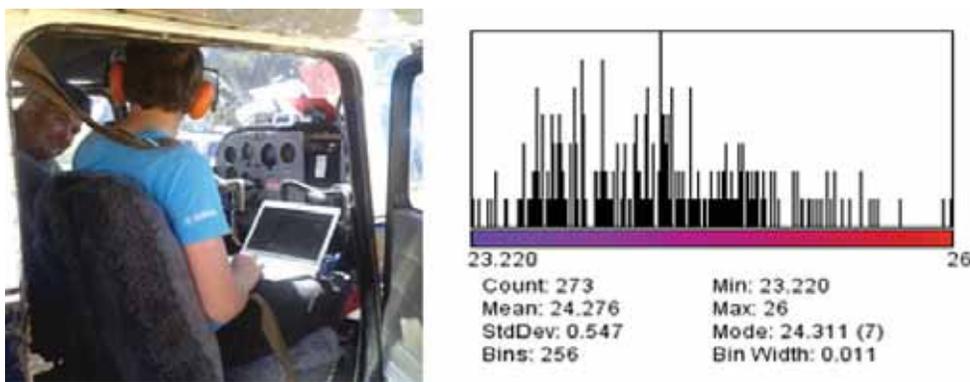
### Developing and applying more-effective ways of measuring canopy temperature

Earlier research conducted at CIMMYT, Obregon, demonstrated that measurements of canopy temperature (CT) can be usefully related to wheat yield potential. That research was conducted using hand-held infrared thermometers and relatively small numbers of plots. Genetic correlations of CT with yield were moderately strong, but the heritability of CT was low. This was mainly due to the strong temporal effects on CT attributed to changes in ambient conditions during the period when measurements were being taken. Within the WYC, both aerial and ground-based platforms for remote-sensing of CT are being developed and tested. Such systems should be capable of quickly collecting CT data repeatedly, on a very large

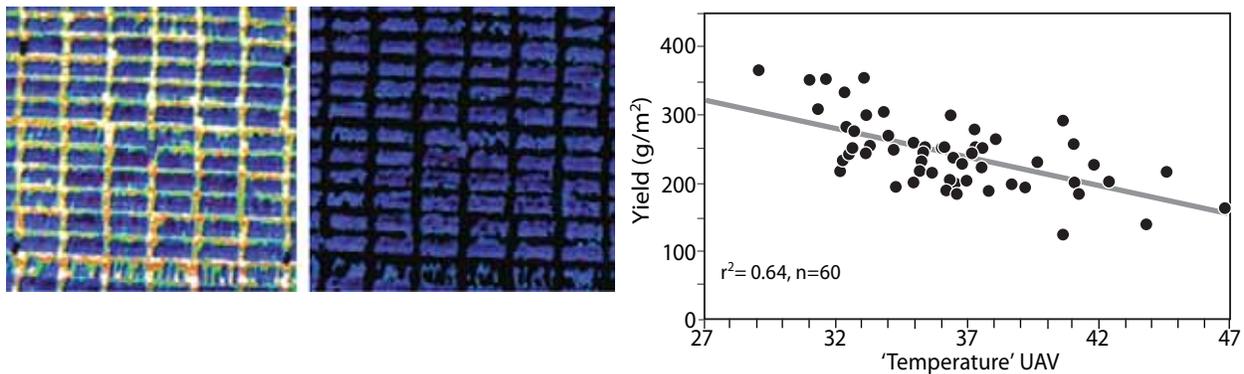
numbers of plots. Aerial imaging has the capacity to capture thermal data from thousands of plots within a few seconds. Repeated measurements within the same day or over longer time spans should result in much higher heritability of canopy temperature estimates. This would be useful not just for use in breeding and selection but also in QTL detection, which becomes much more effective as trait heritability increases.

In the first example (Figure 2), HRPPC staff based in Canberra are testing a fixed-wing system. Even from a height of 200-400m, high-resolution thermal cameras are able to generate ca. 500 pixels per 10m<sup>2</sup> plot. In this case, the right-hand side of the canopy temperature distribution shown is dominated by non-vegetation (soil) pixels that are hotter than the pixels corresponding to vegetation dominating the left-hand side of the distribution. Algorithms are being developed to eliminate “non-canopy” pixels so as to derive accurate estimates of vegetation temperature for each plot (Berni et al. 2009).

An example of how this might be done is shown in Figure 3. Here, measurements of surface temperature were taken by Maria Tattaris at MEXPLAT, Obregon, in late-sown trials of 2012, using a thermal camera mounted on a light, remote-controlled UAV (Astec Falcon 8-rotor Unmanned Aerial Vehicle). An RGB camera was also mounted on the UAV. Subsequently, data from the RGB image was used to mask out “non-canopy” pixels from the thermal image and variation in canopy temperature was estimated using those pixels remaining in the image.



**Figure 2. Aerial infra-red thermography of canopy surface temperature using a fixed-wing aircraft in SE Australia.** Left panel: The co-pilot setting up software for data-capture from a downward-facing FLIR thermal camera. Right panel: A representative frequency histogram of pixel temperature for a single plot canopy. From within the ca. 6m<sup>2</sup> central area of the plot, 273 pixels were captured from 200m above ground. Similar frequency histograms were generated for several thousand plots during a single day’s aerial operation.



**Figure 3.** Left panel: Image of late-sown CIMCOG Subset trial taken from UAV with Tetracam ACD-lite thermal camera (60 plots, two reps, May 11 2012). Center panel: Same image with non-vegetation pixels masked out using thresh-holding of RGB channels. Right panel: Correlation between yield and temperature from UAV. Note that 'temperature' is not the actual surface temperature but is a scalar of temperature, since this very light camera is un-cooled and not calibrated.

Similar canopy temperature data have also been captured in SE Australia using the ground-based "Phenomobile" driven over plots. Although this is faster than using hand-held instruments it still takes considerably longer to capture data from a very large number of plots, compared to aerial thermography, and as a result there is greater exposure to temporal changes in the ambient conditions.

### Next Steps

Substantial advances have been made using LIDAR and other remote-sensing techniques to digitize and extract several important canopy features. Further work will be conducted to refine the detection and measurement of those features already being captured and to extend remote-sensing techniques to the capture of more features, particularly those related to leaf photosynthetic characteristics. Work will also progress on developing algorithms to allow more efficient data extraction and presentation in forms that can be translated directly to breeding, QTL detection, and in silico trait evaluation.

Combining digitization of canopies with modeling and leaf level measurements offers the opportunity to inform not only phenotypic selection in breeding but also QTL detection and candidate gene

identification in transgenic breeding by evaluating traits in silico. Scaling of leaf level biochemistry and physiology to the crop is a major deficiency in canopy level photosynthesis research. To enhance progress in canopy reconstruction and modeling, a "graph-cuts" approach to stereo reconstruction will be implemented at University of Nottingham. Subsequent work will then focus on novel algorithms to generate 3D mesh data from the depth maps generated by this technique. These reconstructions will be used with ray tracing to define dynamic patterns in light throughout the canopy. Modeling the distribution of light will allow prediction of the amount of light striking given surfaces on a plant in the presence of shadows and movement. Between 2013 and 2015, models will be developed for dynamic photosynthesis (parameterized using field data) that will combine with light dynamics to enable prediction of limiting photosynthetic components associated with specific canopy structures.

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# Genetic variation in radiation interception at different canopy levels and in radiation use efficiency

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## Summary

This study presents results on: i) exploration of genotypic variation in radiation interception and radiation use efficiency, ii) variation in plant attributes related to a greater light interception and radiation use efficiency, and iii) screening of outstanding genotypes, using plant characteristics that may help increase radiation interception, radiation use efficiency, and so grain yield. A highly significant result is the genetic variation in light intercepted by spikes, which ranged from 18-44% of incident light during grain filling.

## Major Objectives

Screening for genotypic variation in canopy traits on core CIMMYT germplasm sets.  
Identifying plant traits that will increase radiation interception and radiation use efficiency.  
Outlining useful selection techniques to increase canopy interception and radiation use efficiency.

## Results and Discussion

### Variation in grain yield, harvest index, biomass, yield components, and radiation use efficiency

Two field experiments were conducted at CIMMYT's experimental station, in Ciudad Obregon, Sonora, Mexico, in the winter-spring growing season 2010-2011. A set of 60 high-yielding genotypes of the CIMMYT Mexico Core Germplasm (CIMCOG) panel was chosen for the experiments. All genotypes were sown in one experiment in flat beds (eight rows wide separated at 20 cm and 5 m long) and in another experiment in raised beds (five adjacent beds with two rows separated at 20 cm and 5 m long). Seeding rates used were 65 kg ha<sup>-1</sup> and 78 kg ha<sup>-1</sup> for flat and raised beds, respectively. Fertilizer rates, irrigation, and chemical control of weeds, pests, and diseases were carried out in order to maximize yield potential in all experiments.

Variation in grain yield (GY), biomass at maturity (BM), harvest index (HI), grain yield components, and radiation use efficiency (RUE) among

genotypes was significant in both flat and raised beds. Grain yield, biomass, radiation use efficiency, and grain yield components had greater variation than harvest index (Table 1).

Grain yield was positively and significantly associated with BM and HI in flat and raised beds; genotypes with greater GY had higher BM (Figs. 1a and 1b) and HI (Figs. 1c and 1d). Genotypes with high GY had also a greater HI; some modern genotypes had higher GY and HI than old varieties and varieties released in the 70's, 80's, and 90's in flat (Fig. 1c) and raised beds (Fig. 1d). It was interesting to notice that the relationship between GY and BM (Figs. 1a and 1b) was stronger than between GY and HI in flat (Figs. 1c) and raised beds (Fig. 1b). GY and HI were more strongly associated in the raised (Fig. 1d) than flat beds (Fig. 1c).

### Relationship between grain yield and radiation use efficiency

Grain yield was positively and significantly related to RUE, determined during the grain filling period in flat (Fig. 2a) and raised beds (Fig. 2b). Genotypes with high RUE produced greater grain yield; most of the modern genotypes had higher GY and RUE than old varieties and varieties released in the 70's, 80's, and 90's, in both flat (Fig. 2a) and raised beds (Fig. 2b). The positive relationship between GY and

**Table 1. Ranges of genetic variation (Lds, P≤0.05) for grain yield and its components and radiation use efficiency in flat and raised beds at CIMCOG, winter-spring 2010-11. Obregon, Sonora, Mexico.**

Characteristic	Flat beds	Raised beds
Grain yield (g m <sup>-2</sup> )	534 – 793 (83)	448 – 720 (69)
Biomass (g m <sup>-2</sup> )	1212 – 1998 (220)	1105 – 1560 (153)
Harvest index	0.39 - 0.50 (0.032)	0.40 - 0.51 (0.025)
Heads M <sup>-2</sup>	261 – 476 (47)	222 – 380 (40)
Grains M <sup>-2</sup>	12554 – 22781 (2291)	11357 - 21387 (1804)
Grains Spike <sup>-1</sup>	40 – 62 (4)	36 – 67 (5)
Radiation use efficiency (g MJ <sup>-1</sup> )	1.08- 1.68 (0.18)	0.94-1.31 (0.13)

RUE suggests that the increase in GY by selection in the breeding program at CIMMYT (Mexico) has come about from a significant increase in BM accumulation and crop RUE. Reynolds et al. (2011) pointed out that an increase in yield potential may arise from an increase in any of canopy light interception, radiation use efficiency, and/or harvest index.

On the other hand, RUE determined through the growing season showed that the arrangement of plants in a canopy grown in flat beds is more

efficient at intercepting light and producing dry matter than a canopy in raised beds; this difference was established early in the season and was maintained through the growing season (Fig. 3). RUE at 40 days after seedling emergence in flat beds was about 30 % greater than RUE in raised beds. A greater capture of light by plants grown in flat beds was reflected in a larger production of biomass and RUE, however, an increase in leaf growth in early stages of development, when it is cool and radiation is low, may help achieve a further increase in radiation interception and dry

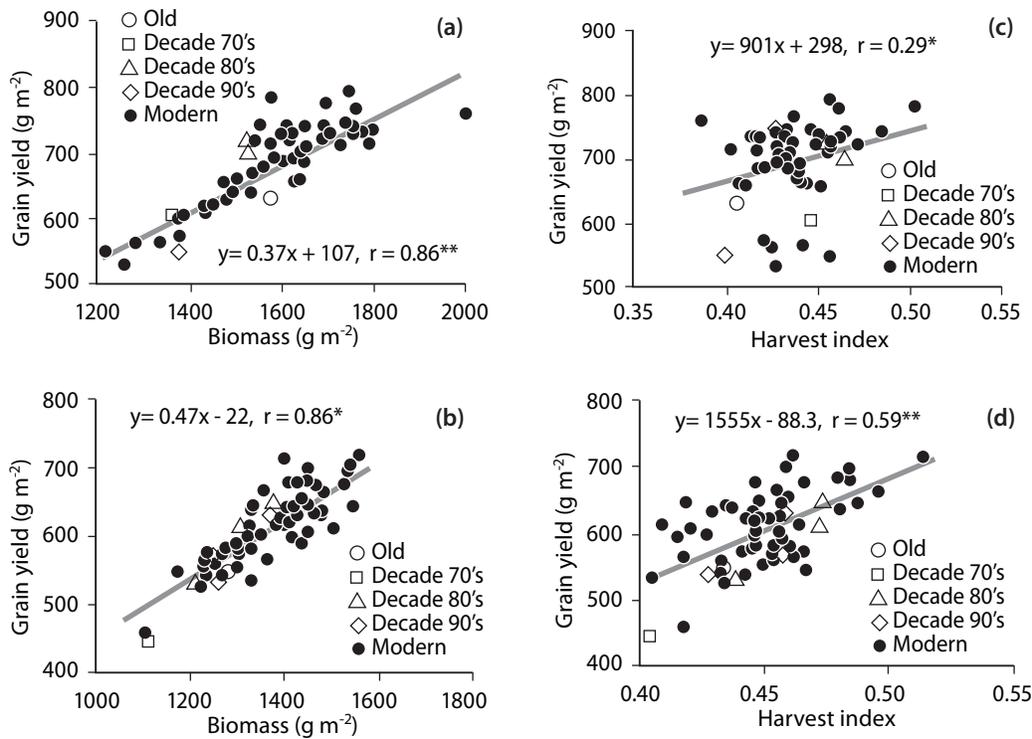


Figure 1. Relationship between grain yield and biomass at maturity in flat (a) and raised beds (b), and harvest index in flat (c) and raised beds (d) for CIMCOG, winter-spring 2010-11, Obregon, Sonora, Mexico.

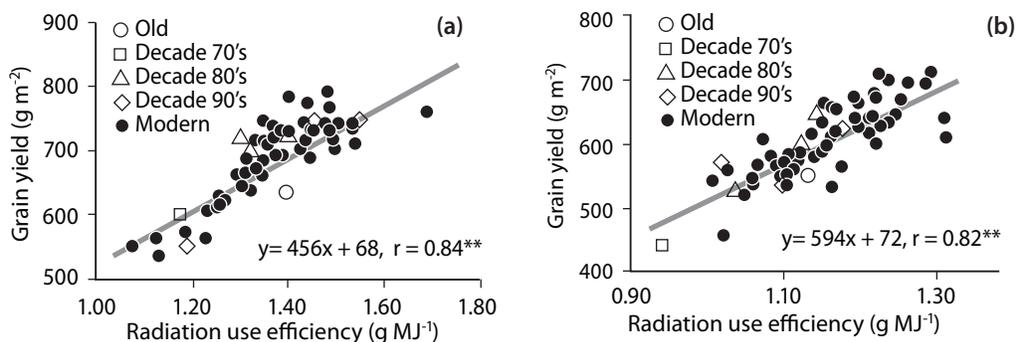
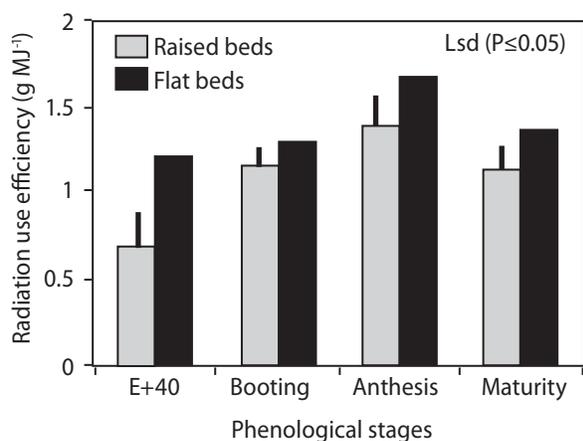


Figure 2. Relationship between grain yield and radiation use efficiency determined between anthesis and physiological maturity in flat (a) and raised beds (b) for CIMCOG, winter-spring 2010-11, Obregon, Sonora, Mexico.

matter accumulation and so a higher RUE. If this advantage in RUE is maintained until the grain filling period, this will be reflected in a higher GY.

### Variation in radiation intercepted by canopy, head and top leaves, and coefficient of extinction

Incident radiation ( $I_o$ ), canopy interception ( $I_c$ ), reflected ( $I_r$ ), and transmitted radiation ( $I_t$ ) were determined at anthesis+7 days in all genotypes.  $I_c$  was calculated as the proportion of light intercepted by the canopy after losses by reflection and transmission into the ground were subtracted ( $I_c = I_o - I_r - I_t$ ).  $I_c$  in flat beds was about 30 % higher than in raised beds due to its greater losses by  $I_t$  (Table 2). Fischer et al. (2005) observed similar results by comparing light interception in flat vs. raised beds at the CIANO Experiment Station



**Figure 3. Radiation use efficiency at 40 days after seedling emergence, booting, anthesis, and physiological maturity in flat and raised beds at CIMCOG, winter-spring 2010-11, Obregon, Sonora, Mexico.** [Vertical bars represent values of the least significant difference (Lsd,  $P \leq 0.05$ )].

and they confirmed this difference in radiation interception was reflected in lower grain yield in raised beds than flat beds.

The coefficient of extinction [ $k = -(\ln(I_t))/LA$ , where  $\ln$  is the natural logarithm of  $I_t$  and  $LA$  is the green leaf area index], green leaf area, and radiation intercepted by the canopy, determined at anthesis+7 days had narrower genetic variation in flat beds than in raised beds (Table 3).

Radiation intercepted by the canopy and its distribution into spikes and top leaves was determined in flat beds only (Table 3). Genetic variation in radiation intercepted by heads, flag leaf, leaf 1, and leaf 2 was 2.4, 3.9, 3.1, and 7.2-fold, respectively. This large genetic variation in light

**Table 2. Incident, reflected, transmitted, and intercepted radiation determined at anthesis+7 days in flat and raised beds for CIMCOG, winter-spring 2010-11, Obregon, Sonora, Mexico.**

Radiation interception	Flat beds	Raised beds
Incident radiation ( $I_o$ , $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	1626 (100) *	1679 (100)*
Reflected radiation ( $I_r$ , $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	81 (5)	82 (5)
Transmitted radiation ( $I_t$ , $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	45 (3)	41(3)+559(32)=600(35)
Intercepted radiation by canopy ( $I_c$ , $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	1508 (92)	997 (60)

\*Numbers between parentheses represent the percentage of  $I_o$ .

**Table 3. Percentage of radiation intercepted by canopy, heads, flag leaf, leaf 1, and leaf 2, plant height used to measure light interception by heads, flag leaf, leaf 1 and leaf 2, and ranges of genetic variation (Lds,  $P \leq 0.05$ ) for extinction coefficient, green leaf area, radiation intercepted by canopy, heads, flag leaf, leaf 1, and leaf 2 in flat and raised beds for CIMCOG, winter-spring 2010-11. Obregon, Sonora, Mexico.**

Radiation interception	(%)	Flat beds Height (cm)	Ranges	Raised beds Ranges
Extinction coefficient	-	-	0.10-0.42(0.21)	0.07-0.57(0.14)
Green leaf area index	-	-	3.6-5.8(1.1)	2.3-4.9(1.3)
Intercepted radiation by canopy ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	1508 (92)	-	72-99(16)	36-83(27)
Heads ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	462 (28)	89	18.3 - 43.7(18.5)	-
Flag leaf ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	442 (27)	73(16)	11.7 - 46.3(23.4)	-
Leaf 1 (Flag leaf-1, $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	436 (27)	49(24)	12.7 - 39.0(13.9)	-
Leaf 2 (Flag leaf-2, $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	168 (10)	32(16)	3.0 - 21.7(8.2)	-

interception indicates the potential for increasing radiation interception during the grain filling period by selecting for specific morphological traits. The amount of light intercepted by spikes was about 30 % of canopy intercepted radiation; similar percentages of radiation interception were determined for flag leaf and leaf 1 (Table 3). It is important to point out that the length of the peduncle and the internode between flag leaf and leaf 1 may play a key role in light penetration to inferior leaves during the grain filling period; these morphological characteristics of the stem architecture may explain why flag leaf and leaf 1 may contribute up to 60 % of the radiation intercepted by the top leaves during the grain filling period.

Plant height was used to determine radiation intercepted by heads, flag leaf, leaf 1, and leaf 2, and was measured from soil surface to the last spikelet in the head, ligule of flag leaf, leaf 1, and leaf 2, respectively. The numbers in parentheses in the plant height column represent the internode length between the spikes base and flag leaf ligule, flag leaf ligule and leaf 1 ligule, and leaf 1 ligule and leaf 2 ligule, respectively.

### **Next Steps**

Light interception at the beginning of the season could be increased by improving early canopy growth when leaf area development and light interception are often slow due to cool conditions.

The large variation in light intercepted by spikes (18-44% of incident light) during grain filling represents a challenge to establish an optimum value for spikes, since they also compete for light with the rest of the canopy.

Evaluation of characters related to the stay green of leaf area during the grain filling period will further increase biomass production and so improve radiation use efficiency and grain yield.

Further exploration of wheat genotypes response to radiation interception and radiation use efficiency in either flat beds or raised beds with three or four plant rows to determine what plant system help maximize radiation interception, biomass accumulation, radiation use efficiency and so, grain yield.

Utilization of some plant traits identified at this stage, namely radiation interception by heads, flag leaf, and leaf 1, may result in the selection of genotypes with higher radiation interception, BM, RUE, and so grain yield.

### **References**

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# Optimizing RuBP regeneration to increase photosynthetic capacity

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## Summary

SP1.5 aims to increase the photosynthetic capacity of wheat by increasing RuBP generation. We received funding to generate initial transgenic lines and analysis of the transformants is underway. Further transgenic events, using alternate promoters, may be needed to optimise expression levels. CIMMYT will conduct crosses of the best lines.

## Results and Discussion

Photosynthesis is co-limited in the canopy by the kinetics of Rubisco and the regeneration rate of RuBP (reflecting light harvesting, electron transport, and photophosphorylation). Increasing RuBP regeneration in model plant species substantially increases photosynthesis. The two limiting enzymes in RuBP regeneration are sedoheptulose-1,7-bisphosphatase (SBPase) and fructose biphosphate aldolase (FBP aldolase) (Raines 2003, 2006), and these are the targets for over-expression in transgenic wheat. The project is high impact with a medium- to long-term delivery timeframe, and therefore has seed funding directly from CIMMYT to allow commencement of transformation experiments.

At Rothamsted Research, we have made constructs to over-express wheat SBPase (pSBPaseT2) and FBP aldolase (pFBPaldT2) under the control of the rice tungro bacilliform virus promoter (RTVP), which targets expression to the leaf lamina. Scutellum of the spring wheat CV Cadenza, and the CIMMYT lines HIST10 and HIST13, have been transformed via biolistics and independent transgenic lines recovered (Table 1).

**Table 1. Transgenic lines produced with CIMMYT, CIRC, and 20:20 Wheat<sup>o</sup> funding**

Wheat line	Gene inserted	Number of independent lines
Cadenza	pFBPaldT2	37
Cadenza	pSBPaseT2	25
HIST10	pFBPaldT2	5
HIST10	pSBPaseT2	5
HIST13	pFBPaldT2	12
HIST13	pSBPaseT2	8

The Cadenza transgenic lines generated are now being screened at the University of Essex to determine the amounts of SBPase and FBPaldolase in each line. The Hist10 and Hist13 lines are being screened at Rothamsted Research for improved photosynthetic properties (Fig. 1).



**Figure 1. Measuring gas exchange and fluorescence in Hist10-SBPase transgenic lines.**

Two new potential photosynthetic tissue-specific (Rubisco activase) promoter fragments from *Brachypodium* were cloned and linked to the  $\beta$ -glucuronidase (GUS) reporter gene. Neither of the two tested promoters successfully drove GUS expression in wheat leaves. Alternative promoters are now being evaluated.

### **Next Steps**

We will confirm gene expression and the amount of proteins in transgenic lines. If increased levels of SBPase and/or FBPaldolase are not observed in the

mesophyll cells, we will redesign transformation constructs to achieve this. Once higher levels of SBPase and/or FBPaldolase are achieved in the mesophyll cells, we will determine the impact of increased RuBP regeneration on photosynthetic performance, in each of the genetic backgrounds. Lines with improved performance will then be tested under controlled environments and field conditions.

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# Improving the thermal stability of Rubisco activase

Elizabete Carmo-Silva<sup>1,2</sup>, Joanna Scales<sup>2</sup>, Pippa Madgwick<sup>2</sup>, Mike Salvucci<sup>1</sup>, Martin Parry<sup>2</sup>, Christine Raines<sup>3</sup>, Matthew Reynolds<sup>4</sup>  
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## Summary

The major objective is to produce wheat germplasm with improved tolerance to heat stress through the modification of the key photosynthetic enzyme Rubisco activase. Current funds have allowed transformation of “best bet” candidate gene constructs into wheat as part of SP1.5. Joanna Scales, a BBSRC PhD student jointly supervised by Martin Parry, Christine Raines, and Mike Salvucci, generated the transformation constructs and will undertake the molecular and biochemical analysis of transgenic lines.

## Results and Discussion

Higher temperatures (a likely result of climate change) induce inactivation of Rubisco, compromising the stability of yield improvements achieved through increases in photosynthetic capacity and efficiency in projects SP1.1, SP1.2, and SP1.7. The heat stability of Rubisco activase, the protein regulating Rubisco activity in wheat leaves, is an important determinant of the heat stability of photosynthesis (Salvucci and Crafts-Brandner 2004). This project will test the hypothesis that transgenic wheat containing an engineered Rubisco activase, previously shown to be heat stable *in vitro*, will possess this trait in planta under field conditions. This project is high impact with a medium- to long-term delivery timeframe.

USDA experiments have confirmed that Rubisco activase from cotton has a high thermal tolerance. Recent experiments have confirmed that, *in vitro*, purified wheat Rubisco (supplied by Rothamsted Research) is successfully activated by recombinant Rubisco activase from cotton. Thus, the duo cotton Rubisco activase and wheat Rubisco is expected to function *in vivo*.

We have designed constructs to introduce the thermally stable cotton Rubisco activase into wheat (Figure 1). This has been used in transformation

experiments and putative transgenic lines are under selection. Lines expressing the transgene will be tested for photosynthetic function at high temperature.

## Next Steps

We will analyse the transgenic lines and confirm that the thermally stable Rubisco activase is expressed, correctly imported, and functional in the mesophyll chloroplasts. If the expression of cotton Rubisco activase is not confirmed, we will redesign the construct to alter the promoter and/or transit peptides, and generate a new construct and transgenic lines. Once transgenic lines successfully expressing the thermally stable Rubisco activase at different temperatures are achieved, we will analyse their photosynthetic performance and test lines with improved thermal tolerance in controlled environment then field conditions.

## Reference

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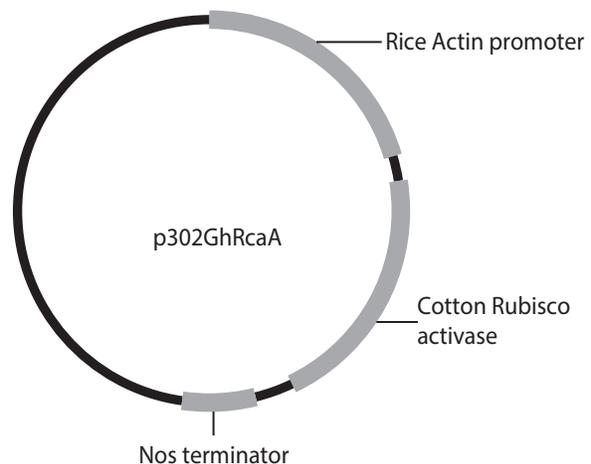


Figure 1. Plasmid p302GhRcaA for expression of cotton Rubisco activase in wheat.

# Replacing the large subunit of Rubisco

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## Summary

The major objective is to exploit natural variation in Rubisco kinetics and introduce the ‘best’ available Rubisco into wheat lines. This requires the identification of better Rubiscos and development of chloroplast transformation to ensure the production of sufficient Rubisco in leaves. Chloroplast transformation has yet to be developed for any cereal.

## Results and Discussion

Rubisco (ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase) enables net carbon fixation through the carboxylation of RuBP. However, some characteristics of Rubisco make it surprisingly inefficient and compromise photosynthetic productivity. Rubisco is a prime target for improvements to supercharge photosynthesis and improve both productivity and resource use efficiency. Although the kinetic constants have only been determined for the Rubiscos of approximately 100 species, representing a diverse range of photosynthetic organisms, considerable variation in the catalytic properties of the enzyme is evident (Parry et al. 2013; Figure 1). Further natural variation (in turnover rate, affinity or specificity for CO<sub>2</sub>) is highly likely, and if such variation can be identified among species closely related to the target crop (wheat), then there will be an improved likelihood of a successful outcome when these genes (or properties) are expressed in wheat.

Nuclear expression of large subunit genes does not give large enough amounts of protein to support high photosynthetic rates. Plastid transformation can deliver large amounts of the introduced protein but is currently only possible in a limited number of species, and homoplasmy has never been achieved in cereals. This project aims to identify ‘better Rubiscos’, to develop plastid transformation in wheat and to use gene constructs encoding better Rubisco large subunits, aimed at improving the enzyme’s kinetic properties.

In a newly-funded CIMMYT initiative, the Rubisco kinetic properties of a diverse array of monocots will be determined, and the most promising variants will be identified for engineering into wheat via chloroplast transformation. The transformed progeny will be screened for improved photosynthetic capacity and yield potential. Germplasm for the initial evaluation of Rubisco kinetics has already been selected on the basis of high productivity in hot, arid environments.

## Next Steps

We will obtain the germplasm for testing and optimize and conduct protocols for the extraction and purification of Rubisco from these diverse genotypes. We will then determine their kinetic constants and specificity factors at 25°C and 30°C.

## Reference

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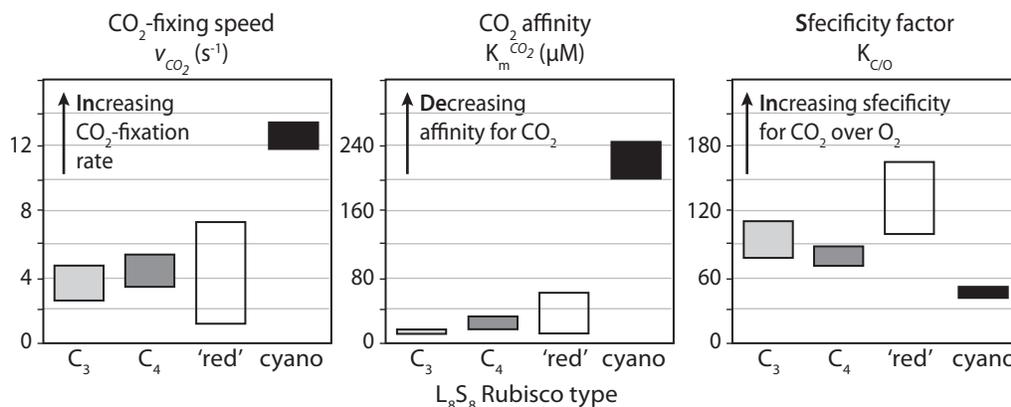


Figure 1. Evidence for natural diversity in L8S8 Rubiscos (Parry et al. 2013).

# Optimizing Partitioning to Maximize Agronomic Yield

## Optimizing partitioning to grain while maintaining physiological and structural integrity

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Improving light interception and radiation use efficiency (RUE) is expected to improve crop biomass, but assimilates must be optimally partitioned during plant growth and development if the benefits are to be fully realized as additional grain yield. This is a complex problem because both photosynthetic capacity and the various demands from plant organs for assimilate continually change in relation to crop development, as well as ambient conditions. The scientific challenge is first to understand the trade-offs necessary to maximize the size of reproductive structures, without under-investing in the roots, stems, and leaves required for maintaining physiological and structural integrity. For example, it is unknown whether the root system of modern wheat is large enough to take up sufficient water and nutrients to support future gains in biomass. The second challenge is related to the fact that the actual environment in which a cultivar will grow is unpredictable, mainly in terms of light regime and temperature profiles. However, to help focus research intended to ensure that harvest index (HI) reaches >0.5 across all major wheat agro-ecosystems in the normal range of seasons, the main factors for consideration are: 1) Optimizing the partitioning of assimilates to different plant organs to maximize investment in reproductive structures without sacrificing functional integrity; 2) Tailoring crop phenology to different environments, e.g. genes of major effect *Ppd* and *Vrn* already determine adaptation to winter vs. spring wheat mega-environments (MEs), but adaptation within these MEs is yet to be fine-tuned to ensure high and stable expression of HI; 3) Modulating sensitivity to environmental cues during the rapid spike-growth phase (when seed number and kernel weight potential are determined) to avoid overly conservative responses that are disadvantageous under favorable agronomic practices; and 4) Lodging resistance to avoid losses of yield and grain quality associated with mechanical damage before harvest, which may occur if both grain yield and aerial biomass are significantly increased. By jointly addressing these factors, genetic improvements in RUE are more likely to be translated in agronomic impacts.

### Optimizing the partitioning of assimilates to different plant organs to maximize HI

There has been no systematic genetic progress in HI since the early 1990s; from values of ca. 0.45-0.50 in spring wheat and ca. 0.50-0.55 in winter wheat (Hay 2008; Foulkes et al. 2009), but of greater concern is that expression of HI is not well controlled, neither in terms of breeding outcomes nor in terms of its expression across environments and years. In fact, large genetic ranges for dry matter (DM) partitioning are reported across all plant organs. In theory at least, by combining the lowest values of DM partitioning observed for each of the alternative sinks (stem, leaves etc), the relative spike mass at anthesis could be increased to 0.35, from the present maximum values of c. 0.30 in spring wheat, which we would expect to boost HI. However, potential trade-offs will need to be carefully assessed to avoid, for example, negative impacts on canopy photosynthetic capacity, root growth, and lodging resistance (Foulkes et al. 2011). In addition, the balance between structural stem and non-structural (soluble) stem carbohydrate must be optimized across environments, taking account of whether assimilate availability during grain filling – and therefore realization of grain weight potential – may be limited by high temperature or light. Reports indicate wide genetic variation in soluble carbohydrate percentage of the stem (Ehdaie et al. 2006; Ruuska et al. 2006), indicating that breeding for this trait should be possible. The complement to spike partitioning in further enhancing HI is the fruiting efficiency (FE; i.e. the number of grains set per unit spike dry weight at anthesis; Lázaro and Abatte 2011). However, trade-offs between these two physiological components (Gonzalez et al. 2011) as well as between grain weight and FE (Bustos et al. 2013) may be observed. Thus identifying mechanisms determining increases in spike partitioning whilst maintaining FE, grain weight, and vice versa should be a priority for future research approaches. Further research is required to understand the genetic bases of these traits for the efficient deployment of existing variation in breeding programs.

### **Tailoring phenology to diverse agro-ecosystems**

Expression of HI is strongly influenced by the timing of different phenological stages and their response to environmental factors (Slafer et al. 2009). The most critical stage of development is the period from initiation of rapid spike growth (generally coinciding with flag leaf emergence) to a few days after anthesis (Fischer 1985; Kirby 1988). This is largely due to the sensitivity of floret development, gamete formation and fertilization, and grain abortion to environmental conditions during the juvenile spike growth phase (Barnabas et al. 2008). Considerable genetic variation (mainly in response to photoperiod and temperature) provides opportunities to tailor phenology to diverse agro-ecosystems (Distefeld et al. 2009) and to optimize the partitioning of time to anthesis to extend the critical phases without altering adaptation (Miralles and Slafer 2007). However, notwithstanding the availability of diagnostic markers associated with development (e.g. Snape et al. 2001; Fu et al. 2005, Beales et al. 2007), and indeed HI itself, expression of HI remains unpredictable in breeding and needs further research to understand its physiological and genetic base.

### **Modulating sensitivity to environmental cues**

With the exception of photoperiod and CO<sub>2</sub> concentration, most abiotic factors that influence crop performance (including temperature, radiation levels, and soil moisture) are unpredictable. Nonetheless, plants must determine their partitioning to reproductive structures in the absence of information on growing conditions during subsequent seed filling. This has presumably led to strong selection pressure for conservative strategies, especially in annual species like wheat. Plant growth regulators (PGRs) are known to help plants adapt to their environment. For example, under high temperatures, ethylene appears to be involved in signaling, leading to kernel abortion in wheat (Hays et al. 2007). In rice, a candidate gene for spike fertility (Gn1a) codes for cytokine oxidase, which, through its regulation of cytokinin levels, influences the number of reproductive organs in the panicle (Ashikari et al. 2005). A better understanding of the physiological and genetic basis of determination of HI by PGRs is expected to play a key role in breeding for higher yielding crops in the future (Zhang et al. 2006).

### **Lodging resistance**

Lodging is a persistent phenomenon in wheat that reduces harvestable yield by up to 80%, as well as reducing grain quality. A validated model of the lodging process has identified the plant traits that determine stem and root lodging risk in wheat and has been used to estimate the dimensions of a wheat plant to make it lodging-proof (Berry et al. 2007). In order to increase lodging resistance, it is predicted that plant breeders must increase the spread of the root plate, stem thickness, and the material strength of the stem wall, whilst minimizing the width of the stem wall. Preliminary work has indicated that wider stems have a greater DM per unit of stem length, and that DM density is positively related to the material strength of the stem wall (Berry et al. 2007), which means there is a significant DM cost associated with increasing stem strength. The extent to which structural requirements compete with yield must be investigated.

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# Optimizing harvest index through increasing partitioning to spike growth and maximizing grain number

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## Summary

In order to raise yield potential we must optimize partitioning of assimilate to spikes, to maximize grain number and harvest index (HI). Across the 2010-11 and 2011-12 seasons, grain yield was positively associated with HI ( $R^2 = 0.37$ ,  $P < 0.001$ ) for 30 CIMCOG (CIMMYT Mexico Core Germplasm) genotypes evaluated at CENEB, Ciudad Obregon, Mexico. Genetic variation in grains  $m^{-2}$  was explained mainly by fruiting efficiency (FE; grains per g spike dry matter (DM) at GS61+7d) rather than by spike partitioning index (SPI; spike DM / above-ground DM) at GS61+7d. True-stem DM partitioning index (PI) and leaf-sheath PI were the most important determinants of genetic variation in SPI. Stronger correlations were found between SPI and true-stem partitioning to internode 2 and/or lower internodes than to the peduncle. Results indicated a positive linear association between each of lemma and palea DM partitioning within the spike and FE ( $R^2 = 0.84$  and  $0.85$ , respectively,  $P < 0.001$ ). The physiological and genetic bases of these relationships are currently under investigation.

## Results and Discussion

Despite a hypothetical limit to HI of ca. 0.65 in wheat (Austin 1980; Foulkes et al. 2011), there has been no systematic progress since the early 1990s from values of ca. 0.50-0.55 (Foulkes et al. 2011; Reynolds et al. 2012). Partitioning to spikes could be increased by reducing competition from alternative sinks, especially during stem elongation when grain number is determined (Fischer 1985; Foulkes et al. 2011; Reynolds et al. 2011). These strategies are complementary to those focused on optimizing phenology (see Slafer et al., these proceedings). The complement trait to SPI, that may be improved to further enhance grain number, is FE at anthesis (Fischer 2011; Foulkes et al. 2011; Lázaro and Abatte 2011). Analysis of these partitioning and spike fertility traits in the CIMCOG panel of advanced lines has been carried out in 2010-11 and 2011-12 at Ciudad Obregon by PhD students Carolina Rivera

and Eliseo Trujillo (registered at Nottingham University, UK, October 2011; CIMMYT-MasAgro/CONACYT/Nottingham University funding).

## Identifying genetic sources of wheat (and close species) expressing favourable values of assimilate partitioning and spike fertility

Averaged across 2010-11 and 2011-12, HI ranged from 0.421-0.522 ( $P < 0.001$ ) and SPI from 0.237-0.313 ( $P < 0.001$ ; Table 1, Fig. 1). Grain yield was positively linearly associated with HI ( $R^2 = 0.37$ ,  $P < 0.001$ ) and harvest biomass ( $R^2 = 0.46$ ,  $P < 0.001$ ) for the 30 genotypes. Overall grains  $m^{-2}$  was not associated with SPI, although a weak positive linear association was apparent ( $P < 0.10$ ), excluding the four cultivars with the highest values of SPI ( $> 0.30$ ). In contrast, there was a strong positive linear association between grains  $m^{-2}$  and FE ( $R^2 = 0.53$ ,  $P < 0.001$ ) among the genotypes. As expected, there was a positive linear association between SPI and spike DM per  $m^2$  at GS61+7d ( $R^2 = 0.54$ ,  $P < 0.001$ ; data not shown). The year  $\times$  genotype interaction for SPI and HI was not significant.

The observed maximum HI (0.52) was much lower than the theoretical maximum of ca. 0.65, indicating that there is significant scope for raising yield potential by improving HI in modern cultivars. The partitioning ranges for plant components at GS61+7d represented novel variation compared to values reported in the literature. For example, the CIMCOG maximum SPI of 0.32 is above values previously reported (0.12-0.29; Foulkes et al. 2011). The variation observed in leaf-lamina PI was relatively narrow (ranging from 0.19-0.22) and at the lower end of the range reported worldwide (0.19-0.31). The observed range for the combined leaf-sheath-and-true-stem PI of 0.48-0.53 was again towards the lower end of the range reported in worldwide investigations (0.48-0.63; Foulkes et al. 2011). By combining the minimum values for alternative sinks (leaf lamina, leaf sheath, and true stem) in the CIMCOG panel, our results indicate that SPI could potentially be increased to 0.33.

### Identifying traits and mechanisms determining genetic variation in spike index and FE and quantify trade-offs

In 2011, SPI was more strongly associated with leaf-lamina PI ( $R^2 = 0.34$ ,  $P < 0.001$ ) and leaf-sheath PI ( $R^2 = 0.33$ ,  $P < 0.05$ ) than true-stem PI ( $R^2 = 0.07$ , ns) at GS61+7d (Fig. 2). In 2012, SPI showed a slightly weaker negative association with leaf-sheath PI ( $R^2 = 0.17$ ,  $P < 0.001$ ), but a much stronger negative

association with true-stem PI ( $R^2 = 0.60$ ,  $P < 0.001$ ). There was no association with leaf-lamina PI amongst the genotypes. Averaging across years, there was a slightly stronger negative association between SPI and true-stem PI ( $R^2 = 0.29$ ,  $P < 0.05$ ) than between SPI and leaf-sheath PI ( $R^2 = 0.21$ ,  $P < 0.05$ ). There was no association between SPI and leaf-lamina PI. Overall, these results indicate that, in modern CIMMYT cultivars, true-stem PI and

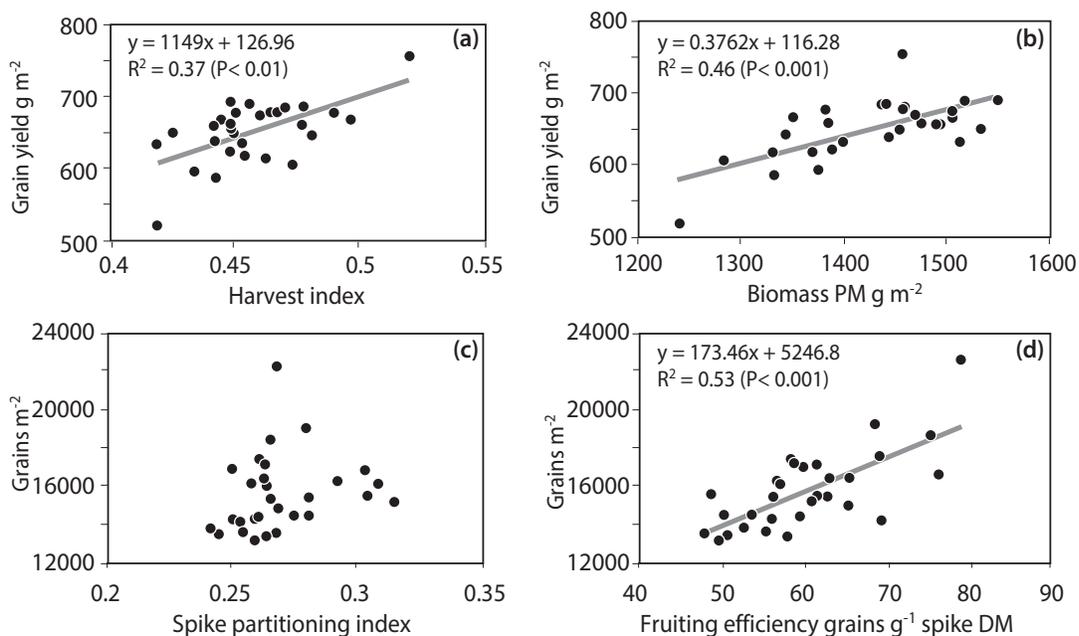


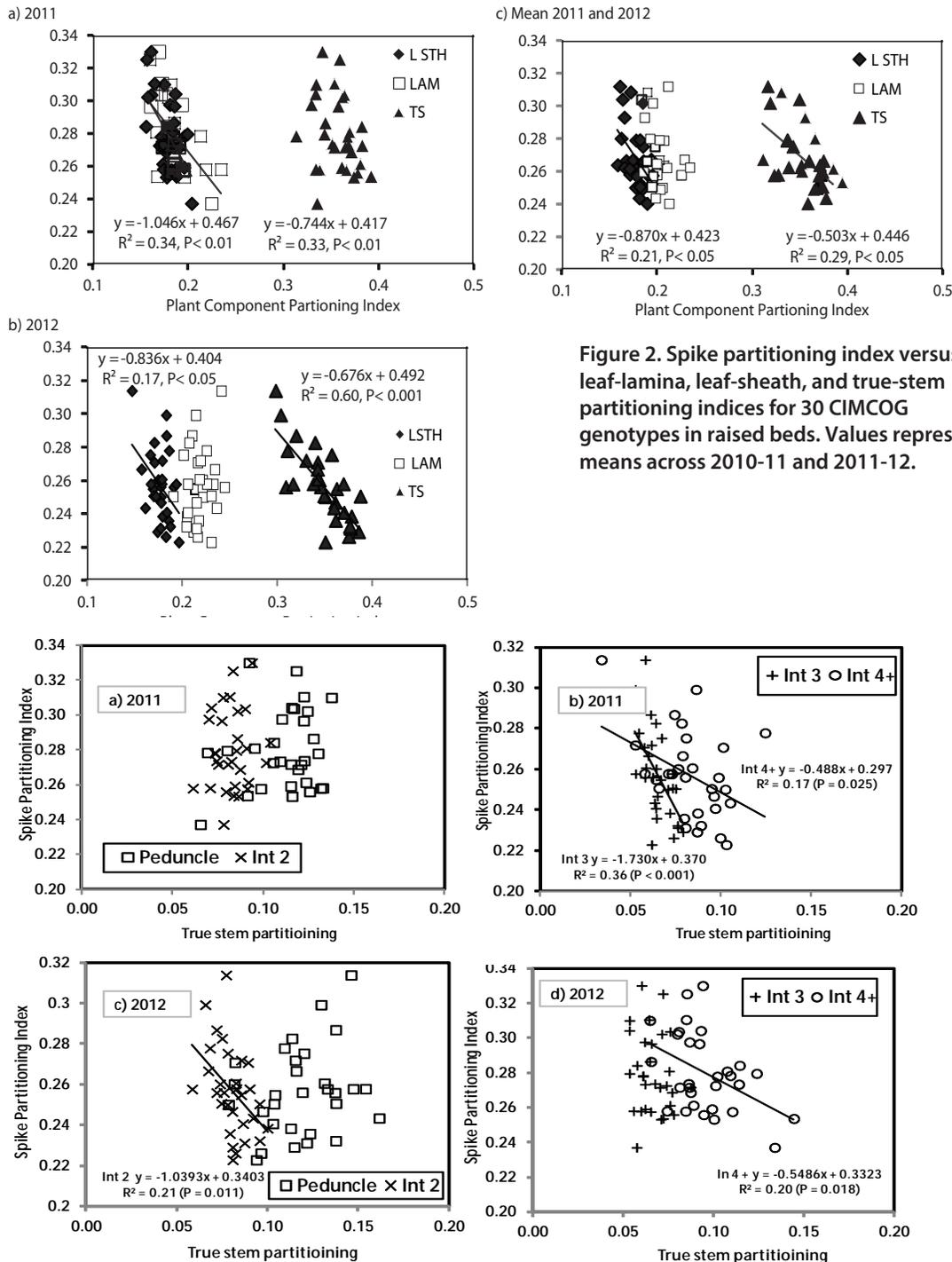
Figure 1. Linear regression of a) grain yield (100% DM) on HI; b) grain yield on above-ground DM at physiological maturity; c) grains  $m^{-2}$  on spike partitioning index; and d) grains  $m^{-2}$  on FE for 30 CIMCOG genotypes. Values are means of 2010-11 and 2011-12 seasons in raised beds.

Table 1. Means, ranges, and LSD of: a) grain yield and yield components at harvest, and b) PIs (spike, leaf lamina, leaf sheath, and true stem), FE, spike DM and above-ground DM at GS61+7d. Values are means of 2010-11 and 2011-12 seasons in raised beds.

a)	YLD $t ha^{-1}$	HI	BM $g m^{-2}$	GM2	SM2	GPS	GW mg
Mean	6.58	0.459	1435	15702	305.6	51.4	42.48
Min	5.36	0.421	1266	13304	236.5	43.6	30.00
Max	7.65	0.522	1552	22397	387.0	68.0	52.47
LSD 5% Gen.	0.346	0.0186	83.4	1267.9	30.82	1.63	2.425
LSD 5% YxGen.	< 0.001	0.507	0.003	0.045	0.308	0.510	0.458
b)	SPI	LLPI	LSPI	TSPI	FE	SDMa $g m^{-2}$	AGDM $g m^{-2}$
Mean	0.254	0.207	0.176	0.353	49.77	323.3	1103
Min	0.237	0.189	0.160	0.316	39.85	270.1	989
Max	0.313	0.222	0.191	0.379	75.94	397.0	1211
LSD 5% Gen.	0.037	0.018	0.0196	0.3473	9.987	58.66	124.7
LSD 5% YxGen.	0.961	0.031	0.598	0.741	0.058	0.091	0.395

leaf-sheath PI are the most important determinants of genetic variation in SPI. Future strategies to increase SPI should therefore focus on the joint optimization of these two partitioning indices. With regard to true-stem PI, we investigated the association of the different internodes on the

extended stem (peduncle and internodes 2, 3, and 4+) with SPI amongst the 30 genotypes (Fig. 3). In general, stronger associations were found between SPI and internode 2 and/or lower internodes than with the peduncle. Indeed, in both years, the association between SPI and the peduncle true-



stem PI (TSPI) was not significant. These results indicate that early events during stem elongation are important in determining spike partitioning at anthesis. Fischer and Stockman (1986) examined tall and dwarf isolines of wheat and observed that competition between spike and stem growth began at a relatively early stage, during stem elongation, and that allometric relationships affecting spike growth may be established shortly after stem elongation. Siddique et al. (1989) also found that the difference in the spike to stem ratio between tall and semi-dwarf Australian isolines was evident soon after the terminal spikelet stage. They observed a constant ratio between relative growth rates of the spike and the stem in tall and semi-dwarf lines. A greater stem:stem ratio at anthesis for the semi-dwarfs was attributed to a bigger intercept of the regression of  $\ln$  ear DM vs  $\ln$  stem DM; semi-dwarf isolines had a lower predicted stem DM per shoot at spike DM (= 1 mg). In our study, the lack of a correlation between SPI and peduncle TSPI suggests that these plant components do not compete strongly for assimilate.

Our results suggest the leaf lamina does not compete with the spike for assimilate, so there may be scope to reduce leaf-lamina PI to increase SPI. However, reducing leaf-lamina PI may be incompatible with raising photosynthetic capacity. To examine the contribution of the leaf lamina to post-anthesis photosynthetic capacity further, a leaf-lamina defoliation treatment was imposed at GS61+10d in 2012. Averaged across cultivars, results showed a 5.5% decrease in grain yield per shoot in defoliated versus control shoots ( $P < 0.001$ , Table 3), and genotypes did not respond differently to the defoliation treatment. The effect of defoliation on true stem and leaf sheath DM per shoot was not statistically significant. The fraction of incident

radiation intercepted by defoliated and control shoots was estimated by applying values for the light extinction coefficient ( $K$ ) and green areas (spike, leaf lamina, and leaf sheath) measured at GS61+7d, and assuming the incident radiation above each leaf layer was the same in defoliated and undefoliated shoots. The fractional radiation interception at GS61+10d was reduced from 92% in the control shoots to 40% in the defoliated shoots. We conclude that spike and/or leaf sheath photosynthesis rate was likely enhanced in the defoliated shoots, since there was no evidence for greater utilization of stem soluble CHO (estimated as stem DM loss from GS61+10d to harvest) in the defoliated shoots, compared to the controls. This result suggests there may be scope to reduce leaf-lamina partitioning whilst maintaining grain growth in the high radiation, irrigated conditions of NW Mexico.

Averaged across 2011 and 2012, genetic variation in grains  $m^{-2}$  was mainly explained by FE rather than SPI. For a subset of nine genotypes, representative of the full range of FE in the 30 genotypes, a detailed analysis of spike partitioning at harvest (rachis, glume, palea, lemma, awn) was carried out. Results indicate a positive linear association between each of palea ( $R^2 = 0.84$ ,  $P < 0.001$ ) and lemma ( $R^2 = 0.84$ ,  $P < 0.001$ ) DM partitioning and FE (Fig. 4.). The genotype with the highest FE was the only unawned line in the subset (Rialto/Bacanora DH line). Omitting this line from the analysis, there was still a positive linear association among the awned lines between palea partitioning and FE ( $R^2 = 0.41$ ,  $P = 0.08$ ), although the association between lemma partitioning and FE was not significant ( $R^2 = 0.36$ ,  $P = 0.11$ ). The basis of these relationships is being investigated in ongoing work.

**Table 3. Means, ranges, and P-values of grain weight, grain DM per spike, chaff DM per spike, leaf sheath DM, and true stem DM per shoot in the defoliation treatment and control.**

	Defoliation treatment			Control			P-value		
	Mean	Max	Min	Mean	Max	Min	Genotype	Treat.	GxT
Individual grain (mg)	39.8	49.8	33.4	42.1	57.1	35.2	<0.001	0.019	0.294
GrainDW per spike (g)	2.61	3.09	2.05	2.89	3.73	2.4	<0.001	<0.001	0.294
Chaff per spike (g)	0.918	1.22	0.787	0.898	1.079	0.772	<0.001	0.339	0.474
Sheath per shoot (g)	0.862	1.08	0.606	0.829	0.978	0.548	<0.001	0.325	0.977
True stem per shoot (g)	1.847	2.45	1.437	1.77	2.32	1.42	<0.001	0.309	0.995

There is an interdependence between targets for assimilate partitioning to boost spike fertility and the minimum support DM for lodging resistance. The identification of the traits for increased spike partitioning to boost spike fertility and HI will therefore link closely with the analysis of lodging traits (see Berry et al., these proceedings) and the ideotype design and comparison with CIMCOG (see Sylvester-Bradley et al., these proceedings).

### Next steps

Our third objective is to: *“Estimate the contribution of genes of major effect (Rht, Ppd, Vrn, Lr19, 1B/1R) on SPI, grains m<sup>2</sup>, HI, and related traits, and develop genetic markers for these traits using an association genetics panel and bi-parental populations.”*

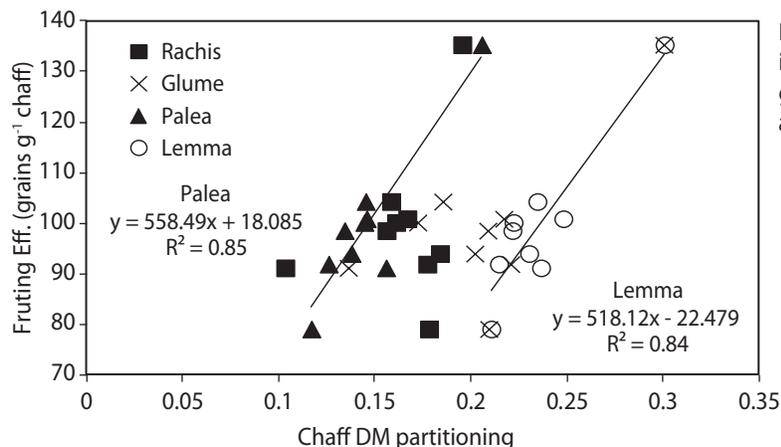
The present CIMCOG data set will be analyzed according to the markers for major genes for which the 30 genotypes have been characterized (see Slafer et al., these proceedings), to estimate the contribution of these genes to partitioning, spike fertility traits, and HI in the high radiation, irrigated environment of Ciudad Obregon. However, further research activities will be required to:

- Identify genetic variation in a wider range of genetic resources, including exotics, for ideotype target traits not found in CIMCOG and for which the 2<sup>nd</sup> CIMCOG is being assembled
- Characterize traits in multi-location field trials (UK, Spain, Mexico, Argentina) to quantify environmental interactions.
- Conduct genetic analyses of traits in association panels and bi-parental populations in multi-location trials to identify loci and alleles

influencing these traits and their interactions with the environment. For this, a number of mapped elite by elite populations already exist, including Seri/Babax and Kauz/Weebil. For association analysis, the Wheat Association Mapping Initiative (WAMI) panel is also available. A considerable international data set of yield and other agronomic traits already exists for these populations (Lopes et al. 2012; Bustos et al. 2013), and some relevant QTL have already been identified (McIntyre et al. 2010).

In 2012-13, a wider set of 240 spring and winter wheat genotypes (including landraces, synthetic derivatives, and elite cultivars) will be phenotyped at Nottingham for partitioning at anthesis (leaf lamina, stem-and-leaf-sheath, and spike), FE and grain yield, HI, and yield components. Additionally, funding will be sought to:

- Phenotype wider germplasm in multi-environments, including advanced lines, related species (AB genome, D genome, triticale), synthetics, and mutants (e.g. multi-ovary). Measurements will include DM partitioning (leaf, structural stem, non-structural stem stem, spike and infertile tillers), with a particular focus on allocation of assimilate during the rapid spike growth phase from booting to anthesis.
- Phenotype appropriate association panels and bi-parental populations, where these are available within the consortium (and new panels/populations developed where suitable panels/populations cannot be identified). In collaboration with Theme 3, genotyping will be implemented through SSR, Dart, and SNP markers to identify new genomic locations for traits underlying optimal partitioning and improved grain number and HI through QTL and association genetics analysis.



**Figure 4. FE vs. chaff partitioning indices (at harvest) for nine CIMCOG genotypes.** Values represent means of 2010-11 and 2011-12 in raised beds.

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# Dynamics of floret development determining differences in spike fertility within the CIMCOG population

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## Summary

We aimed to determine variation in dynamics of floret generation/degeneration responsible for differences in spike fertility among a subset of the CIMCOG panel. We found genotypes differed in spike fertility mainly due to differences in floret survival, which were far more relevant than floret generation in determining the differences in number of fertile florets per spikelet. The genotypic differences in floret survival was related to the ability to maintain development progressing normally in relatively distal florets (3-5, depending on the spikelet position), which seemed related to a longer duration of floret development.

## Introduction and Methodology

Yield in wheat is more related to grain number than to grain weight (Fischer 2011; Sadras and Slafer 2012), and grain number is largely determined during the stem elongation (SE) phase (Slafer and Rawson 1994). Therefore improvements during SE are required to further increase grain number. Beyond increasing crop growth rate and further improving biomass partitioning before anthesis, it may be also relevant to optimise the developmental

attributes to maximize spike fertility. This involves two different aspects of development: i) The pattern of partitioning of time to anthesis into different phases (Slafer 2003; Slafer et al. 2009), as lengthening the duration of the SE phase may increase yield (Slafer 2003; Miralles and Slafer 2007), and ii) The dynamics of floret development (Kirby 1988), as grain number is the consequence of the developmental process of floret generation/degeneration resulting in a number of fertile florets (González et al. 2011).

Within the overall objective of this research (to clarify physiological, and to identify genetic, bases of spike fertility associated with the pattern of development in wheat), a field study was conducted over two consecutive growing seasons<sup>1</sup>, to quantify differences in dynamics of floret development (generation and degeneration of floret primordia) responsible for differences in number of fertile florets (the basis of spike fertility). Due to the difficulties associated with the determination of floret developmental processes, this work was carried out with a subset of 10 genotypes selected from the CIMMYT Mexico Core Germplasm (CIMCOG) Panel.

Entry	Name	Trait
1	BACANORA T88	high grains/m <sup>2</sup>
2	BCN/RIALTO	late development
3	BRBT1*2/KIRITATI	large seed
4	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2	high floret number
5	ATTILA/PASTOR	high floret number with late development
6	PFAU/SERI.1B//AMAD/3/WAXWING	early development
7	SERI M 82	wide adaptation
8	SIETE CERROS T66	benchmark
9	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	wide adaptation
10	WHEAR/SOKOLL	wide adaptation

<sup>1</sup> 2010/11 and 2011/12 in the MEXPLAT at the CENEB (near Cd. Obregon, Mexico), sown on 06 Dec. 2010 and 08 Dec 2011. There are other research works of SP2.2 developed mainly at John Innes Centre (UK), Univ. of Lleida (Spain), Univ. of Nottingham (UK), and CONICET (Argentina), unfunded by the WYC so far (as 'in-kind' contribution by scientists of this SP), studying genetic and physiological determinants of spike fertility with other genetic populations as well as with the CIMCOG panel in other environments. Scientists involved in these works, beyond those authoring this paper, are in the groups led by J. Foulkes (Nottingham, UK), F. González (Pergamino, Argentina) and D. Miralles (B. Aires, Argentina)

## Results and Discussion

Yield and its determinants of the selected genotypes for the floret development analysis did represent well the whole CIMCOG panel (Table 1). Therefore conclusions achieved with the subset might be trustworthily extrapolated to the population.

The genotypes of the subset analysed showed differences in number of grains per spikelet which were reasonably consistent among years, with the unique exception of line 2 (Figure 1).

Within the subset analysed, line 8 was within the lines exhibiting the highest levels of spike fertility in both experiments, and line 9 was within those exhibiting the lowest values (Figure 1).

The number of fertile florets per spikelet was much stronger related to the survival of floret primordia initiated than to the maximum number of floret primordia produced (Fig. 2).

These data are in agreement with those reported by Ferrante et al. (2010) and Gonzalez et al. (2011) when

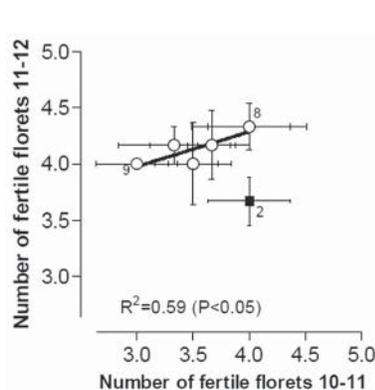
they exposed the wheat plants to different N regimes and different photoperiods, respectively. It seems then that the differences between elite genotypes are also more associated with survival than initiation processes.

To further understand the processes involved in the genotypic differences within the CIMCOG population, we studied the dynamics of generation and survival of floret primordia in apical, central, and basal spikelets. The general dynamics was expectedly similar in all cases (genotypes x spikelet positions). During stem elongation number of floret primordia firstly increased rapidly, reaching a particular peak representing the maximum number of floret primordia and finally decreased also sharply until a certain number of fertile florets is established as the balance of the generation and degeneration process.

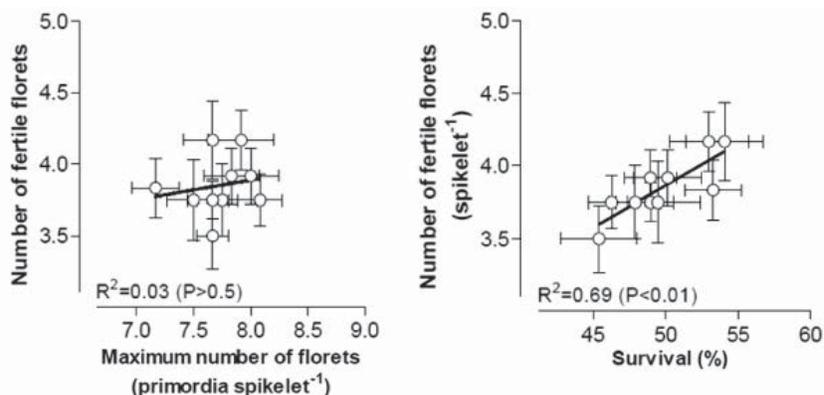
To illustrate the issue we compared here the curves corresponding to the genotypes exhibiting the lowest values of spike fertility (line 9) and one of lines exhibiting the highest values (lines 8) for the apical, central and basal spikelets (Fig. 3).

**Table 1. Comparison of yield and its determinants between the CIMCOG population and the subset.**

Trait	Average		CIMCOG		Subset	
	CIMCOG	Subset	Range	LSD <sub>0.05</sub>	Range	LSD <sub>0.05</sub>
Yield (Mg ha <sup>-1</sup> )	6.11	6.05	4.48 - 7.20	0.68	5.55 - 6.52	0.68
Biomass (Mg ha <sup>-1</sup> )	13.58	13.41	11.05 - 15.60	2.24	12.37 - 15.04	1.57
Harvest index	0.45	0.45	0.40 - 0.51	0.02	0.41 - 0.49	0.03
Number of grains (m <sup>-2</sup> )	14379	15801	11357 - 21387	1622	12853 - 21387	2085
Number of grains (spike <sup>-1</sup> )	47	47	36 - 67	5.50	39 - 57	4.14
Grain weight (mg grain <sup>-1</sup> )	42.9	39	28 - 53	2.08	28 - 45	1.43
Days to anthesis	89	88	79 - 96	2.20	79 - 96	2.00



**Figure 1. Fertile florets per spikelet in both experiments for the subset of CIMCOG panel**



**Figure 2. Number of fertile florets per spikelet related to either the maximum number of floret primordia initiated (left panel) or the percentage of these primordia which survived to produce fertile florets at anthesis. Data are averaged across both experiments.**

The genotypes had a similar maximum number of floret primordia initiated in the apical and central spikelets, whilst in the basal spikelets, genotype 9 (that with lowest spike fertility) had a slightly lower maximum, and in all spikelets the decrease in this number (floret mortality) was more noticeable in genotype 9. Interestingly it seemed that in all spikelets this genotype reached the maximum number of floret primordia closer to anthesis than genotype 8, implying that the time for floret survival at anthesis was shorter (Fig. 3).

When analysing the development of the particular florets that established the difference in number of fertile florets per spikelet between the genotypes and spikelets (florets 3, 4, and 5 [Fig. 4], as florets 1 and 2 were always -in all spikelets and all genotypes- fertile and florets 6 or more distal were never fertile) it becomes clear that:

1. Floret 3 became fertile in the three genotypes and in all the spikelets, though it seemed to have been developed with some delay in genotype 9 compared to genotype 8 (Fig. 4, left panels).
2. Floret 4 was also a) fertile in all genotypes for the central spikelets (but again it seemed that this floret developed with some delay in genotype 9), b) fertile only in some of the replicates in genotypes 8 but never fertile in genotype 9 in apical spikelets, and c) always fertile in basal spikelets of genotype 8 but never in these spikelets of genotype 9 (Fig. 4, middle panels).
3. Floret 5 was only fertile for some replicates of genotype 8 in the central and basal spikelets and in none case in genotype 9 and in none genotype in the apical spikelets (Fig. 4, right panels).

Even in the case of the floret\*spikelet positions in which primordia did not continue developing normally to achieve the stage of fertile florets, there was a clear trend in genotype 8 to be more developed than genotype 9 (Fig. 4). It seems therefore that much of the differences between genotypes of the CIMCOG panel in terms of spike fertility can be traced back to the developmental processes of floret development.

It seemed possible to speculate that advancing development progress of labile florets increase the likelihood of a floret to progress towards becoming a fertile floret and that influences spike fertility, in this case apparently associated with genotypic differences in fruiting efficiency.

Selecting lines exhibiting this property as prospective parents may help in further raising yield potential in wheat, in addition to any other improvements associated with improved partitioning to spike growth, or improved crop growth, during pre-anthesis.

### Next Steps

The work described above is been complemented with the genotyping of the CIMCOG population (at least for Rht-D1, Rht-B1, Rht8, 1BL1RS, Ppd-D1, Ppd-B1, Ppd-A1, Vrn-A1, Vrn-B1, Vrn-D1, Vrn-B3, and for earliness per se and stem extension QTLs identified in Griffiths et al. 2009 and Griffiths et al. 2011) with SNP markers in order to establish levels of kinship among cultivars. A new bi-parental population is been grown at JIC by crosses made with Paragon, a UK variety used to develop ten

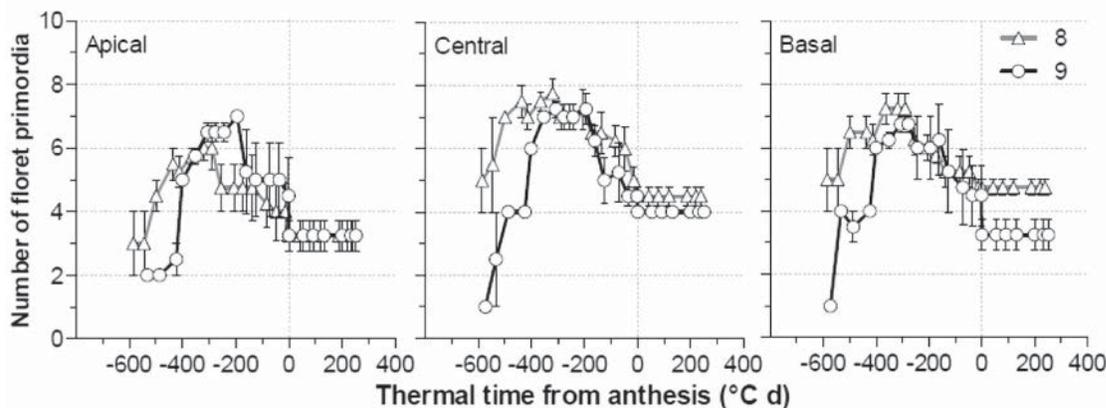


Figure 3. Dynamics of the number of living floret primordia (those developing normally at the time of measurement) from the onset of stem elongation onwards, plotted against thermal time from anthesis in genotypes 8 and 9 of the subset analysed from the CIMCOG population in the apical (left panel), central (middle panel), and basal spikelets (right panel).

single seed descent (SSD) populations, and with Weebil, a CIMMYT standard, in order to constitute a nested association mapping panel.

A complementary study on NIL's from Weebil x Bacanora is being prepared to be sown and analysed in other environmental conditions (a Mediterranean environment in Spain) in addition to the UK. Further funding will allow a deeper comprehensive study by including other germplasm pools and other environmental conditions.

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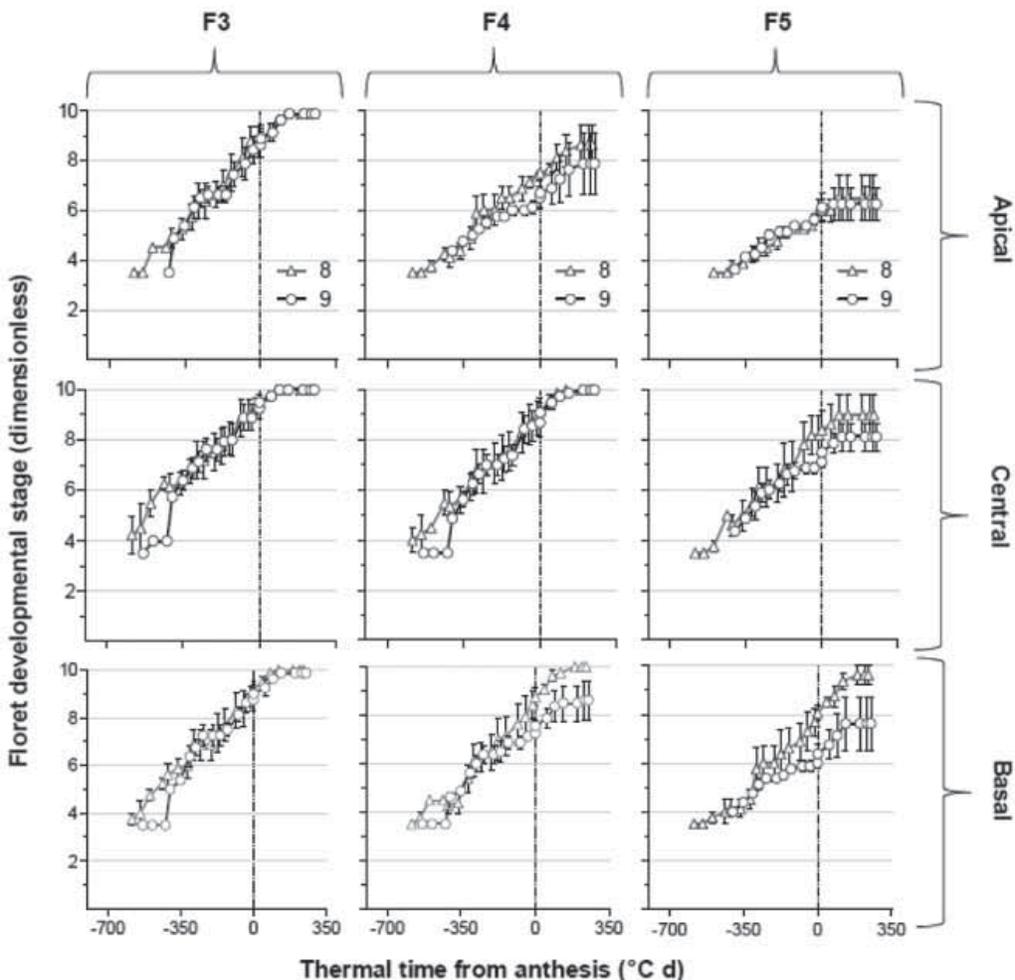


Figure 4. Developmental progress of floret primordia 3, 4, and 5 (from left to right panels) in apical, central and basal spikelets (from top to bottom panels) from the onset of stem elongation onwards, plotted against thermal time from anthesis in genotypes 8 and 9 of the subset analysed from the CIMCOG population. The florets are fertile when achieving the score 10 in the scale developed by Waddington et al. (1983).

# Improving spike fertility by understanding and modifying its sensitivity to environmental cues

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## Summary

Our recent research has shown that several plant drought stress responses can be explained most effectively as functions of the actions of abscisic acid (ABA), a second hormone, ethylene, and interactions between these agents. This information is important for understanding plant functioning under environmental stress, because plants in the field generate a range of environmentally-dependent ABA-ethylene concentration combinations. We argue that this synergy can confer highly plastic plant functioning and development in variable environments, and that it provides a sound basis for crop improvement strategies.

## Results

### Separating the effects of grain water status from the effects of mild soil drying

We achieved this by applying different concentrations of a combination of hormones, hormone precursors, and synthesis and sensitivity inhibitors to both well-watered and droughted plants in the lab.

### Assessing meristem/spikelet hormone concentrations in plants supplied with sufficient water and nutrients, and 'optimal' temperature and radiation

Meristem/spikelet hormone concentrations were measured at key stages of plant development by immunoassay and gas chromatography-mass spectrometry (GC-MS). Experimentation was performed on the Lancaster phenotyping platform (LanPlat) where we can monitor performance of 100 genotypes simultaneously. We are currently extending this platform to process twice this number of plants. Biologically significant variations in hormone balances and ratios were detected between plant parts and between genotypes.

### Quantifying hormonal signals in plants exposed to environmental challenges

To characterize hydraulic signalling, midday leaf and grain water potential (and osmotic potential to calculate leaf turgor) were measured using thermocouple psychrometers. These manipulations

and measurements will determine the relative importance of hydraulic, carbohydrate, and hormone signalling. Significant genetic variation in hormone signalling has been detected in response to both drought and heat

### Assessing signalling and crop yield in field grown crops

To complement the mechanistic studies in the laboratory (described above), a wheat crop was grown at Lancaster's field site (Myerscough College), either exposed to UK weather or undercover. Using the cover, water availability and temperature were manipulated. Plants were either well watered throughout development (with dielectric sensors allowing feedback control of soil water status), or the soil was allowed to dry from the beginning of stem elongation. Samples were taken at regular intervals from developing meristem and leaves to assess spike hormone and water status. Spikelet fertility index (assessed as number of florets at anthesis) and grain size (at harvest) will be related to hormone balance. These experiments are currently being repeated in the field in Mexico and on the LanPlat..

## Discussion

We have proposed (Wilkinson and Davies 2010; Wilkinson et al. 2012) that stomatal response patterns can be interpreted to be a function of the combined influence of both ABA and ethylene, which is crucial given that these hormones often exhibit different patterns of accumulation in response to environmental perturbation (Sharp and LeNoble 2002; Sobeih et al. 2004). Stomatal behaviour is selected here as a model response for a range of physiological/developmental perturbations that are sensitive to these hormones and will impact yield. We argue that a given plant has the capability both to reduce gas exchange in response to ABA or ethylene accumulation, and to exhibit ABA-ethylene antagonism that results in higher rates of gas exchange, dependent on prevailing conditions. This pattern of response can be of adaptive significance and our hypothesis is that peak functioning at

intermediate ratios, accompanied by sensitive responses to both increased and decreased ratios, is an effective means of achieving high water productivity. Here we perceive opportunities for gene discovery.

To test this hypothesis, we have generated a range of ABA-ethylene concentration combinations relevant to field-grown plants, by exposing wheat plants to a variable rooting environment and/or to combinations of externally applied hormones and hormone precursors. Effects on stomatal conductance ( $g_s$ ) were tested in intact plants (where higher rates of  $g_s$  equate to more open stomata).

We selected ACC (1-aminocyclopropane-1-carboxylic acid) treatment concentrations that gave rise to ethylene concentrations within the range occurring in leaves of plants a) experiencing soil water deficit, b) recovering from soil water deficit, or c) growing in compacted soil (e.g. Gomez-Cadenas et al. 1996; Yang et al. 2006). When ACC/ethylene was applied/generated in this manner, it induced a reduction in  $g_s$  that was concentration-dependent. This is consistent with data of Desikan et al. (2006), derived from experiments in isolated epidermal tissue of *Arabidopsis*. The reductions in  $g_s$  were weaker, or absent, when plants were pre-treated with the ethylene perception inhibitor 1-MCP (1-methylcyclopropane), demonstrating that endogenously produced ethylene, rather than externally applied ACC, triggered the associated stomatal closure.

In identical sets of plants, or in some cases within the same experiments that tested effects of ACC/ethylene on  $g_s$ , we applied solutions containing a range of ABA concentrations to foliage. In intact plants, these leaf ABA concentrations would indicate soil moisture deficit; in our wheat plants they reduced  $g_s$ . Endogenous ABA accumulation generated by imposing a soil drying (drought) treatment was also associated with reduced  $g_s$ . Such effects of drought and ABA on stomata have frequently been documented (e.g. Hussain et al. 2009; Wilkinson and Davies 2010). However, when ACC was co-applied in the foliar spray solution,  $g_s$  was not reduced to the extent routinely observed in the presence of either applied ABA, or the soil-drying treatment; ACC antagonised the stomatal closing effect of ABA/drought. This is consistent with the stomatal responses described by Tanaka et al. (2005) in *Arabidopsis* epidermal tissue and intact plants exposed to ethylene gas. We also observed

a corresponding effect whereby applied ABA and/or early-stage soil drying antagonised ACC-induced reductions in  $g_s$ . Thus, in contrast to the traditionally accepted view that ABA accumulation in plants in drying soil induces stomatal closure, we have shown that an increase in ABA concentration and/or a reduction in soil moisture can actually be associated with more open stomata, in plants accumulating ethylene.

Within the same, or identical, set(s) of plants, we have identified four stomatal 'response classes' to ABA and ethylene, which can occur independently of species, tissue type or, in some cases, experimental environment: a) reduced stomatal aperture via ABA, b) reduced aperture via ethylene, c) antagonism, by ethylene, of the closing effect of ABA effect, or d) antagonism, by ABA, of the ethylene 'closing' effect. Crucially, by demonstrating that ethylene accumulation can both reduce stomatal aperture and antagonise ABA- and drought-induced stomatal 'closure', and that neither effect precludes the other, we have established that there is a broad-ranging role for ethylene in mediating stomatal responses (and other physiological and developmental changes impacting yield). This may be one explanation for the apparent lack of responsiveness of stomata to ABA often observed in the field (Auge et al 2000; Tardieu and Davies 2003).

Differing accumulation responses to the fluctuating soil moisture availability, between ABA and ethylene, resulted in dynamic, wide-ranging changes in the ABA-ethylene concentration ratio over the course of the experiments. Critically, the data clearly show that  $g_s$  (the model response selected for this work) can be explained more effectively by the hormone interactions than by the action of one or other hormone in isolation, over a broad range of hormone combinations and soil moisture conditions. We see a dramatic increase in the responsiveness of  $g_s$  to changes in ABA concentration when the shoots could no longer perceive the ethylene that they produced; that is, in the presence of 1-MCP. This is because ethylene curtailed the magnitude of changes in  $g_s$  in response to ABA, depicted as an ethylene-induced "smoothing" of the curve describing the relationship between ABA and  $g_s$ , in the absence of 1-MCP. Again this is relevant to the reduced effectiveness of ABA in controlling gas exchange in the field, where ethylene concentrations are known to fluctuate with many aspects of the environment. In stressed

plants producing ethylene, restoration of stomatal sensitivity to the environment by 1-MCP has also been demonstrated under ozone pollution, increased planting density, and drought. Our data show that ethylene effects may, in fact, impact many stomatal responses to the changing external environment, such that its involvement in the regulation of plant gas exchange is likely to be much more important, and prevalent, than has previously been suspected.

We have defined a relationship between an ABA-ethylene concentration 'ratio' and  $g_s$  in a changeable environment, which is described by a parabolic curve. Peak  $g_s$  (required for 'maximum' carbon gain) occurred at low to intermediate ABA-ethylene concentration combinations, however  $g_s$  could be reduced from this point either when the relative concentration of ABA decreased (a left shift to a lower ratio) or when the relative concentration of ABA increased (a right shift to a higher ratio). There are thus several means of "escape", in terms of reduced transpiration (with a C penalty) under a harsh environment, for genotypes that are able to respond by altering either ABA accumulation/sensitivity, ethylene accumulation/sensitivity, or both. Crucially, in variable environments, positive selection for stomatal sensitivity and plasticity has been demonstrated (Dunn et al. 2007; Nicotra and Davidson 2010; Piovani et al. 2011).

Our data elucidate a mechanistic basis for the plasticity often observed in this major plant response to an environment where climatic extremes are becoming more common. Plant environmental responses are more accurately explained by corresponding changes in an ABA-ethylene concentration 'ratio' than in the concentration of each hormone taken individually. Such integrated multi-hormone signalling systems seem generally to provide greater flexibility in plant responses (Sankar et al. 2011). Significant genetic variability exists in ethylene and/or ABA accumulation in response to stress and in the sensitivity of plant responses to ABA; and developmental stage and past environment also alter production of, and/or sensitivity to these hormones. Our data thus allow us to propose that ABA-ethylene synergy in the control of plant gas exchange can dynamically integrate broadly different combinations of genetic (G), developmental stage (D), and environmental (E) complexity (GxDxE). We suggest that ABA-ethylene concentration ratios can represent novel targets for plant selection and breeding programs for stress avoidance or for increased yielding.

Interactions between ABA and ethylene may also effectively regulate grain filling rate (Yang et al. 2006), root and shoot extension growth (Sharp and LeNoble 2002; Pierik et al. 2007), and seed germination (Gasseman et al. 2000). We need to determine whether relationships between ABA-ethylene synergism and several physiological responses are conserved, such that systemic changes in hormones induce multiple effects.

### Next Steps

- Test the hypothesis that ABA-ethylene concentration ratios can represent novel targets for plant selection and breeding programs for stress avoidance or for increased yielding.
- Define impact of variation in ABA-ethylene concentration ratio on grain filling and root growth
- Investigate the hypothesis that the parabolic relationship between growth and functioning and hormone ratio is of adaptive significance and that 'peak function' at an intermediate ratio is an appropriate strategy for a productive but stress resilient genotype.
- Investigate the effect of late season heat on hormone ratios and spikelet sterility

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# Improving grain filling and potential grain size

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## Summary

Our experiments show a curvilinear relationship between grain yield and grain number, highlighting the need to increase grain weight to counteract the trade-off between the two main yield components. The positive association between grain weight and grain length reinforces previous findings and suggests that grain length is a key driver of grain weight determination. The evaluation of 92 doubled haploid lines gave promising indications for developing molecular markets of both grain weight and length. Finally, the association between grain weight and crop growth rate per grain supports the period bracketing anthesis as a key phase for both grain number and weight determination.

## Results

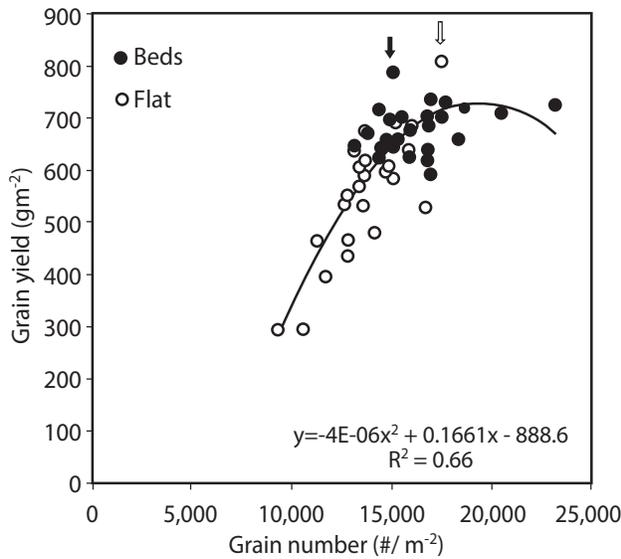
It is widely accepted that wheat breeding has improved grain yield potential by increasing grain number (GN) per unit area, while thousand grain weight (TGW) was negatively or little affected (Austin et al. 1980; Slafer et al. 1994; Calderini et al. 1999; Shearman et al. 2005). However, recent studies evaluating CIMCOG genotypes (Calderini et al. 2012) or assessing doubled haploid lines (Bustos et al. 2013; García et al. Submitted) have shown curvilinear associations between grain yield and GN. As a consequence, the efficiency of breeding programs and the improvement of grain yield will be limited in the near future if increases in GN are not complemented with improvements in TGW. Thus, in order to increase wheat yield potential, or improve grain weight per se, whilst avoiding a trade-off between TGW and GN, it has become necessary to study the trade-offs between the two main yield components, the physiological bases, and the mechanisms accounting for both potential and actual grain weight. This is supported by the fact that wheat is scarcely limited by the source of assimilates after anthesis in favorable wheat growing regions (e.g. Slafer and Savin 1994; Borrás et al. 2004), and even under Mediterranean conditions (Cartelle et al. 2006).

The data reported here aim to: i) identify the physiological and genetic determinants of potential grain weight and size and ii) quantify trade-offs between GN and grain weight. We recorded these results in Ciudad Obregón during the 2011-12 growing season, when 30 CIMCOG genotypes grown in beds and flat plot systems were evaluated. This report also includes information from an experiment sown in Valdivia (Chile) on September 10 2012. In this experiment, nine selected genotypes (from the 30 CIMCOG genotypes) were assessed (the same nine as chosen for detailed measurements in C. Obregón). Additionally, the analysis of 92 doubled haploid lines, derived from the cross between Bacanora x Weebil, is included. These lines were phenotyped during two seasons in Valdivia under different source-sink and plant rate treatments. Two hundred and fifty-two genetic markers (SSR and DArT) were used to create the population linkage map of the 92 Bacanora x Weebil double haploid lines (John Innes Centre, UK). QTL analysis of phenotypic traits was performed using the R/QTL module (Broman et al. 2003), version 1.18-7.

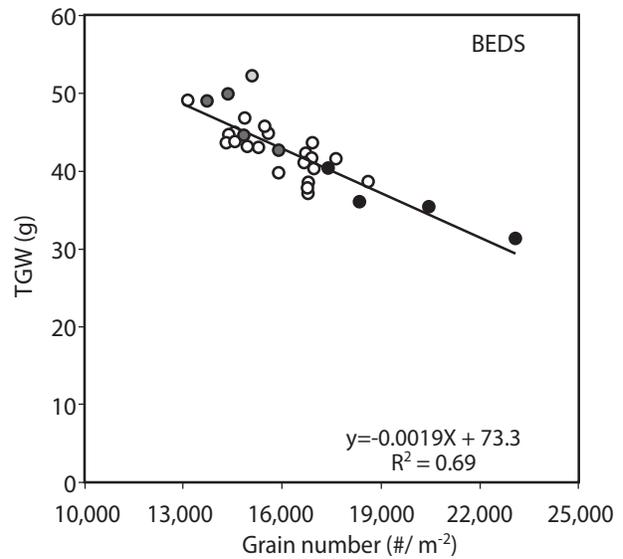
Trade-off between grain weight and grain number and its consequences on grain yield  
Wide variation in yield was recorded among the 30 CIMCOG genotypes in both bed (590-787 g m<sup>-2</sup>) and flat (295-809 g m<sup>-2</sup>) plots in Ciudad Obregón. Similarly, yield components also showed differences among genotypes. GN in beds ranged between 13,100 and 23,000 m<sup>-2</sup>, while in flat plots these values were lower (between 9,300 and 17,500 m<sup>-2</sup>). TGW showed similar ranges between the two cropping systems (31-52 and 28-50 g in beds and flats, respectively). Remarkably, a curvilinear association was found between grain yield and GN (Fig. 1), where the highest yield in both sowing systems was reached by the durum wheat cultivar Cirno 2008 (Fig. 1). Taking into account that almost half of the genotypes had unexpected low grain yields and GN in flat plots, the analysis of results will focus on data collected in bed plots.

When the trade-off between TGW and GN was assessed in beds, a clear negative relationship was found between these yield components (Fig. 2). Compensation between TGW and GN was almost complete as no relationship ( $r=0.33$ ,  $p>0.05$ ) was found between grain yield and GN in beds (Fig. 1). The negative relationship shown in Fig. 2 cannot be

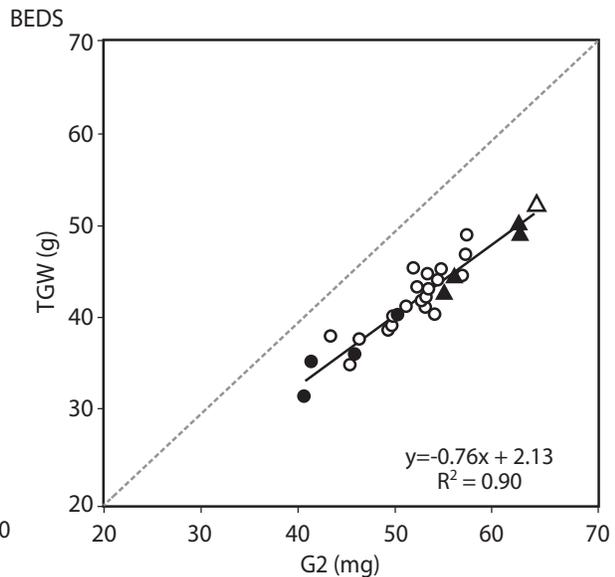
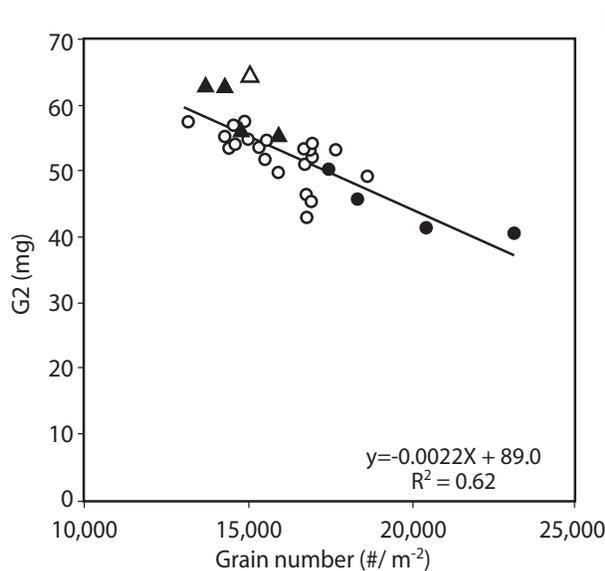
ascribed to the setting of more, and smaller, grains in distal positions of the spikes, as there were both: i) a negative relationship between individual grain weight at specific grain positions (e.g. G2) and GN and ii) a positive association between TGW and G2 (Fig. 3). In addition, phenology has little, if any, effect on grain yield (Fig. 4).



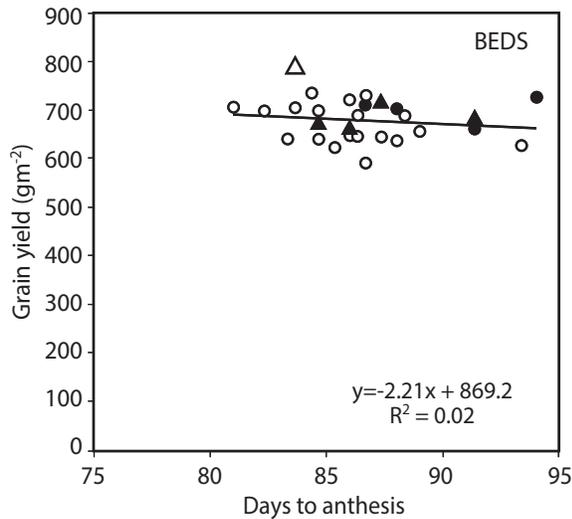
**Figure 1. Relationship between grain yield and grain number in bed and flat plots of 30 CIMCOG wheat genotypes grown in Ciudad Obregon during the 2011-12 season. Arrows show data for the durum wheat cultivar Cirno 2008.**



**Figure 2. Relationship between thousand grain weight and grain number in bed plots. Nine genotypes, with contrasting TGW and selected in the previous experiment in C. Obregon (2010-11), are tagged: durum wheat Cirno 2008 (green circle) and four high (blue circles) and low (red circles) TGW from the CIMCOG genotypes**



**Figure 3. Relationship between the individual grain weight of a proximal grain (G2) and grain number (left panel), and between (TGW) and individual grain weight of G2 (right panel) in bed plots. Durum wheat Cirno 2008 (green circle) and four high (blue circles) and low (red circles) TGW from the CIMCOG genotypes are shown.**

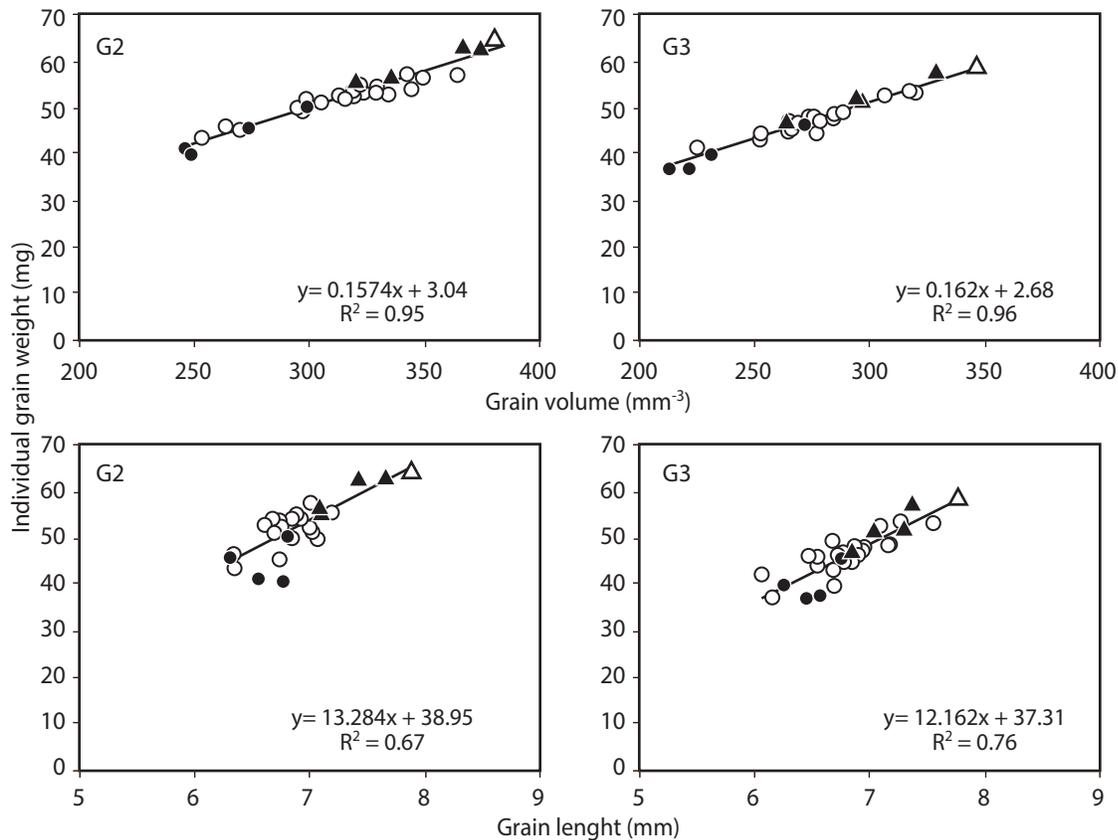


**Figure 4. Relationship between grain yield and days to anthesis in bed plots.** Durum wheat cultivar Cirno 2008 ( $\Delta$ ) and four high ( $\blacktriangle$ ) and low ( $\bullet$ ) TGW from the 30 CIMCOG genotypes are shown.

### Grain weight, associated traits, and QTLs

Individual grain weights at specific positions within the spike were closely associated with grain volume at harvest (Fig. 5). Grain dimension (length, width, and height) was also assessed in this experiment. Length reached its final value before the other grain dimensions and early in the grain filling period. Positive associations were also found between individual grain weight and grains length (Fig. 5). The time course of grain enlargement was followed in Valdivia by evaluating the same tagged genotypes as in C. Obregón (i.e. Cirno 2008 and the high and low TGW genotypes). The high TGW genotypes and Cirno 2008 had greater grain length than the low TGW group. Most of these genotypes reached the stabilized grain length by 20 days after anthesis (Fig. 6). The analysis of expansins expression in the experiment carried out in Valdivia is beginning.

In another experiment carried out in Valdivia, a wide range of grain weights was recorded when 92 lines corresponding to the Bacanora x Weebil



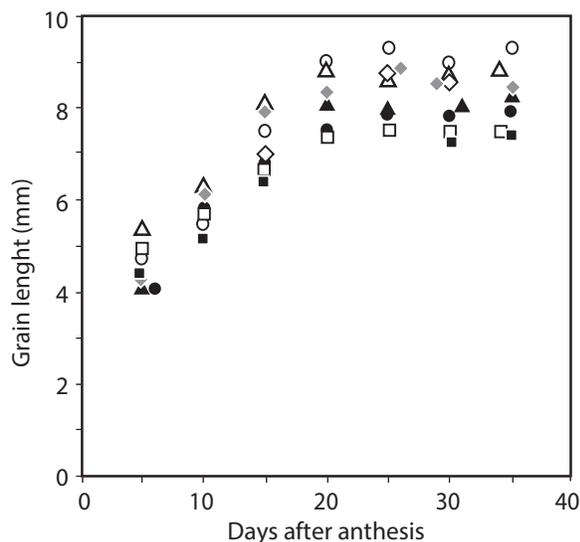
**Figure 5. Relationship between individual grain weight and grain volume (upper panels) and grain length (lower panels) in grain positions 2 (G2) and 3 (G3) in bed plots.** Durum wheat cultivar Cirno 2008 ( $\Delta$ ) and four high ( $\blacktriangle$ ) and low ( $\bullet$ ) TGW from the 30 CIMCOG genotypes are shown.

doubled haploid population were evaluated across eight growing conditions (source-sink and seed rate treatments in two growing seasons). For G2grains, individual grain weight varied between 43- 74 mg. QTL analysis for traits associated with grain weight was assessed in this experiment using 252 genetic markers (SSR and DArT). Interestingly, several markers for both individual grain weight and grain length were found on chromosome 6A and more so on 7Bb (Table 1), therefore supporting the hypothesis that grain length is a key trait for grain weight.

Finally, the relationship between TGW and the crop growth rate per grain was evaluated in C. Obregón, taking into account previous evidence showing that growing conditions bracketing anthesis affect both grain number and grain weight potential. A positive association ( $p < 0.001$ ) was found (Fig. 7), therefore confirming that grain weight depends on the availability of resources to the growing reproductive organs.

### Discussion and Next Steps

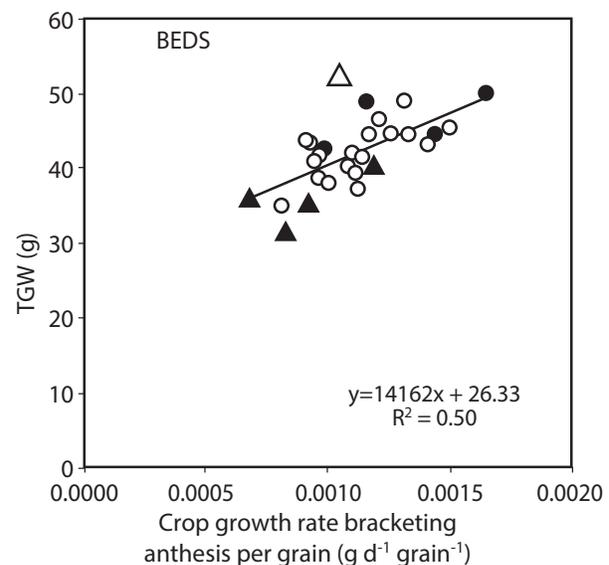
Understanding the determinants of grain weight potential and the mechanisms accounting for the trade-off between grain weight and number remain elusive in wheat and other grain crops. Previous studies (Wardlaw 1994; Calderini et al. 1999; Calderini and Reynolds 2000) have shown that the period preceding anthesis is important for grain weight. The phase immediately before anthesis,



**Figure 6. Time course of grain length of Cirno 2008 ( $\Delta$ ), high ( $\blacktriangle$ ), and low ( $\circ$ ) TGW from the 30 CIMCOG genotypes measured in Valdivia.**

**Table 1. Identified QTL for grain weight and grain length in 92 doubled haploid lines (Bacanora x Weebil) evaluated under eight different environmental conditions (years, source-sink treatments and sowing rates) in Valdivia.**

Trait	Chr.	Grain growing condition	LOD	Var (%)	Additive effect
Grain weight	6A	Pr09	2.8	9.9	-1.686
	7Ab	PD08	3.2	10.2	1.507
	7Bb	PD08	2.9	9.3	-1.727
	7Bb	PD09	3.1	11.8	-2.164
	7Bb	Po08	2.6	10.1	-1.670
	7Bb	Po09	2.6	9.0	-1.671
	7Bb	Pr08	3.0	11.2	-2.024
	7Bb	Pr09	3.7	13.3	-1.884
	7Bb	Co09	3.2	11.6	-1.844
Grain length	1B	Po08	3.9	10.4	0.110
	1B	PD08	3.2	9.4	0.136
	1B	Co08	3.2	8.5	0.104
	2Ba	Po09	3.8	9.6	-0.095
	2Bb	Co08	3.0	8.0	-0.097
	2Bb	PD08	3.2	7.9	-0.116
	6A	Pr09	3.9	12.6	-0.126
	6A	Po09	5.4	14.1	-0.136
	7Bb	Po09	4.2	10.5	-0.096
	7Bb	Pr08	2.8	8.9	-0.120
	7Bb	Co08	3.5	9.4	-0.094
	7Bb	PD09	5.3	16.6	-0.137



**Figure 7. Relationship between TGW and crop growth rate around anthesis, per grain of CIMCOG genotypes.** Durum wheat cultivar Cirno 2008 was not considered in the regression analysis. Colors of circles as in previous figures 2-6.

i.e. when the floret carpels grow (eventually becoming the external structures of the grain), has been highlighted as a key window for grain weight potential (Calderini et al. 1999; Ugarte et al. 2007; Hasan et al. 2011). Therefore, studying the trade-off between GN and TGW could be facilitated by considering the overlap between the determinations of both main yield components, which has scarcely been attempted in the past.

Uncovering the importance of particular genes in the determination of complex traits as grain weight and interactions between grain weight and number represents a key challenge for multidisciplinary efforts to improve the efficiency of wheat breeding. Previous studies showing a close relationship between grain growth dynamic and the expression of five putative expansins genes (Lizana et al. 2010) emphasize the possible role of these proteins as drivers of the grain growth, particularly considering that grain length, which is set early in the grain filling period, is closely related to carpel weight at pollination and to the final grain weight (Hasan et al. 2011). Recent studies reporting the interaction between GhRDL1 and expansins genes in *Arabidopsis thaliana* (Xu et al. 2012) and TaGW2 encoding an E3 ligase that positively regulates grain size in wheat (Bednarek et al. 2012) encourage the study of grain weight in light of an integrated view of traits developed at both the pre- and post-anthesis periods, a subject that few works have addressed thus far.

This study confirms previous evaluations reporting curvilinear relationships between grain yield and GN (Calderini et al. 2012; Bustos et al. 2013; García et al. Submitted). Moreover, results from this study reinforce previous findings highlighting the need to improve grain weight in wheat breeding programs, to counteract the trade-off between both main yield components. The requirement of grain weight increase is clearly shown by the trade-off between TGW and GN recorded in beds, which hampered the improvement of yield of high grain number genotypes (Fig. 1).

Whilst the setting of small grains in distal positions of the spikes has been proved the leading explanation for the trade-off between TGW and GN (Miralles and Slafer 1995; Acreche and Slafer 2006), this is not be the cause of the negative relationship between the main yield components found in CIMCOG genotypes. Similar to previous

experiments carried out in C. Obregón, the trade-off between weight and number of grains was verified in both TGW and individual grain weight at specific positions within the spike (Calderini et al. 2012). Interestingly, the relationship between TGW and G2 found in our experiment (Fig. 3) confirms G2 as an accurate descriptor of TGW, guaranteeing the use of G2 as a surrogate to TGW, as proposed in the previous experiment (Calderini et al. 2012). This supports, in addition, that plant and environmental factors affecting the weight of G2 could be assumed as the main drivers of CIMCOG's TGW.

Grain volume is closely associated with individual grain weight across different positions within the spike. As volume, grain length, which is the earliest dimension of the grain to be stabilized, also showed positive association with individual grain weight. This trait could therefore be considered key for grain weight determination, as previously proposed (Tashiro and Wardlaw 1990; Lizana et al. 2010; Hasan et al. 2011). The study of expansins associated with grain enlargement is a central objective and next step of this project. The discovery of QTLs for both grain weight and length on both chromosomes 6A (also the location of TaGW2; Yang et al. 2012) and 7Bb, not only supports the importance of grain length, but is also promising for facilitating wheat breeding aimed at increasing both grain weight and GN.

The relationship between TGW and crop growth rate per grain around anthesis encourages the study of grain weight determination and the trade-off between the two main yield components by an integrated view of the pre- and post-anthesis periods. In light of this result, resources allocated to the reproductive organs when the florets are growing fast affects both GN and grain weight, though GN is more sensitive (Sadras and Slafer 2012). The yield stagnation found in this experiment could be solved by improving the understanding of mechanisms regulating the allocation of resources to reproductive organs. We hope to conduct research to further understand these mechanisms.

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# Identifying traits and developing genetic sources for lodging resistance

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## Summary

Wide genetic diversity has been identified in elite germplasm for stem strength and anchorage strength traits. A preliminary investigation has indicated that these lodging traits are under complex genetic control. To achieve lodging-proofness, new germplasm must be identified with the requisite traits, especially root plate spread. Ongoing work is developing rapid phenotyping methods.

## Results and Discussion

Objective 1) Identify sets of traits for different wheat growing environments to maximize lodging resistance with the least investment in structural dry matter.

and

Objective 4) Assess whether the target traits exist within breeders' germplasm and if necessary identify novel germplasm with the target traits that could be used in wide crosses.

## Trait sets

The mechanistic model of lodging, developed and tested by Berry et al. (2003), has been used to calculate the minimum stem strength required for the bottom internode and the root plate spread to avoid stem and root lodging, for crops grown in different wind environments and with yield potentials of 5 t/ha or 10 t/ha (Table 1). Low- and high-wind speed environments of 15 m/s and 20 m/s gust speeds have been assumed. For reference, the one in 25-year wind gust for the UK has been calculated at 18 m/s (Berry et al. 2003).

The minimum stem strength required was calculated as ranging from 115 Newton millimeters (Nmm) for the low yielding crop grown in the low wind environment to 373 Nmm for the high yielding crop in the high wind environment (Table 1). Analysis of 60 (2010/11) and 30 (2011/12) spring wheat varieties (CIMCOG panel) grown in Ciudad Obregon, Mexico, indicated that some existing germplasm could achieve a stem strength of up to 290 Nmm, and therefore the minimum stem strength required for the low wind environment could be achieved. However, a wider range of germplasm needs to be evaluated to find the stem strength required to support high yielding crops grown in a high wind environment. A root plate spread of 48 mm was required to provide sufficient anchorage to prevent root lodging for a low yielding crop in a low wind environment. However, this was not found within the CIMCOG germplasm.

Analyses of the CIMCOG panel for 2010/11 and 2011/12 indicated statistically significant genetic variation for all lodging-associated traits. There were also significant year x variety interactions for the majority of the lodging traits. Berry et al. (2007) reported reasonably high heritability values for many of the lodging traits in winter wheat. Nevertheless, the observed GxE interactions demonstrate the importance of multiple environment selection to improve lodging resistance.

If the lodging traits have a large requirement for biomass, and this competes with yield formation (see next section), then it is possible that a greater risk of lodging must be accepted in order to

**Table 1. Calculated stem strength and root plate spread required to avoid lodging for crops grown in different wind environments and with different grain yields.**

Maximum wind gust speed	15 m/s		20 m/s		CIMCOG variety range	
	5 t/ha	10 t/ha	5 t/ha	10 t/ha	2010/11	2011/12
Grain yield						
Stem strength (Nmm)	115	210	204	373	98 – 189	149 – 290
Root plate spread (mm)	48	59	58	71	28 – 37	28 – 47

Calculations assume a crop height of 0.7m, 200 plants/m<sup>2</sup>, 2.5 shoots per plant, and sufficient rain or irrigation to bring the top 10cm of soil to field capacity for a period of at least one day between flowering and harvest.

maximize yield. In order to quantify the effect of lodging on yield, multiple lodging datasets have been analyzed to develop the equation for lodging-induced yield losses ( $Y_{LOSS}$ , below), which can be used to estimate the trade-offs between lodging resistance and yield.

$$Y_{LOSS} = \frac{\sum_i^f (L_{90} \times 0.7 + L_{65} \times 0.3 + L_{25} \times 0.1)}{n}$$

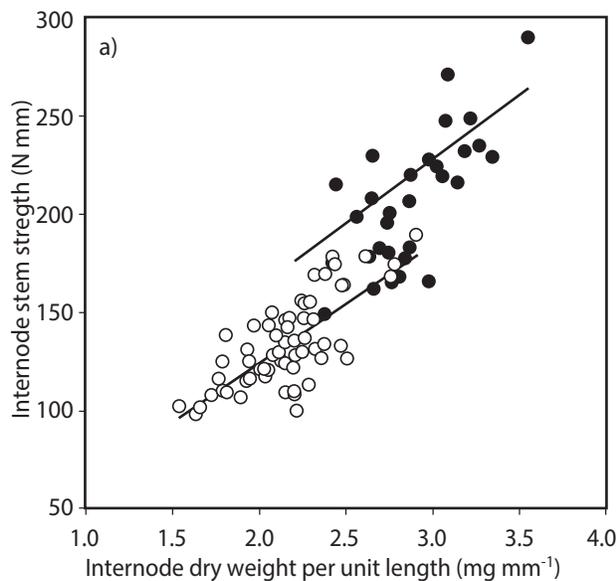
In this equation,  $i$  and  $f$  are the 1st and last days of grain filling,  $L_{90}$  is the proportion of crop area lodged at 85-90° from the vertical,  $L_{65}$  is the proportion of crop area lodged between 46° and 84°,  $L_{25}$  is the proportion of crop area lodged between 5° and 45°, and  $n$  is the number of days of grain filling. This equation was published in Berry and Spink (2012).

#### Dry matter cost of increasing lodging resistance

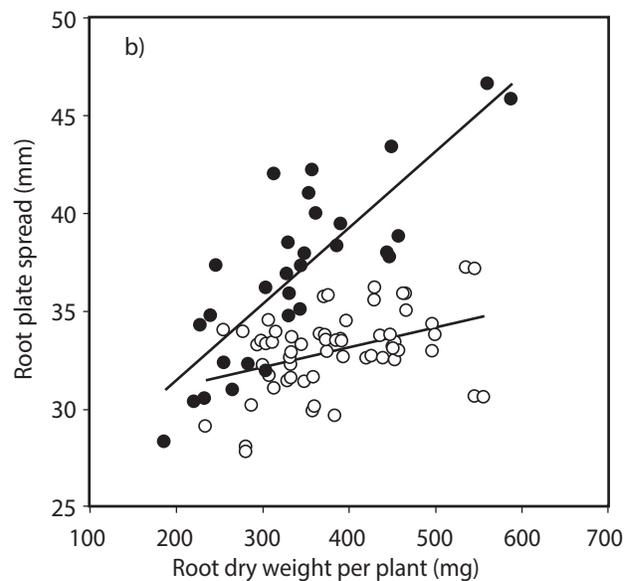
Across varieties there was a significant and positive relationship between stem strength and dry weight per unit of stem length (Figure 1). There was a weaker positive relationship between root plate spread and dry matter of the surface roots. These analyses show that breeding for stronger stems and a wider root plate is likely to incur a biomass cost.

Whether or not the additional biomass required for stronger stems and anchorage will compete with yield formation will depend on during which phase of growth the stem strength and root plate develop. This has been investigated within experiments conducted in the UK, which showed that winter wheat stems accumulate a significant amount of structural carbohydrate during the 20-day period prior to anthesis, when grain number is being determined (Figure 2). Development of structural roots is likely to offer lower levels of competition with grain yield (Figure 2).

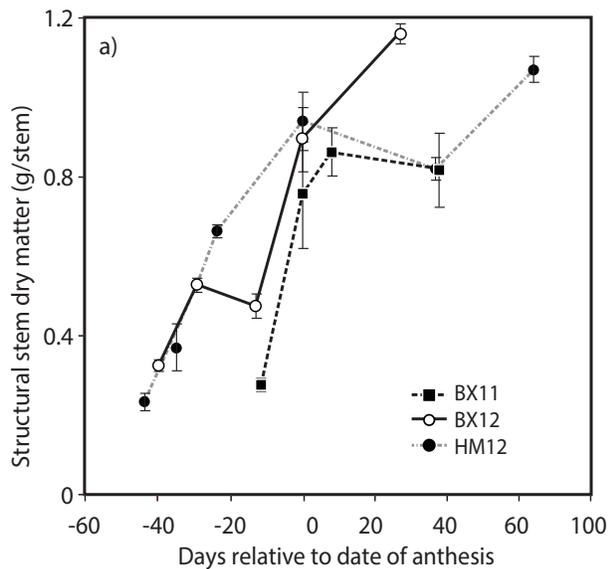
The work described above demonstrates the advantage of minimizing the amount of biomass invested in stems and roots to achieve target strength, in order to minimize competition against the developing ear. For the bottom internode 1, significant varietal differences within the CIMCOG panel were found for stem dry weight per unit length expressed per unit of stem strength ( $P < 0.05$ ). A stem strength of 100 Nmm could be achieved with a stem dry weight per unit length of 1.5-2.1 mg/mm. These results indicate that, despite the generally positive relationship between stem strength and stem dry weight per unit length, it should be possible to find varieties with strong and relatively light stems. Geometric principles



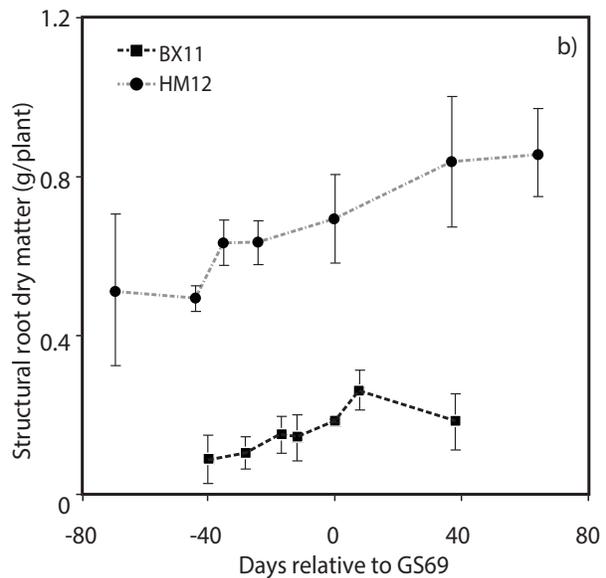
**Figure 1a. Relationship between stem strength and dry weight per unit length for internode one during (○) 2010-11 with 60 CIMCOG varieties ( $y = 68.5x - 14.2$ ); and (●) 2011-12 with 30 CIMCOG varieties ( $y = 68.5x - 5.00$ );  $R^2 = 0.82$  ( $P < 0.001$ ).**



**Figure 1b. Relationship between root plate spread and dry weight of roots during (○) 2010-11 with 60 CIMCOG varieties ( $y = 0.0098x + 29.3$ ;  $R^2 = 0.15$ ;  $P < 0.001$ ); and (●) 2011-12 with 30 CIMCOG varieties ( $y = 0.038x + 24.7$ ;  $R^2 = 0.67$ ;  $P < 0.001$ ).**



**Figure 2a.** Development of structural stem dry matter, after subtracting soluble stem carbohydrate, for three UK experiments. Error bars +/- sed.



**Figure 2b.** Development of structural root dry matter in top 10cm of soil for two UK experiments. Error bars +/- sed.

postulate that the width of the stem wall should be minimized and the stem diameter and material strength of the stem wall maximized, in order to minimize the dry matter required to achieve a particular strength (assuming stem wall density remains the same). There were several genetic inter-relationships between stem strength traits, including: a negative relationship between stem diameter and material strength, a positive relationship between material strength and stem wall density, and negative relationships between stem diameter or wall width with stem wall density. Some of these correlations are antagonistic for achieving ideal stem dimensions for light weight stem strength. However, these correlations generally had  $R^2$  values of less than 0.5, and should therefore not prevent the ideal stem from being bred, although they may act to slow progress.

Objective 2) Test the effects of the optimum trait sets on lodging risk and structural dry matter within different wheat growing environments.

The work plan of the first two years does not include this objective.

Objective 3) Understand the genetic basis of the key lodging traits and develop genetic markers and phenotypic screens that will enable breeders to rapidly select these traits.

#### Genetic basis

A separate project, led by ADAS with Limagrain UK Ltd, has conducted a preliminary investigation of the genetic basis of traits associated with lodging resistance using two winter wheat doubled haploid mapping populations, each grown for three seasons in the UK. It showed that breeders germplasm contains a very wide range of each lodging trait, including those associated with stem strength and anchorage strength. All of the lodging associated traits were shown to be under complex genetic control. Individual quantitative trait loci (QTL) with the largest estimated effects on lodging resistance were for height, stem diameter, stem material strength, stem strength, root plate spread, and root plate depth. A paper describing this project is being submitted to a peer reviewed journal.

### Rapid trait assessment

Lodging traits for stem strength have traditionally been measured for the bottom two internodes of each plant, as these are the most likely to fail. A linear regression for the biophysical properties related to lodging between these two internodes gave correlation coefficients ranging from 0.69-0.97 ( $P < 0.01$ ), indicating enough similarity to measure only one of the internodes. Furthermore, height at centre of gravity was strongly correlated with height to the ear tip and could be omitted. The effect of reducing the number of plants measured per plot on the statistical analysis has been tested using the CIMCOG panel datasets. The similarities of the coefficients of variation and P values using five, ten, and fifteen plants, and the high correlation coefficients ( $r = 0.82-0.99$ ,  $P < 0.001$ ) between the variety means calculated with these sub-sample sizes indicate that lodging traits could be measured on less than 10 plants per plot. Overall, it was estimated that the rate of measuring lodging associated traits could be increased from two genotypes per person per day to between four and five genotypes per person per day. However, it is recognized that significantly more improvement will be required before breeders can regularly screen their germplasm for lodging traits.

### Next steps

- Investigating how genetic variation in stem strength and stem material strength affects the chemical composition of stems including lignin, cellulose, and hemicellulose.
- Analyze the inter-relationships between stem strength and its components with stem wall density and stem dry weight per unit length, to identify the optimum combination of stem diameter, wall width, and material strength for maximizing stem strength with minimum investment in biomass.
- Specify the minimum plant sample size for measuring lodging traits.
- Quantification of lodging-proof ideotype targets for spring wheat in northwest Mexico.
- Phenotype mapping populations to better understand the genetic basis and identify QTLs for lodging target traits in spring and winter wheat.
- Develop new rapid assessment methods. These will include an investigation of remote sensing methods such as spectral reflectance and possibly ground penetrating radar, simple pull-up and measure tests, and instrumentation for measuring stem and anchorage strength in the field.

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# Breeding for Yield Potential and Research Support Platforms

## An integrated approach to breeding for yield potential

Simon Griffiths, Matthew Reynolds, Ian King, Tom Payne, David Bonnett, Hans Braun

In order to accelerate genetic gains from their current rate of under 1% per year, the breeding effort for the foreseeable future will depend on three main strategies that will complement conventional approaches: 1) Strategic hybridization to combine traits associated with RUE and partitioning; 2) High throughput phenotyping and molecular marker assisted breeding, including whole genome selection (WGS), to permit the efficient deployment of yield and other trait-linked markers as they are identified through gene discovery and WGS modeling; 3) Use of exotic germplasm (including wild relatives and in some cases transgenes) to complement levels of expression in conventional gene pools.

### Strategic Hybridization

Using all information available on photosynthetic and partitioning traits and their interaction, hybridization schemes will be designed to combine yield potential traits in such a way that the main drivers of yield potential are combined deterministically with the view to achieving cumulative gene action. Given limited understanding of the genetic basis of complex traits it is impossible to predict the outcome of combining albeit theoretically complementary characteristics. Nonetheless, it is axiomatic that a good level of trait expression in a given genotype indicates a favorable (albeit unspecified) combination of positive alleles, and selecting for traits is thereby a practical means of achieving cumulative gene action, as demonstrated by recent impacts in breeding for drought adaptation (Rebetzke et al. 2009; Reynolds et al. 2009). This approach will be complemented by marker assisted crossing and selection as more information from genetic dissection of complex physiological traits becomes available (Pinto et al. 2010; Snape et al. 2007). Diagnostic markers are widely used for genes where the functional polymorphism has been identified. These include homoeoallelic series at: *Ppd-1* (Beales et al. 2007; Turner et al. 2005); *Vrn-1* (Yan et al. 2003); *Vrn-3* (Yan et al. 2006); and *Rht-1* (Peng et al. 1999). Diagnostic assays exist for alien introgression segments including 1BL1RS rye translocation (Koeberl 1995) and *Lr19 Agropyron* translocation (Prins et al. 2001).

### High throughput phenotyping and molecular marker assisted selection

While a number of high throughput phenotyping approaches can be applied in progeny screening (including thermal imaging and spectral radiometry) diagnostic markers are not yet available for complex traits ruling out the immediate and widescale application of trait-specific marker assisted selection (MAS). Effective marker assisted selection depends on cloning genes that control the priority traits of the WYN so that the genetic markers used to track alleles in breeding programs are based on functional polymorphisms, as is the case now for *Ppd-1*, *Rht-1*, and *Vrn-1*. A watershed for efficient gene cloning in any species is the completion of whole genome sequencing for that species. The first plant genome sequence to be completed was the model dicot *Arabidopsis thaliana* (Kaul 2000). The first of the worlds staple crop species to be sequenced was rice (Yu 2002; Goff 2002). As shown in figure 1, the number of completed genomes has accelerated rapidly since 2005. In spite of the challenges for bread wheat (18 Gb of DNA, 85% of which is repetitive and most genes are triplicated due to allohexaploid genome structure) the encouraging trajectory of Fig. 1 means we expect a usable draft genome assembly of wheat well within the timeframe of WYN. This objective is being pursued by members of the international wheat genome sequencing consortium (<http://www.wheatgenome.org/>).

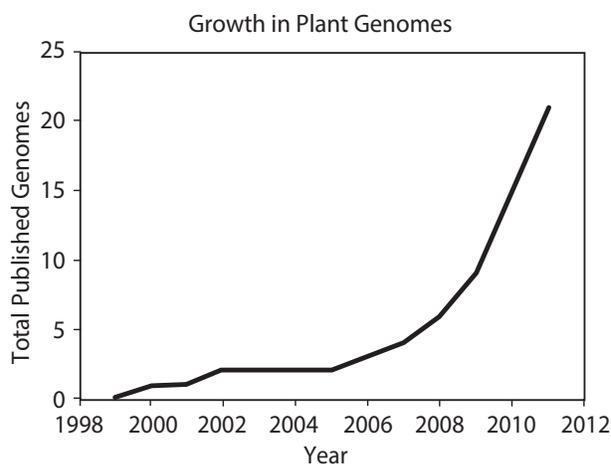


Figure 1. Number of sequenced plant genomes over time (figure from CoGePedia ([http://genomeevolution.org/wiki/index.php/Sequenced\\_plant\\_genomes](http://genomeevolution.org/wiki/index.php/Sequenced_plant_genomes))).

The prospect of a wheat genome sequence, resequenced varieties, and the very significant shotgun sequence and SNP data available for bread wheat already (eg <http://www.cerealsdb.uk.net/>) raises a most critical question for the wheat community over the next ten years :‘When we have the wheat genome sequence, what genes do we want to clone, and how can we deploy them?’. Even for established post genomic crops like rice and maize, the answer to this question is not yet clear, but for all crops it is likely to lie at the interface of physiology and genetics. The complex traits that are integrated to achieve genetic gain in yield potential can be compartmentalized into major gene effects and cloned, just as has been done in rice (Ashikari and Matsuoka 2006). This is a key objective for the WYN. The identification of genes underlying these traits will allow WYN to test the effect of MAS for these genes within the CIMMYT breeding program. It will also facilitate the efficient interchange of alleles between breeding programs and those that lie with genebank and mutant collections, yet to be deployed in elite breeding efforts.

Looking ahead, whole genome selection (WGS) offers considerable promise to increase breeding efficiency by capitalizing on the empirical association between expression of yield per se and high density markers across the entire genome (Bernardo and Yu 2007), assuming all of the representative genetic sources are present in the “training” population (Heslot et al. 2012). Using data from CIMMYT advanced spring wheat lines, genomic prediction models have been developed that have good correlations with phenotypic performance within a large and diverse cohort of CIMMYT advanced breeding lines. Ongoing efforts are focused on extending predictions to other cohorts of CIMMYT advanced breeding lines and for application in early generation selection and rapid cycling. Populations have been developed and are now being progressed to validate genomic selections made in early generation material (see Bonnett et al. these proceedings). It is important to note, however, that GWS will not inform gene discovery or the identification of new and useful variation from diverse germplasm. To push genetic gains further upwards, our understanding of the physiological basis of yield will be used to focus exploration of genetic resources on specific yield limiting traits associated with RUE, partitioning and lodging resistance.

### **Expanding the genetic base of wheat**

Wheat’s wild relatives have already been used extensively in breeding (Ortiz et al. 2008; Trethowan and Mujeeb-Kazi 2008) and represent a key resource in the pipeline for increasing yield potential. A good example is the 7Ag.7DL chromosome translocation that is associated with increased yield and biomass in most backgrounds into which it has been introduced (Singh et al. 1998). Although the value of genetic variation from alien species has been clearly demonstrated, only a fraction of their full potential in breeding has so far been exploited in breeding programs (see Table 4 in Reynolds et al. 2009a). In the past this has been as a direct result of the lack of robust high throughput screening procedures that enable the rapid identification and characterization of introgressed chromosome segments. However, recent advances in conventional and next generation sequencing platforms and technology in combination with the sequencing of the model plant genomes, e.g. rice and *Brachypodium* are now enabling the development of strategies to fully exploit the potential of alien species for crop improvement. The forage grasses are one example where modern technology is being exploited to introgress interspecific genetic variation into an important crop species, i.e. the *Lolium perenne/Festuca pratensis* introgression system (Harper et al. 2011).

### **Phenotyping Networks**

Conventional breeding depends on multi-location yield trials so that both locally adapted lines can be selected and broadly adapted material identified for subsequent rounds of crossing. To this end, CIMMYT coordinates the International Wheat Improvement Network (IWIN) consisting of hundreds of partners in wheat growing environments worldwide (Manes et al. 2012; Sharma et al. 2012), a model that has been adapted for targeted selection including the CSISA project (see Pask et al. these proceedings). In previous cycles subsets of IWIN locations were used to evaluate the Wheat Association Mapping Initiative (WAMI) panel and the Seri/Babax mapping population (Lopes et al. 2012; Lopes et al. these proceedings), and other elite panels including the CIMCOG (Chavez et al. these proceedings). In 2013 the 1st WYCYT (a panel of new lines selected for good expression of yield potential traits) was also circulated. These initiatives will be expanded into a dedicated phenotyping network focused on raising wheat yield potential.

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# Pre-breeding for yield potential

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## Summary

In order to accelerate genetic gains from their current rate of approximately 0.6% per year in favorable environments (Sharma et al. 2012), breeding efforts in the foreseeable future will depend on four main strategies:

- Strategic hybridization to combine traits and alleles associated with favorable expression of radiation use efficiency (RUE) and partitioning (Reynolds et al. 2012).
- Use of exotic germplasm (including transgenes) to complement levels of expression in conventional gene pools (Trethowan and Mujeeb-Kazi 2008; Parry et al. 2011).
- High throughput phenotyping and molecular marker assisted breeding (including whole genome selection; see Bonnett et al. these proceedings) to permit the efficient deployment of yield and other trait-linked markers as they are identified through gene discovery and whole genome selection (WGS) modeling (Tester and Langridge 2010).
- Conventional breeding including individual plant selection for heritable traits and multi-location yield testing.

## Results and Discussion

### Developing conceptual models

Yield potential (YP) can be expressed as a function of the light intercepted (LI) and RUE, whose product is biomass, and the partitioning of biomass to yield, i.e. harvest index (HI). Physiological trait (PT)-based breeding focuses on improving all three of these components. The PTs related to LI, such as stand establishment and canopy architecture, are relatively straightforward to phenotype on a routine basis. Although PTs related to RUE are generally more challenging to measure, the potential to increase RUE is supported by theory (Zhu et al. 2010) as well as observations of increased biomass in recent cultivars (Shearman et al. 2005), in some cases stemming from introgression of exotic germplasm (Singh et al. 1998). Approaches for increasing RUE include modifying the specificity, catalytic rate and regulation of Rubisco, up regulating Calvin cycle enzymes, introducing CO<sub>2</sub>-concentrating

mechanisms in chloroplasts, optimizing light and N distribution of canopies while minimizing photo-inhibition, and increasing the contribution of spike photosynthesis (See *Increasing photosynthetic capacity and efficiency of wheat*, these proceedings).

Although HI has increased steadily during the 20th century, with the exception of the (generally imprecise) deployment of major-effect alleles at the *Rht*, *Ppd* and *Vrn* loci (Slafer and Rawson 1994), optimal expression of HI is still achieved empirically within major agro-ecosystems and is subject to seasonal effects (Ugarte et al. 2007). As a result, HI varies from 0.4-0.55 in elite cultivars worldwide (see Shearman et al. 2005; Zheng et al. 2011). Improving light interception and RUE will increase crop biomass, but maximum yield expression will require dynamic optimization of source:sink ratios. This will require a better understanding of how to maximize dry matter partitioning to reproductive structures without under-investing in roots, stems, and leaves, on which both grain yield and lodging resistance are also dependant. Crop phenology needs to be optimized to favor spike fertility and should be tailored to different photoperiod and temperature regimes. Moreover, sensitivity to unpredictable environments, including extreme weather, must be modulated to prevent overly conservative responses that reduce seed set and harvest index (see Davies et al. these proceedings).

Using information available on photosynthetic and partitioning traits, hybridization schemes have been implemented that combine PTs (Fig. 1) in such a way that the main drivers of yield potential are combined deterministically with the view to achieving cumulative gene action (see below). With limited understanding of the genetic basis (and less the gene action) of traits that contribute to yield, it is impossible to predict the outcome of combining albeit theoretically complementary characteristics. Nonetheless, it is axiomatic that a good level of PT expression in a genotype indicates a favorable (albeit unspecified) combination of positive alleles, and selecting for PTs is thereby a practical means of achieving cumulative gene

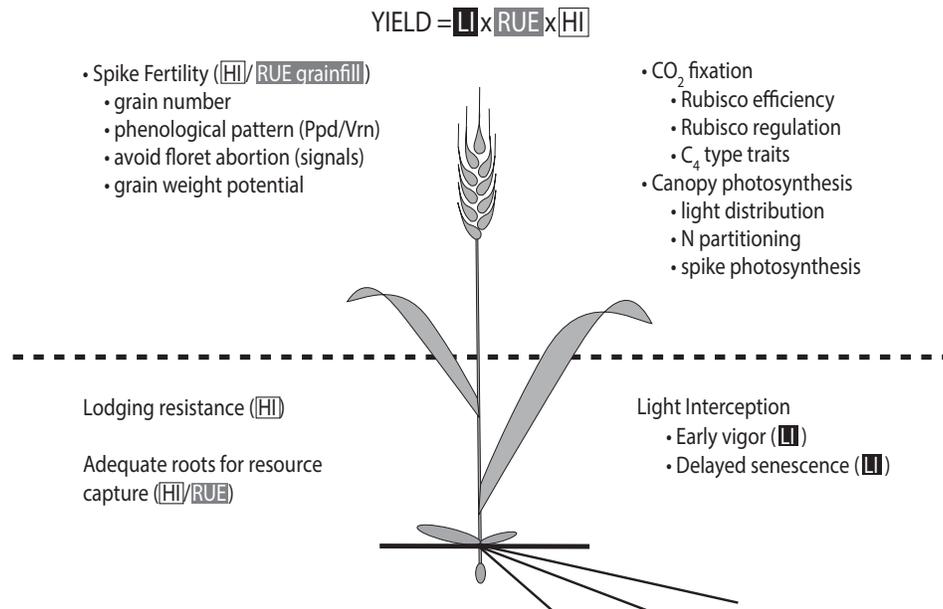


Figure 1. A conceptual platform for designing crosses that combine complementary yield potential traits in wheat (based on traits reviewed in Reynolds et al. 2009).

action, as demonstrated by recent impacts in breeding for drought adaptation (Rebetzke et al. 2009; Reynolds et al. 2009). This approach will be complemented by marker assisted crossing and selection initially using diagnostic markers for genes where the functional polymorphism have been identified; predominantly for major effect genes including the homoeoallelic series at: Ppd-1 (Beales et al. 2007; Turner et al. 2005), Vrn-1 (Yan et al. 2003), Vrn-3 (Yan et al. 2006), and Rht-1 (Peng et al. 1999). Assays also exist for alien introgression segments including *1BL1RS* rye translocation (Koebner 1995) and *Lr19* Agropyron translocation (Prins et al. 2001). As yield and other complex physiological traits are genetically dissected, new diagnostic markers will be added to models that predict gene action and interaction with environment, and subsequently deployed in breeding.

#### Assembling a crossing block in Mexico and making strategic crosses

The CIMMYT Mexico Core Germplasm (CIMCOG) Panel, established from landmark cultivars and advanced breeding lines that express high yield potential, has been comprehensively evaluated for the following classes of traits at MEXPLAT in four environments:

- Canopy photosynthesis, including N and pigment distribution
- Spike photosynthesis and respiration

- Source and sink balance
- Partitioning of assimilates among different organs.
- Developmental patterns
- Grain filling and potential grain size
- Lodging resistance

The data set has been used to form crossing blocks for yield potential pre-breeding work. The first round of crosses focused on high yield and biomass, response to source/sink treatments, high floret number, spike index, leaf and spike photosynthesis, and lodging resistance. A set of synthetic lines were included in one crossing block for their expression of high biomass under irrigated conditions. Based on these data, 45 crosses were made in three different crossing blocks and the F1 generation is currently growing at MEXPLAT.

#### Applying marker-assisted selection and early generation selection tools to enrich for favorable alleles

Diagnostic markers are not yet available for the majority of PT, ruling out widescale application of trait-specific marker assisted selection. Nonetheless, a few high throughput phenotyping approaches can be applied in progeny screening. For canopy temperature (CT), airborne remote sensing platforms can be used to measure a range of spectral indices high throughput, including the

Normalized Vegetative Difference Index and the Water Index, both of which can estimate relative differences in biomass among plots at similar growth stages and are measured in the same timeframe as CT (Babar et al. 2006). Other spectral indices are sensitive to photosynthetic pigments, and the possibility of estimating photosynthetic rate has been proposed (Serbin et al. 2012). Looking ahead, WGS offers considerable promise for increasing selection efficiency by capitalizing on the empirical association between expression of yield *per se* and high density markers across the entire genome (Bernardo and Yu 2007), assuming that all of the representative genetic sources are present in the “training” population (Heslot et al. 2012). It is important to note, however, that GWS will not inform gene discovery or the identification of new and useful variation from diverse germplasm (see Bonnett et al. these proceedings).

To date, gene discovery work has focused on two main populations: 1) Kauz/Weebil double haploids, which have generated lines of exceptional yield potential by combining YP traits (Bustos et al. 2013; Garcia et al, these proceedings), and 2) the Wheat Association Mapping Initiative (WAMI), for which agronomic data is already available from 30 environments in 10 countries and is being analyzed for markers linked to yield. A few consistent markers associated with grain yield have been identified on chromosomes 2A, 2B, 3B, and 4B.

#### **Designing hybrids using physiological and genetic information**

Research on hybrid wheat has been supported through a project with Syngenta. The first phase of this project aimed to identify heterotic groups within elite CIMMYT germplasm. Lines were selected that had a combination of good yield performance, good pollen production characters, and spanned the greatest possible breadth of genetic diversity within CIMMYT’s advanced breeding lines. Diversity was based on pedigree but also spanned differences in physiological characters and estimates of diversity based on marker genotyping corresponded with the pedigree-based estimates. Experimental wheat hybrids were produced using a gametocide supplied by Syngenta and hybrids were tested in yield trials in Obregon for two years. Promising levels of heterosis were identified as well as parents with good combining ability.

#### **Testing advanced lines in multi-location yield trials**

Data from the CIMCOG panel distributed to major high yield potential wheat agro-ecosystems in Asia and Africa in the 2011/12 wheat cycle has been analyzed (see Chavez et al. these proceedings).

A panel of 30 new advanced lines encompassing high yield and/or biomass (based on strategic crosses to combine complementary source and sink traits) were delivered to 30 international sites in 14 countries under the name of the 1<sup>st</sup> Wheat Yield Consortium Yield Trial (1<sup>st</sup> WYCYT) and are being evaluated for yield and agronomic traits in the 2013 wheat cycle.

#### **Next Steps**

- Update conceptual model using CIMCOG global data base, as well as data from other trials, with high yield germplasm.
- A second CIMCOG is being assembled using the latest CIMMYT advanced lines as well as information from genetic resource screening. For example, high biomass Mexican landraces have been identified for crossing and/or further characterization. Requests for high YP germplasm from other major wheat producing countries have resulted in the receipt of accessions from South Asia and Iran. Additional yield potential material received from Mexican national programs such as INIFAP has been included for evaluation in MEXPLAT. All this material will be either multiplied or evaluated for YP characteristics in Mexico, starting in 2013, and added to the Wheat Yield Consortium/ Network Crossing Block.
- Further crosses are being designed using combined analyses, including agronomic data from CIMCOG trials worldwide (see Chavez et al. these proceedings).
- QTL analysis on Kauz/Weebil double haploids and the WAMI will be continued, including meta-analysis. Other populations will be phenotyped and genotyped (See Sivakumar et al. these proceedings).
- The 2<sup>nd</sup> WYCYT is being assembled for international distribution

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## Expanding the genetic base of wheat

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The distant relatives of wheat provide a vast reservoir of genetic variation for agronomically important traits and, given that wide crossing is already a mature discipline in wheat (Ortiz et al. 2008; Trethowan and Mujeeb-Kazi 2008), represent a key resource in the pipeline for increasing yield potential. Examples of alien introgressions include those from *Aegilops umbellulata* (Sears 1956, 1972) which saved US wheat production from catastrophic failure due to leaf rust in 1960; resistance to a range of diseases, tolerance to acid soils, increased yield and yield stability from rye (Ammar et al. 2004; McIntosh 1983) [in the late 1990s a 1B/1R translocation from rye was present in the majority of world wheat varieties and a number of the current global top varieties, e.g. “Rialto”, still carry it]; a gene from *Ae. ventricosa* (Garcia-Olmedo et al. 1977) conferring resistance to eyespot is present in several wheat varieties; many of the top wheat varieties in Europe, e.g. “Robigus” are derived from unknown introgressions from *Triticum dicoccoides*; over 30% of all wheat varieties currently produced at CIMMYT are derived from crosses between normal wheat and “synthetic” wheat (Dreisigacker et al. 2008) (synthetic wheat is derived from crosses between *Ae. squarrosa*, DD genome, and tetraploid wheat, AABB genomes followed by chromosome doubling via colchicine).

Alien introgression in crop species, in its simplest form, involves the sexual hybridization of different species to form an inter-specific F1 hybrid. Alien introgression occurs in the F1 hybrid (or its derivatives) when related chromosomes from the two parental species (i.e. chromosomes that carry orthologous genes in essentially the same order) recombine at meiosis resulting in the generation of inter-specific recombinant chromosomes. These recombinant chromosomes are then transmitted to the next generation through the gametes. The repeated backcrossing of the F1 hybrid to one of the parental genotypes results in the generation of lines which carry the majority of the genome of one species but also carry one or more chromosome segments from the other

parental species. In wheat, alien introgression is more complex because of the presence of pairing control genes. Normal wheat carries the Ph1 locus (located on the long arm of chromosome 5B), which restricts recombination to homologous (identical) chromosomes, thus preventing recombination between related (homoeologous) wheat and alien chromosomes. Thus in order to induce wheat/alien homoeologous recombination, wheat/alien hybrids (amphidiploids), or lines of wheat which carry an alien chromosome(s), need first to be crossed to lines of wheat which lack the Ph1 locus, i.e. ph1 mutant lines.

A further complication in wheat is that once an alien segment has been introgressed into wheat it is very difficult to reduce the size of the segment even when the Ph1 pairing control genes have been removed. Since many wheat/alien introgressions are large and thus carry deleterious genes along with the target gene an additional strategy has to be employed before an alien chromosome segment can be used in commercial breeding programmes. Sears (1956, 1972) developed a successful strategy to isolate smaller alien chromosome segments only carrying the target gene involving the inter-crossing of two lines with different but overlapping alien chromosome segments where the target alien gene lies within the overlap. As a result of recombination between the two overlapping alien segments (in the presence of the normal Ph1 pairing control genes), some of the progeny produced are recombinant and will carry the target gene on a reduced alien chromosome segment lacking deleterious genes.

Although the value of genetic variation from alien species has been clearly demonstrated, only a fraction of their full potential in breeding has so far been exploited in breeding programs (see Table 4 in Reynolds et al. 2009). In the past this has been as a direct result of the lack of robust high throughput screening procedures that enable the rapid identification and characterization of introgressed chromosome segments. However, recent advances in conventional and next generation sequencing

platforms and technology in combination with the sequencing of the model plant genomes, e.g. rice and *Brachypodium* are now enabling the development of strategies to fully exploit the potential of alien species for crop improvement. The forage grasses are one example where modern technology is being exploited to introgress interspecific genetic variation into an important crop species, i.e. the *Lolium perenne*/*Festuca pratensis* introgression system (King et al. 2007; Harper et al. 2011). Here genetic markers have been used to introgress the entire genome of *F. pratensis* into *L. perenne* in overlapping chromosome segments. Assuming the chromosomal regions associated with traits of interest are known, introgression into a modern cultivar may take around 8 years. However, for complex traits where several regions may be involved, it would be necessary first to identify those regions, adding 2-5 years to the process.

While the introduction of genes from outside of the Triticeae tribe is not a routine procedure in wheat breeding, chromatin from  $C_4$  species, maize and *Tripsacum dactyloides*, has been introduced into wheat but so far not proven to be stably integrated and transmitted (Laurie and Bennett 1989; Comeau et al. 1992; Li et al. 1996; Brazauskas et al. 2004). Greater success has been achieved in oat (*Avena sativa* L.) with the production of a complete set of disomic additions of each of the maize chromosomes (Kynast et al. 2001). Expression of  $C_4$  photosynthetic enzymes in some of these oat-maize chromosome addition lines has been reported (Knowles et al. 2008). These precedents and the

availability of advanced molecular techniques allowing earlier, higher throughput screening and identification of putative introgressions, suggest that with appropriate investment, wide crossing may be able to introduce all of the chromatin into wheat required for full expression of  $C_4$  photosynthesis although this would clearly require considerable effort. It remains to be seen whether wide crossing or the transgenic approach will have impacts sooner in terms of increasing crop biomass.

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# Genomic selection to increase breeding efficiency

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## Summary

Genomic prediction models have been developed that have good correlations with phenotypic performance within a large and diverse cohort of CIMMYT advanced breeding lines. Ongoing efforts focus on extending predictions to other cohorts of CIMMYT advanced breeding lines and applications in early generation selection and rapid cycling. Populations have been developed and are now being progressed to validate genomic selections made in early generation material.

## Results and Discussion

### Designing, implementing, and evaluating wheat breeding strategies involving marker-assisted recurrent selection and genome-wide selection

This project aims to couple the power of the large breeding program at CIMMYT, which generates and phenotypes large numbers of high-performing breeding lines each year, with high density, highly multiplexed marker technology to develop genomic selection strategies to increase genetic gain for yield at the breeding program scale in a cost-effective, logistically feasible manner.

Combining phenotypic and genotypic data sets in a training set, GS models simultaneously estimate effects for *all* markers, even permitting capture of those with small effects. The GS model is then used to predict the performance of lines outside the set, based on marker data alone.

### Development and validation of prediction models within advanced breeding lines

An initial indication of the predictive ability of models can be achieved by 5-fold validation, in which lines are randomly and evenly divided into five groups (Figure 1). All lines have both genotypic and phenotypic data. A model can then be built using data from four of the five groups, and this model is used to predict performance of the 5<sup>th</sup> group, based solely on genotype. Phenotypic data is available for all lines so predictions can be validated. This process is then repeated with different groupings.

Models have so far been built and tested using a training set of 1361 advanced CIMMYT lines, spanning a range of SAWYT, SAWSN and IBWSN nurseries. Multi-year and multi-environment yield data is available for all lines and GBS genotypes, and around 30,000 loci were generated.

A feature of GBS datasets is that while data is generated across many loci, for a substantial number of these there is a high proportion of missing data, that is, no genotypic data for a high proportion of the lines screened. This characteristic of GBS datasets presents a range of issues and approaches to resolve them. Basically they are to either only use data from loci where there is a low proportion of missing data, or to try to impute the correct genotype of the missing data. Imputation has not led to substantial improvements in model predictive ability thus

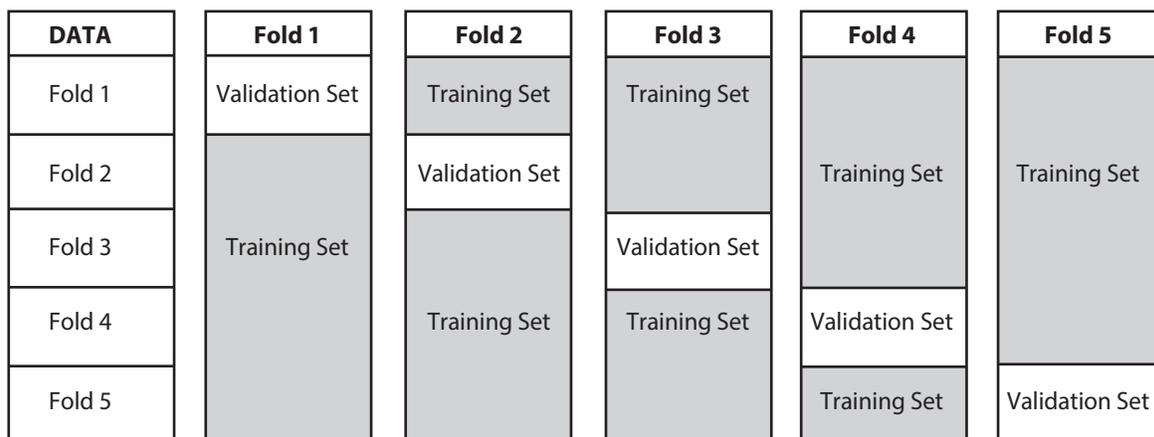


Figure 1. Graphic representation of the concept of 5-fold validation of GS models.

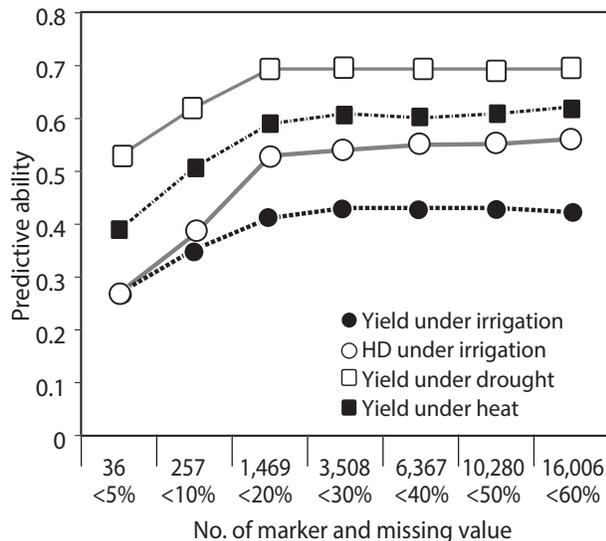
far (data not shown), but this is perhaps because methods are limited by not yet having good map positions for most GBS loci. It is also possible to make modifications to the GBS process to reduce the amount of missing data.

Building prediction models using a smaller number of loci with a lower proportion of missing datapoints generated predictions as good as those with a larger number of loci with and higher levels of missing data (Figure 2). Importantly, predictions did not deteriorate with higher numbers of loci and missing data.

Predictions using the 5-fold method achieved high levels of correlation with observed data (Table 1). A range of statistical models, including genomic best linear unbiased prediction (GBLUP), Bayesian Lasso (BL), Bayesian Ridge Regression (BRR), and Reproducing Kernel Hilbert Space (RKHS, not shown), were used for predictions, but all produced very similar results within the training set.

**Table 1. Correlation between predicted and observed phenotypes using 5-fold cross validation.**

Models	Yield-Irrigated	DH-Irrigated	Yield-Drought	Yield-Heat
(9K GBS loci, <60% missing data)				
GBLUP	0.51	0.63	0.70	0.65
BL	0.50	0.62	0.70	0.65
BRR	0.50	0.61	0.71	0.65



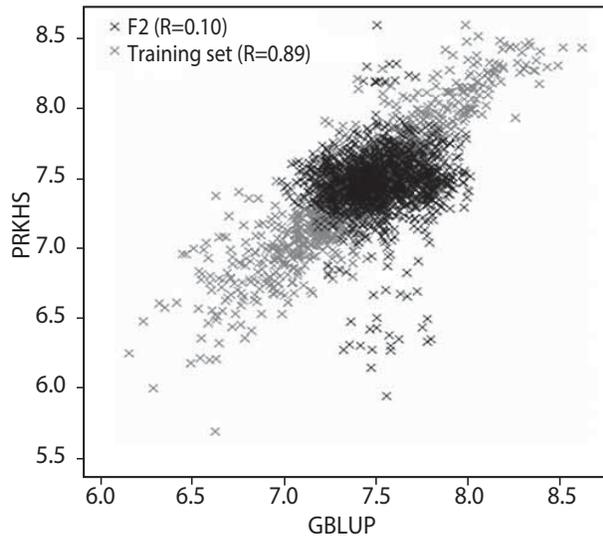
**Figure 2. Effect of marker number and proportion of missing data on predictive ability in 5-fold validation**

### Testing genomic predictions in other cohorts of advanced lines and in rapid cycling

Validation within a training set is only a start and needs to be extended to other scenarios such as predicting performance of a later cohort of advanced lines or making early generation selections. Efforts are ongoing in testing predictions in later cohorts of advanced lines and are revealing the importance of family structure and levels of relatedness, as well as effects of GxE (data not shown).

One of the key objectives of the GS project has been to make early generation predictions in F2, and intercross F1 populations. Selected individuals are intercrossed in an effort to pyramid favorable alleles without having to wait until F6 or later to phenotype lines before intercrosses. The great reduction in breeding cycle time offers the potential to dramatically increase genetic gain per unit of time. Crosses have been made between high performing lines in the initial training set and progressed through two cycles of genomic selection and intercrossing in F2 and the following intercross F1 generations. The performance of derivatives of the initial F2s, and the subsequent 1st and 2<sup>nd</sup> cycle intercross populations, will allow us to validate the accuracy of our predictions and quantify any difference in rate of genetic gain. Derivatives of the initial F2 population are growing in field trials this Obregon cycle (2012-13) to give the first validation of response to selection from genomic prediction in this project.

Genomic predictions in early generations may not behave in the way predicted within the training set of inbred lines. One possible issue is the low level of heterozygosity identified by GBS in early generations (much lower than expected), which means that the genotyping is less accurate than in inbred lines. Different statistical methods, based on the same genotypic and phenotypic data from the training set, produced quite different predictions in the F2 (Figure 3), although all were quite similar in the inbred lines of the training population (Table 1). The differences in predictions present problems in making selections and intercrossing selected individuals, but large numbers of intercrosses were made and many progeny generated to maintain a high level of diversity. This should allow validation of a range of early generation prediction models.



**Figure 3. Correlation between Genomic Estimated Breeding Values (GEBVs) using two different prediction models in a set of inbred lines (Training set) and a set of F2 populations (1924 plants) generated from 19 parents selected from the training set.**

### Next Steps

#### Enlarge training set and predict between original and new additions to training sets

After the initial 1361 lines, a further 1305 lines from the 31SAWSN and 46IBWSN, representing the next cohort of advanced lines, have had GBS genotypes generated. As well as increasing the size of the training set, they represent a slightly different

set of environments because not all years and environments overlapped with those of the original set. They also include lines with varying levels of relatedness to the original set. Using this enlarged dataset we are investigating how to best make predictions between different cohorts of lines that may not have been tested in common environments and how to best use levels of relatedness in predictions.

#### Validating early generation genomic selections

Populations developed by crossing lines from the first training set and then intercrossing at F2 and 1<sup>st</sup> intercross F1 stages, based on genomic prediction, are being progressed to more inbred generations to allow validation of predictions in yield trials. The first set of material, F2:4 lines from the original F2s, are in yield trials this Obregon cycle (2012-13) to provide initial data to begin the process of validation and refinement of models for use in early generation material.

The growing body of material and the timeline to develop lines suitable for conducting accurate yield trials means much of this work will not take place within the timeframe of the current project funded by the Bill & Melinda Gates Foundation. It is therefore critical that other sources of funding are found within the next year to continue this important and promising area of research that could make very large increases in yield gain responses over time, compared to current methods.

# China Update

## Wheat Yield Consortium update from China

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### Summary

Gains in yield potential and associations with physiological traits in Shandong and Henan provinces have been completed. The cell wall invertase gene *TaCwi-A1* on the common wheat chromosome 2A was characterized by comparative genomic approach, and two functional markers for the *Cwi* gene, associated with kernel weight, were developed and validated.

### Results and Discussion

The establishment of the International Wheat Yield Consortium (WYC) has provided an excellent platform for Chinese scientists to work with international colleagues on yield improvement. Major activities for WYC in China, funded by the National Natural Science Foundation of China and Ministry of Agriculture, included four aspects. (1) Understanding the yield improvement between 1980 to present of historical cultivars in two largest producers Henan and Shandong Provinces, and its association with physiological traits. (2) Identifying QTL/genes for yield and yield components using RILs from Zhou 8425B/Chinese Spring and Dumai/Shi 4185, and leading and historical cultivars through association analysis, and gene cloning and development of functional markers for kernel weight. (3) Evaluating the potential of 7DL.7Ag translocation on improving yield potential in Chinese winter wheat, and development and extension of high yield cultivars with seed companies. (4) Training postgraduate students and workshop.

#### Gains in yield potential and its association with physiological traits in Shandong Province

Shandong, located in the northern part of the Yellow and Huai Valleys, is the province with the second largest wheat production in China, with a sowing area of 3.6 million ha and production of 21 million tones. The objective of this study was to assess the improvement in yield potential and associated agronomic and physiological traits in Shandong province. Thirteen milestone cultivars and two advanced lines released from 1969-2006 were examined over three years at Tai'an during 2006-2009. The mean annual genetic gain in grain yield was 62 kg/ha (0.85% per year), largely associated

with increased kernels/m<sup>2</sup>, above-ground biomass, and harvest index (HI), and reduced plant height. Significant positive genetic changes were observed especially for apparent leaf area index (LAI) at heading and anthesis, and also for chlorophyll content (Chl) at anthesis, photosynthesis rate during grain filling, and stem water-soluble carbohydrate (WSC) content at anthesis. In comparison to genotypes with the *Rht-D1b* marker, genotypes with *Rht-D1b+Rht8c* showed increased grain yield, thousand kernel weight (TKW), kernels/spike, kernel weight/spike, HI, canopy temperature depression (CTD), and Chl at anthesis, and LAI at heading, but no difference in height. These results suggest that genetic gains in grain yield in Shandong were mainly contributed via increases in kernels/m<sup>2</sup> and biomass, which were achieved by improving crop photosynthesis at and after heading. Additionally, the source for grain filling may have benefited from increased WSC in stems at anthesis (Xiao et al. 2012).

#### Gains in yield potential and its association with physiological traits in Henan Province

Henan, located in the southern part of the Yellow and Huai Valleys, is the province with the largest wheat production in China, with a sowing area of 5.3 million ha and production of 31 million tones. This study aimed to identify the agronomic and photosynthetic traits associated with genetic gains in grain yield of facultative wheat in Henan between 1981-2008. During the 2006-2007 and 2007-2008 crop seasons, a yield potential trial comprising 18 leading and new cultivars released between 1981-2008 was conducted at two locations. Results showed that the average annual genetic gain in grain yield was 0.60% or 51.30 kg/ha, and that this significant genetic improvement was directly attributed to increased TGW, which also contributed to a significant increase in HI. The genetic gains in rates of net photosynthesis at 10, 20, and 30 days after anthesis were 1.10% ( $R^2 = 0.46$ ,  $P < 0.01$ ), 0.68% ( $R^2 = 0.31$ ,  $P < 0.05$ ), and 6.77% ( $R^2 = 0.34$ ,  $P < 0.05$ ), respectively. The rates of net photosynthesis at 10 ( $r = 0.58$ ,  $P < 0.05$ ), 20 ( $r = 0.59$ ,  $P < 0.05$ ), and 30 ( $r = 0.65$ ,  $P < 0.01$ ) days after anthesis were closely and positively correlated with grain yield. A slight decrease in leaf temperature and increased stomatal

conductance after anthesis were also observed. Grain yield was closely and positively associated with stomatal conductance ( $r^2 = 0.69$ ,  $P < 0.01$ ) and transpiration rate ( $r = 0.63$ ,  $P < 0.01$ ) at 30 days after anthesis. Therefore, improvement in these traits was the likely basis of the increased yields in Henan during 1981-2008. Genetic improvement in yields was primarily attributed to the utilization of two elite parents: viz Yumai 2 and Zhou 8425B. The future challenge of wheat breeding in this region is to maintain genetic gains in grain yield and to improve grain quality, without increasing inputs for the wheat-maize double cropping system. For details, see Zheng et al. 2011.

#### **Characterization of the cell wall invertase gene *TaCwi-A1* on common wheat chromosome 2A and development of functional markers**

Cell wall invertase (*Cwi*) is a critical enzyme for sink tissue development and carbon partition, and has a high association with kernel weight. Characterization of *Cwi* genes and development of functional markers are important for marker-assisted selection in wheat breeding. In this study, the full-length genomic DNA sequence of a *Cwi* gene located on wheat chromosome 2A, designated *TaCwi-A1*, was characterized via in silico cloning and experimental validation. *TaCwi-A1* comprises seven exons and six introns (3676 bp in total) and an open reading frame of 1767 bp. A pair of complementary dominant markers, CWI21 and CWI22, was developed based on allelic variations at the *TaCwi-A1* locus. A 404-bp PCR fragment was amplified by CWI21 in varieties with lower kernel weights, whereas a 402-bp fragment was generated by CWI22 in the varieties with higher kernel weights. The markers CWI21 and CWI22 were located on chromosome 2AL using an  $F_{2:3}$  population from Doumai/Shi 4185 cross, and a set of Chinese Spring nullisomic-tetrasomic lines. They were linked to the SSR locus Xbarc15-2AL with a genetic distance of 10.9 cM. QTL analysis indicated that *TaCwi-A1* could explain 4.8% of phenotypic variance for kernel weight over two years. Two sets of Chinese landraces and two sets of commercial wheat varieties were used to validate the association of CWI21 and CWI22 with kernel weight. Results indicated that the functional markers CWI21 and CWI22 were closely related to kernel weight, and could be used in wheat breeding for improving grain yield. For details, see Ma et al. (2012).

#### **Germplasm development**

The Ministry of Agriculture officially released Zhongmai 895 for the southern part of Yellow and Huai Valleys, which has high yield potential, lodging resistance, high TKW (around 50g), and staygreen associated with a fast grainfilling rate and tolerance to high temperatures. Demonstration fields were arranged to promote this new cultivar. Zhongmai 875, a sister line of Zhongmai 895, with higher yield potential, is expected to be released in Henan Province in 2013.

A limited backcross approach was used to transfer the 7Ag/7DL translocation from Wheatear into three leading facultative wheat cultivars through marker-assisted selection. Twenty lines were sown for yield trials in the 2012-13 season to understand its potential application for yield improvement in China.

#### **Next Steps**

1. A yield trial with 25 leading and historical cultivars from 1950 to present was sown in 2012-13 season to reevaluate the yield gains in Henan province.
2. QTL mapping for yield components in Zhou8425B/Chinese Spring; Zhou 8425N is an elite parent used for breeding in Henan province over the last 20 years.
3. Identification of SNPs associated with yield and yield components in Chinese cultivars through association mapping.
4. Characterization of *TaGS* gene at chromosome 7D associated with kernel weight and development of functional markers.
5. Characterization of various alleles associated with kernel weight in Chinese and CIMMYT germplasm by available functional markers.
6. Development and promotion of new cultivars with high yield potential.

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# Germplasm Evaluation and Delivery

## CIMCOG: Mexico and international data summary

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### Summary

The CIMMYT Mexico Core Germplasm (CIMCOG) panel was established in 2010 in consultation with wheat breeders and physiologists (Reynolds et al. 2011). Along with other advanced lines, it was distributed as part of CIMMYT's international nursery (IN) system, which in turn is part of the International Wheat Improvement Network (IWIN), the central delivery tool for WYC products.

### Main objectives

1. Distribute WYC germplasm panels and nurseries to WYC stakeholders.
2. Establish standard protocols for physiological and agronomic characterization.
3. Statistically analyze and report data, including trait expression and its genotype by environment interaction (G x E).

### Results

The CIMCOG panel was distributed to 14 countries during the 2011/12 wheat cycle and data from 10 countries were returned (Table 1). Table 2 shows the list of genotypes participating in this international nursery.

A total of 60 CIMCOG spring wheat genotypes were tested in 10 of the mega-environments (ME) worldwide and a total of 14 countries, grown during the 2011/2012 cropping cycle. A ME is defined as a broad, not necessarily contiguous area, occurring in more than one country and frequently transcontinental, defined by similar biotic and abiotic stresses, cropping system requirements, consumer preferences, and, for convenience, by a volume of production (Hans and Payne 2011). The

Table 1. Participants in CIMCOG international nursery during 2011/2012 cycle under full irrigation for yield, whose data has been included in the present analysis.

Country	Location	Institute Name	Latitude & longitude		Altitude m a sl	ME	Average Yield TM/ha
ARGENTINA	BUENOS AIRES	Universidad de Buenos Aires	34o35'N	58o28' W	30	4B	2.04
BANGLADESH	BARI	Wheat Testing Station	25o00'N	89o00'E	28	5A	3.68
BANGLADESH	JESSORE	University of Rajshahi	23o13'N	89o13'E	8	5A	3.16
CHINA	BEIJING	CIMMYT-China	43o46'N	87o35'E	870	10A	6.49
CHINA	CHENGDU	CIMMYT-China	30° 40' N	104° 4' E	640	6	5.40
EGYPT	GIZA	Field Crop, ARC	28o36'N	31o38' W	61	1	4.55
INDIA	HARYANA	DWR	29o40'N	77o2'E	300	1	4.59
INDIA	INDORE	IARI	22o37'N	75o50'E	600	4A	4.33
INDIA	KARNATAKA	University of Agricultural Scs.	15o28'N	75o7'E	678	5A	5.30
INDIA	LUDHIANA	Punjab Agric. Univ.	30o56'N	75o52'E	247	1	6.09
INDIA	NEW DELHI	PUSA Farms	28o35'N	77o12'E	228	1	6.30
INDIA	VARANASI	Banaras Hindu University	25o18'N	83o13'E	128	1	4.14
IRAN	KARAJ	CIMMYT-Iran	35o50'N	50o58'E	1321	7B	6.90
MEXICO	BAJA CALIFORNIA	INIFAP	32o39'N	115o28' W	3	1	5.21
MEXICO	GUADALAJARA	INIFAP	21o18'N	102o30' W	1541	2	4.98
MEXICO	GUANAJUATO - IRAPUATO	INIFAP	20o40'N	101o20' W	1730	2	6.97
MEXICO	GUANAJUATO - CELAYA	INIFAP	20o32'N	100o49' W	2640	2	6.58
MEXICO	SINALOA	INIFAP	25o45'N	108o48' W	14	1	4.52
MEXICO	SONORA	INIFAP	32o18'N	115o4' W	37	1	6.57
MEXICO	TOLUCA	UAEM	19o16'N	99o51' W	2680	2	6.48
MEXICO	CHIHUAHUA	INIFAP	28o10'N	105o29' W	1178	9	5.90
NEPAL	BHAIRAHAWA	NWRP	27o30'N	83o27'E	105	5A	2.75
PAKISTAN	ISLAMABAD	CIMMYT-Pakistan	28o24'N	71o40'E	170	1	3.34
PAKISTAN	ISLAMABAD	CIMMYT-Pakistan	31o25'N	73o7'E	117	1	7.77
PAKISTAN	ISLAMABAD	NARC-Islamabad	33o45'N	73o6'E	683	1	5.56
SOUTH AFRICA	BETHLEHEM	Small Grain Institute	28o12'S	28o12'E	1687	2	4.69
SUDAN	WAD MEDANI	Gezira Research Station, ARC	14o24'N	33o29'E	411	5B	2.20

Table 2. List of genotypes assessed in the CIMCOG international nursery during 2011/2012 cycle. An analysis number was given to each genotype due to differences in entry number found across countries.

CID	SiD	Entry	Genotype Cross name	Analysis number
477630	14	40	ARMENT//2*SOOTY_9/RASCON_37/4/CNDO/PRIMADUR//HAI-OU_17/3/SWITAN	1
8890	34	1	ATTILA	2
313200	45	2	ATTILA*2/PBWB	3
520844	25	3	ATTILA*2/PBWB*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUAR ROSA (213)//PGO/4/HUITES	4
363214	50	4	ATTILA//PGO/SERI/3/PASTOR	5
325362	63	31	ATTILA/PASTOR	6
435275	67	5	BABAX/LR42//BABAX/3/ER2000	7
512111	79	6	BABAX/LR42//BABAX/3/VORB	8
7896	254	7	BAGANORA T88	9
8626	465	8	BAVIACORA M82	10
485002	407	9	BCN/RIALTO	11
467519	358	10	BCN/WBLL1	12
448409	101	11	BECARD	13
448409	101	55	BECARD	14
520227	43	12	BECARD/KACHU	15
448418	52	13	BRST1*2/KIRI TATI	16
509700	20	14	C80.1/3*BATAVIA//2*WBLL1/5/RH/HARE//2*BCN/3/CROC_1/AE.SQUAR ROSA (213)//PGO/4/HUITES	17
425852	42	17	CHIR3/4/SIREN//ALTAR 84/AE.SQUAR ROSA (205)/3/2*8 UC/2/PFAU/WEAVER	18
426041	54	18	CHWLB9/6/FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUAR ROSA (TAUS)/4/WEAVER	19
459206	190	20	CIRNO C 2008	20
485004	542	19	CMH79A.535/4/AGA/3/4*SNB4/CNO67//1NIAB6/5/NAQ6/RIALTO	21
435266	116	21	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUAR ROSA (TAUS)/4/OCI/3/PASTOR/6/TEMPORALERA M87/ROMO96	22
135029	263	22	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUAR ROSA (TAUS)/4/WEAVER/5/2*KAUZ	23
516463	88	23	CNO78//PF70354/MU5/3/PASTOR/4/BAV92*2/5/FH6-1-7	24
428600	39	24	CROC_1/AE.SQUAR ROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#3/4/FRET2	25
506830	99	25	GK ARON/ AG SBEO 7846//2130/4/2*MILAN/KAUZ//PRINI A/3/BAV92	26
521064	14	27	KFA/3/PFAU/WEAVER//BRAMBUNG/4/PFAU/WEAVER*2//BRAMBUNG	27
520543	41	26	KINGBIRD#1//INQALAB 91*2/TUKURU	28
473281	85	28	MEX84.27.120/3/SOKOLL//ATTILA/3*BCN	29
342454	62	29	MILAN/KAUZ//PRINI A/3/BAV92	30
414815	55	33	MISR 1	31
448400	62	30	MUNAL #1	32
366109	122	32	OASI/5*8ORL95/5/CNDO/R143//ENTE/MEXI_2/3/AESQ/4/2*OCI	33
521017	12	34	OASI/5/KAU2//4*8ON/3/2*PASTOR/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/6/SAUAL #1	34
520287	53	35	PANDORA//WBLL1*2/8RAMBLING	35
428648	78	36	PASTOR/3/UR ES/JUN//KAUZ/4/WBLL1	36
7624	7	37	PAVON F 76	37
516587	41	38	PBW343*2/KUKUNA*2//FRTL/PI FED	38
459355	73	39	PFAU/SERI.18//AMAD/3/WAKWING	39
520698	31	41	QUAIU #3//MILAN/AMBEL	40
373334	61	42	RL6043/4*NAQ/2*PASTOR	41
520762	43	43	ROLP07*2/5/RH/HARE//2*BCN/3/CROC_1/AE.SQUAR ROSA (213)//PGO/4/HUITES	42
516777	76	15	SAUAL/4/CROC_1/AE.SQUAR ROSA (205)//KAUZ/3/ATTILA/5/SAUAL	43
517040	33	16	SAUAL/WHEAR//SAUAL	44
7691	50	44	SERIM82	45
6831	33	45	SIETE CERROS T66	46
507101	61	46	SOKOLL*2/3/8AB AX/LR42//8AB AX	47
507064	90	47	SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR	48
520765	33	49	TACUPETO F2001/8RAMBLING*2//KACHU	49
520758	38	48	TACUPETO F2001/SAUAL/4/BABA/XLR42//BABA*2/3/KURUKU	50
437242	76	50	TG70344/GUI//TEMPORALERA M87/AGR/3/2*WBLL1	51
509138	32	51	TRAP#1/BOW/3/VEE/PIN//2*TU/1/4/8AV92/RAYON/3/KACHU #1	52
510201	41	52	TRCH/SRTU//KACHU	53
516615	84	53	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	54
473251	30	54	W1392/4/PASTOR//HXL7573/2*8AU/3/WBLL1	55
516641	60	56	WBLL1*2/4/BABA/XLR42//BABA/X/3/8AB AX/LR42//8AB AX	56
516689	38	57	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUAR ROSA (213)//PGO/4/HUITES	57
516383	29	58	WBLL1*2/TUKURU*2/4/CROC_1/AE.SQUAR ROSA (205)//BORL95/3/2*MILAN	58
487192	32	59	WHEAR/SOKOLL	59
520820	43	60	YAV_3/5CO//IOBB/CR A/3/YAV79/4/AE.SQUAR ROSA (488)/5/LINE 1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAQ/5/KAU2/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343	60

ME to which wheat breeding stations participating in IWIN are assigned are shown in Figure 1 (Hodson and White 2007). Particularly for Mexico, representatives of central, north, and northern Mexican wheat production regions were tested.

Significant variability was found among genotypes for different traits measured. The wheat genotypes showed large variations for grain yield, biomass, days to heading, days to maturity, thousand grain weight (TGW), and harvest index (HI). Nevertheless, TGW varied slightly among countries within ME, in contrast with its variation among ME. Environmental conditions resulted in large differences in yield and yield components, indicating presence of GxE effects (data not shown). From the principal components analysis (PCA), it was observed that variations in yield are linked to the genotype more strongly than to environmental interactions. Figure 2 shows the results for yield of the principal component analysis (PCA) for the CIMCOG international nursery grown during the 2011-12 cycle, according to the location of the testing area. Notice that the Yaqui Valley (where the

CIMMYT station is), in the Sonora State of Mexico, is located in the middle of the PCA distribution, as well as Mexico (Mexico Valley), confirming its relevance as a breeding platform for wheat. The cluster distribution tree shows the linking relativeness among locations, where it can be seen that the Yaqui Valley's yield from the 60 genotypes is closely related to other Mexico testing areas, such as Sonora, Guanajuato and Sinaloa, which were the highest yielding areas for Mexico.

Table 3 presents the values of the parameters measured for every location in Mexico, and Table 4 shows yield data for the 60 genotypes grown across Mexico. Guanajuato obtained the highest yield and biomass. Guanajuato also showed the longest cycle, with 93 days to heading and 147 days to physiological maturity, and the lowest HI, which could be related to the high amount of above ground biomass produced by plants, without modifying significantly the plant height. In turn, Sinaloa and Guadalajara obtained the lowest values for yield, biomass, and cycle length (duration).

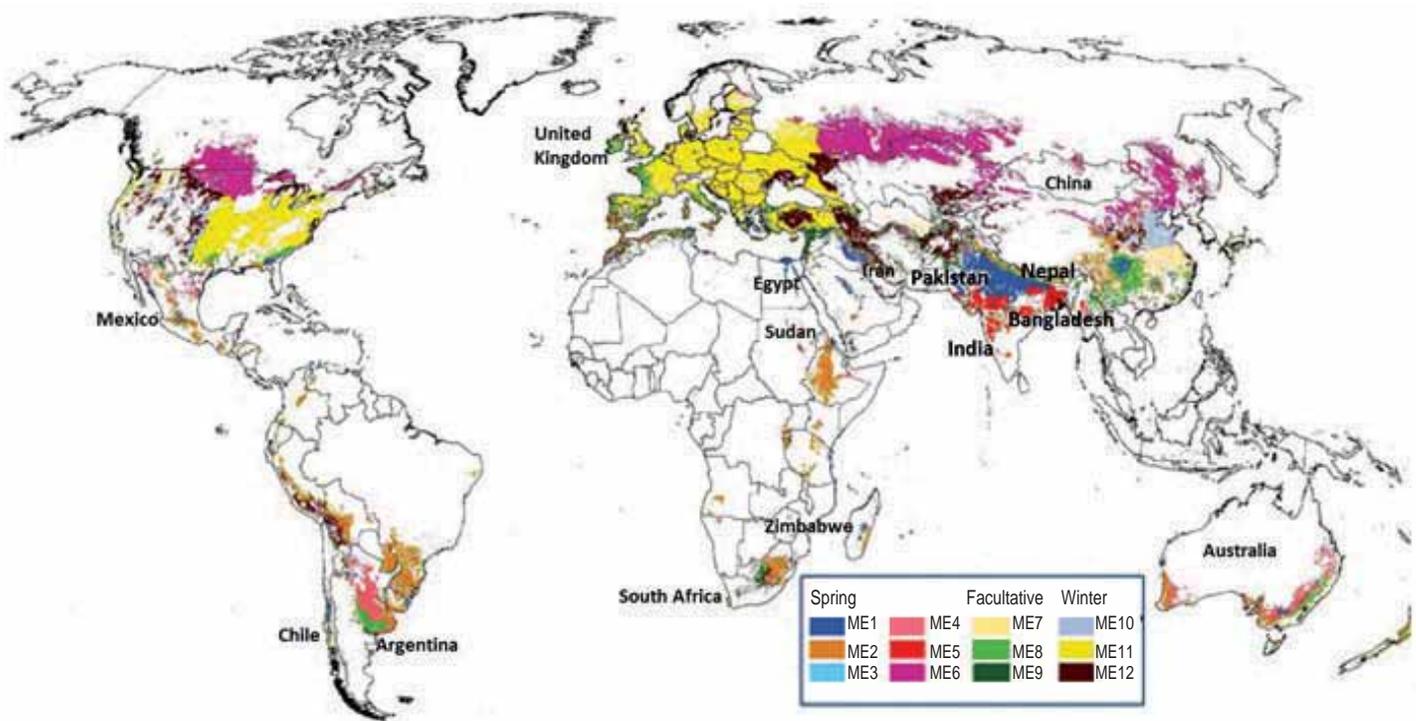
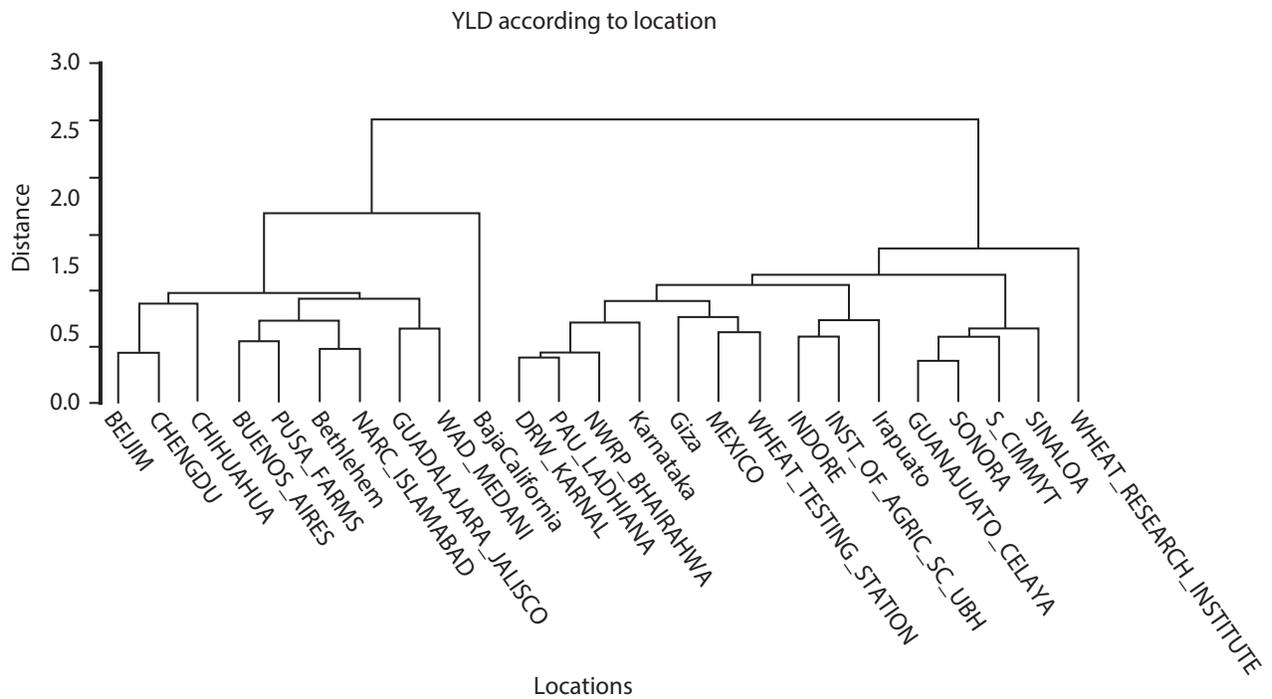
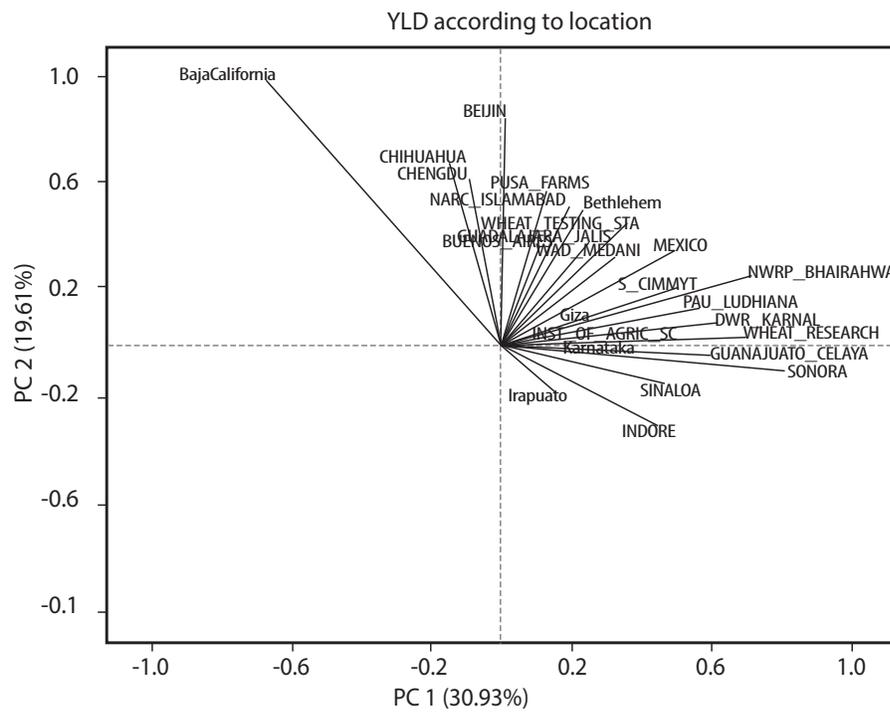


Figure 1. Countries participating in CIMCOG international nursery according to CIMMYT defined wheat production and breeding targeted mega environments (ME).



**Figure 2. PCA analysis for the CIMCOG international nursery grown during the 2011-12 cycle.** Yield (YLD) is presented according to the location. Notice that S\_CIMMYT (Yaqui Valley, CIMMYT, Sonora) is located in the middle of the PCA distribution – as well as Mexico (Mexico Valley), confirming its relevance as a breeding platform for wheat. The cluster distribution tree shows the linking relativeness among locations, where it can be seen that S-CIMMYT's yield from the sixty genotypes is closely related to other Mexico testing areas, as Sonora, Guanajuato and Sinaloa.

Table 3. Average values from 60 genotypes grown in Mexico during 2011/2012 cycle under full irrigation for yield and yield components.

Location	Days to Heading	Height (cm)	YLD (TM/ha)	TGW (g)	DAYS TO MATURITY	Biomass (t/ha)	HI (%)
GUANAJUATO	92.72	107.33	6.77	46.08	147.33	16.87	41.45
CHIHUAHUA	82.37	90.05	5.90	40.68	-	-	-
GUADALAJARA	71.62	97.43	4.98	47.85	119.38	8.91	56.01
BAJA CALIFORNIA	94.52	74.04	5.21	46.29	133.33	9.77	53.48
SONORA	111.92	93.32	6.67	44.49	136.80	13.81	47.87
MEXICO	-	98.96	6.48	44.39	-	-	-
SINALOA	74.20	86.33	4.52	44.61	109.53	8.95	49.71
Mean	87.89	92.50	5.78	44.91	129.27	11.66	49.70
Corr (r)	0.71	0.63	1.00	0.21	0.92	0.97	0.78
CV	13.71	9.79	0.84	2.08	13.32	3.17	5.01
<b>P level</b>	<b>0.006</b>	<b>0.0002</b>	<b>&lt; 0.0001</b>	<b>0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>

Table 3. Average values from 60 genotypes grown in 11 different countries during 2011/2012 cycle under full irrigation for yield and yield components.

YIELD (TM/ha)												
Genotype	ARGENTINA	BANGLADESH	CHINA	EGYPT	INDIA	IRAN	MEXICO	NEPAL	PAKISTAN	SOUTHAFRI	SUDAN	Mean total
1	1.49	3.63	5.83	4.71	5.20	7.12	6.20	2.60	5.47	3.95	2.11	5.11
2	2.92	3.95	6.53	4.57	4.98	6.41	5.95	2.84	5.08	3.90	2.49	5.06
3	3.00	6.64	6.99	4.77	4.94	6.94	6.02	3.00	5.83	4.70	2.50	5.46
4	3.58	3.52	6.58	4.96	4.72	5.87	6.25	3.09	5.87	5.80	3.08	5.28
5	1.99	3.47	6.60	3.07	5.47	6.46	5.94	3.42	5.42	4.55	2.15	5.13
6	1.54	3.35	6.97	4.86	5.51	8.24	6.16	2.84	5.77	4.25	2.54	5.35
7	1.57	3.36	7.01	3.43	5.35	7.15	6.08	2.92	5.95	3.70	2.24	5.18
8	3.00	4.11	6.38	5.43	5.69	7.36	6.46	3.42	5.34	5.10	2.71	5.55
9	2.38	3.82	5.50	4.79	4.91	7.42	5.95	2.42	5.74	4.60	2.56	5.07
10	2.79	3.20	5.94	6.10	5.39	6.53	6.32	2.67	5.20	3.95	1.68	5.20
11	1.73	2.73	6.02	4.36	4.45	7.95	6.05	2.08	5.78	4.50	2.58	4.93
12	3.12	2.72	5.76	4.93	5.99	7.17	6.33	3.42	5.41	4.75	2.79	5.32
13	1.09	3.66	5.61	3.71	5.40	6.18	6.16	3.08	5.97	4.95	2.71	5.18
14	3.96	3.30	5.65	4.86	5.58	8.09	6.32	2.84	5.69	4.95	2.09	5.40
15	2.22	2.97	5.42	4.79	5.35	6.14	6.23	2.85	6.28	5.15	2.48	5.23
16	2.62	2.93	5.99	4.64	5.25	6.41	5.71	2.50	5.10	4.45	1.51	4.88
17	0.86	3.37	6.20	4.43	5.11	6.18	5.53	3.27	4.90	4.50	1.68	4.80
18	1.63	2.91	6.36	4.43	4.99	6.35	5.28	2.92	4.83	4.10	2.16	4.70
19	2.43	3.64	6.08	4.50	4.76	6.58	5.48	2.17	6.14	4.75	2.17	4.92
20	2.35	3.38	5.14	4.50	5.23	6.98	5.82	2.20	5.09	3.40	2.59	4.90
21	2.72	2.84	5.67	4.21	4.31	8.25	5.38	0.95	4.86	4.45	1.70	4.55
22	2.30	3.06	5.49	4.63	4.16	6.07	4.79	2.25	5.18	4.30	1.77	4.34
23	2.67	3.74	5.68	5.71	4.60	7.60	5.73	1.92	5.31	4.05	2.10	4.90
24	1.83	2.79	5.89	3.86	5.41	6.50	5.52	3.00	5.87	4.65	2.19	4.95
25	3.63	3.27	6.42	5.14	5.68	8.43	5.98	3.00	6.36	4.70	1.22	5.43
26	1.86	3.12	6.17	3.50	5.26	6.78	5.86	3.00	6.02	5.45	2.53	5.12
27	1.95	3.43	5.58	5.29	4.94	6.72	6.00	2.92	6.22	4.75	2.35	5.12
28	2.27	2.69	6.13	3.64	4.74	6.71	5.77	2.25	5.54	4.50	2.71	4.85
29	1.20	3.74	6.28	3.50	5.55	6.76	5.66	2.83	5.29	5.60	2.02	5.05
30	2.69	3.48	5.48	5.14	5.01	6.25	5.35	3.09	5.84	5.15	1.74	4.90
31	1.99	3.59	5.73	5.73	5.38	7.29	5.98	2.69	5.34	4.40	2.31	5.16
32	1.20	3.26	6.62	4.21	5.54	7.04	5.96	3.17	5.36	4.55	2.14	5.15
33	2.08	3.42	5.48	5.00	5.64	6.88	6.21	2.92	5.82	5.10	1.82	5.28
34	0.72	3.44	5.57	4.36	5.04	6.96	5.95	2.58	5.69	4.30	2.02	4.96
35	2.45	3.63	6.04	5.71	5.42	7.77	6.68	3.09	5.72	5.85	1.97	5.33
36	1.16	3.75	6.08	5.07	5.61	7.51	6.33	2.68	5.74	5.50	1.87	5.37
37	1.77	3.23	6.14	4.57	4.94	5.91	5.84	2.27	4.65	5.85	2.33	4.88
38	2.71	2.76	5.72	3.71	4.95	6.26	5.92	2.50	5.21	4.65	2.31	4.88
39	3.34	3.94	5.72	4.00	5.94	7.74	5.75	3.17	5.61	5.85	2.19	5.34
40	1.04	3.86	6.89	3.86	5.57	7.11	5.90	3.27	5.46	4.95	2.46	5.23
41	1.57	3.84	5.58	4.00	5.05	7.55	5.66	2.92	6.62	5.65	1.56	5.10
42	2.56	3.28	6.00	5.36	5.05	5.92	6.13	2.92	5.68	5.15	2.12	5.15
43	1.10	2.78	7.15	6.00	4.49	6.43	5.52	2.25	5.47	4.70	2.02	4.81
44	2.54	3.06	5.40	5.79	4.90	7.18	5.36	2.37	5.82	4.00	2.47	4.85
45	1.09	3.05	4.90	5.17	4.67	7.49	5.66	2.35	4.67	4.35	1.89	4.65
46	1.83	3.20	5.08	1.99	4.56	6.92	5.62	2.75	5.43	1.65	1.60	4.51
47	1.10	3.34	5.77	3.21	4.68	7.00	6.38	2.59	5.06	5.55	2.16	4.97

(Cont'd on next page)

(Table 3. Average values cont'd...)

YIELD (TM/ha)												
Genotype	ARGENTINA	BANGLADESH	CHINA	EGYPT	INDIA	IRAN	MEXICO	NEPAL	PAKISTAN	SOUTHAFRI	SUDAN	Mean total
48	2.32	3.87	6.05	5.36	5.54	6.73	6.11	3.09	5.71	4.05	2.47	5.30
49	1.85	2.78	5.66	5.00	4.97	6.44	6.16	2.69	6.48	5.30	2.05	5.14
50	1.56	3.50	5.95	4.86	5.18	7.06	5.88	2.84	5.15	4.95	2.14	5.03
51	1.87	3.76	5.74	4.87	5.78	7.00	6.01	2.92	5.41	3.95	2.57	5.23
52	1.83	3.83	5.42	4.79	5.60	5.95	5.79	3.00	6.21	3.95	2.76	5.16
53	0.97	3.39	5.99	3.21	5.13	6.31	5.88	3.02	5.79	4.90	2.46	4.99
54	1.84	3.70	5.90	4.67	4.94	7.17	5.96	2.59	6.11	4.75	2.06	5.11
55	2.31	3.80	5.94	5.93	5.12	6.60	5.38	3.25	5.18	5.05	2.08	4.96
56	2.50	2.95	5.68	3.29	4.62	6.81	6.01	2.60	5.60	4.80	2.06	4.89
57	0.56	3.13	5.96	2.93	4.58	7.01	5.49	2.42	5.12	4.60	2.27	4.62
58	1.55	3.65	6.22	3.93	5.26	6.72	5.48	3.09	4.78	5.15	1.96	4.89
59	1.48	3.31	5.09	4.86	5.33	6.79	6.29	2.53	5.90	4.50	2.19	5.11
60	1.97	3.31	6.32	4.93	4.62	6.86	6.61	2.78	5.51	5.70	2.83	5.24
Mean	2.04	3.42	5.95	4.55	5.13	6.90	5.90	2.75	5.55	4.69	2.20	5.06
Genotypes	***	***	Ns	Ns	***	Ns	***	***	***	***	Ns	***
Environment	-	***	**	**	***	***	***	-	***	-	***	***
GxE	-	***	**	**	***	***	***	-	***	-	***	***
Std.Dev.	0.7585	0.5614	0.5475	0.8452	1.0679	0.2204	1.1684	0.4240	0.6131	0.7059	0.7059	0.7059

Table 4. Average values for yield from 60 genotypes grown in Mexico during 2011/2012 cycle under full irrigation according to th

GENOTYPE	GUANAJUATO	CHI HUAHUA	GUA DA LA JARA	IRA PUATO	MEXICAU	SONORA	TO LUCA	SINALOA	Total YLD
Number	YLD(TM/ha)	YLD(TM/ha)	YLD(TM/ha)	YLD(TM/ha)	YLD(TM/ha)	YLD(TM/ha)	YLD(TM/ha)	YLD(TM/ha)	(TM/ha)
1	8.85	7.25	5.85	7.84	2.55	6.92	4.85	5.46	6.20
2	6.41	6.10	5.27	5.86	7.18	6.32	6.10	4.25	5.93
3	5.67	6.53	5.26	5.63	7.53	6.31	6.82	4.39	6.02
4	7.31	6.73	5.42	6.22	6.34	6.59	6.39	5.02	6.25
5	8.05	6.15	4.81	6.10	5.75	6.45	6.20	4.04	5.94
6	6.80	6.03	6.02	7.41	5.54	6.60	6.39	4.52	6.16
7	7.28	5.62	4.99	6.27	5.92	6.42	7.47	4.32	6.03
8	8.02	6.27	5.18	6.92	5.44	7.17	8.07	4.64	6.46
9	6.62	5.81	5.18	5.82	5.91	6.57	6.89	4.63	5.93
10	6.03	5.60	5.36	5.99	8.33	6.85	7.59	4.84	6.32
11	6.73	6.13	5.44	5.67	6.41	6.16	7.49	4.39	6.05
12	8.17	5.52	6.21	7.30	4.37	7.38	6.47	5.23	6.33
13	6.86	6.59	5.31	7.04	4.33	7.04	7.51	4.63	6.16
14	7.89	5.82	6.49	6.82	4.35	6.94	7.23	5.05	6.32
15	8.08	5.86	4.66	7.02	5.70	7.47	6.42	4.62	6.23
16	7.64	6.27	3.88	4.62	5.65	6.41	7.03	4.20	5.71
17	6.91	6.38	4.81	5.53	3.99	6.28	6.72	3.63	5.53
18	5.23	4.87	4.46	6.22	3.94	6.42	6.76	4.36	5.28
19	6.30	4.66	4.38	5.66	5.43	6.21	6.88	4.30	5.48
20	7.92	6.35	3.69	6.56	2.68	7.64	6.32	5.42	5.82
21	5.40	6.69	4.46	5.96	5.85	5.59	4.12	4.99	5.38
22	6.01	4.50	4.01	6.54	2.59	5.50	6.39	2.80	4.79
23	7.23	7.10	4.31	5.78	5.23	6.47	5.64	4.10	5.73
24	7.16	5.23	3.48	6.34	4.77	6.59	6.49	4.15	5.52
25	6.36	6.07	4.23	7.21	6.07	7.17	6.59	4.15	5.98
26	7.40	6.25	3.54	7.60	6.00	6.70	6.26	3.18	5.86
27	7.24	5.81	5.58	6.12	4.43	7.29	7.15	4.37	6.00
28	5.42	6.71	6.82	5.98	5.35	5.58	6.16	4.14	5.77
29	7.87	5.82	5.05	5.84	3.58	6.44	6.47	4.24	5.66
30	7.25	4.60	3.72	6.34	4.74	6.27	5.57	4.29	5.35
31	6.71	5.92	5.05	6.45	4.88	6.93	6.75	5.17	5.98
32	6.70	5.95	5.92	6.94	3.70	6.78	7.42	4.32	5.96
33	8.71	5.93	6.02	6.39	4.58	7.54	6.20	4.34	6.21
34	7.17	5.31	4.66	6.81	5.18	6.44	7.06	4.99	5.95
35	7.52	6.75	6.41	7.09	6.45	6.94	6.91	5.39	6.68

(Cont'd on next page)

(Table 4. Average values for yield cont'd...)

GENOTYPE Number	GUANAJUATO YLD(TM/ha)	CHIHUAHUA YLD(TM/ha)	GUADALAJARA YLD(TM/ha)	IRAPUATO YLD(TM/ha)	MEXICALI YLD(TM/ha)	SONORA YLD(TM/ha)	TOLUCA YLD(TM/ha)	SINALOA YLD(TM/ha)	Total YLD (TM/ha)
36	6.29	5.55	6.48	7.66	6.86	6.66	6.90	4.28	6.33
37	4.98	5.96	5.48	7.32	7.24	5.94	6.15	3.64	5.84
38	9.14	4.61	5.05	6.41	4.63	6.85	5.59	5.11	5.92
39	6.54	5.36	5.28	7.04	2.77	7.14	6.61	5.28	5.75
40	6.45	6.05	4.53	7.72	5.21	5.72	7.19	4.34	5.90
41	6.35	4.99	4.31	7.31	5.10	6.62	6.32	4.33	5.66
42	7.79	6.15	5.91	6.87	4.89	6.19	6.86	4.37	6.13
43	6.38	6.73	5.81	5.10	4.94	6.24	5.39	3.61	5.52
44	6.89	5.27	4.53	5.84	4.69	6.95	4.37	4.33	5.36
45	5.50	6.28	4.75	6.38	5.84	6.21	5.22	5.13	5.66
46	5.35	5.14	4.64	6.80	6.46	6.30	5.39	4.87	5.62
47	7.44	6.52	5.39	7.31	6.46	6.72	6.49	4.74	6.38
48	7.88	5.13	5.01	7.50	5.35	6.84	6.17	5.00	6.11
49	7.64	5.80	5.13	6.71	6.35	6.72	6.15	4.74	6.16
50	6.22	5.77	5.33	6.09	4.57	7.13	6.89	5.07	5.88
51	7.05	6.24	5.50	7.95	4.53	6.70	5.60	4.48	6.01
52	6.94	5.46	4.78	7.14	4.48	6.85	6.13	4.52	5.79
53	7.55	5.57	4.51	7.13	4.13	6.63	6.38	5.14	5.88
54	6.45	5.52	4.60	7.02	5.68	6.56	6.95	4.90	5.96
55	6.23	6.00	4.61	6.13	3.83	5.97	6.04	4.24	5.38
56	7.73	6.18	3.60	6.24	6.76	5.75	6.82	5.00	6.01
57	6.50	5.86	3.35	5.84	5.53	6.11	6.94	3.77	5.49
58	6.33	5.43	3.76	5.98	6.51	5.80	5.25	4.80	5.48
59	7.89	6.01	4.95	7.65	3.47	6.55	9.37	4.51	6.29
60	7.92	7.50	5.50	8.04	5.58	6.98	6.86	4.54	6.61
Mean	6.97	5.90	4.98	6.58	5.21	6.57	6.48	4.52	5.90
C.V.	0.92	0.64	0.80	0.74	1.22	0.49	0.84	0.53	0.35
P level	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

## Conclusions

ME 7B and 10A showed the highest yield, with 6.90 and 6.49 TM/ha, respectively. Results shown that Mexico wheat yields are among the highest around the 10 analyzed ME, especially those located in the ME 1 and 2A, as Guanajuato and Sonora presented yields over 6.90 and 6.67 TM/ha respectively, exceeded only by Iran and Pakistan with 6.90 and 7.77 TM/ha, respectively. Therefore, the Yaqui Valley in Sonora, Mexico, represents one of the best platforms for wheat research and physiological breeding.

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# The CSISA wheat phenotyping network

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## Introduction

The Cereal Systems Initiative for South Asia (CSISA) was established in 2009 to support regional and national efforts on improving cereal production growth in South Asia's most important grain baskets. In recent years the annual growth of cereal production in South Asia has slowed in terms of both grain yields and residue production, with rates two to three times higher during 1966-94 than during 1995-2005, whilst demand continues to rise. New input-responsive wheat varieties are demanded which will have higher yield potential along with being well buffered against increasing heat and drought stresses (resulting from climatic change and receding water tables), which are serious challenges to enhancing wheat production to maintain food security across this region.

## Description of activities related to physiological breeding

CIMMYT's cooperative evaluation network established across South Asia has been used to distribute a large number of new wheat lines for breeding programs and for the characterisation of various agronomic and physiological traits. These physiological data collected by scientists have been used to augment selection criteria in national breeding programs, whilst feeding back valuable information to CIMMYT's wheat physiology program. Scientists in NARS programs across the region have been actively encouraged to implement physiological breeding approaches through the distribution of instruments (principally infrared thermometers and chlorophyll meters), the publication of two new phenotyping manuals by CIMMYT in 2012, and a program of onsite training, lectures and workshops by CIMMYT scientists.

## Objectives

Extensive field-based testing across South Asia is being performed to select and cultivate high-yielding and stress-tolerant wheat varieties using physiological breeding approaches, with the main objectives to: (1) establish physiological and

environmental limitations to yield, including (a) the identification of phenological stages sensitive to heat and drought stress, and (b) dissection of the expression of genotype and trait by environment interaction; (2) optimize a range of heat- and drought-adaptive traits to increase yield potential; (3) characterize potential parents for strategic crosses directed at specific target environments; (4) develop phenotyping tools for early-generation selection; (5) screen genetic resources at hot spots for heat and drought to be used to develop germplasm with better resilience to climate changes; and (6) identify (a) which genomic regions are most likely to provide marker-assisted selection in conventional gene pools and (b) potential allelic diversity among genetic resources for adaptation to future hotter and drier climates.

## Plant material

Within CSISA, five international trials have been prioritised for physiological characterization:

1. Semi-Arid Wheat Yield Trial (SAWYT) used to establish physiological and environmental limitations to yield using stable lines under drought stress grown under limited irrigation; the 17<sup>th</sup> SAWYT grown in 2010 included advanced lines developed at CIMMYT using physiological criteria to design complementary crosses.
2. Stress Adaptive Trait Yield nursery (SATYN) constituting lines selected from genetic resource collections that show favourable expression of heat adaptive traits; these are planted through late sowing to increase exposure to heat stress
3. Wheat Association Mapping Initiative (WAMI) used to characterise lines for heat and drought stress tolerance traits and QTL identification for fine marker development and gene discovery (see Lopes and Reynolds, these proceedings)
4. CIMMYT core-germplasm (CIMCOG) panel including lines identified with high biomass, and high yield component expression under optimal conditions of sowing and irrigation
5. 1st Wheat Yield Consortium Yield Trial (1st WYCYT), a panel of 30 new advanced lines encompassing high yield and/or biomass -based on strategic crosses to combine complementary source and sink traits.

## Growing conditions

Of the trials sown to date, the most relevant to improving yield potential has been the CIMCOG trial grown at 12 locations in 2012 across South Asia (comprising Bangladesh, India, Nepal, and Pakistan; see Table 1 and Figure 1). Experiments were conducted in the spring wheat season to correspond with recommended sowing (November to early December) and harvesting (March to April) times depending on individual environments. At all sites, appropriate irrigation and fertilization was implemented to avoid yield limitations, whilst weed, disease, and pest control was applied according to local best practice. Experimental design was a randomized alpha lattice of 60 entries with two replications per entry giving a total of 120 plots. Plants were sown on flat beds of 4-6 m long with 4 to 6 crop rows (20 to 25 cm between rows), providing a total harvestable area of around 4-7 m<sup>2</sup>. A local check representing the best locally adapted germplasm was included at each site, this entry varied among locations. The 1st WYCYT is being grown in the current wheat cycle.



Figure 1. Map of CIMCOG testing locations.

Table 1. Locations and NARS partners used for CSISA physiological trials in South Asia.

Country	City	Institution	Principal scientist	Contact email
1 Bangladesh	Joydebpur	Bangladesh Agricultural Research Institute	Dr. Naresh Chandra Deb Barma	ncdbarma@gmail.com
2 Bangladesh	Jessore	Bangladesh Agricultural Research Institute	Mr. MahubRahman	mahubwrc@yahoo.com
3 India	Delhi	Indian Agricultural Research Institute	Dr. Gyanendra P Singh	gyanendrapsingh@hotmail.com
4 India	Dharwad	University of Agricultural Sciences, Dharwad	Dr. Ishwar K. Kalappanavar	ikkyashu@gmail.com
5 India	Indore	IARI Regional Research Station	Dr. Venkata Sai Prasad Sukaru	sprasad98@yahoo.com
6 India	Karnal	Directorate of Wheat Research	Dr. Ravish Chatrath	r.chatrath@gmail.com
7 India	Ludhiana	Punjab Agricultural University	Dr. Virinder Singh Sohu	sohuvs@yahoo.com
8 India	Ugar	University of Agricultural Sciences, Dharwad	Dr. Ishwar K. Kalappanavar	ikkyashu@gmail.com
9 India	Varanasi	Banaras Hindu University, Inst. of Agri. Sci.	Dr. Vinod K. Mishra	vkmbhu@gmail.com
10 Nepal	Bhairahwa	Nepal Agriculture Research Council	Dr. Janmjai Tripathi	tripathijanmejai@yahoo.com
11 Pakistan	Islamabad	Pakistan Agricultural Research Council	Dr. Muhammad Yaqub Mujahid	yaqubmujahid@hotmail.com
12 Pakistan	Faisalabad	Ayub Agricultural Research Institute	Dr. Makhdoom Hussain	makhdoomhussain@yahoo.com

All sites are high radiation environments for which climatic data are summarised in Table 2. Data for sites were collected on a daily basis by meteorological stations located within a range of 1 km and at a similar altitude, minimum, maximum, and mean air temperatures, and mean relative humidity.

### Agronomic and physiological measurements

The number of days to heading (DH) was recorded as the number of days for more than 50% of plants to exhibit heading (Zadoks stage 59, Zadoks et al. 1974). Shortly after heading and at around anthesis, the chlorophyll content of the flag leaf (CHL; in chlorophyll concentration index units) was measured in three leaves per plot using a hand-held field portable chlorophyll meter (e.g. SPAD-502 Minolta; Spectrum Technologies Inc., Plainfield, IL, USA). Canopy temperature was measured several times

during pre-heading (CTV) and during grain-filling (CTG) using a portable infrared thermometer (e.g. Sixth Sense LT300, Sixth Sense Technologies, USA). When all plots reached physiological maturity, plant height (PH) was determined by measuring the distance between the base of the stem and the top of the spike excluding awns. Grain yield, grains per square metre (GNO) and thousand kernel weight (TKW) were determined using standard protocols (see Pask et al. 2012).

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Table 2. CIMCOG grain yields and long-term mean weather data for the period 01 November to 30 April (ten years; 2001/2-2010/11). Values shown are the minimum, maximum, and mean air temperatures, and mean relative humidity.

Country	City	Yield (t/ha)	Days to heading	T_min	T_max	T_mean	RH (%)	
1	Bangladesh	Joydebpur	3.66	68	14.7	27.8	21.3	84.1
2	Bangladesh	Jessore	3.08	72	17.6	29.0	22.8	64.3
3	India	Delhi	6.30	92	11.5	26.6	19.6	62.4
4	India	Dharwad	2.82	66	16.9	32.1	24.5	54.2
5	India	Indore	4.33	69	14.1	31.0	22.5	75.7
6	India	Karnal	4.59	101	11.0	25.6	18.3	65.9
7	India	Ludhiana	7.34	106	10.9	25.2	18.1	67.3
8	India	Ugar	5.27	67	21.9	31.9	26.9	70.1
9	India	Varanasi	4.14	77	14.1	28.6	21.4	56.0
10	Nepal	Bhairahwa	2.58	66	13.3	28.0	20.7	81.1
11	Pakistan	Islamabad	5.52	139	7.4	23.0	15.2	66.1
12	Pakistan	Faisalabad	1.50	106	-	-	-	-
		<b>mean</b>	<b>4.32</b>	<b>87</b>	<b>13.9</b>	<b>28.1</b>	<b>21.0</b>	<b>67.9</b>

\*Data for Faisalabad, Pakistan not available at time of printing.

# Gene Discovery Platforms

## Advances in QTL discovery using two wheat populations designed to minimize confounding agronomic effects

Marta Lopes and Matthew Reynolds  
CIMMYT

### Summary

There has been difficulty in identifying consistent QTL for yield, not only in wheat but also in maize (Lu et al. 2012). In wheat especially, the exercise of identifying QTL has been complicated by confounding effects of phenology, whereby genes of major effect have been shown to mask the identification of minor effects (Reynolds et al. 2009). Most adaptive traits interact with agronomic traits, so the precision of QTL discovery will be affected by the degree to which experimental populations are controlled for heritable traits like phenology and plant height (Pinto et al. 2010; Lopes et al. 2013). For this reason, CIMMYT started developing biparental populations whose progeny showed a relatively restricted range of these traits, starting with the elite by elite cross Seri/Babax. Subsequently, advanced lines were selected from CIMMYT's international nurseries to form the Wheat Association Mapping Initiative (WAMI) panel. These populations have been phenotyped internationally (see Sukumaran et al. these proceedings), and while the emphasis has been on heat and drought adapted environments, both populations are well suited to genetic dissection of yield potential traits since they constitute elite high yielding lines and their progeny.

### Results and Discussion

Yield can be dissected into its physiological and developmental components over the course of the wheat crop cycle, including adaptive strategies to cope with drought and heat stress. For example, earliness has been recognized as one of the most important strategies for optimizing water use and escaping terminal heat stress. Other traits such as cooler canopy temperatures (CT) associated with deeper roots, stay-green associated with robust delayed senescence, and spike fertility, also significantly contribute to yield under stress conditions. Adaptive traits were measured for both populations in trials conducted in Mexico and several countries in South Asia and the WANA (West Asia and North Africa) region. Genotyping on the SBS population has been developed in McIntyre et al. (2010) and additional SSR markers have been added to the previous map (Lopes et al. 2013). Extensive genotyping has been developed on the

WAMI population with DArT and SNP markers, and Genotyping by Sequencing (GBS) markers will soon be available.

Different QTL were associated with grain number and size, indicating the potential for improvement of both traits. However, pleiotropy was observed for QTL regulating both grain size and days to heading. CT can be measured early in the cycle and is linked or pleiotropic to yield genes. Stay-green or lower senescence rate showed lower genetic complexity. Some QTL were common to stay-green and CT and this was possibly related to increased water extraction capacity in more stay-green phenotypes. Prediction accuracy of yield was improved when using marker scores of component traits.

In the Seri/Babax population, a yield QTL located on 4A-a explained 27 and 17% of variation under drought and heat stress, respectively (Pinto et al. 2010; Lopes et al. 2013). At the same location, a QTL explained 28% of the variation in CT under heat, while 14% of CT variation under drought was explained by a QTL on 3B-b. Common QTL for drought and heat stress traits were identified on 1B-a, 2B-a, 3B-b, 4A-a, 4B-b, and 7A-a, confirming their generic value across stresses (Pinto et al. 2010).

### Next Steps

These and other populations (see Sukumaran et al. these proceedings) that have been developed explicitly for genetic dissection of complex traits (by controlling genes of major effect) will be deployed to collaborators in high yield potential environments for precision phenotyping and genetic analysis.

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# Phenotyping and genotyping of an elite wheat double haploid population: grain yield strategies in contrasting environments and QTL analysis

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## Summary

This work describes the physiological performance of an elite wheat mapping population derived from modern cultivars with similar phenology but different and stable combinations of grain number per unit area (GN) and grain weight (GW), which was grown in four contrasting environments. The population has been genotyped using 322 genetic markers: 151 DArT markers, 96 SSR markers, 66 SNP markers, and seven gene based markers. Of these markers, 272 could be put into linkage groups, resulting in a map length of 1191 cM. All wheat chromosomes, except 5D, are represented in the map. An important phenotypic variation was observed in physiological and numerical yield components, in combination with favorable expression of all agronomic traits. Therefore, this population represents a valuable resource for fine phenotyping in order to understand the physiological and genetic bases of key traits linked with grain yield.

## Results and Discussion

This work synthesizes the main results obtained from the phenotyping of an elite wheat population in four environments: Buenos Aires 2009 (BA), Ciudad Obregon 2009/10 (CO), and Valdivia 2008/09 (V1) and 2009/10 (V2). This population (105 DH lines) was derived from the spring cultivars Bacanora and Weebil at CIMMYT and genetically characterized by the John Innes Centre. All field trials were conducted without water, nutritional, or biotic constraints. The phenotyping was based on two simple approaches to understand grain yield variations: i) above ground dry matter (AGDM) and harvest index (HI), and/or ii) GN and GW. Other related traits were also considered.

Before discussing the strategies for grain yield potential, it is important to consider the variations in flowering time and plant height among individuals within the population which should be reduced in order to obtain significant results for breeding (Reynolds et al. 2009). Although parental lines were elite cultivars with similar flowering

time, transgressive segregation was expressed not only in this trait, but also in plant height. However, in all environments, the flowering time range of 50% of DH lines (i.e. DH lines included between the first and third quartile) was lower than five days and plant height of whole population was within the optimal range for grain yield (ca. between 0.7 and 1 m; Miralles and Slafer 1995; Table 1).

Main effect of environment on grain yield was significant ( $p < 0.0001$ ) but the genotypic component was larger than genotype by environment interaction (30%). DH population showed transgressive segregation in grain yield and its components at each environment (Table 1). Both grain yield and biomass production were similar between parental lines. These elite cultivars showed similar values of HI at each trial. As expected, Bacanora set a greater GN in all environments, while Weebil had heavier grains in agreement with the basis for the initial choice as parents.

Across all environments, a significant and positive relationship between grain yield and biomass was observed ( $r^2 > 0.82$ ,  $p < 0.01$ ). This robust and positive relationship was consistent with the suggestion that a genetic gain in biomass production is a way to improve wheat grain yield potential (Calderini et al. 1999; Reynolds et al. 2009). However, a necessary condition is to maintain the current levels of partitioning to grain (Miralles and Slafer 2007). In this way, HI ranged from 0.34-0.57 (Table 1) and the maximum HI were higher than the maximum values reported in modern spring wheat cultivars (ca. 0.45; Fischer 2007). These results reinforce the idea that enhancing biomass production within optimal plant height range, while avoiding penalties in HI, is a viable way to genetically increase grain yield (Miralles and Slafer 2007). In this line, different evidences suggest that improvements in biomass production in current wheat germplasm will most likely be achieved by focusing on radiation use efficiency (RUE) rather than on radiation interception (Parry et al. 2011). On the other hand, and as expected, grain yield

**Table 1. Descriptive statistic of time to anthesis (At), plant height (PH), above ground dry matter (AGDM), harvest index (HI), grain number (GN), and grain weight (GW) for double haploid (DH) population and parental lines (Bacanora and Weebil) in the different environments.**

			At(°Cd)	PH(m)	GY(g m <sup>-2</sup> )	AGDM(g m <sup>-2</sup> )	HI	GN (grains m <sup>-2</sup> )	GW(mg)
Buenos Aires 2009	DH population	min	1081	0.59	438	1125	0.34	12497	24
		Q1	1145	0.72	617	1478	0.41	19929	29
		mean	1179	0.76	683	1610	0.42	21726	32
		Q3	1213	0.80	772	1758	0.44	23852	34
		max	1365	0.91	977	2161	0.48	29352	43
	Bacanora	mean	1161	0.70	713	1672	0.42	25261	28
	Weebil	mean	1162	0.82	663	1533	0.43	17221	38
Obregon 2009/10	DH population	min	1163	0.69	482	975	0.42	10887	31
		Q1	1295	0.81	545	1145	0.46	13659	37
		mean	1335	0.84	583	1242	0.47	14899	39
		Q3	1388	0.86	618	1318	0.48	16135	42
		max	1479	0.94	718	1575	0.51	19341	47
	Bacanora	mean	1280	0.79	583	1190	0.49	14972	39
	Weebil	mean	1282	0.88	589	1246	0.47	12868	46
Valdivia 2008/09	DH population	min	840	0.70	591	1705	0.35	14196	35
		Q1	872	0.79	1105	2246	0.48	24592	42
		mean	913	0.82	1192	2396	0.50	27177	44
		Q3	946	0.86	1295	2539	0.52	30119	47
		max	1030	0.95	1504	3166	0.57	37132	55
	Bacanora	mean	864	0.75	1174	2226	0.53	30144	39
	Weebil	mean	927	0.84	1289	2444	0.53	28410	45
Valdivia 2009/10	DH population	min	803	0.63	608	1658	0.37	14684	35
		Q1	854	0.72	1127	2356	0.47	24760	42
		mean	885	0.76	1215	2510	0.48	27303	45
		Q3	909	0.79	1324	2667	0.51	29783	47
		max	984	0.86	1617	3309	0.56	37723	57
	Bacanora	mean	850	0.72	1180	2406	0.49	30719	39
	Weebil	mean	905	0.81	1271	2630	0.49	27645	46

Minimum (min), maximum (max), mean, and first (Q1) and third (Q3) quartiles values are included.

was mainly explained by changes in GN across all environments ( $r^2 > 0.51$ ,  $p < 0.01$ ), rather than in GW, highlighting the importance of GN for grain yield potential improvements (Fischer 2008). However, in this population, GW seemed to be more plastic than generally reported (Sadras 2007), with similar relative variability as for GN. Similarly, GN and GW tended to be negatively associated, but this trade-off was not completely competitive, indicating that more grains would increase grain yield without important reductions in GW.

To analyze the population grain yield strategy, two mega-environments (ME) were defined: (i) relative low-yielding ME including CO and BA and (ii) relative high-yielding ME including Val-1 and Val-2. In general terms, the main trait relationships

within each ME were similar to the general trends across environments (Table 2). Thus, above ground dry matter (AGDM) was the variable that better explained the variations in grain yield in both ME, while HI was associated with grain yield only in the high-yielding ME. Variations in grain yield were better associated with GN in the high than in the low-yielding ME. In addition, both numerical components were more negatively correlated in low than in high-yielding ME. Variations in GN were strongly associated with grain number per spike in high yielding ME, although in low-yielding ME both traits were poorly correlated, and GW was positively associated to grain filling rate in both ME. It is important to highlight that flowering time and plant height did not explain the differences observed in grain yield in any ME.

In line with the proposal of optimizing developmental pattern to maximize spike fertility (Foulkes et al. 2011), a longer stem elongation phase relative to anthesis (SEPr) has been proposed as a way to increase the spike dry weight and, in turn, GN and grain yield potential (Slafer et al. 2001; Miralles and Slafer 2007). However, no close relationship was observed between GN and SEPr in the population evaluated here (Table 2), suggesting that a longer SEPr is a subtle way to increase spike dry weight, resulting in unclear effects (García et al. 2011). Other traits related to GN set, according to the physiological approach proposed by Fischer (1983; 2008), were evaluated in BA and CO. In this case, the fruiting efficiency (i.e. grains per gram of spike at flowering) was the main GN physiological component and an important variability in this trait was registered in both environments (data not shown).

After population phenotyping trials, an extra experiment was carried out in Valdivia (Bustos et al. 2013). In this case, the two highest yielding DH lines recorded in V1 and V2 were evaluated together with two local cultivars (Pandora INIA and Invento-BAER). Similar phenology was recorded in the evaluated genotypes (Table 3). Both DH lines showed higher yield than the Chilean cultivars ( $p < 0.01$ ), being on average 45% higher than the mean of the checks (Table 3). In spite of the grain yield consistency of the DH lines, different strategies were observed between them to achieve the higher yield. For instance, DH2 gain was reached mainly by higher biomass, while HI was the key trait in DH1 (Table 3). DH2 also showed higher HI than Pandora-INIA but similar to Invento-BAER. QTL analysis for grain weight was carried out in Valdivia using 252 genetic markers (SSR and DArT) during

**Table 2. Correlation coefficients obtained from a multivariate analysis in low and high-yielding mega environments between different variables: Grain yield (GY), time to anthesis (At), plant height (PH), above ground dry matter (AGDM), grains per unit area (GN), spike number (SN), grains per spike (GNS), stem elongation phase relative to anthesis (rSEP), grain weight (GW), grain filling rate (GFR), and grain filling duration (GFD).**

		High-yielding mega environment											
		GY	At	PH	AGDM	HI	GN	SN	GNS	rSEP	GW	GFD	GFR
Low-yielding mega environment	GY		0.22*	0.29*	0.92*	0.82*	0.82*	0.00	0.79*	0.19*	0.03	0.1	0.00
	At	0.09		0.41*	0.32*	0.01	0.32*	0.00	0.38*	0.45*	-0.25*	0.26*	-0.25*
	PH	-0.01	0.29*		0.46*	-0.03	0.21*	0.00	0.25*	0.32*	0.05	0.13	0.00
	AGDM	0.89*	0.31*	0.15		0.54*	0.76*	0.00	0.73*	0.29*	0.03	0.12	0.00
	HI	0.27*	-0.37*	-0.23*	-0.15		0.68*	0.00	0.65*	-0.01	0.03	0.03	0.00
	GN	0.55*	0.31*	-0.02	0.7	-0.22*		0.00	0.94*	0.37	-0.53*	0.13	-0.32*
	SN	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00
	GNS	0.22*	0.29*	0.11	0.17	0.32*	0.35*	0.00		0.37*	-0.49*	0.15	-0.32*
	rSEP	0.23*	0.27*	0.33*	0.4	-0.30*	0.44*	0.00	0.22*		-0.37*	0.22*	-0.36*
	GW	0.10	-0.27*	0.06	-0.13	0.55*	-0.70*	0.00	-0.13	-0.34*		-0.08	0.60*
	GFD	0.08	0.09	-0.06	0.09	-0.03	0.03	0.00	0.05	0.03	0.01		-0.73*
	GFR	0.03	-0.31*	0.07	-0.17	0.47*	-0.57*	0.00	-0.16	-0.29*	0.78*	-0.59*	

Asterisks indicate significant values at  $p < 0.05$ .

**Table 3. Grain yield (GY), above ground dry matter (AGDM), harvest index (HI), grain number per area unit (GN), grain weight (GW), plant height, and days to anthesis (At) recorded in the doubled haploid lines (DH) selected from previous phenotyping in Valdivia as well as in the check cultivars Pandora-INIA and Invento-BAER. Genotypes are shown according with the ranking of grain yield.**

Genotype	GY(g m <sup>-2</sup> )	AGDM(g m <sup>-2</sup> )	HI	GN m <sup>-2</sup>	GW(mg)	At(days)	PH(m)
DH2	1656 a	3573 a	0.47 ab	38829 a	42.7 d	100	0.82 ab
DH1	1524 a	2990 b	0.51 a	33502 a	45.5 c	93	0.75 cd
Pandora-INIA	1116 a	2684 b	0.41 c	23656 b	47.2 b	98	0.79 bc
Invento-BAER	1075 b	2516 b	0.43 bc	20876 b	51.5 a	100	0.85 a
	**	*	**	***	***	n.s.	***
S.E.M	84.4	142	0.01	2338	1.05		1.2

Different letters indicate significant differences (LSD test,  $p < 0.05$ ) between genotypes. n.s., \*, \*\* and \*\*\* mean no significant difference or difference at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively.

two growing seasons and under four growing conditions at each season. Several markers for both individual grain weight and grain length were found on chromosome 6A and especially on 7Bb (Hasan 2011; Calderini et al. 2013).

### Next Steps

To maintain or even improve current levels of expression for HI, extra assimilates must be partitioned to more grains and/or higher potential GW (Reynolds et al. 2009). In this line, the next steps should be related to fine-tuning studies in this elite population including aspects as fertile flowers establishment, for improving GN and its trade-off with GW. Other physiological traits that should be more explored, to evaluate the possibility to use in breeding programs, are fruiting efficiency and RUE during the critical period.

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# Wheat mapping populations available at CIMMYT for yield potential research

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## Summary

The physiological breeding group in CIMMYT has assembled a number of wheat mapping populations for characterizing yield potential and adaptation of wheat lines to climate change, especially drought and heat stress. Phenotyping and genotyping of these populations were done in collaboration with public and private institutions worldwide. Mapping populations developed in CIMMYT consist of populations assembled for screening purposes and for dissecting the genetic basis of simple as well as complex traits through linkage mapping and association mapping. The linkage mapping populations are from bi-parental crosses of contrasting phenotypes that are created to select lines and molecular markers for adaptation to heat, drought, and irrigated environments (eg. Seri/Babax). An association mapping panel of WAMI (wheat association mapping initiative) consists of lines selected from the CIMMYT program based on 30 years of research data. A key emphasis in the development of these populations has been the

recognition of confounding effects of flowering time differences. For this reason, most of the populations including bi-parental and diversity panels were selected for a narrow range in phenology. Extensive multi-location characterization data already exists for many of these populations including data on physiological component traits relevant to yield potential and yield under drought. Genotyping of these populations has been conducted in collaboration with Seeds of Discovery (<http://seedsofdiscovery.org/>) using simple sequence repeats (SSR), Diversity Array Technology (DArT), and genotyping-by-sequencing (GBS). A description of the CIMMYT populations available with its characteristics, excerpt on genotyping and phenotyping, and markers identified are provided in the tables below.

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 McIntyre, C.L. et al. 2010. *Theoretical & Applied Genetics* 120:527-541.  
 Pinto, R.S. et al. 2010. *Theoretical & Applied Genetics* 121:1001–1021.

**Table 1. Summary of mapping populations**

Cross/ Population	Characteristics	Environments	Genotyping
Seri/Babax	Elite × Elite cross, Phenology Controlled RILs	YP, MH, IH, and D	SSR + GBS markers
Wheat Association Mapping Initiative (WAMI)	Advanced lines, phenology controlled	RF, IR, and H	DArT, SNP (90K Chip), GBS underway
Atil/Dicoccum	RILs, high yielding wide cross with heat tolerant parents, phenology controlled	IR and H	DArT and SSR
Sokoll/Weebil1	PT crosses (drought) progeny excelled in international yield trials (17 SAWYT)	YP, H, D	Will be mapped by Limagrain and GBS
Pastor//HXL7573/2*Bagula/3/Weebil1	PT crosses (drought) progeny excelled in international yield trials (17 SAWYT)	YP, H, D	Will be mapped by Limagrain and GBS
Vorb//Parus/Pastor	PT crosses (drought) progeny excelled in international yield trials (17 SAWYT)	YP, H, D	Will be mapped by Limagrain and GBS

*(cont'd on next page)*

**Table 1. Summary of mapping populations cont'd...**

<b>Cross/ Population</b>	<b>Characteristics</b>	<b>Environments</b>	<b>Genotyping</b>
Jnrb.5/Pifed/5/Bjy/ Coc//Prl/Bow/3/Sara/ Thb//Vee/4/ Pifed	Screened 200 for lines with similar anthesis date and GxE for anthesis, Diagnostic markers, crossed best matched, phenology 10-12 days.	H	GBS
KS940935.7.1.2/2*Pastor/4/ Frame//Milan/Kauz/3/Pastor	Heading date and diagnostic markers	H	GBS
Worrakatta/2*Pastor/6/ Chir3/4/Siren//Altar84/ Ae.squarrosa(205)/3/3*Buc/ 5/ Pfau/ Weaver	Heading date and diagnostic markers	H	GBS
Sokoll/Westonia	Heading date and diagnostic markers	H	GBS
Kauz/Weebil	Kauz= high yield and biomass, small spikes, and small grains; Weebil= high yield, high biomass, large grains, and large spikes, Broke yield barrier in Chile	YP	Mapped at JIC by Snape and Griffiths
Dicoccum/Sooty/Rascon	Wide cross between heat tolerant lines, contrast in spike photosynthesis	H	DArT, Mapped at ACPFG (Diane Mather & Dan Mullan)
Croc_1/Ae.Squarrosa (224)//Opata/3/Weebil1	Three day phenology range, good for heat + drought treatments as not confounded by phenology	YP, H, D, and IR	SSR, GBS, 90K SNP
Synthetic/Opata	ITMI populations plus four DH populations, semi-dwarf in height and early flowering.	H, D, and IR	GBS genotyping at Kansas State, 90 lines Diversity Array GBS
Genomic Selection population	Lines included the 17th and 18th SAWYT, Candidates for the 29th, and 31st SAWSN and Candidates 30th for the 45th and 46th IBWSN, 2600 lines	YP	Diversity Array and Kansas State GBS platforms
3D NAM	Forty four synthetics crossed with a set of elite, mostly CIMMYT bread wheat, phenology controlled, 5000 lines	H, D, and IR	GBS
Pavon-rye introgression lines	Single arm translocations, 8 different rye chromosome arms, various rye sources, translocations of the same rye arm onto a different wheat homologue, 67 lines	YP	C-banding, SSRs specific for rye chromosome arms

RIL = Recombinant inbred lines; DArT = Diversity Array Technology; SNP = Single nucleotide polymorphism; GBS = Genotyping by sequencing; JIC = John Innes Centre, UK; ITMI = International Triticeae mapping initiative; YP = Yield potential; MH = Moderate heat stress; IH = Intense heat stress; D= Drought stress; IR = Irrigated; H = Heat stress; RF= Rainfed; SAWYT = Semi-arid wheat yield trial; 3DNAM = Three-dimensional nested association mapping populations

**Table 2. WAMI: Environments grown (Lopes et al. 2012)**

<b>Country</b>	<b>Year</b>	<b>Location</b>	<b>Environment</b>
Iran	2009-10	Ahwaz	YP
		Darab	YP
Sudan	2009-10	Dongola	YP
		Hudeiba	YP
		Wad Medani	YP
Egypt	2009-10	Sohag	YP
		El Mataana	YP
Bangladesh	2009-10	Gazipur	YP
India	2009-10	Ludihana	YP
		Varanasi	YP
		Karnal	YP
		Dharwad	YP
		Delhi	YP
		Bha	YP
		Ludihana	YP
		Varanasi	YP
		Karnal	YP
		Dharwad	YP
Pakistan	2009-10	Islamabad	YP
	2010-11	Islamabad	YP
Ethiopia	2011	Melkassa	Wet and dry soils
Mexico	2009-10	Obregon	IR
		Obregon	D
		Obregon	H
		Obregon	HD
USA	2010-11	Greeley	IR
		Greeley	IR and RF
USA	2010-11	College Station, Texas	IR
		College Station, Texas	Drought stress at flowering
	2010-11	Uvalde, Texas	IR
		Uvalde, Texas	Drought stress at flowering
	2010-11	Chillicothe	IR
		Chillicothe	Drought stress at flowering

YP = Yield potential; D= Drought stress; H = Heat stress; RF= Rainfed; IR = Irrigated

**Table 3. WAMI: Markers identified through association analysis**

<b>Trait</b>	<b>Markers Identified</b>
GY	rPt0079, rPt2869, rPt2940, rPt3642, rPt4523, rPt6561, rPt6965, rPt7959, rPt8894, tPt0136, tPt0602, tPt0734, tPt1051, tPt1586, tPt2076, tPt2240, tPt2326, tPt2440, tPt2550, tPt3696, tPt4377, tPt4566, tPt5515, tPt5755, tPt6015, tPt7559, tPt7918, tPt8109, tPt8754, tPt9585, wPt1116, wPt1251, wPt1346, wPt1541, wPt1620, wPt1637, wPt1708, wPt1781, wPt1862, wPt1997, wPt2577, wPt2696, wPt2866, wPt3465, wPt3533, wPt3908, wPt4199, wPt4306, wPt4343, wPt5096, wPt5209, wPt5506, wPt5522, wPt5680, wPt5836, wPt5878, wPt6457, wPt6643, wPt664964, wPt669404, wPt7024, wPt7185, wPt732423, wPt740897, wPt743523, wPt7491, wPt8930, wPt9251, wPt9277, wPt9350, and wPt9496.
PH	wPt0189, wPt0419, wPt10071, wPt2564, wPt2810, wPt5704, wPt5816, wPt667618, wPt671947, wPt7063, wPt729839, wPt8226, wPt8340, and wPt9654.
DH	rPt4471, wPt3198, wPt4664, wPt7238, wPt730618, wPt7672, wPt9094, wPt9230, and wPt9256.
CT(gf) D	tPt0325, tPt1663, tPt8504, wPt0419, wPt2810, wPt5265, wPt5694, wPt666861, wPt729839, wPt7394, wPt7807, wPt8292, wPt9196, wPt9268, wPt9515, and wPt9992
CT(gf) H	rPt5341, rPt9057, wPt0189, wPt0831, wPt0902, wPt11278, wPt1634, wPt1637, wPt1726, wPt1842, wPt1972, wPt2744, wPt5385, wPt5562, wPt6643, wPt664792, wPt665036, wPt666937, wPt7242, wPt730063, wPt730772, wPt732355, wPt732636, wPt734140, wPt743211, wPt743523, wPt7765, wPt7848, wPt7857, wPt8340, wPt8737, wPt8854, wPt9251, wPt9422, and wPt9654
CT(gf) HD	rPt9057, wPt8854, wPt9422, wPt7765, wPt1634, wPt1637, wPt7242, wPt1842, wPt8737, wPt9654, wPt664792, wPt732355, wPt7848, rPt5341, wPt732636, wPt730772, wPt0189, wPt6643, wPt7857, wPt5385, wPt743211, wPt0902, wPt2744, wPt8340, wPt1726, wPt666937, wPt11278, wPt9251, wPt5562, wPt665036, wPt730063, wPt743523, wPt1972, wPt0831, and wPt734140
SPAD	wPt798970, wPt665480, wPt1325, wPt8776, wPt666459, wPt0935, wPt9205, wPt0959, wPt9913, wPt0288, wPt664022, wPt8446, wPt0139, wPt733641, wPt7984, wPt0936, wPt667763, wPt0289, wPt8094, wPt733856, wPt1011, wPt0694, wPt7127, and wPt6381
Se D	wPt9586, wPt8473, wPt6502, wPt9299, wPt9277, wPt3468, wPt672158, wPt664400, wPt5234, wPt732423, wPt744960, wPt8015, wPt7754, wPt0538, wPt665687, and wPt3950
Se H	wPt1348, wPt8449, wPt2838, wPt8803, wPt669693, wPt1510, wPt8246, tPt8569, wPt5816, wPt5642, wPt8418, wPt2933, wPt1826, wPt9992, wPt0866, wPt4140, wPt9006, wPt6967, wPt8015, wPt0808, wPt6654, wPt5246, wPt5234, wPt4960, wPt742051, wPt4778, wPt8267, wPt0884, wPt3445, and wPt744217
Se HD	wPt5816, wPt0100, wPt2838, wPt9992, wPt669693, wPt11177, wPt9094, wPt10179, rPt4471, wPt7489, wPt1348, wPt1304, wPt0866, wPt6661, wPt8449, wPt3733, wPt0419, wPt4300, wPt667015, wPt11400, wPt8246, and wPt2933

GY = Grain yield; PH = Plant height; DH = Days to heading; CT(gf) = Canopy temperature at grain filling;

SPAD = Chlorophyll content; Sc = Senescence; D = Drought stress; H = Heat stress; DH = Drought + heat stress

**Table 4. Seri/Babax environments grown**

Country	Year	Location	Environments
Iran	2008–09	Darab	YP and D
		Sohag	
Egypt	2008–09	Dongola	YP and D
Sudan	2008–09	Wad Medani	D and H
Syria	2008–09	Tel Hadya	H
India	2008–09	Karnal	H
		Ludhiana	
Mexico	1999-00	Obregon	YP and D
	2000-01		YP and D
	2001-02		YP and D
	2004-05		D
	2005-06		YP and D
	2004-05		MH
	2005-06		H
	2009-10		IH
	2010-11		IH
Australia	2002-06	Gatton	YP and D
	2002-06	Queensland	

YP and D YP = Yield potential; D= Drought stress; H = Heat stress; MH= Moderate heat stress; IH = Intense heat stress

**Table 5. Seri/Babax: Markers identified through QTL mapping (Pinto et al. 2010).**

Trait	Marker identified
GY	4A-act/cag-3, 4A-barc070, 4B-gwm375, 5A-barc040, 6B-agg/ctg-8, and 7D-acc/cat-10
TKW	6A-wmc0163, 6D-gwm325, and C29P13
GM2	C1P48, C14P6, 4B-aag/cta-5, and 6D-cfd0188
DH	2B-act/ctc-11, 3A-wPt-2478, 4A-wmc048d, 5D-wPt-5505, 6A-wPt-7599, 6B-agg/ctg-8, and C29P13
DM	1D-gdm0111, 2B-act/ctc-11, 4A-gwm397, C16P7, C20P6, 6A-barc0113, 6B-aac/ctc-3, 7D-aca/cag-11, 7D-acc/cat-10
PH	1D-gdm0111, 2B-aag/ctg-5, C9P62, 2D-aac/ctg-6, 2D-aac/ctg-6, and 4A-agg/cta-12, 4B-aag/cta-5, 4D-wmc048b, 5A-gwm617a, C19P17, and 7D-gwm130
CT(vg)	4A-wmc048d
CT(gf)	6A-gwm617b and C29P13
EGC	7D-acc/ctc-7 and 7D-acc/cat-10

GY = Grain yield; TKW = Thousand kernel weight; GM2 = Grain number / m<sup>2</sup>; DH = Days to heading; DM = Days to maturity; PH = Plant height; CT = Canopy temperature; EGC = Early ground cover.

**Table 6. Atil /Triticum dicoccum 259 RILs summary**

Country	Environments	Year	Genotyping/ Map	Marker identified
Mexico	Drought (Ob)	2006-07	Genome coverage 2192cM (A genome 799cM and B genome 1330cM: Total number of markers 853 (322 on A and 531 on B); No. of linkage groups 27 (14 on A genome and 13 on B genome); average chromosome length 79cM (57cM for chromosome on A genome and 102 for chromosome on B genome)	Major QTLs for GY, GN, TGW, CT, SPAD, V, P, and W
	Heat (Ob)	2007-08		
		2008-09		
	Irrigated (Ob)	2007-08		
		2008-09		

Ob = Obregon; GY= Grain yield; GN= Grain number; TKW = Thousand kernel weight; CT = Canopy temperature; SPAD = Chlorophyll content; V= Vigor; P = Phenology; W= Waxiness.



