

Exploring genetic diversity for harvest index and identifying improved selection approaches

A step change in the expression of harvest index (HI) underpinned the dramatic yield gains of the Green Revolution, and steady genetic gains in HI were associated with yield improvements of semi-dwarf wheat until the late 1990s (Reynolds et al., 1999). Despite this, HI still expresses significant genetic variation in the most modern cultivars (e.g. Aisawi et al., 2015) –generally in the range of 0.40 to 0.55 depending on both genetic background and environment. This variation in the ability to partition resources to grain represents significant untapped yield potential, especially given the generally negative association between HI and biomass in the most modern cultivars (Aisawi et al., 2015). For example, Table 1 shows potential yield gains associated with more optimal expression of HI in elite CIMMYT material, from the currently achievable level of 0.5 up to a level of 0.6 which is close to the theoretical limit (Foulkes et al., 2011). These figures indicate that step changes in yield are attainable at current levels of biomass if HI can be better exploited.

Table 1. New Lines from the pre-breeding pipeline showing highest biomass in replicated yield trials, NW Mexico 2013

Cross	Yield g/m ²	Maturity days	Biomass g/m ²	HI	Norm Biom** g/m ²	% of check Biom** (%)	Yield (g/m ²) with theoretical HI		
							HI=0.5	HI=0.55	HI=0.6
WORRAKATTA/2*PASTOR	684	127	1766	0.39	1801	116	883	971	1059
WBLL1//PUB94.15.1.12/WBLL	718	133	1803	0.40	1753	113	901	992	1082
HE1/2*CNO79//BAV92/3/ROLF07	732	128	1728	0.42	1749	113	864	950	1037
SOKOLL//PUB94.15.1.12/WBLL1	685	126	1703	0.40	1746	113	851	936	1022
PASTOR//HXL7573/2*BAU/3/WBLL1	749	128	1720	0.44	1737	112	860	946	1032
WBLL1//YANGLING SHAANXI/ESDA/3/ROLF07	731	129	1719	0.43	1732	112	860	946	1031
D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM	734	134	1791	0.41	1730	112	896	985	1075
BCN/WBLL1//PUB94.15.1.12/ WBLL1	757	133	1766	0.43	1719	111	883	971	1060
WBLL1//PUB94.15.1.12/WBLL	741	134	1773	0.42	1710	111	887	975	1064
CHEN/AE.SQ//WEAVER/3/SSERII	730	129	1689	0.43	1698	110	844	929	1013
SOKOLL (Check in synthetics)	677	124	1609	0.42	1678	108	804	885	965
Check	710	131	1560	0.46	1548	100	780	858	936
Mean	721	129.7	1719	0.41	1717	111	859	945	1031
CV	4.6	1.2	5.9	5.0	-	-	-	-	-
LSD (5%)	64.5	3.2	188	0.04	-	-	-	-	-

*Best elite line from bread wheat breeding program (3 year data)

**Biomass normalized to average cycle length

To ensure that HI is expressed in the future at >0.5 across all major wheat agro-ecosystems, the main factors to consider are:

- Optimizing partitioning of assimilates to reproductive structures (without sacrificing functional integrity of the roots and above ground canopy).

- Tailoring crop phenology to different environments. Genes of major effect *Ppd* and *Vrn* (conferring photoperiod and vernalization sensitivity, respectively) already determine adaptation to winter versus spring wheat mega-environments but adaptation within these mega-environments is yet to be fine-tuned to ensure high and stable expression of harvest index, and must consider genes of minor effect such as *Eps* (earliness *per se*) alleles.
- Modulating sensitivity to environmental cues, for example during rapid spike-growth phase when seed number and kernel weight potential are determined, to avoid conservative responses that reduce grain set.
- Adaptation to potential stresses post-anthesis that may truncate the period of grain-filling and reduce seed size.

Evidence for genetic diversity

While HI still shows significant genetic variation, it is no longer generally associated with yield in the most recent cultivars (e.g. Aisawi et al., 2015), indicating that breeders have not been able to fix the trait. There are two main reasons for this:

- 1) Harvest index is strongly influenced by flowering behavior which is very sensitive to latitude and sowing date. The genetic basis of flowering is controlled to a large extent by two genes of major effect, *Ppd-1* and *Vrn-1*, and significant allelic diversity exists for both these genes and explains much of the variation in germplasm collections. However, there are also additive effects of *Eps* alleles which contribute to the fine tuning of flowering behavior (Griffiths et al., 2009). To date, no systematic attempt has been made to associate any of these alleles with HI expression.
- 2) The physiological and genetic basis for assimilate partitioning among different plant organs is relatively poorly understood. Nonetheless, in recent studies of wheat yield potential under the MasAgro-TRIGO project, large genetic ranges for dry matter partitioning among plant organs have been reported and some promising leads have been identified (Foulkes et al., 2015).

State of the art of phenotyping and genotyping tools.

The project will capitalize on three main recent advances in genetic and physiological understanding:

- 1) Collaborative work between the John Innes Center (JIC) and CIMMYT has shown that all of the known allelic variants at *Ppd-1* and *Vrn-1* are represented in two CIMMYT panels of elite lines -the CIMMYT Mexico Core Germplasm (CIMCOG) panel (Chavez et al., 2013), and the Wheat

Association Mapping Initiative (WAMI) panel (Lopes et al., 2015; Sukumaran et al., 2014)-. The WAMI and CIMCOG panels have also been genotyped with a series of allele-specific markers for *Rht-1* genes (plant height), as well as *Vrn-1* and *Ppd-1*. There is a significant opportunity to make genetic analysis of HI expression in these panels in relation to all three loci. Furthermore, alternative sources of variation in flowering time, such as the additive effects of *Eps* alleles *Eps*, for which markers are now available (Zikhali et al. unpublished), present new opportunities to understand how changes in flowering behavior influence HI. Different combination of these alleles not only determine flowering time, but also more subtle aspects of phenological pattern such as the relative duration of the rapid spike growth phase which has been linked to determination of grain number (Whitechurch et al., 2007; Garcia et al., 2011). The study of these phenological patterns not only helps better understand the genetic bases of adaptation of reproductive growth, but can also identify promising new sources of diversity for pre-breeding.

- 2) In terms of phenotyping, intensive growth analysis studies in elite spring germplasm have so far identified two main phenotypic characteristics associated with optimal expression of HI (Foulkes et al., 2015). These are:
 - Reduced structural dry matter investment to stem internodes 2 and 3 to enhance spike growth during stem elongation.
 - Increased fruiting efficiency (FE); in other words reducing chaff dry weight cost per unit of grain (Slafer et al., 2015).

- 3) In terms of the sensitivity of HI to environmental factors (beyond photoperiod, vernalization, and cumulative effects of temperature) it is becoming increasingly apparent that plant growth regulators (PGRs) play an active role in transducing external stimuli to growth responses, ultimately determine partitioning of assimilates to the spike and other organs (Wilkinson et al., 2012; Nuccio et al., 2015). However, these responses are not easy to measure, so neither high throughput tools nor molecular markers have yet been established. Nonetheless, statistical approaches can be used to pinpoint genotypes with robust responses to the environment in terms of HI and related traits. While direct measurement of PGRs will not be possible within this project, lines identified by statistical approaches will be made available to collaborators with PGR capability with the view to understanding mechanistic bases.

Research Objectives and Methodology

Increasing and stabilizing HI in wheat is too big for any one project to tackle comprehensively given its complex genetic and physiological basis. Nonetheless, based on progress made to date, some

significant opportunities for near-term genetic gains exist, as well as increasing genetic and physiological understanding which can lead to better breeding tools.

These opportunities are based in large part on the fact that breeding programs have not generally measured HI, as it is time consuming to measure, and even less information is available on traits related to partitioning, as described above. Therefore, our effort will focus on characterizing elite materials (Appendix 1) with the view to identifying genetic resources with promising expression of HI and related traits for use in pre-breeding, as well as physiological and genetic analysis that will lead to improved screening protocols. Specific research objectives in relation to exploring HI and related traits will include:

- 1) **Phenotypic screening of elite genetic resources.** A wide range of elite genetic resources (Appendix 1) will be screened to identify diversity for HI and related traits including:
 - a. Harvest index
 - b. Fruiting efficiency
 - c. Spike Partitioning index
 - d. Length of stem internodes 2 and 3
 - e. Flowering time and phenological pattern including relative duration of
 - i. Rapid spike growth phase (from initiation of booting to anthesis)
 - ii. Grainfilling phase (from anthesis to physiological maturity).
- 2) **Genetic analysis.** Associations will be examined between the traits outlined under Objective 1) and *Ppd*, *Vrn*, *Eps*, and *Rht* alleles, as well as other markers in populations for which genetic maps are available. The outcome will be used to identify lines with particular constellations of *Ppd*, *Vrn*, *Eps*, and *Rht* alleles that are associated with favorable expression of HI levels. The information will also be used to develop a MAS strategy that can be applied on the progeny of crosses between sources of high biomass, and lines identified above with favorable expression of HI and related traits
- 3) **Statistical modelling.** While Objective 1 will identify genotypes that express constitutively high HI and related traits, Objective 3 will consider their GxE in order to identify sources that are robust to conditions that generally decrease their expression in most backgrounds. This information can be inferred by examining the response of HI and related traits where sufficient genetic diversity is examined in conjunction with meteorological data. Advanced statistical approaches will be employed for dissecting genotype by environment interaction as outlined in Reynolds et al. (2004). The outputs of such statistical approaches can pinpoint lines whose HI is relatively stable even when sensitive growth stages are affected by high temperature or low radiation for example, namely conditions that typically reduce partitioning of assimilates to the developing spike. The same approaches can be used to pinpoint genotypes that are less affected by factors that tend to reduce expression of HI due to

adverse conditions during grainfilling. This exercise will be carried out once data is available from more than one year on the panels described, as well as on extant data sets where HI data is available from the the MasAgro-TRIGO project. For example, the Wheat Yield Consortium Yield Trial (WYCYT) now entering its third cycle is distributed annually to international sites, of which approximately 10 return international data (Reynolds et al., 2015); the CIMCOG panel was grown at 25 international sites between 2011-2012 (Chavez et al., 2013).

- 4) **Pre-breeding.** Lines identified by Objectives 1-3 will be used in crosses with sources of high biomass identified in another project that have additive effects. Outputs of Objective 2, namely identification of favorable constellations of *Ppd*, *Vrn*, *Eps*, and *Rht* alleles, will also be used to pre-screen high biomass parents in an attempt to match favorable allelic combinations associated with high HI expression.

Appendix 1. Panels and population available for screening of HI related traits at the IWYP Platform

Progeny of Physiological Trait Crosses + Parents (PT+Pads) (~300 lines). CIMMYT has recently distributed -through the IWIN- a number of nurseries comprising elite novel germplasm designed to combine specific physiological traits associated with increased yield potential (1st to 3rd WYCYT, Wheat Yield Consortium Yield Trails), and adaptation to heat and drought (1st to 5th SATYN, Stress Adaptive Traits Yield Nursery). International trialing has identified lines with significant genetic gains over elite checks for both yield and biomass.

Wheat diversity panels (350 and 200 lines). Association panels of selected wheat genetic resources (350 bread wheat; 200 durum wheat) have been assembled from spring wheat sources after screening approximately 60,000 lines for heat and drought adaption in the Sonoran desert. They were derived from the following sources:

- International nurseries. Every year approximately 1,000 new, high yielding, disease resistant wheat lines with appropriate end-use quality are generated by CIMMYT and delivered via IWIN to most public and private wheat improvement programs worldwide, where they are tested at approximately 200 sites annually. Now in its fifth decade, the IWIN database provides a valuable starting point to identify modern varieties with outstanding adaptive characteristics in specific environments (Braun et al., 2010; Gourdjji et al., 2012).
- Wheats with ancestral chromosomal introductions. Many elite lines already contain alien introgressions from the Triticeae tribe that are linked to improved yield potential and disease resistance (Ortiz

el al., 2008). CIMMYT's genebank holds around 500 accessions with specific translocations (e.g. 7Ag.7DL, 1B.1R, Lr34, Lr42), while thousands of elite lines are derived from crosses with synthetic wheat (see below).

- Landraces. Approximately 15,000 spring wheat landraces from the World Wheat Collection have recently been pre-screened under high temperature stress. Several hundred landraces were selected for superior yield and biomass (the latter evaluated visually).
- Focused Identification of Germplasm Strategy (FIGS) are landrace panels that have been selected based on their origin being in regions with abiotic stress (Sehgal et al., 2015).

High Biomass Association Panel (HiBAP)(150 lines). Systematic screening of genetic resources from the World Wheat Collection has identified genotypes with favorable expression of biomass. Selected material will form the High Biomass Association Panel (HiBPAP) of aApproximately 150 spring types, including elite high yield material, pre-breeding lines crossed and selected for high yield and biomass, synthetic derived lines, and appropriate checks. The material has a restricted range of maturity to avoid confounding effects associated with extreme phenology.

Mapping Populations and Parents. Several populations (bi-parental, association mapping, genomic selection and NAM populations) are available at CIMMYT for the genetic dissection of complex traits. A number of suitable RILs populations are available whose parents are elite high yield/biomass lines, some of whose progeny express transgressive segregation for these traits while not expressing a large range of phenology. Some data already exists from previous projects for yield and NDVI at different growth stages which can be made available, and it is expected to phenotype at least two more populations depending on the demands put on IWYP-PLAT by successful projects. Among the extant populations, the most relevant include

- **CIMCOG:** The panels of 60-100 lines represent a full range of genetic diversity in elite high yielding backgrounds and provide a common research platform for yield potential research. CIMCOG is renewed biennially using elite materials from various sources. The 1st CIMCOG has already been evaluated at 25 international sites in Asia and Africa, as well as at different Mexican environments (Chavez et al. 2013).
- o **The WAMI panel** consisting of 287 genetically diverse elite lines previously released by IWIN, was selected for a restricted maturity class to avoid confounding the expression of yield potential traits with phenology. WAMI has been mapped and genetic analyses have been conducted under high yield conditions (Lopes et al. 2015; Sukumaran et al., 2015). Further genetic analyses are pending using phenotypic data from almost 31 international environments (to date) making this a valuable resource for extrapolating research results to target wheat growing regions.
- o **The Kauz/Weebil double haploid population** combines yield potential traits to generate lines with exceptional yield potential compared to either parent, as recently shown in southern Chile (Bustos et al. 2013).

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