# Developing a high throughput screen for source:sink balance to tap photosynthetic potential

It is well established that there is a dynamic interaction in plants between 'source' and 'sink'. As seen in economic models of supply and demand, experiments in cereals have shown that a high demand for assimilates — determined by sink strength of the grains—can stimulate the supply of photo-assimilates (Reynolds et al., 2005), and vice-versa (Calderini et al. 2001). These studies have demonstrated that well-managed wheat crops carry excess photosynthetic capacity, a probable conservative response to the natural risk of losing leaf area or photosynthetic function. As a result, increasing photosynthetic potential does not necessarily optimize grain number (Sadras 2007) as shown by the negative association observed between harvest index and biomass (e.g. Bustos et al. 2013). Therefore, to achieve full expression of yield potential, it will be necessary to optimize the source:sink dynamic by ensuring that expression of grain set matches the photosynthetic potential of current and future genotypes.

## *Evidence for untapped genetic diversity*

Evidence for genetic variation in source:sink balance (SSB) and its importance in boosting yield and radiation use efficiency (RUE) has come from various sources, including studies with cytogenetic stocks. Substitution of the long arm of chromosome 7D in hexaploid wheat with the homologous chromosome from *Agropyron elongatum* resulted in a significant increase in yield and biomass in six elite lines (Reynolds et al., 2001). While there were interactions with genetic background, the average yield increase was 13%, associated with a larger number of grains per spike that resulted in an average 15% increase in grains $m^{-2}$. The effect was demonstrably a consequence of an improved SSB since, (i) differences in grain number were associated with increased partitioning of assimilates to spike growth at anthesis (13%), (ii) both flag-leaf photosynthetic rate and RUE measured during grain filling were increased in the substitution lines, and (iii) in addition to yield, final biomass was increased by an average of 10% in five of the six lines. In follow up studies, it was found that RUE and flag leaf photosynthetic rate could be boosted in elite lines by increasing sink-strength artificially using light treatments at the rapid spike-growth stage when grain number is fixed (Reynolds et al., 2005; 2009).

More recently, a cross designed to combine different genes for high sink strength in high RUE backgrounds resulted in doubled haploid lines expressing exceptional yield and biomass in a high yielding environment in Southern Chile (Bustos et al., 2013), further supporting the idea that the right combination of sink traits can permit increased expression of RUE.

Finally, results of the Wheat Yield Consortium Yield Trial (WYCYT) have shown step changes in yield potential (average 7% above elite checks across 26 international sites) in crosses that were explicitly designed to combine favorable sources of sink traits with high RUE lines (Reynolds et al., 2015). Biomass was also increased in the same materials by up to 12% depending on genetic background and environment.

***State of the art of phenotyping and genotyping tools.***

While this body of work provides clear evidence that yield potential can be boosted by improving SSB, it has remained an elusive trait, principally because it is complex to measure directly as it necessitates a rather precise and somewhat costly light treatment. However, recent work focusing on genetic variation for adaptation to agronomic planting density (Skumaran et al., 2015a) has demonstrated a high throughput alternative based on a planting system that permits different rows of the same plot to be evaluated under distinct light regimes (Fig. 1).

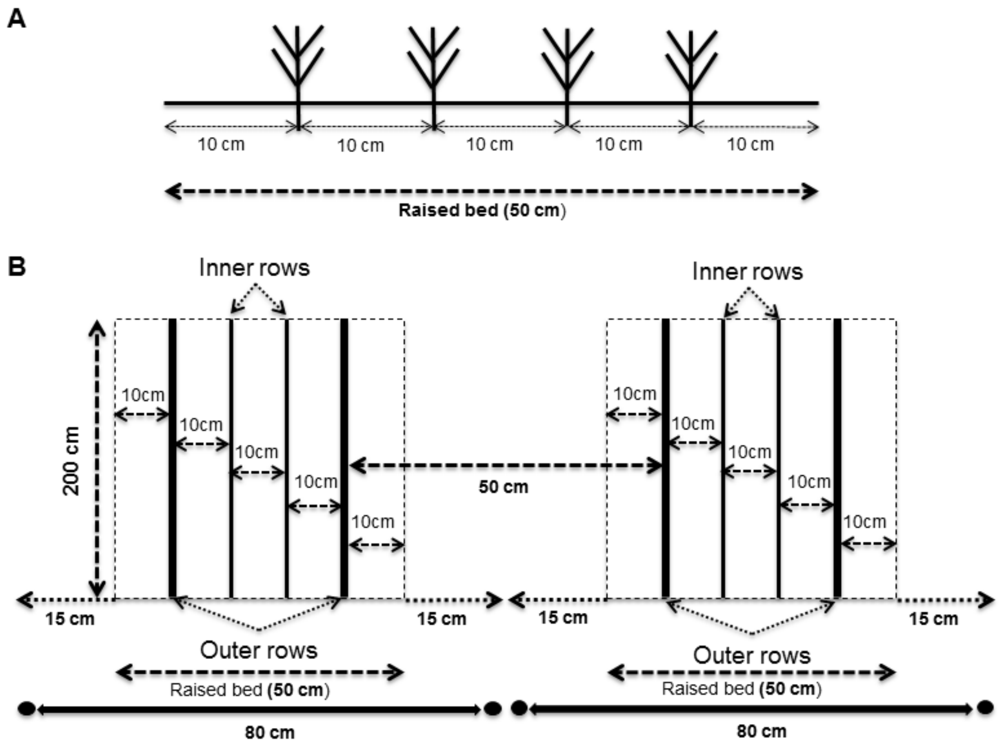

**Figure 1.** Diagram showing the planting of the experiment (**A**) raised bed (50cm wide) with four row planting 10cm spacing between the rows and (**B**) two raised beds with 30cm spacing between the outer row of each bed. Inner rows in each bed are under density stress and outer rows were at low plant density allowing more light penetration to the canopy.

In both wheat and maize systems, it has been shown that the ability to perform well at high planting density is also a key component of yield potential (Duvick 1992, Reynolds et al., 1994; Sukumaran et al., 2015a). Recent analysis of a panel of elite spring wheat lines (WAMI) has shown that adaptation to plant density gives a specific advantage in high yield potential environments (Fig. 2), and a genetic basis has been shown for an adaptation to density index (*ADi*). Adaptation to density index (*ADi*) was calculated for grain yield ($ADi_{\mathrm{YLD}}$) as:

$$ADi = \left(YLD_O - YLD_I\right) \times \frac{\mu}{\mu l}$$

where *ADi* is adaptation to density index, *YLD*o is the grain yield of outer rows, and $YLD_I$ is the grain yield of inner rows, $\mu$ is the grand mean of all data when conducted for multiple years, and $\mu l$ is the mean of the specific trial in and year. Genome-wide association analysis was able to detect a consistent locus for *ADi* for grain yield and grain number. This locus was also associated with the grain yield *per se* (Sukumaran et al., 2015a). The results indicated that adaptation to density is at least partly a function of favorable SSB; lines showing the adaptation to density characteristic are able to set a relatively large grain number at high plant density while showing no loss of kernel weight, indicating a favorable supply of photo-assimilates associated with the maintenance of good sink strength. In fact, the protocol for measuring adaptation to plant density (Fig 1) is a variation on the ‘extra light treatment’ designed to measure SSB (Reynolds et al., 2005), but with a considerably higher throughput. Since the two methods for estimating SSB (i.e. response to extra light and adaptation to density) both show association with yield potential, and given that the latter has an established genetic basis as well as being amenable to breeding scale throughput, the opportunity exists to launch a large scale screening of genetic resources.

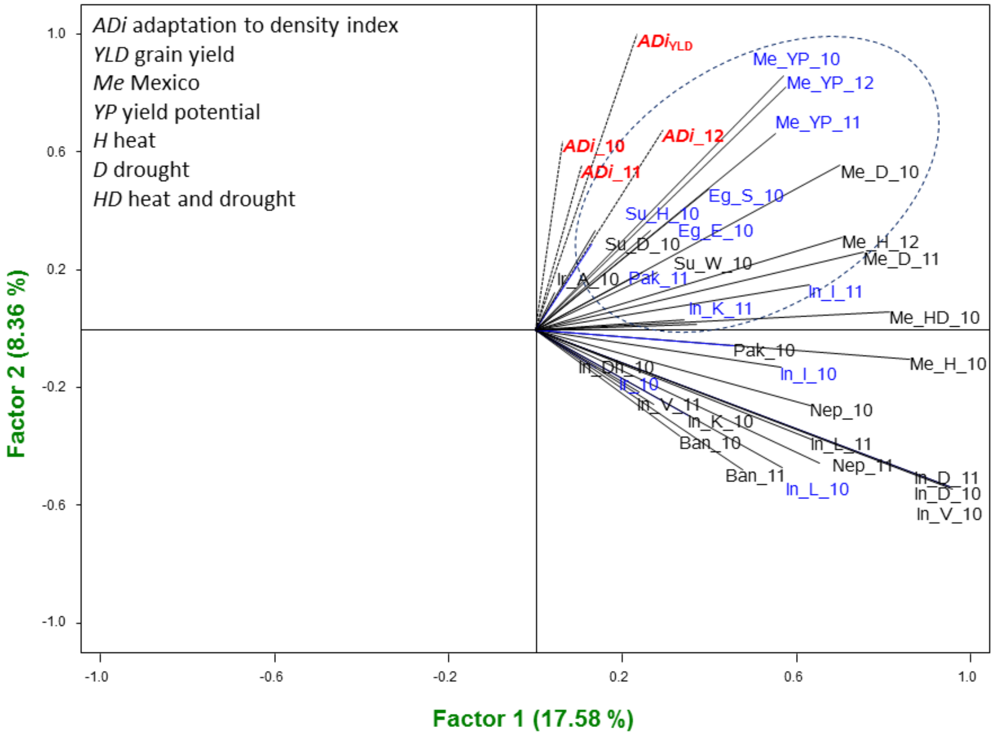

**Figure 2.** Biplot of adaptation to density index (*ADi*) with the yield of 31 international environments (South Asia, North Africa, and Mexico) in 2010, 2011, and 2012. Biplot indicates that adaptation to density index ($ADi_{YLD}$) is correlated with the grain yield in high yielding environments. Locations with high yield are colored in blue and low yielding locations in black. *ADi* estimated from data in Mexico is shown in red colors. Dotted lines area indicates maximum number of locations with high yield.

## *Research Objectives and Methodology*

The main objective of this project is to select lines that, under high plant density, show both good grain set and good grain-filling characteristics as indicators of a favorable SSB. At the same time, extra light treatments will be applied on subsets of selected lines to verify directly their capacity for increased RUE in response to increased grain set. While screening will ultimately be conducted right across CIMMYT's spring wheat genepool as resources permit, experiments will focus initially on the two genetically mapped populations mentioned earlier:

- The population of 106 doubled haploid lines generated between Bacanora and Weebill, which are both high yield, high biomass lines contrasting in their yield components; the former has small spikes at high density with relatively small grains, while the latter expresses the reciprocal traits. This population shows transgressive segregation for most agronomic traits, including yield in a range of environments (Bustos et al. 2013; García et al. 2013; Griffiths et al., 2015).
- The Wheat Association Mapping Initiative (WAMI) panel of 287 highly diverse elite lines, which was designed to avoid the masking effect of major genes— vernalization (*Vrn*) and photoperiod (*Ppd*)—in genetic analysis, permitting easier identification of genes of minor effect, as demonstrated by recent genetic analysis under high yield potential conditions in NW Mexico (Sukumaran et al., 2015b), and a wider range of international environments (Lopes et al., 2015).

Detailed phenotypic data from these two well mapped populations will permit a better understanding of SSB with the view to fine tuning the screening protocol. Specific objectives of the project are:

1) *Genetic and physiological dissection*. By measuring yield components on the two genetically mapped populations in response to both density (Fig 1) and extra light treatments (Reynolds et al., 2005), it will be possible to:
    a. Determine the genetic architecture of SSB, and its relationship to other agronomic and physiological traits.
    b. Compare genomic regions identified by QTL analysis and GWAS to gage the degree of genetic diversity likely to be encountered in the wider wheat genepool.
    c. Compare genetic bases of the two methods (i.e. adaptation to density and response to extra light treatments) for estimating SSB, to estimate the extent to which the two responses have overlapping genetic bases.
    d. Identify candidate regions for future application in marker assisted selection and gene identification.

2) *Screening spring wheat diversity*. Outputs from 1) will permit high throughput screening to be fine-tuned for maximum resolution of SSB. Screening for SSB in years 2 and beyond will be conducted on a range of germplasm that encompass broad genetic diversity.

3) *Breeding for yield potential.* Based on the outcomes of 2), lines identified with favorable SSB will be used in the ongoing hybridization program to increase yield potential (Reynolds et al., 2015). In particular, lines with favorable SSB will be crossed with sources of high biomass in an attempt to fully exploit extra RUE in yield production.

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