

# Proceedings of the 2<sup>nd</sup> International **TRIGO (Wheat) Yield Potential** WORKSHOP 2016

CENEBC, CIMMYT, Cd. Obregón, Sonora, México  
9-10<sup>th</sup> March, 2016

Matthew Reynolds, Gemma Molero and Emma Quilligan (Editors)



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 **CIMMYT**<sub>MR</sub>  
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SECRETARÍA DE AGRICULTURA,  
GANADERÍA, DESARROLLO RURAL,  
PESCA Y ALIMENTACIÓN

  
**CONACYT**

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**Abstract:** The abstracts herein are of presentations by crop experts for the “TRIGO (Wheat) Yield Potential Workshop”. Sponsored by SAGARPA’s international strategic component for increasing wheat performance, under the Sustainable Modernization of Traditional Agriculture Program (MasAgro); and CRP WHEAT.

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 MasAgro TRIGO project and CRP WHEAT 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.

## Table of contents

List of participants .....	v
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### Breeding methods

Pedigree-based genotype × environment prediction of grain yield in multi-environment trials of WYCYT and SATYN .....	1
<i>Sivakumar Sukumaran, Jose Crossa, and Matthew Reynolds</i>	
Evaluation of the Stress Adaptive Trait Yield Nursery (SATYN) in irrigated wheat growing locations in Mexico during the 2015-16 growing season .....	10
<i>Ernesto Solís-Moya, Miguel A. Camacho-Casas, Pedro Figueroa-López, Javier Ireta Moreno, Jorge I. Alvarado-Padilla, Alberto Borbón-Gracia, and Gemma Molero</i>	
International performance of physiological trait based lines of the 4th Stress Adaptive Trait Yield Nursery (4th SATYN) .....	15
<i>S. Sukumaran, M.P. Reynolds, A.J.D. Pask, G. Molero, A. Torres, Y.N. Quiche, T. Payne, R. Ernesto Solís-Moya, M.A. Camacho-Casas, P. Figueroa-López, J. Ireta Moreno, J.I. Alvarado-Padilla, A. Borbón-Gracia, R. Chatrath, G.P. Singh, V.S. Sohu, G.S. Mavi, V.S.P. Sakuru, R. Naik, S. Biradar, V.K. Mishra, B. Arun, R. Ud-din, S. Kundu, M. Hussain, S.R. Upadhyay, D. Pandey, N.C.D. Barma, A. Hakim, A. Ahmed Hamada, M. Dastfal, A.R. Nikzad, S. M. Tabib-Ghaffary, M. Khodarahmi, H. Akbari Moghaddam, M.R. Jalal-Kamali, and A.K. Joshi</i>	
Traits determining differences in yield potential among elite lines of a spring wheat panel with the view to accelerating genetic gains .....	25
<i>Gemma Molero, Gustavo A. Slafer, Alma C. Rivera-Amado, Francisco J. Pinera-Chavez, Eliseo Trujillo-Negrellos, Alejandro Quintero, Oscar E. Gonzalez, Daniel F. Calderini, M. John Foulkes, Pete. M. Berry, Simon Griffiths, Cándido Lopez-Castañeda, Ravi Singh, and Matthew P. Reynolds</i>	
State of the art and current situation of wheat production in Mexico .....	35
<i>Anabel Molero, Enrique Autrique, Roberto J. Peña, Xiomara Chavez, Jenny Nelson, Bram Govaerts, Matthew P. Reynolds, and Hans Braun</i>	
Wheat breeding in Mexico: Delivering newly developed advanced lines .....	42
<i>Enrique Autrique, Carlos Guzman, Pawan K. Singh, Caixia Lan, Mandeep S. Randhawa, Julio Huerta, and Ravi P. Singh</i>	

### Partitioning

Key molecular and physiological traits/mechanisms affecting the setting of potential grain weight in two contrasting spring wheat cultivars.....	53
<i>Alejandro Quintero, Paola Montecinos, Gemma Molero, Matthew Reynolds, and Daniel F. Calderini</i>	

Wheat spike fertility and fertile tillers number are enhanced by exogenous application of Cytokinin and Auxin .....	60
<i>Arnauld A. Thiry, Matthew P. Reynolds, and William Davies</i>	
Achieving increases in spike growth, fruiting efficiency, and harvest index in high biomass wheat cultivars.....	70
<i>Carolina Rivera-Amado, Eliseo Trujillo-Negrellos, Roger Sylvester-Bradley, Gemma Molero, Aleyda Sierra-Gonzalez, Matthew Reynolds, and John Foulkes</i>	
Avoiding lodging in wheat through the development of genetic resources and the genetic dissection of lodging proof traits.....	77
<i>Francisco J. Piñera-Chavez, Peter M. Berry, Michael J. Foulkes, Sivakumar Sukumaran, Gemma Molero, and Matthew P. Reynolds</i>	
Variation in developmental patterns among elite wheat lines and relationship with spike fertility .....	83
<i>Oscar E. Gonzalez-Navarro, Simon Griffiths, Gemma Molero, Matthew P. Reynolds, and Gustavo A. Slafer</i>	

## **Photosynthesis**

Potential to include spike photosynthesis in breeding programs: Genetic variation for spike photosynthesis and identification of molecular markers.....	89
<i>Gemma Molero, Sivakumar Sukumaran, and Matthew P. Reynolds</i>	
Predicting physiological traits in wheat landraces using hyperspectral reflectance .....	96
<i>Viridiana Silva-Pérez, John R. Evans, Anthony G. Condon, Gemma Molero, Robert T. Furbank, and Matthew P. Reynolds</i>	
Partitioning photosynthetic limitation and yield diversity in wheat cultivars grown at CIMMYT .....	102
<i>Luis Robledo-Arratia, Gemma Molero, Elizabete Carmo-Silva, Matthew P. Reynolds, Martin Parry, and Howard Griffiths</i>	
Nitrogen contribution of the ear to grain filling in wheat .....	107
<i>Rut Sanchez-Bragado, Gemma Molero, Matthew P. Reynolds, and Jose Luis Araus</i>	

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## BREEDING METHODS

### Pedigree-based genotype × environment prediction of grain yield in multi-environment trials of WCYT and SATYN

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#### Abstract

Genomic selection is the most promising marker-assisted selection method when genes with minor alleles are associated with the trait of interest, especially in physiological pre-breeding where loci with minor effects govern the trait of interest. Thanks to large-scale worldwide screening of the Wheat Yield Consortium Yield Trial (WCYT) and Stress Adaptive Trait Yield Nursery (SATYN), CIMMYT is able to access a unique combination of data related to grain yield and physiological traits, which can serve as training populations in genomic selection or pedigree based prediction. This study evaluated the prediction accuracy of different genomic models based on the pedigree derived matrix ( $A$  matrix) as an alternative to genomic based relatedness matrix. We used a genotype × environment (G × E) model on the WCYT (39 environments) and SATYN (48 environments) nurseries for checking models' prediction accuracy. A higher predictive ability was obtained by modelling G × E interaction to the genomic selection models; these can be used for prediction as well as selecting for target environments.

#### Introduction

Genomic selection (GS) and prediction is a new genetic tool that helps to shorten the breeding cycle and thus accelerate genetic gains (Lado et al. 2016). GS can also make phenotyping evaluation more cost efficient as wheat lines that are genotyped can be phenotyped in fewer plots and predicted with great precision in environments that were not evaluated. Furthermore, the combination of GS with high throughput phenotyping platforms (HTPP) – where correlated traits are measured in early stages of the crop – is a valuable tool for increasing selection intensity (thousands of plots can be measured rapidly) and shortening the breeding cycle (two basic components of the breeder's equation) (Lorenz et al. 2012; Rutkoski et al. 2015).

This study used available international nursery data from the Wheat Yield Consortium Yield Trial (WCYT) and Stress Adaptive Trait Yield Nursery (SATYN), which are phenotyped worldwide through the International Wheat Improvement Network (Table 1). Analysis of data from international environments are often confounded by high G×E, hindering the selection of high yielding stable lines across the environment. Recently, Perez-Rodriguez et al. (2015) demonstrated the usefulness of the pedigree based matrix (COP) in predicting multi-environmental traits in cotton. Here we predict the yield of WCYT and SATYN international trials on COP-based best linear unbiased predictions (BLUPs) using EC and GEI modelled into the GS models.

**Table 1. Phenotyped environments and data returned for WCYT and SATYN nurseries. W = WCYT; S = SATYN**

Code	Site	Country	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
			W 2012	W 2013	S 2009-10	S 2012	S 2013
BGLD D	BARI Dinajpur,Rangpur	Bangladesh	*	*			*
BGLD J	BARI Joydebpur, Dhaka	Bangladesh	*	*	*	*	*
BGLD R	BARI Rajshahi,	Bangladesh		*			*
China L	Laomancheng	China		*			
Egypt A	ARC Assiut	Egypt	*	*			*
Egypt S	Sohag, Shandawheel	Egypt					*
India D	IARI Delhi	India	*	*	*	*	*
India H	UAS Dharwad, Karnataka	India	*	*		*	*
India I	IARI Indore, Madhya Pradesh	India	*	*		*	*
India K	IIWBR Karnal, Haryana	India	*	*	*	*	*
India L	PAU Ludhiana, Punjab	India	*	*	*	*	*
India U	Ugar Khurd, Karnakata	India	*	*		*	*
India V	BHU, Varanasi, Uttar Pradesh	India	*	*	*	*	*
Iran DH	Darab-Hassan-Abad, FARS	Iran		*			*
Iran DS	Safiabad ARC, Dezful	Iran	*	*		*	*
Iran K	SPII Cereal Res STN, Karaj, Tehran	Iran	*	*		*	*
Iran SZ	Shiraz, Zargan, FARS	Iran		*			*
MEX CM	CIMMYT CENEB	Mexico	*	*	*	*	*
MEX SON	INIFAP_Valle del Yaqui, Sonora	Mexico		*		*	*
Mex Bajio	INIFAP-Bajio	Mexico		*		*	*
MEX BC	INIFAP-Mexicali Baja California	Mexico		*		*	*
MEX JAL	INIFAP-Tepatitlan Jalisco	Mexico		*		*	*
MEX SIN	INIFAP-Valle del Fuerte, Sinaloa	Mexico		*		*	*
Nepal B	NWRP, Bhairahawa, Rupandehi	Nepal	*	*	*	*	*
PAK B	RAR, Bahawalpur, Punjab	Pakistan		*		*	
PAK F	WRI, Faisalabad, Punjab	Pakistan	*	*		*	*
PAK I	NARC, Islamabad	Pakistan	*	*	*	*	*
PAK P	CCRI, Pirsabak, Khyber	Pakistan	*	*			*
PAK R	RAR, Bahawalpur, Punjab	Pakistan					*
SAB	SGI Bethlehem, Free State	South Africa	*				
Sudan D	Wad Medani GEZIRA STN, GEZIRA	Sudan					
Sudan H	El Damer Hudeiba, River Nile State	Sudan					
Sudan D	Dongola Research Station, Northern State	Sudan					

\*indicates sites data returned and analyzed.

## Methods

### *Germplasm*

Panels used for the BLUPs estimation were 1<sup>st</sup> WCYT, 2<sup>nd</sup> WCYT, 1<sup>st</sup> SATYN, 2<sup>nd</sup> SATYN, and 3<sup>rd</sup> SATYN. WCYT international nurseries are the result of research and breeding conducted to raise the yield potential of spring wheat through strategic crossing of traits related to source and sink potential of a plant (Reynolds et al. 2010; 2015), whereas SATYN nurseries are bred for stress adaptive traits (Pask et al. 2014).

### *Estimation of COP and BLUPs*

For the WCYT and SATYN the unique identifiers are GID in the CIMMYT IWIS system. This information can be used for estimating coefficient of parentage (COP) between lines. COP gives the probability of two genes being identical by descent, and when individuals inherit copies of the same alleles they tend to show phenotypic resemblance because of their genetic relationship. Thus, the additive relationship matrix  $A$  equals two times the COP ( $A=2\text{COP}$ ), and this is directly related to the response to selection and genetic gains.

### *GBLUP $G \times E$ models for traits measured in different environments*

Response variables were grain yield measured in all environments across years for both type of trials, SATYN and WCYT. We used the multiplicative reaction norm models of Jarquín et al. (2014) with pedigree-based relationship matrices as used by Pérez-Rodriguez et al. (2014). Two models included only the main effects of environment, lines, and pedigree information, and three models included the main effects and different types of interactions. A brief description of the models is given below.

#### *Main effect model 1 ( $Y=E+L+\text{error}$ )*

This model considers the response of the  $j^{\text{th}}$  line in the  $i^{\text{th}}$  environment ( $y_{ij}$ ) as a function of a random effect model that accounts for only the effect of the environment ( $E_i$ ), the lines ( $L$ ), plus a residual ( $\varepsilon_j$ ):

$$y_{ij} = \mu + E_i + L_j + \varepsilon_{ij} \quad (1)$$

where  $\mu$  is an intercept,  $E_i \sim N(0, \sigma_E^2)$  is the random effect of the  $i^{\text{th}}$  environment,  $L_j \sim N(0, \sigma_L^2)$  is the random effect of the  $j^{\text{th}}$  line, and  $\varepsilon_{ij} \stackrel{iid}{\sim} N(0, \sigma_\varepsilon^2)$  is a model residual. In this model the lines are independent random variables, the environments are also independent random variables as well as the residual terms.

#### *Main effect model 2 ( $Y+E+L+A+\text{error}$ )*

In the other main effect model, the random effect of the  $j^{\text{th}}$  pedigree  $a_j$  considers a random variable that accounts for the pedigree relationship. Specifically, the vector  $\mathbf{a} = (a_1, \dots, a_j)'$  contains the pedigree values of the lines and is assumed to follow a multivariate normal density with zero mean and covariance matrix  $\text{Cov}(\mathbf{a}) = \mathbf{A}\sigma_a^2$ , where  $\mathbf{A}$  is the pedigree relationship matrix and  $\sigma_a^2$  is the additive variance. Therefore, model (1) becomes

$$y_{ij} = \mu + E_i + L_j + a_j + e_{ij} \quad (2)$$

The term  $a$  approximates the true genetic values of the  $L_j$  line.

### *Main effect and interaction model 3 (Y+E+L+A+EL+error)*

Model 3 is similar to model 2 but it includes the interaction of the  $j^{\text{th}}$  line with the  $i^{\text{th}}$  environment,  $EL_{ij}$ , such that

$$y_j = \mu + E_i + A_j + g_j + EL_{ij} + e_j \quad (3)$$

where the term  $EA_{ij}$  denotes the interaction of the  $j^{\text{th}}$  line in the  $i^{\text{th}}$  environment with  $\mathbf{EL} \sim N(\mathbf{0}, (\mathbf{Z}_L \mathbf{I} \mathbf{Z}'_L)^\circ (\mathbf{Z}_E \mathbf{Z}'_E) \sigma_{EL}^2)$ , where  $\mathbf{Z}_L$  and  $\mathbf{Z}_E$  are the incidence matrices for lines and environments, respectively, and  $\sigma_{EL}^2$  is the variance component of the interaction term  $EL$ .

### *Main effect and interaction model 4 (M4) (Y+E+L+A+AE+error)*

Model 4 is similar to model 3 but the response of the trait is measured in the  $j^{\text{th}}$  line in the  $i^{\text{th}}$  environments ( $y_{ij}$ ) it includes also the random effects of the interaction between the  $i^{\text{th}}$  environment ( $E_i$ ) and the  $j^{\text{th}}$  additive relationship ( $a$ )  $Ea_{ij}$

$$y_j = \mu + E_i + A_j + g_j + Ea_{ij} + e_j \quad (4)$$

where  $\mathbf{Eg} \sim N(\mathbf{0}, (\mathbf{Z}_a \mathbf{A} \mathbf{Z}'_a)^\circ (\mathbf{Z}_E \mathbf{Z}'_E) \sigma_{Eg}^2)$  is the interaction of the pedigree with environment, with  $\mathbf{Z}_a$  being the incidence matrix for the effects of the additive values of and  $\sigma_{Ea}^2$  is the variance component of  $Ea$  and ‘ $\circ$ ’ stands for the Hadamard product between two matrices. Matrix  $\mathbf{Z}_E$  is the incidence matrix for environments.

### *Main effect and interaction model 5 (Y+E+L+A+AE+EL+error)*

Model 5 is similar to model 4 but the response of the trait is measured in the  $j^{\text{th}}$  line in the  $i^{\text{th}}$  environments ( $y_{ij}$ ). It includes the two type of interactions the  $j^{\text{th}}$  line with the  $i^{\text{th}}$  environment ( $EL_{ij}$ ) the interaction between the  $i^{\text{th}}$  environment ( $E_i$ ) and the  $j^{\text{th}}$  additive relationship ( $a$ )  $Ea_{ij}$

$$y_j = \mu + E_i + A_j + g_j + EL_{ij} + Ea_{ij} + e_j \quad (5)$$

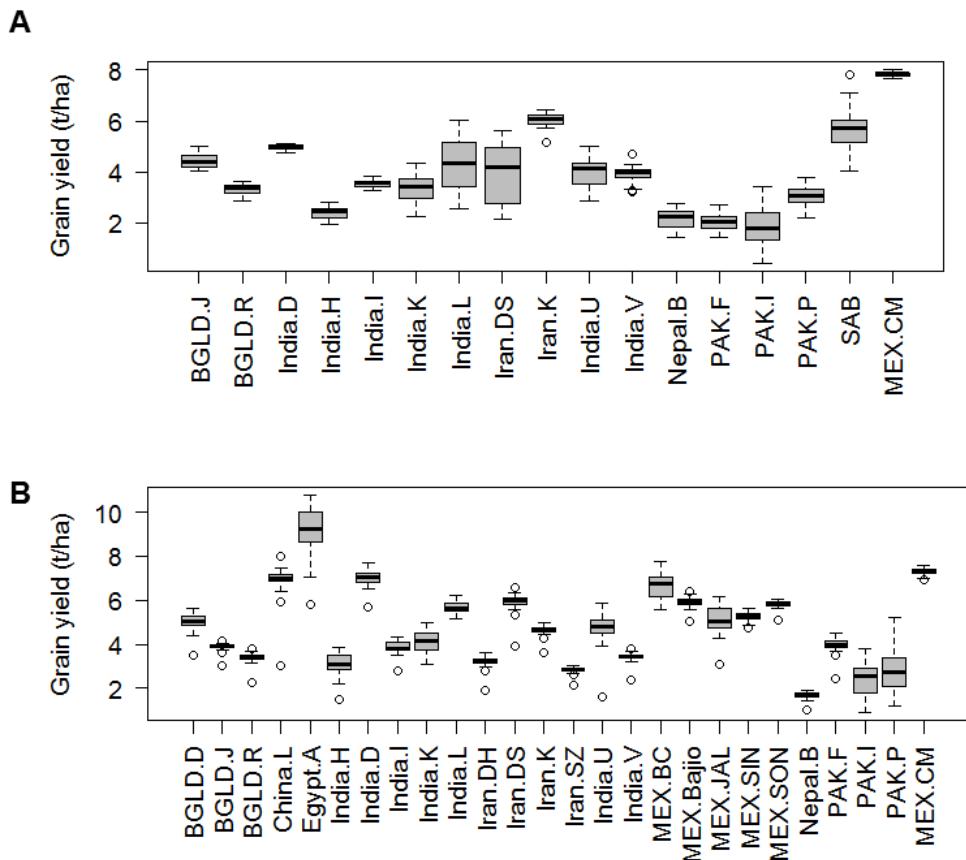
All the terms have been already defined.

The models described were all fitted using the BGLR R-package (de los Campos and Pérez-Rodríguez 2013; Pérez-Rodríguez and de los Campos 2014).

## **Results/Discussion**

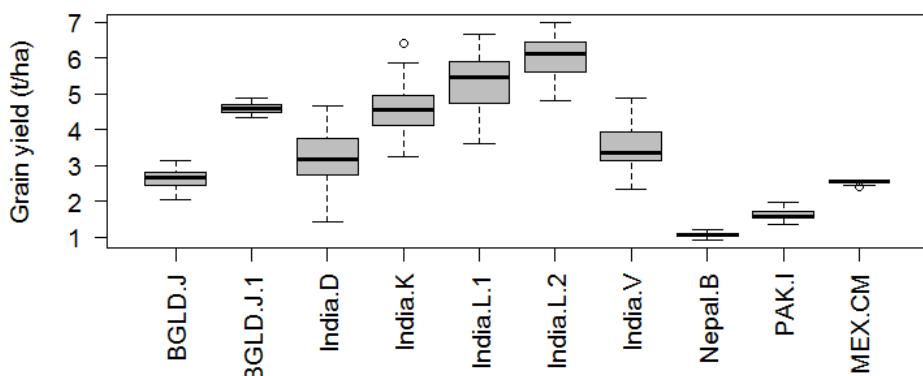
### *Predicted yields*

The yield of the 1<sup>st</sup> WCYT at international sites ranged from 1.8 t/ha to 7.8 t/ha, with a mean yield of 3.9 t/ha across the environments, and highest yield of 7.8 t/ha observed at CNEB location (Fig. 1A). Yields of the 2<sup>nd</sup> WCYT nursery ranged from 1.6 t/ha to 7.2 t/ha; the highest yield was again recorded at CNEB (7.2 t/ha), followed by India Delhi (7.0 t/ha; Fig. 1B).

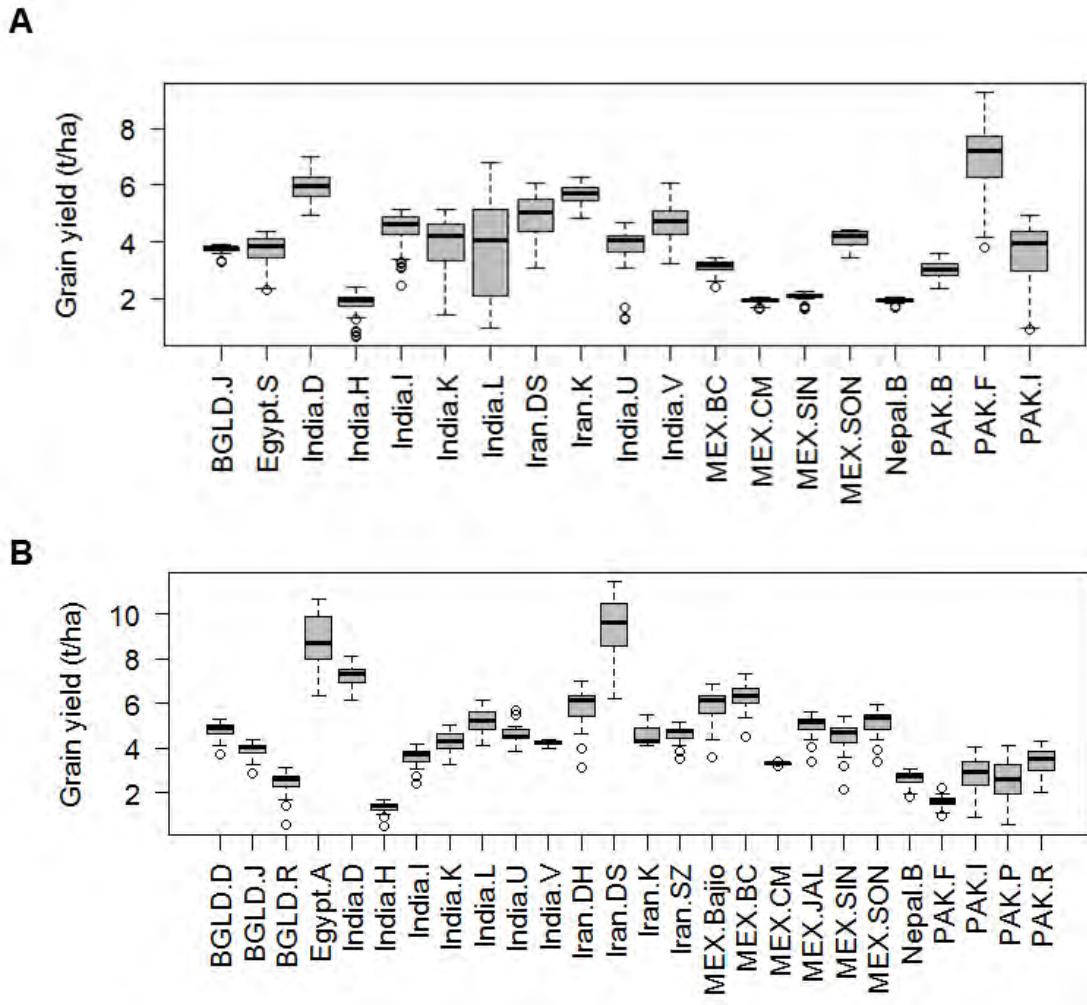


**Figure 1. Box plot of the BLUP estimates of grain yield from the (A) 1<sup>st</sup> WCYT and (B) 2<sup>nd</sup> WCYT data from different environments.**

The SATYN international nursery is bred for stressed environments at CENEB, Mexico, and has average yields 3-4 t/ha (Figs. 2, 3). At some sites (e.g. Mexico, India), the SATYN is grown under irrigation, and these environments recorded yields >5 t/ha. The 1<sup>st</sup> SATYN was grown in 10 environments, with grain yield ranging from 1.0 to 6 t/ha. The highest yielding site was IP12 and the average yield of the nursery at CENEB was 2.5 t/ha. BLUPs across the sites had a mean yield of 3.5t/ha (Fig. 2).



**Figure 2. Box plot of the BLUP estimates for grain yield of the 1<sup>st</sup> SATYN from different environments.**

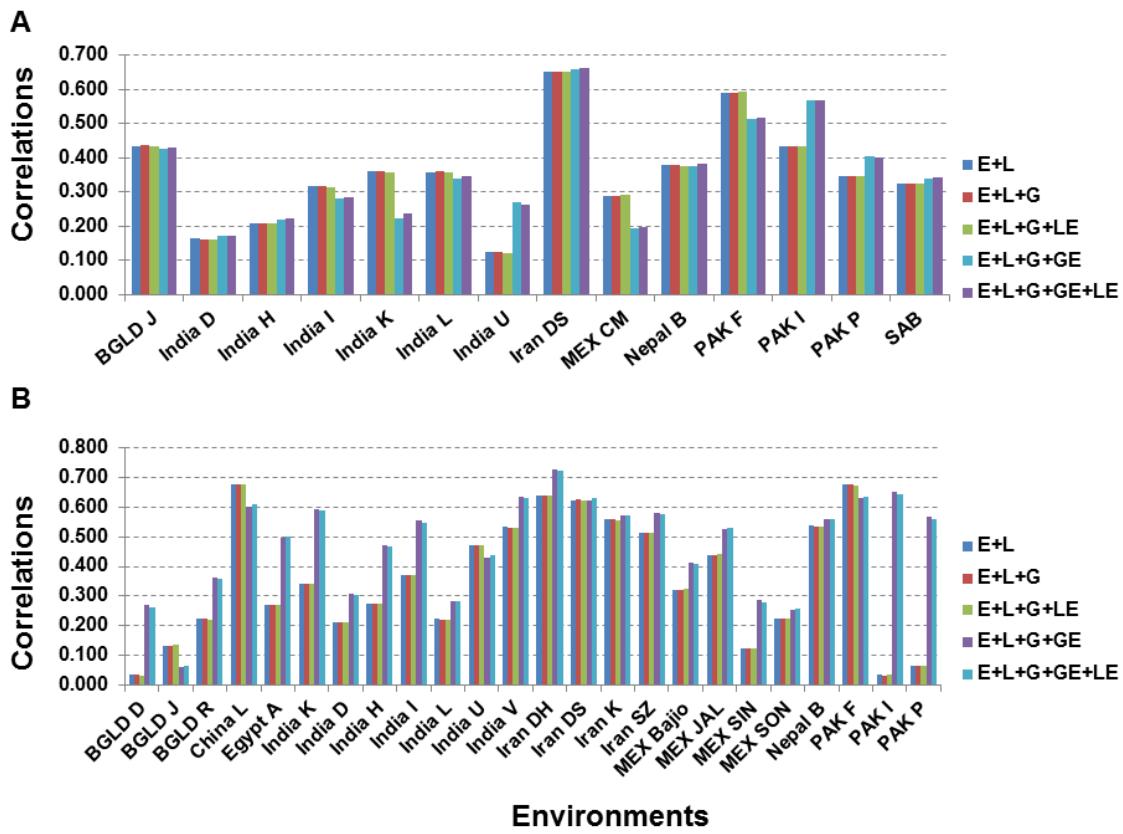


**Figure 3. Box plot of the BLUP estimates for grain yield of the (A) 2<sup>nd</sup> SATYN and (B) 3<sup>rd</sup> SATYN from different environments.**

The 2<sup>nd</sup> SATYN was grown at 19 different sites and grain yield ranged from 1.8 to 7.1 t/ha. The average yield across sites was 3.9 t/ha (Fig. 3A). The 3<sup>rd</sup> SATYN (24 entries) was grown in 26 environments and the highest yield was recorded in India Delhi. The yield at CENEBC was 3.2 t/ha (Fig. 3B).

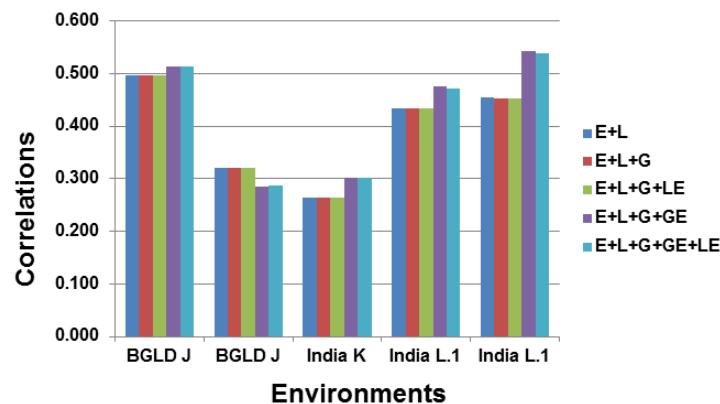
#### *Prediction accuracy*

The average prediction accuracy when models 1 and 2 were used in WYCYT was 0.35, whereas the additional terms in models 3, 4, and 5 increased the prediction accuracy to 0.43. This is evident in most of the environments (Fig. 4).

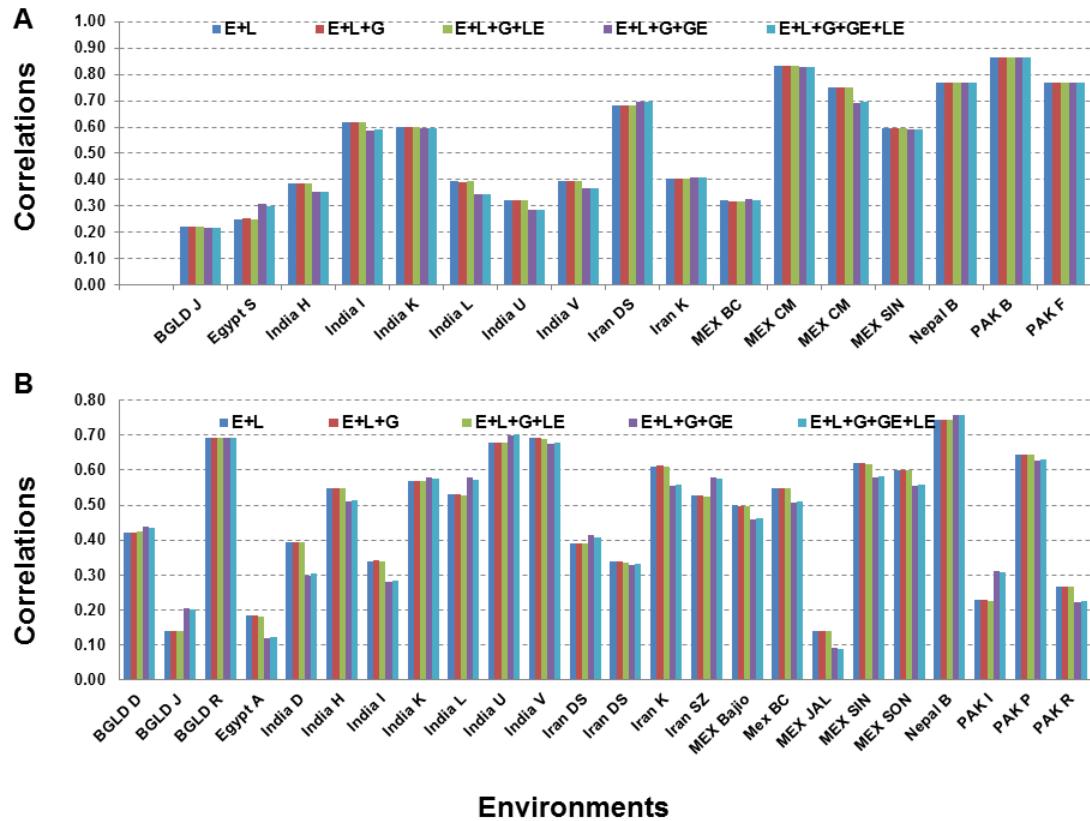


**Figure 4. Correlation between the predicted and observed values using different models: model 1(E+L), model 2 (E+L+G), model 3 (E+L+G+LE), model 4 (E+L+G+GE), and model 5 (E+L+G+GE+LE) for (A) prediction accuracy of sites in the 1<sup>st</sup> WCYT and (B) prediction accuracy of sites in the 2<sup>nd</sup> WCYT.**

Prediction accuracy for SATYN ranged from 0.10 to 0.50. The prediction accuracy values were higher for models 4 and 5 in the 1<sup>st</sup> SATYN, compared to models 1, 2, and 3 (Fig. 5). Average prediction accuracy was 0.48 using models 1, 2, and 3, where was the value remained the same with the addition of further factors into models 4 and 5 in the 2<sup>nd</sup> and 3<sup>rd</sup> SATYN (Fig. 6).



**Figure 5. Correlation between the pedigree based prediction and the observed values in the 1<sup>st</sup> SATYN.**



**Figure 6. Correlation between the pedigree based prediction and the observed values in 2<sup>nd</sup> and 3<sup>rd</sup> SATYN.**

The reaction norm model proposed by Jarquin et al. (2014) was adopted in this paper and applied to five international nurseries grown in different environments. These models can predict grain yield in different environments with different prediction accuracies. Models 4 and 5 showed increased prediction accuracy in the 1<sup>st</sup> WCYT grain yield predictions, whereas the prediction accuracy remained the same even with the environmental terms in the SATYN nurseries. The analysis also indicated a high degree of correlation between the tested sites, and these can be used for making decisions on the international testing sites and release of variety (data not shown).

#### Potential for genomic selection

CIMMYT has the unique advantage of running international trials in thousands of environments across the world. This phenotypic data, together with sequence evaluation of the wheat lines across a large number of environments, provide the two most important elements for assessing line adaptability and stability and for a precise assessment of G × E, phenotypic, and genetic information. Furthermore, environmental variables can be incorporated into statistical models to combine these highly dimensional data and thus improve the prediction of lines in biotic and abiotic stress environments. It will be worth comparing the GS models with marker based matrix and the COP based matrix once the genotypic data become available.

#### Conclusions

- The additive relationship matrix ( $A$ ) based on COP matrix is an excellent complement to genomic marker based matrix for genomic selection and predictions. While pedigree accounts for between family relationships, markers use within family relations and thus account for Mendelian sampling, therefore saving one of two cycles of phenotyping,
- The reaction norm models for assessing G x E interactions predict well some environments and allows compilation and combination of highly unbalanced data from different environments used in international breeding trials.

## References

- de los Campos, G. and Pérez-Rodríguez, P., 2013. BGLR: Bayesian generalized linear regression. R package v.1.0.3. Available at <http://CRAN.R-project.org/package=BGLR> (verified 16 Feb. 2016).
- Jarquín, D., Crossa, J., Lacaze, X., Cheyron, P., Daucourt, J., Lorgeou, J., Piraux, F., et al. 2014. A reaction norm model for genomic selection using high-dimensional genomic and environmental data. *Theoretical and Applied Genetics* 127(3):595–607.
- Lado, B., Barrios, P. G., Quincke, M., Silva, P., and Gutiérrez, L., 2016. Modeling Genotype $\times$  Environment Interaction for Genomic Selection with Unbalanced Data from a Wheat Breeding Program. *Crop Science* 56: 1-15.
- Lorenz, A., Smith, K., and Jannink, J., 2012. Potential and optimization of genomic selection for Fusarium head blight resistance in six-row barley. *Crop Science* 52:1609–1621.
- Pask, A., Joshi, A. K., Manès, Y., Sharma, I., Chatrath, R., Singh, G. P., ... and Mishra, V. K., 2014. A wheat phenotyping network to incorporate physiological traits for climate change in South Asia. *Field Crops Research* 168, 156-167.
- Pérez-Rodríguez, P. and de los Campos, G., 2014. Genome-wide regression and prediction with the BGLR statistical package. *Genetics* 198: 483-495.
- Pérez-Rodríguez, P., Crossa, J., Bondalapati, K., De Meyer, G., Pita, F., and de los Campos, G., 2015. A Pedigree-Based Reaction Norm Model for Prediction of Cotton Yield in Multi-environment Trials. *Crop Science* 55(3): 1143-1151.
- Rutkoski, J., Singh, R.P., Huerta-Espino, J., Bhavani, S., Poland, J., Jannink, J.L., and Sorrells, M.E., 2015. Efficient use of historical data for genomic selection: A case study of stem rust resistance in wheat. *Plant Genome* 8: 1–10.
- Reynolds, M., Bonnett, D., Chapman, S. C., Furbank, R. T., Manès, Y., Mather, D. E., and Parry, M. A., 2010. Raising yield potential of wheat. I. Overview of a consortium approach and breeding strategies. *Journal of Experimental Botany* 62: 439–452.
- Reynolds et al., 2015. Pre-Breeding for Yield Potential Traits: Summary of International Data from 2nd WCYT and Performance of Pipeline Material. In: Reynolds, M. P., Molero, G., Mollins, J., and Braun, H.J., "International TRIGO (Wheat) Yield Potential, Proceedings; Cd. Obregon, Sonora, Mexico; 24-26 Mar 2015.".

## **Evaluation of the Stress Adaptive Trait Yield Nursery (SATYN) in irrigated wheat growing locations in Mexico during the 2015-16 growing season**

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### **Abstract**

The SATYN (Stress Adaptive Trait Yield Nursery) was established as a uniform trial in five locations of Mexico (Baja California, Guanajuato, Jalisco, Sinaloa, and Sonora) during the 2014-15 crop season. It included 28 outstanding bread wheat lines derived from, or having Parula, Pastor, Veery, synthetic lines, SOKOLL, or WEEBIL 1 in their parentage, with the final two and Roelfs F2007 included as checks. Grain yield, plant height, days to heading, days to maturity, harvest index, biomass, spikes per square meter, thousand kernel weight, and grains per square meter were evaluated. Independent analyses of variance by location and a combined analysis of variance with the data from all the locations were performed for each variable. Significant differences were observed among locations, genotypes, and the genotype-by-environment interaction for all variables studied. From the combined analysis, two sister lines derived from the cross Sokoll/Weebil 1 (genotypes 25 and 23) showed better average yields than the best check, by 7.9 and 6%, respectively, and a tendency to interact positively with environments. Their good performance can be explained, at least partially, by their outstanding TKW. Similarly, line 4 (JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED) deserves special attention for its tendency to outyield the checks in Sonora and Sinaloa, environments characterized this season by exceptional heat stress. Its good performance seems to be associated with maintaining good values of biomass and thousand kernel weight and this type of material could be more valuable in the future, as global warming increases.

### **Introduction**

Wheat is an important staple food in Mexico, but is mainly grown under irrigation (Aguilar 1991; Solís and Rodríguez 2000). Yet the crop is produced in a variety of Mexican environments, offering a good opportunity to accurately evaluate the yield potential and adaptability of new genotypes, since usually under irrigation there is less uncontrolled variation among experimental plots than under rainfed conditions.

This is the summary of results of the uniform assay of the SATYN (Stress Adaptive Trait Yield Nursery), established during the 2015-16 crop season in five of the main wheat growing regions of México (Baja California, Guanajuato, Jalisco, Sinaloa, and Sonora).

### **Methods**

The uniform trial was established at the environments shown in Table 1. The SATYN consisted of 28 outstanding bread wheat lines derived from, or having Parula, Pastor, Veery, synthetic lines, SOKOLL or WEEBIL 1 in their parentage, with the final two and Roelfs F2007 included as checks.

**Table 1. Environments where the SATYNs were established in Mexico, 2014-15 season.**

Location	Planting date	Coord.	Altitude (masl)	Fertilization (N-P-K)	Irrigation (DAP)	Medium Temp °C	Max. Temp °C	Min Temp °C	Harvest Date
<b>Baja California</b>	Jan/07/15	32° 18' N 115° 4' W	8.6	280-80-00	0-35-65- 85-110	18.90	27.90	10.20	May/18/15
<b>Guanajuato</b>	Dec/18/14	20° 32' N 100° 49' W	1752	240-60-00	0-45-75- 100	16.75	25.92	8.24	May/15/15
<b>Jalisco</b>	Jan/05/15	21° 18' N 102° 30' W	1541	310-40-40	0-30-55- 80	21.73	31.02	12.44	May/09/15
<b>Sinaloa</b>	Dec/12/14	25° 45' N 108° 48' W	14	204-52-00	0-46-75- 98	20.14	29.83	12.88	May/20/15
<b>Sonora</b>	Dec/22/14	27° 21' N 109° 55' W	37	253-46-00	0-46-70- 100-111	18.33	27.36	10.73	May/18/15

Traits evaluated were: grain yield (GY), plant height (PH), days to heading (DH), days to maturity (DM), harvest index (HI), biomass (BIO), spikes per square meter (SPSM), thousand kernel weight (TKW), and grains per square meter (GPSM). Independent analyses of variance by location and a combined analysis of variance with the information obtained from all the locations were performed for each variable and Tukey's tests were performed to detect significant differences among average values. Genotype-environment interaction was also analyzed using the AMMI1 method when significant interactions were detected in the combined analysis (Vargas and Crossa 2000).

## Results/Discussion

Table 2 shows that most of the environments were useful for detecting differences among genotypes in each of the variables evaluated. Although the expression of such differences may be an inherent characteristic of the genotypes and the environments, it is important to pinpoint a) that some variables are more sensitive to changes than others, and b) that the inability to detect differences in a particular environment may be associated with the ability to reduce uncontrolled variation. Considering that there is some association between the highest coefficients of variation and the lower amount of significant differences in the location of Jalisco, it would be important to find out how to improve the accuracy in that location (investment in the facilities may be required), and how to improve the data obtained for variables like HI and BIO, as differences among genotypes were observed as statistically significant for these variables only in two of the five locations.

**Table 2. Significance of the differences from the analyses of variance applied individually to data of every location/variable, SATYN México, 2014-15.**

Loc	DH	DM	PH	HI	GY	BIO	SPSM	TKW	GPSM
BC	**	*	**	NS	*	NS	NS	**	**
GTO	**	NS	**	**	**	**	**	**	**
JAL	**	NS	**	**	NS	NS	NS	**	NS
SIN	**	**	NS	NS	*	*	**	**	**
SON	**	**	**	NS	*	NS	*	**	**
CV=									
BC	1.83	4.09	7.02	7.85	13.88	16.64	17.04	6.58	16.15
GTO	2.4	0.86	3.19	8.77	14.61	16.9	18.9	6.87	18.41
JAL	2.06	1.01	3.76	7.66	21.42	22.6	21.9	6.05	19.87
SIN	1.5	0.82	7.22	8.86	15.6	15.43	18.71	5.37	16.01
SON	1.2	0.8	4.5	7.07	6.24	9.93	10.45	5.39	7.66

\*\* = Highly significant differences ( $p \leq 0.01$ ); \* = significant differences ( $p \leq 0.05$ ); NS = Non significant differences; LOC = location; DH = days to heading; DM =days to maturity; PH = plant height; HI = harvest index; GY = grain yield; BIO = biomass; SPSM = spikes per square meter; TKW = thousand kernel weight; GPSM = grains per square meter; BC = Baja California; GTO = Guanajuato; JAL= Jalisco; SIN = Sinaloa; SON = Sonora; CV = Coefficients of Variation.

Independently of such observations, the combined analysis detected significant differences among locations, genotypes, and the genotype by environment interaction in every one of the evaluated variables (table 3).

**Table 3. Mean square values from the five locations combined analysis, for every variable evaluated.**

SV	DF	DH	DM	PH	HI	GY	BIO	SPSM	TKW	GPSM
LOC	4	4617.9**	10307.5**	4259.9**	0.262**	113505185.7**	537.8**	172050.1**	2240.1**	825123735**
REP	5	5.41	19.59	49.39	0.0050	2151942	5.01	3608.30	10.73	9960342
GEN	27	71.6**	22.2**	622.7**	0.0023*	2692277.8**	12.6**	15263.1**	118.7**	17055572**
LOC*GEN	108	9.5**	9.9**	44.6**	0.0023**	1983852.2**	9.3**	4341.7*	15.7**	9055143**
ERROR	135	2.02	5.03	27.49	0.0013	667106.60	4.38	2859.90	6.49	3974150
TOTAL	279									
C.V.		1.92	1.94	5.23	7.98	16.30	18.55	18.63	6.13	16.20

SV = source of variation; DF = degrees of freedom; DH, DM, PH, HI, GY, BIO, SPSM, TKW, GPSM, LOC, and CV as in Table 1; REP = repetition; GEN =genotypes.

Table 4 shows that TKW was one of the most important variables associated with GY; in Baja California TKW had a positive effect on GY, whereas it had a negative effect in Guanajuato, in spite of the fact that the Guanajuato environment allowed an excellent stand of the genotypes evaluated. The good average yields obtained in Jalisco can be attributed to a good density of spikes and grains formed, as well as good BIO production. For Sinaloa and Sonora, poor yields were associated with a short season affecting the stand of the plants, which was observed through DH, DM, PH, BIO, SPSM, and GPSM, and for Sinaloa, also through HI. These results are explained by the fact that the 2014-15 crop season was characterized by exceptional heat stress, particularly in Sonora and Sinaloa. If climate change is likely to cause more of

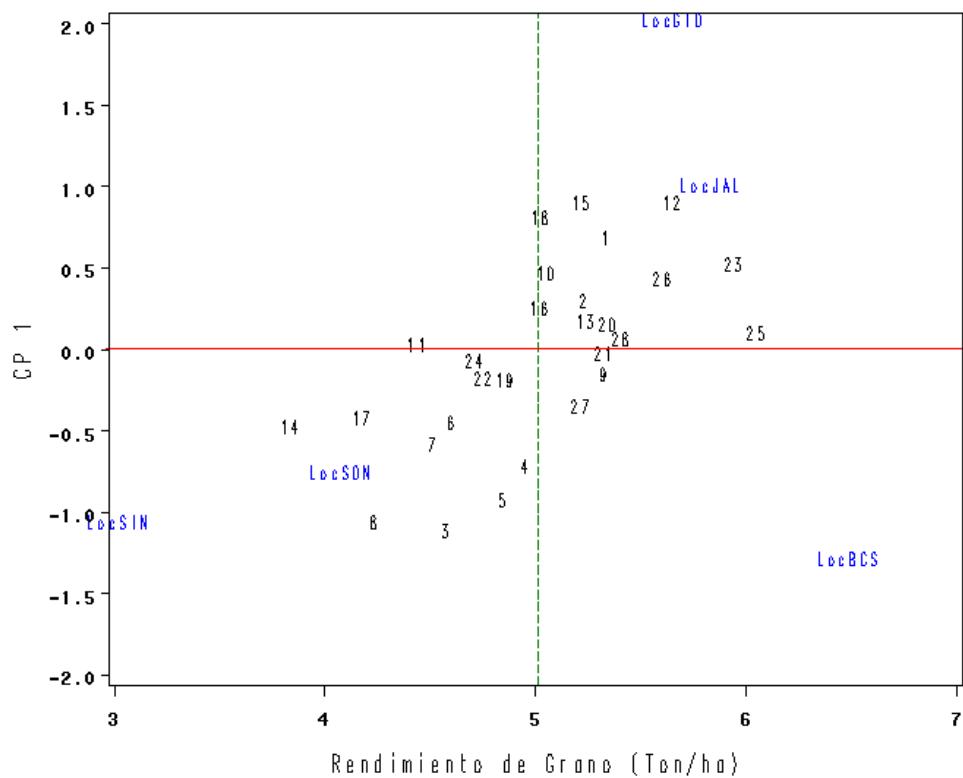
this type of environment in the future, the information obtained this season could be particularly valuable for selecting the most adapted genotypes and knowing their characteristics.

**Table 4. Comparisons of means among locations for the variables evaluated.**

LOC	DH	DM	PH	HI	GY	BIO	SPSM	TKW	GPSM
<b>BC</b>	81(B)	112(C)	92(C)	0.51(A)	6482(A)	12.8(B)	283(B)	50.8(A)	12888(B)
<b>GTO</b>	86(A)	138(A)	108(A)	0.40(B)	5649(B)	14.2(A)	332(A)	34.5(E)	16190(A)
<b>JAL</b>	68(C)	117(B)	111(A)	0.42(B)	5830(B)	13.5(B)	345(A)	37.9(D)	15357(A)
<b>SIN</b>	67(C)	107(D)	97(B)	0.37(C)	3015(D)	8.1(C)	206(C)	44.7(B)	6839(D)
<b>SON</b>	68(C)	104(E)	94(BC)	0.53(A)	4072(C)	7.7(C)	270(B)	40.1(C)	10229(C)
<b>HSD</b>	1	1	3	0.02	427	1.1	28	1.3	1042

HSD = Honest Significant Difference. Other abbreviations as per Table 2. Values in the same column and with the same letter are not statistically different.

Genotypes 25 and 23 (sister lines from the cross Sokoll/Weebil 1) showed positive interactions with the environment (Fig. 1), outyielding the best check by 7.9 and 6.0%, respectively (data not shown). Unfortunately, in Sonora these genotypes were not outstanding, but it will be interesting to evaluate them again, as environmental conditions as harsh as those experienced this season are not expected to be as representative of the near future. It will also be interesting to analyze and reevaluate genotype 4 (JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED), since it had a tendency to outyield the checks in Sonora and Sinaloa under the heat stressed conditions described above, apparently due to better BIO production and TKW (data not shown).



**Figure 1. Grain yield biplot AMMI Graph for the SATYN genotypes evaluated in five locations in Mexico.**

## **Conclusions**

The uniform assay enabled us to identify overall outstanding lines and the way they interact with the environment, as well as a line with good performance under the exceptional heat stress conditions present this season in Sonora and Sinaloa. The latter may be particularly important if global warming continues.

This study was also useful for gaining a better understanding about the level of association of several variables with GY, with the importance of TKW a particular interest. The level of increase of GY in the most outstanding lines, with respect to the checks (7.9 and 6%) is encouraging for maintaining the required pace in genetic gains in breeding programs to satisfy the requirements of this cereal in the future.

## **References**

- Aguilar, G. J. J., 1991. *El trigo mexicano y el tratado de libre comercio. XI Seminario de Economía Agrícola del Tercer Mundo.* UNAM. México, D.F. pp. 1-16.
- Solís-Moya, E. and Rodríguez Guillén, A. 2000. *Trigo de riego: origen, variedades, manejo del cultivo, calidad industrial.* Celaya, Gto., México. SAGAR INIFAP, Campo Experimental Bajío. 139 p. (Libro Técnico No. 2).
- Vargas, M. J. and Crossa, J., 2000. The AMMI analysis and the graph of biplot in SAS. Available at:  
<http://www.cimmyt.org/biometrics>.

## **International performance of physiological trait based lines of the 4<sup>th</sup> Stress Adaptive Trait Yield Nursery (4<sup>th</sup> SATYN)**

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### **Abstract**

Lines of the 4<sup>th</sup> Stress Adapted Trait Yield Nursery (4<sup>th</sup> SATYN) were developed by phenotyping diverse genetic resources from the World Wheat Collection for physiological traits (PT) associated with performance under hot irrigated environments, and then crossing the best complementary sources as parents. Selected progeny were delivered to collaborators at 24 locations worldwide through the International Wheat Improvement Network (IWIN). Analysis of the data indicated the following:

- At most sites, new PT lines outperformed checks: of 25 new lines, an average of 13 and 10 beat the local and CIMMYT checks, respectively, when all sites were considered.
- The mean yield of the best three new PT lines at any given site was on average 22% and 8% over the local and best CIMMYT checks, respectively.
- When considering yield averaged across all 24 sites, 8 of 25 new PT lines outperformed the local check, and one PT line (SOKOLL/WBLL1; entry no. 9423) showed the highest yield across all sites.
- When considering biomass averaged across all sites, 8 and 6 lines outperformed the local check and best CIMMYT check, respectively.

### **Introduction**

CIMMYT has produced several pre-breeding nurseries, including for yield potential (1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> Wheat Yield Consortium Yield Trials; WCYT) and for stress adaptive traits (1<sup>st</sup>-5<sup>th</sup> Stress Adaptive

Trait Yield Nurseries; SATYN), all of which have been distributed through the IWIN (Payne 2004). These nurseries have not only delivered new, genetically diverse germplasm to national wheat programs worldwide, but have also expressed significant genetic gains in spring wheat, providing sources of novel alleles for stress tolerance (Reynolds et al. 2015a) and yield potential (Reynolds et al. 2015b). For example, in the 2<sup>nd</sup> WYCYT, new PT lines were the highest yielding at 23 of 26 international sites, by an average of 10% over the best CIMMYT check at each site, and by as much as 20% at any given site. One PT line (pedigree SERI/BAV92//PUB94.15.1.12/WBLL1) expressed the highest average yield across all sites. Above-ground biomass was reported for 13 sites and PT lines expressed the highest biomass at all 13 sites, by an average of 14% above the best CIMMYT check, and by up to 25% at the best site (Reynolds et al. 2015b).

Here we present results from the 4<sup>th</sup> SATYN, developed for hot irrigated environments.

### **Results (summary)**

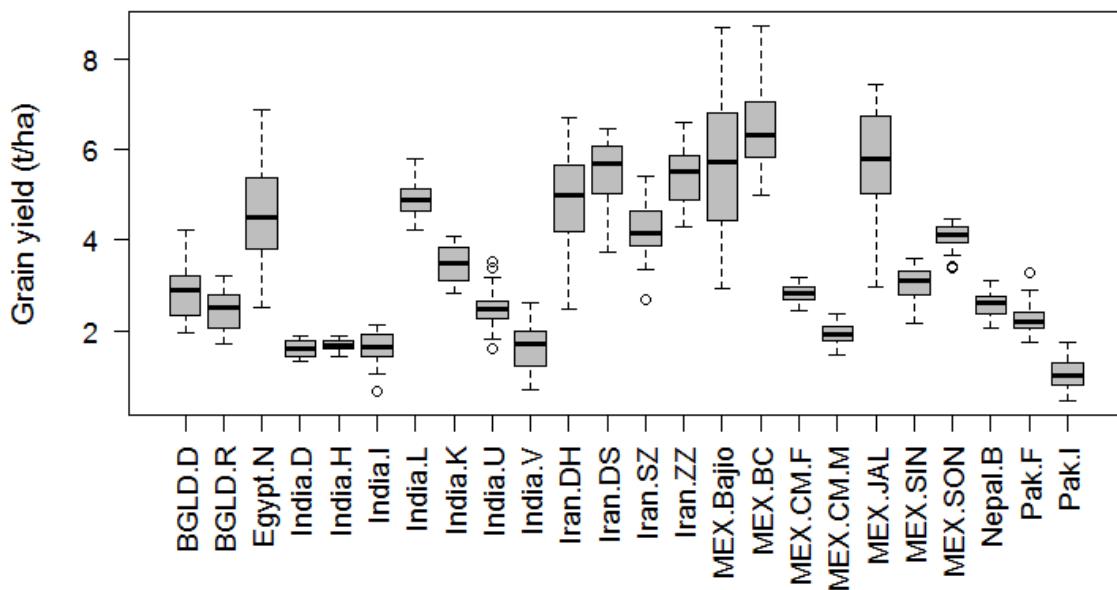
The 4<sup>th</sup> SATYN was grown at 24 international sites in 2015-16 (Table 1) as yield plots of approximately 8-10 m<sup>2</sup> in a replicated lattice design. The trial consisted of 25 new PT lines, two CIMMYT checks (SOKOLL and Weebil), and a local check. This international nursery is the result of PT crosses where parents were selected based on well-established heat adaptive traits (Cossani and Reynolds 2012), including high above-ground biomass, cool canopy temperature, stay green canopy, and ability to store and remobilize stem water soluble carbohydrates. In many of the crosses, new alleles were introduced from primary synthetic hexaploids expressing one or more of the aforementioned PTs, since synthetic derived lines have been shown to express excellent heat tolerance (Cossani and Reynolds 2015).

**Table 1. Sites returning data from 4<sup>th</sup> SATYN (to date)**

No	Abbreviation	Site	Country
1	BGLD D	BARI Dinajpur, Rangpur	Bangladesh
2	BGLD R	BARI Rajshahi A.R.S	Bangladesh
3	Egypt N	ARC Nobaria	Egypt
4	India D	IARI Delhi	India
5	India H	UAS Dharwad, Karnataka	India
6	India I	IARI Indore, Madhya Pradesh	India
7	India K	IIWBR Karnal, Haryana	India
8	India L	PAU Ludhiana, Punjab	India
9	Iran DH	FARS Darab Hassan Abad	Iran
10	Iran DS	Dezfoul Safiabad ARC, Khuzestan	Iran
11	Iran SZ	Shiraz Zargan, FARS	Iran
12	Iran ZZ	Zabol Zahak STN, Sistan and Balochistan	Iran
13	India U	Ugar Khurd, Karnataka	India
14	India V	BHU Varanasi, Uttar Pradesh	India
15	MEX BC	INIFAP-Mexicali Baja California	Mexico
16	MEX Bajio	INIFAP-Bajio	Mexico
17	MEX CM F	CIMMYT CENEB February	Mexico
18	MEX CM M	CIMMYT CENEB March	Mexico
19	MEX JAL	INIFAP Tepatitlan Jalisco	Mexico
20	MEX SIN	INIFAP Valle del Fuerte, Sinaloa	Mexico
21	MEX SON	INIFAP Valle del Yaqui	Mexico
22	Nepal B	NWRP Bhairahawa, Rupandehi	Nepal

23	PAK F	WRI Faisalabad, Punjab	Pakistan
24	PAK I	NARC Islamabad	Pakistan

As expected, considerable G x E variation was observed for grain yield (Fig. 1). Average yield of the SATYN varied from 1.04 t/ha (PAK I) to 6.48 t/ha (MEX BC). The lowest yield recorded for a line was at PAK I (0.6 t/ha average yield), with the highest yield of an SATYN line recorded at MEX BC (8.72 t/ha). In general, sites in Egypt, Iran, and Mexico (MEX BC, MEX Bajio, and MEX JAL) were higher yielding. For example, in Egypt A, one PT line (JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED; entry no. 9407) yielded 6.8 t/ha, compared to a SOKOLL yield of 4.5 t/ha. At one of the highest yielding sites in Iran (Iran DS), SOKOLL (5.33 t/ha) was outperformed by 1.1 t/ha by the PT line JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED (entry 9405) with a mean yield of 6.46 t/ha (Table 2).



**Figure 1. (A) Box plot of grain yield of 4th SATYN presented in each site.**

**Table 2. Summary data of the lines in 4<sup>th</sup> SATYN averaged across heat stressed IWIN sites (24). Lines that yielded higher or had higher biomass than SOKOLL are shaded grey. Lines that yielded higher than the local check are shaded yellow.**

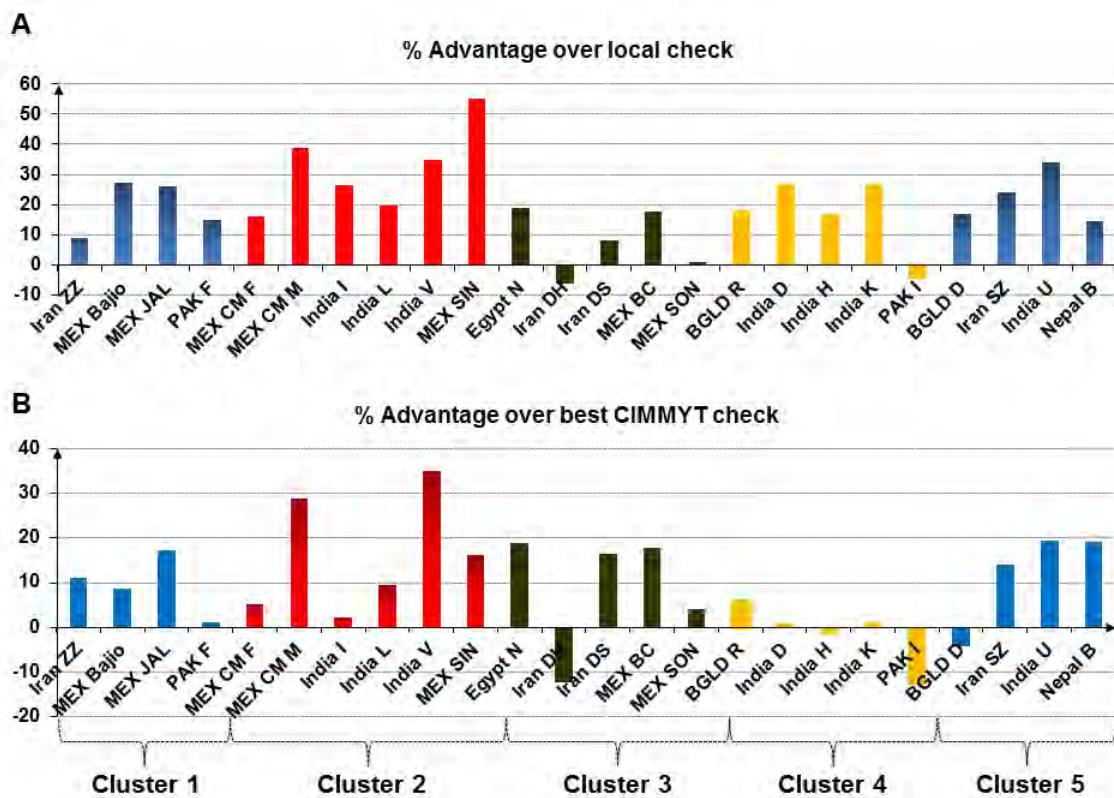
Entry	Cross name	Grain yield*(t/ha)	Biomass	Days to heading	Days to maturity	Plant height
9401	KS940935.7.1.2/*PASTOR/4/FRA MILAN/KAUZ/3/PASTOR	<b>3.55</b>	1029.8	71.0	103.8	85.8
9402	KS940935.7.1.2/*PASTOR/4/FRA MILAN/KAUZ/3/PASTOR	<b>3.67</b>	1037.8	74.8	106.7	86.9
9403	JNRB.5/PIFED/5/BJY//COC//PRL/BOW/3/SARA/THB//V EE/4/PIFED	3.23	880.0	75.7	106.2	82.4
9404	JNRB.5/PIFED/5/BJY//COC//PRL/BOW/3/SARA/THB//V EE/4/PIFED	<b>3.52</b>	962.7	77.1	108.4	91.5
9405	JNRB.5/PIFED/5/BJY//COC//PRL/BOW/3/SARA/THB//V EE/4/PIFED	<b>3.62</b>	1019.9	76.9	107.4	91.2
9406	JNRB.5/PIFED/5/BJY//COC//PRL/BOW/3/SARA/THB//V EE/4/PIFED	3.31	1005.1	76.9	108.0	95.1
9407	JNRB.5/PIFED/5/BJY//COC//PRL/BOW/3/SARA/THB//V EE/4/PIFED	3.48	950.5	75.6	106.7	95.4
9408	JNRB.5/PIFED/5/BJY//COC//PRL/BOW/3/SARA/THB//V EE/4/PIFED	3.24	1001.5	76.7	106.4	84.9
9409	WORRAKATTA/2/*PASTOR/6/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/5/PFAU/WEAVER	3.60	1065.1	78.4	110.4	95.4
9410	WORRAKATTA/2/*PASTOR/6/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/5/PFAU/WEAVER	3.38	973.1	76.2	107.5	90.8
9411	SOKOLL/WESTONIA	3.34	1025.4	75.0	105.9	99.2
9412	SOKOLL/WESTONIA	<b>3.50</b>	944.9	73.9	106.0	89.7
9413	SOKOLL/WESTONIA	<b>3.57</b>	1062.4	74.4	106.1	100.0
9414	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	2.96	943.7	75.3	106.4	101.3
9415	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	3.38	926.5	75.2	105.3	97.6
9416	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	3.38	839.9	76.4	107.4	96.2
9417	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	3.20	916.7	75.3	106.0	97.9

9418	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	3.27	895.3	75.9	107.7	98.5
9419	PASTOR//HXL7573/2*BAU/3/WBLL1	3.41	918.2	73.9	105.6	91.4
9420	PASTOR//HXL7573/2*BAU/3/WBLL1	<b>3.56</b>	910.7	76.1	106.1	86.2
9421	PASTOR//HXL7573/2*BAU/3/WBLL1	3.48	964.5	77.1	106.7	85.2
9422	SOKOLL/WBLL1	3.40	1009.3	77.9	109.0	90.0
9423	SOKOLL/WBLL1	<b>3.74</b>	976.9	77.2	108.1	88.6
9424	SOKOLL/WBLL1	3.44	985.2	77.6	107.5	85.9
9425	SOKOLL/WBLL1	3.58	963.2	80.1	110.5	93.1
9426	<b>SOKOLL</b>	<b>3.72</b>	<b>1010.2</b>	<b>76.3</b>	<b>106.2</b>	<b>89.0</b>
9427	WEEBILL1	3.56	982.3	76.1	108.1	87.4
9428	<b>LOCAL CHECK</b>	<b>3.48</b>	<b>918.1</b>	<b>76.3</b>	<b>107.8</b>	<b>90.7</b>

A combined analysis of the data using “PROC GLM” and “lsmeans”, considering genotypes and environments as random effects in Statistical Analysis Software (SAS), showed that several lines in the SATYN had higher average yield across the sites than the local checks. As expected, the highest biomass line (WORRAKATTA/2\*PASTOR/6/CHIR3/4/SIREN//ALTAR84/AE.SQUARROSA(205)/3/3\*BUC/5/PFAU/WEAVER) had a synthetic parent. In the combined analysis of environments, six lines had higher biomass than the CIMMYT check SOKOLL and eight lines yielded higher than the local checks. SOKOLL/WEEBIL (entry no 9423) was the highest yielding line on average across the sites.

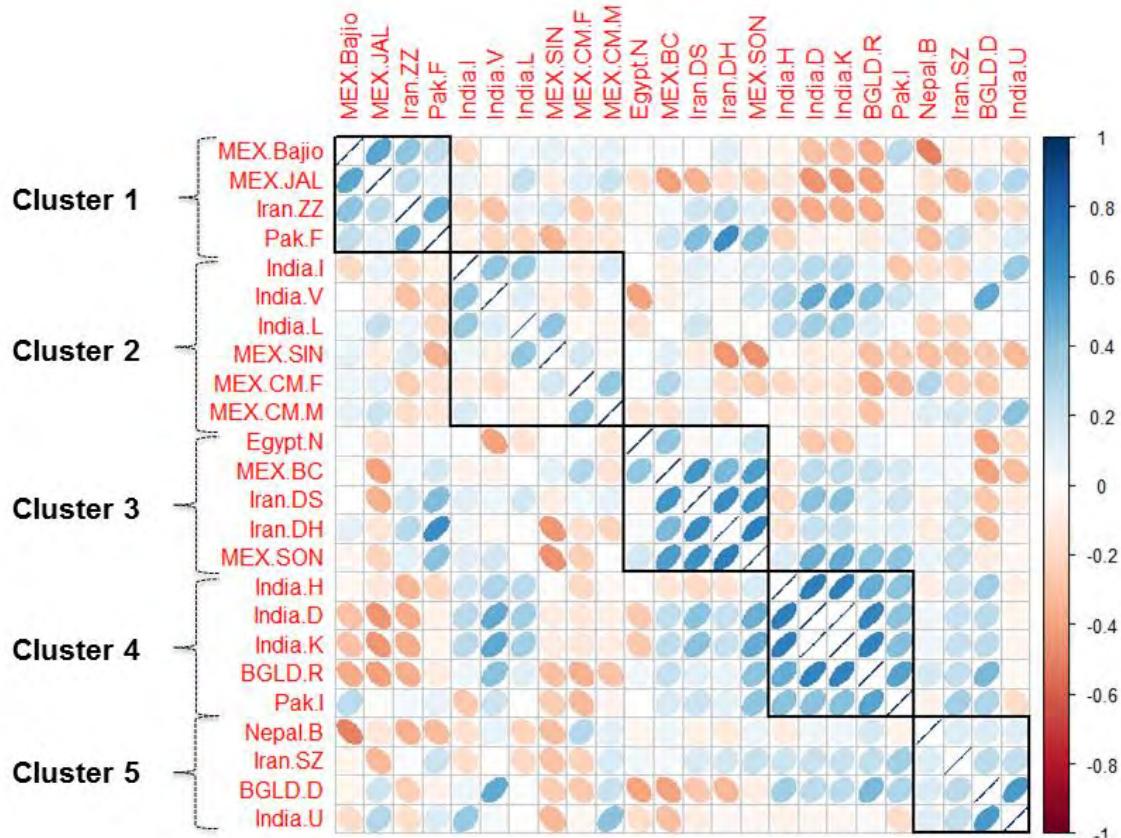
On average, the yield of best performing PT line in a site ranged from 1.7 t/ha (PI) to 8.2 t/ha (MEX BC). The minimum yield of a PT line at a given site ranged from 0.45 t/ha (PI) to 5 t/ha (MEX BC) across the sites. Thus the most stressed environment was Pakistan Islamabad (PAK I), and the least stressed site was Mexico Baja California (MEX BC). Days to heading ranged from 71 to 80 days, with the earliest line in maturing 100 days and the latest line maturing 110 days after emergence.

The performance of PT lines compared to local and CIMMYT checks at all individual sites are shown in figure 2. In several locations (BGLD D, India I, India U, and India V), the maximum yield of PT lines were higher. On average, 13 of 28 lines out-yielded the local check in each environment, and nine lines out-yielded SOKOLL.



**Figure 2. Percentage advantage of the mean of the best three PT lines in each environment over the (A) local check and (B) best CIMMYT check.**

To avoid any bias in the comparison of PT lines with the checks, we also did a cluster analysis of the sites based on the “corrplot” package in R, which indicated that the sites can be grouped into five clusters, based on the correlations for grain yield. Clusters 3 and 4 had the highest correlation for grain yield between the sites (Fig. 3). Clusters 1, 2, 3, 4, and 5 had mean site yields of 4.81, 2.66, 5.80, 2.09, and 2.62 t/ha, respectively. Cluster 3 was the cluster with high yield that included sites from Mexico, Egypt, and Iran. Within each cluster, we compared the performance of PT lines in comparison to SOKOLL and local checks. Clusters 1, 2, 3, 4, and 5 had 3, 21, 13, 0, and 5 lines, respectively, that outperformed SOKOLL (Table 3).



**Figure 3. Clustering of sites based on the correlation of grain yield between them.**

The 4<sup>th</sup> SATYN was also grown at the Norman E. Borlaug Experimental Center (CENEB), Mexico, under heat stress conditions with two sowing dates: February (MEX CM F) and March (MEX CM M). March sown plots experienced more heat stress (average site yield 1.93 t/ha) than plots sown in February (average site yield 2.81 t/ha). In a combined analysis of the 4<sup>th</sup> SATYN, 13 lines (grain yield range 2.42 t/ha to 2.68 t/ha) yielded better than SOKOLL (2.36 t/ha; Table 4).

Table 3. Mean yield (t/ha) of each line in the cluster, based on correlations between sites. Several lines performed better than SOKOLL in each cluster are shaded.

Entry	Pedigree	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5
9401	KS940935.7.1.2/2*PASTOR/4/FRAME//MILAN/KAUZ/3/PASTOR	5.3	2.6	4.6	2.1	3.8
9402	KS940935.7.1.2/2*PASTOR/4/FRAME//MILAN/KAUZ/3/PASTOR	5.7	2.4	5.3	2.0	3.8
9403	JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED	3.6	2.5	5.3	2.1	2.9
9404	JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED	4.2	2.7	5.6	2.4	3.1
9405	JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED	3.9	2.9	5.9	2.4	3.4
9406	JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED	4.1	2.6	4.8	2.3	3.0
9407	JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED	4.0	2.7	5.5	2.3	3.1
22	JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED	3.2	2.6	5.6	2.2	3.0
9408	WORRAKATTAA/2*PASTOR/6/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/5/PFAU/WEAVER	5.1	2.8	5.3	2.3	2.9
9409	WORRAKATTAA/2*PASTOR/6/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/5/PFAU/WEAVER	5.2	2.7	4.5	2.0	2.8
9410	SOKOLL/WESTONIA	4.3	2.6	4.5	2.3	3.5
9411	SOKOLL/WESTONIA	6.0	2.6	5.1	1.8	2.3
9412	SOKOLL/WESTONIA	5.1	2.9	4.7	2.3	3.2
9413	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	4.0	2.4	4.3	1.6	3.1
9414	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	5.5	2.8	4.3	1.7	3.1
9415	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	5.1	2.5	4.8	1.8	3.0

		4.2	2.7	4.6	1.7	3.1
9417	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	5.2	2.7	4.2	1.8	2.9
9418	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	4.7	3.0	4.7	2.1	2.8
9419	PASTOR//HXL7573/2*BAU/3/WBLL1	5.0	2.9	5.4	1.8	3.1
9420	PASTOR//HXL7573/2*BAU/3/WBLL1	5.3	2.6	5.5	1.7	2.9
9421	PASTOR//HXL7573/2*BAU/3/WBLL1	4.6	2.8	4.9	2.3	2.8
9422	SOKOLL/WBLL1	5.7	2.9	5.7	2.1	2.6
9423	SOKOLL/WBLL1	4.7	2.6	5.2	2.0	3.3
9424	SOKOLL/WBLL1	5.5	2.5	6.2	1.7	2.6
9425	SOKOLL/WBLL1	5.5	2.5	5.2	2.5	3.3
9426	SOKOLL	4.8	2.6	5.8	2.0	2.9
9427	WEEBILL1	5.2	2.2	5.8	2.1	2.6
9428	LOCAL CHECK	<b>4.8</b>	<b>2.7</b>	<b>5.1</b>	<b>2.1</b>	<b>3.1</b>
<b>Mean cluster yield</b>						

**Table 4.** Lines yielding higher than SOKOLL at CENEB, Sonora, Mexico.

No	Lines	Feb*	March	†LS mean
9420	PASTOR//HXL7573/2*BAU/3/WBLL1	3.00	2.37	2.68
9401	KS940935.7.1.2/2*PASTOR/4/FRAME//MILAN/KAUZ/3/PASTOR	2.88	2.36	2.62
9425	SOKOLL/WBLL1	3.03	2.12	2.57
9405	JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED	2.94	2.19	2.56
9423	SOKOLL/WBLL1	3.16	1.92	2.54
9414	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	2.98	2.10	2.54
9404	JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED	2.99	2.07	2.53
9422	SOKOLL/WBLL1	2.87	2.18	2.52
9418	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	2.97	2.00	2.48
9415	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	2.92	2.03	2.48
9421	PASTOR//HXL7573/2*BAU/3/WBLL1	2.78	2.16	2.47
9417	CROC_1/AE.SQUARROSA (224)//OPATA/3/WBLL1	2.93	1.92	2.43
9424	SOKOLL/WBLL1	2.83	2.00	2.42
9426	SOKOLL	2.92	1.79	2.36

\*Feb (MEX CM F) and March (MEX CM M) indicates sowing date of the trials in CENEB, Mexico, †LS mean indicates least square mean of the two trials.

## Conclusions

- In general, PT lines were higher yielding and expressed higher biomass than local and elite CIMMYT checks at most international sites.
- When considering the average yield of lines across all sites for heat stressed environments, PT lines KS940935.7.1.2/2\*PASTOR/4/FRAME//MILAN/KAUZ/3/PASTOR and JNRB.5/PIFED/5/BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED expressed the highest yields.
- Many of the best performing PT lines showed synthetics in their pedigrees.

## References

- Cossani, C.M. and Reynolds, M.P., 2012. Physiological traits for improving heat tolerance in wheat. *Plant Physiology* 160: 1710-1718.
- Cossani, C.M. and Reynolds, M.P., 2015. Heat Stress Adaptation in Elite Lines Derived from Resynthesized Hexaploid Wheat. *Crop Science* 55:1-17.
- Payne, T., 2004. The international wheat improvement network (IWIN) at CIMMYT.
- Reynolds, M.P., Tattaris, M., Cossani, C.M., Ellis, M., Yamaguchi-Shinozaki, K., Saint-Pierre, C., 2015a. Exploring genetic resources to increase adaptation of wheat to climate change. In: Ogiara, Y., Takumi, S., and Handa, H. (Eds.), *Advances in Wheat Genetics: From Genome to Field*. Springer, Japan.
- Reynolds M.P., A. Pask, et al., 2015b. *Pre-Breeding for yield potential: Summary of International Data from 2nd WCYT and Performance of Pipeline Material*. In: Reynolds, M.P., Molero, G., Mollins, J., and Braun, H.J. (Eds.). Proceedings of the International TRIGO (Wheat) Yield Potential Workshop 2015, CENEB, CIMMYT, Cd. Obregon, Sonora, Mexico.

## Traits determining differences in yield potential among elite lines of a spring wheat panel with the view to accelerating genetic gains

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### Abstract

It is generally assumed that there is restricted genetic diversity for traits contributing to yield potential in modern wheat, though few studies have systematically checked this assumption. In order to assess genetic variation for yield potential related traits, a representative set of elite material that breeders would realistically use in their strategic crosses to raise yield potential was evaluated. Over a three-year period, a subset of 27 genotypes (22 recent elite bread wheat lines, four landmark historical bread wheat cultivars, and one durum line) selected from the CIMMYT Mexico Core Germplasm (CIMCOG) panel were evaluated in northwest Mexico. The results from the 27 genotypes, and additional analysis considering only the 22 elite lines (disregarding the four historic cultivars and the one durum wheat line) show a substantial level of genotypic variation in most variables analyzed, indicating that a good combination of traits into strategic crosses can drive yield potential. Future increases in yield potential must include improvements in source- and sink-related traits in an integrated strategy. Our results indicate than an increase in water soluble carbohydrate after anthesis and chlorophyll content in flag leaf can potentially increase sink demand. However, high chlorophyll content in the flag leaf was negatively correlated with radiation use efficiency, likely associated with better light distribution in the crop canopy and less photochemical damage to leaves with less chlorophyll content. On the other hand, higher biomass at initiation of booting or anthesis could result in more spike dry weight at anthesis, increasing grain set and yield.

### Introduction

Yield potential is defined as the yield of an adapted cultivar when grown with the best agronomical management and without any biotic or abiotic stress (Evans 1993). During the Green Revolution, yield increases were associated with the improvement of sink-related traits such as harvest index (HI) and grain number (GNO). These came from the dwarfing genes that reduced plant height (Fischer and Stockman 1986; Miralles and Slafer 1995; Sayre et al. 1997; Shearman et al. 2005), thus increasing biomass partitioning to the juvenile spikes growing during the second half of the stem elongation phase (Siddique et al. 1989; Miralles et al. 1998). Since the Green Revolution, yield progress has continued with genetic gains in both sink- and source-related traits. For instance, there have been improvements in photosynthetic rate and increased stomatal conductance in bread and durum wheats (Fischer et al. 1998; Fischer 2007), as well as in other source-related traits such as stem water soluble carbohydrate (WSC) content, above-ground biomass, crop growth rate, and radiation use efficiency (RUE) (Shearman et al. 2005; Acreche et al. 2009; Aisawi 2011; Aisawi et al. 2015).

Better knowledge of the physiological traits associated with genetic gains in yield potential, and the study of synergies and trade-offs that may be expected from combining different physiological mechanisms, is essential for improving our understanding of yield-limiting factors (Reynolds et al. 2012). In order to accelerate rates of genetic gains in the near future, complex physiological traits must be incorporated as additional criteria in breeding programs. Breeders aiming to increase yield (and other complex traits) by pyramiding related attributes are keen to limit their programs to crosses between elite material, thus determining the range of genotypic variation in these traits within elite material is of paramount importance. In this sense, a general conceptual platform for identifying variation of critical traits among elite germplasm that permits designing crosses combining complementary physiological traits for yield potential has been recently developed (Reynolds et al. 2009, 2011).

This study used a set of the latest elite spring bread wheat lines developed for high yield irrigated environments from the CIMMYT Core Germplasm (CIMCOG) panel, which also includes historical lines and two elite durum wheat lines. The study aimed to: i) determine the genetic variation of the main yield potential related traits with a specific focus on source and sink; ii) quantify the phenotypic and genetic correlations of these traits with yield/biomass, as well as their heritability; and iii) using principal component analysis, compare the associations among and between source- and sink-related traits to suggest trait combinations that could potentially result in cumulative gene action for yield, as an outcome of strategic crossing. By the nature of the CIMCOG, it served primarily to analyze variation within the elite material, but also may shed light on differences that may be expected when the variation widens through the inclusion of other materials (historical lines and durum wheat).

## Methods

The CIMCOG panel is the primary germplasm studied thus far by the Wheat Yield Consortium (Reynolds et al. 2011) and is comprised of 60 elite wheat lines (58 *T. aestivum* and 2 *T. turgidum* var. *durum*). These lines were primarily selected to represent lines derived from the most recent CIMMYT selections (from 1999 to 2009), including some synthetic-derived wheat material, six older cultivars that represent landmarks in past genetic gains, and two high-yielding durum wheats. They represent an actual set of elite material breeders would realistically use in their strategic crosses to further raise yield potential (51 of the 60 lines). The 60 lines were evaluated in two consecutive growing seasons (2010/11 and 2011/12) and a subset of 27 lines was evaluated in an additional year (2012/13). This subset of 27 lines comprised 22 elite lines, four landmark historical cultivars of *T. aestivum*, and one *T. turgidum* var. *durum* line. Traits evaluated included: biomass measured at canopy closure ( $BM_{T1}$ ); biomass at initiation of booting ( $BM_{T2}$ ); biomass seven days after anthesis ( $BM_{T3}$ ); biomass at physiological maturity (BM); canopy temperature at canopy closure ( $CT_{T1}$ ); canopy temperature during grain filling ( $CT_{GF}$ ); days to anthesis (DTA); days to booting (DTB); days to heading (DTH); days to initiation of booting (DTInB); days to maturity (DTM); days to terminal spikelet (DTTS); fruiting efficiency (FE); number of florets per spikelet (Florets SPKL<sup>-1</sup>); grains per square meter (GNO); grain number per spike (GSP); grain weight per spike (GWSP); Harvest Index (HI); number of infertile spikelets per spike (Infertile SPKL SP<sup>-1</sup>); light interception at canopy closure ( $LI_{T1}$ ); light interception at initiation of booting ( $LI_{T2}$ ); light interception seven days after anthesis ( $LI_{T3}$ ); light interception during grain filling period ( $LI_{GF}$ ); NDVI at canopy closure (NDVI<sub>T1</sub>); NDVI during grain filling (NDVI<sub>GF</sub>); percentage of grain filling (PGF); rapid spike growth phase (RSGP); RUE, from emergence until canopy closure (RUE<sub>T1-T0</sub>); RUE from canopy closure to initiation of booting (RUE<sub>T2-T1</sub>); RUE from initiation of booting to seven days after anthesis (RUE<sub>T3-T2</sub>); RUE from seven days

after anthesis until physiological maturity ( $\text{RUE}_{\text{GF}}$ ); RUE from canopy closure to physiological maturity ( $\text{RUE}_{\text{T}}$ ); spikes per square meter (SNO); SPAD units measured in flag leaf seven days after anthesis ( $\text{SPAD}_{\text{A7}}$ ); spike index (SPI); spike length (Spike); number of spikelets per spike ( $\text{SPKL SP}^{-1}$ ); thousand grain weight (TGW); percentage of stem WSC measured at heading ( $\text{WSC}_{\text{H}}$ ); percentage of stem WSC measured seven days after anthesis ( $\text{WSC}_{\text{A7}}$ ); percentage of stem WSC measured fourteen days after anthesis ( $\text{WSC}_{\text{A14}}$ ); percentage of stem WSC measured twenty one days after anthesis ( $\text{WSC}_{\text{A21}}$ ); percentage of stem WSC measured twenty eight days after anthesis ( $\text{WSC}_{\text{A28}}$ ); and percentage of stem WSC measured at physiological maturity ( $\text{WSC}_{\text{Mat}}$ ).

## Results/Discussion

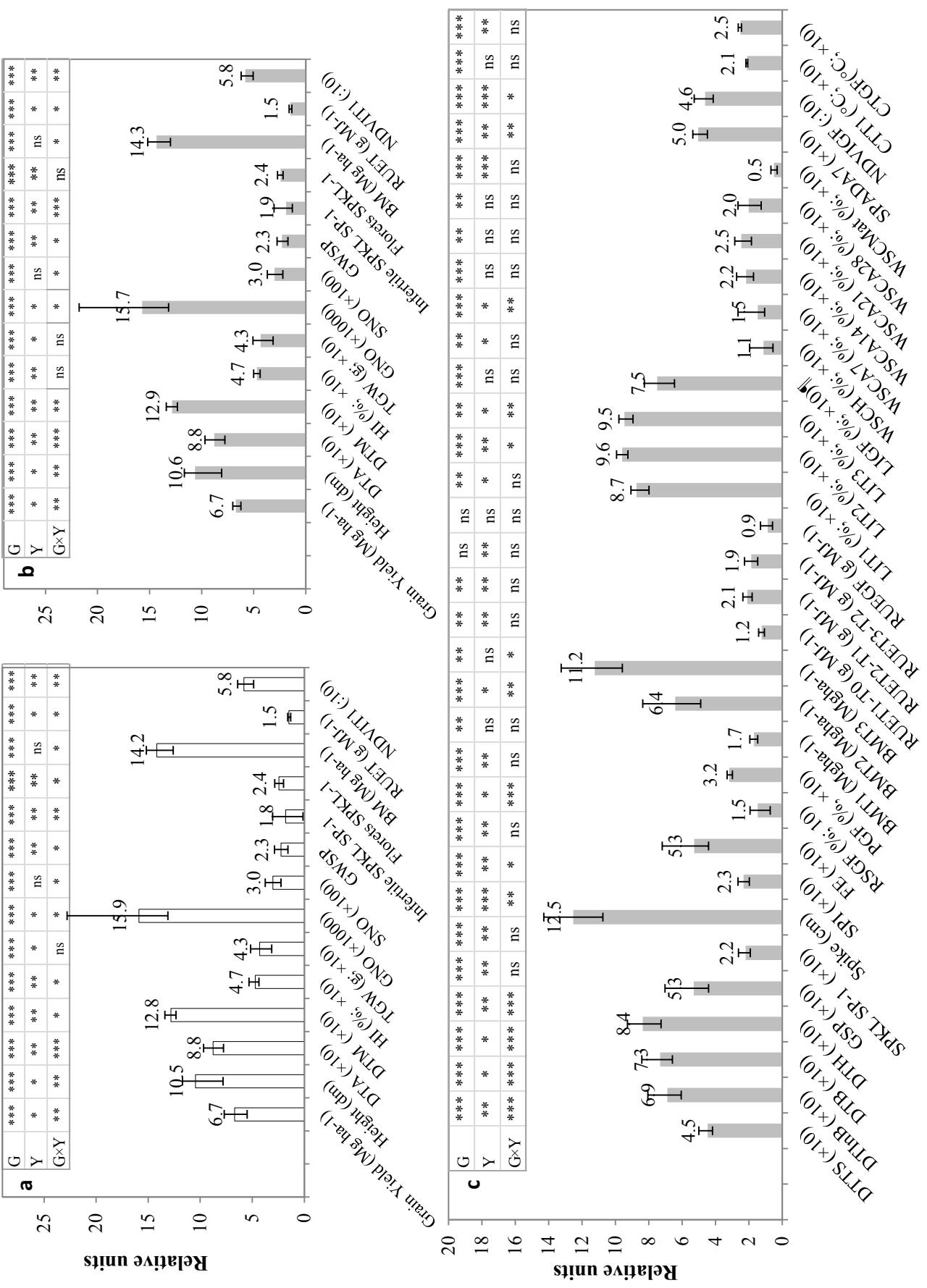
Only the 27-line subset was grown in all three experiments carried out in this study; the other 33 lines were included in the first two years of evaluation (2010/11 and 2011/12). The subset was carefully selected to reliably represent the whole panel of 60 genotypes (data not shown). The analysis conducted here considered the whole subset of 27 lines, and additional analysis was conducted on the 22 elite lines of this subset (disregarding the four historic cultivars and the durum wheat line). When using the whole subset, it is possible to recognize variations and associations considering the complete CIMCOG panel, whereas when analyzing only the 22 elite hexaploid lines, any ranges of variations or relationships reflect the influence of the historic lines and/or the durum wheat in the overall analysis.

### *Genetic variation in yield, source- and sink-related traits*

Although the CIMCOG panel largely comprises well adapted and high-yielding elite lines, there was a substantial level of genotypic variation in most variables analyzed, as revealed by consistently highly significant genotypic effects. This occurred not only for the whole subset of the 27 lines (Fig. 1a) but also when the analysis was restricted to exclude the historic lines and durum wheats (Fig. 1b, c). As expected, the phenotypic range of yield was reduced for the subset of 22 lines, compared to the subset of 27 lines. Yet even within the elite hexaploid wheats, genotypic differences in yield were highly significant but also reasonably consistent across environments, as the broad-sense heritability was reasonable ( $h^2 \approx 0.53$ ) and the genotype-by-year interaction was less significant than the genotypic differences (Fig. 1b).

Considerable differences were observed in all yield components and in all cases the genotypic effect and consistency across environments was noticeable (Fig. 1). Genotypes also differed highly significantly and rather consistently across seasons in biomass, light interception at the onset of stem elongation (assessed by NDVI around GS31), and RUE (from then to maturity) in both the 27 and 22 lines studied.

**Figure 1. Main agronomic, yield components and phenology, source and sink related traits (a, b) together with additional traits measured (c) in of the subsets of 27 (white bars) and 22 genotypes (grey bars, not considering the durum line and the histories) across three years for CIMCOG panel grown in northwest Mexico under fully irrigated conditions. To include all variables in the same figure, the actual values of some of them were divided by 10, 100, or 1000, or multiplied by 10. For each variable the bar indicates the average value, and error bars indicate minimum and maximum values. The significance of interactions among experimental factors is shown above the graphs, where \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , and not significant (ns). For trait codes, see material and methods section.**



The germplasm used in this study represents a selection of genetically diverse recent advanced lines from CIMMYT's spring wheat breeding program for yield potential under optimal conditions. One of the purposes of assembling this germplasm set was to provide parents that could be used as sources of specific yield potential related traits for both pre-breeding and genetic studies. It is therefore necessary to identify traits that can drive genetic yield potential and assess their genetic variation (Ghanem et al. 2014).

#### *Physiological determinants of yield and biomass*

Less variation among sink and source traits was explained when the 27 lines were analyzed, compared to when the analysis was restricted to the 22 elite hexaploid lines (Fig. 2). In both instances, biomass in the early stages (represented here by NDVI and LI measurements in the same period), were positively correlated with the number of infertile spikelets per spike and negatively related with the number of floret per spikelet. In the 27-line subset, the number of infertile spikelets was negatively correlated with yield ( $r = -0.35$ ) whereas the number of floret per spikelet and GWSP were positively correlated with yield ( $r = 0.43$  and  $r = 0.40$ , respectively). No correlations were observed when the analysis was restricted to the 22 elite hexaploid lines, indicating a strong influence of the durum line (CIRNO, number 6 in the biplot) in the analysis, which presented the largest number of floret per spikelet and no infertile spikelets per spike (data not shown). Yield was significantly correlated with biomass at maturity (considering the whole subset of 27 and only the 22 elite hexaploid lines) and with RUE<sub>T</sub>. On the other hand, yield was significantly correlated with HI only when considering the whole subset; when the analysis was restricted to the 22 elite hexaploid lines no associations were observed (Fig. 2). This can be attributed to yield increases since 1966 (year of release of a historic line in the subset, Siete Cerros T66, number 1 in the biplot), as HI has improved in the last 50 years (Fischer and Stockman 1986; Miralles and Slafer 1995; Sayre et al. 1997; Shearman et al. 2005). However, it is clear that improvements in the last 10 years have not increased HI.

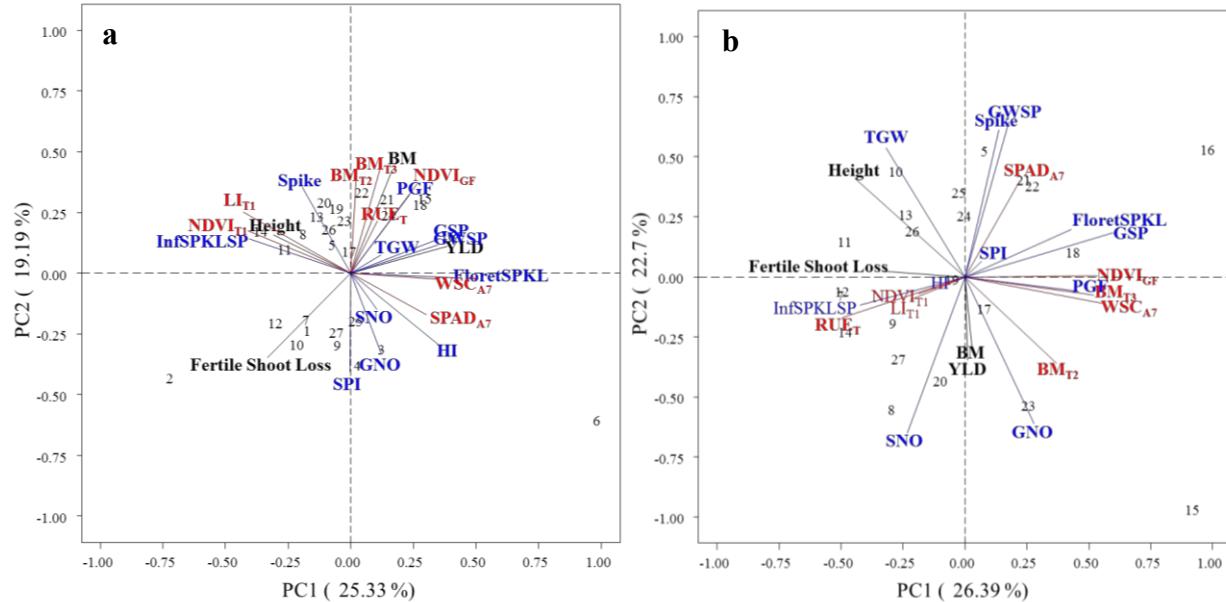
As with the correlations observed for HI, the relationship between yield and its major components varied if the analysis included all lines of the panel, and was restricted by eliminating historical cultivars and durum wheat. Yield was correlated to TGW when considering the whole subset of 27 lines ( $r = 0.42$ ), but there was no correlation when the analysis only included the 22 lines. In contrast, yield was unrelated to GNO when considering the whole subset, but the relationship became clear ( $r = 0.40$ ) when restricting the analysis to the 22 elite hexaploid lines (Fig. 2). These differences indicate that yield improvements in the last 10 years of breeding (from 1999 until 2009) were associated with an improvement in GNO.

For the physiological determinants of GNO, yield was clearly unrelated to fruiting efficiency when considering the whole subset of 27 lines (phenotypic correlations,  $rp = -0.21$ ; genetic correlations,  $rg = 0.34$ ), but there was a correlation when only the 22 elite hexaploid lines were considered ( $rp = 0.35$ ;  $rg = 0.59$ ). Similarly, GSP was relevant for determining differences in yield (and higher number of spikes/m<sup>2</sup> seemed rather negative) when considering all the lines (Fig. 2), but when disregarding the historical cultivars and the durum wheat, SNO seemed relevant (and GSP became unsuitable) to explain differences in yield between elite hexaploid lines (Fig. 2).

Focusing on source-related traits, it seemed that the level of WSCs during grain filling was positively related to yield when considering the whole dataset of 27 lines (Fig. 2a), but was only loosely related to yield when considering the 22 elite hexaploid wheats (Fig. 2b). RUE during grain filling was related to

yield when the 27 lines were analyzed, but the relationship became only marginally significant (data not shown) when the analysis was restricted to the 22 elite hexaploid lines (Fig 2).

WSC during grain filling was positively related to number of florets per spikelet, yield, GSP, GWSP, SPADA7 (chlorophyll relative units), and even with HI when considering the whole dataset of 27 lines (Fig. 2a), but it was only related to number of floret per spikelet and GSP when considering the 22 elite hexaploid wheats. Furthermore, when considering the 22 elite hexaploid wheats, WSC was correlated with PGF, biomass seven days after anthesis, and NDVI during grain filling (Fig. 2b).



**Figure 2. Biplot of agronomical, sink, and source related traits of the 27 (a) and 22 (b) genotypes from the CIMCOG subset grown for three years in northwest Mexico. In order to reduce the number of traits included in Figure 1 and 2, sink traits where selected when they were phenotypically correlated with at least three source traits, source traits where selected when they were phenotypically correlated with at least three sink traits. For trait codes, see material and methods section.**

Yield potential is usually represented as a function of its numerical yield components, grain number, and grain weight (Fischer et al. 2014). No significant correlations were observed between grain number per unit area with yield for the subset of 27 lines, even though grains per unit area is the component considered most closely correlated with grain yield (Slafer et al. 1994; Fischer 2008; Foulkes et al. 2009). However, when the analysis was restricted to only the 22 hexaploid elite lines, a correlation among yield and GNO was obtained, indicating that part of the genetic gains of the historical material were related with TGW. This is reinforced by the positive correlation of TGW with yield observed when historic and the durum lines were included in the study, and this has been reported in recent decades in investigations on genetic progress of wheat (Calderini et al. 1995; Sadras and Lawson 2011; Aisawi et al. 2015). The strong negative correlation observed between grain number and grain weight, as previously reported for other environments (Sadras and Lawson 2011; Slafer et al. 2014), highlights the importance of reducing trade-offs between these two yield components and the need to identify mechanisms to improve both simultaneously.

### *Balancing source and sink strength*

Future increases in yield potential must include improvements in structural and reproductive aspects of the crop, together with increased RUE (Reynolds et al. 2011). In this sense, source- and sink-related traits must be improved in parallel in an integrated strategy, as has been proposed by Wheat Yield Consortium (Foulkes et al. 2011; Parry et al. 2011; Reynolds et al. 2011, 2012). Balancing source and sink strength is a complex genetic challenge, since a crop will change between source and sink limitation as conditions vary during the day, and with phenological stage (Reynolds et al. 2000). Our results indicate than an increase in WSC after anthesis and chlorophyll content in flag leaf can potentially increase sink demand. However, high chlorophyll content in the flag leaf was negatively correlated with RUE, likely associated with better light distribution in the crop canopy and less photochemical damage to leaves with less chlorophyll content (Hamblin et al. 2014). With this information, strategic crosses combining genotypes with enhanced source traits (e.g. WSC pre- and post-anthesis, chlorophyll content in flag leaf, RUE during grain filling, considering high spike photosynthesis and high total light intercepted during grain filling) with genotypes with larger biomass production during the stem elongation phase, can deliver lines with a reduced trade-off between grain number and grain weight, and increased sink strength.

On the other hand, higher biomass at initiation of booting or anthesis could result in more spike dry weight at anthesis, thus increasing grain set and yield (Parry et al. 2011; Reynolds et al. 2012) as observed here. It has been proposed that increased duration of the spike growth period (or more broadly, the duration of the stem elongation phase) at a constant rate of growth and level of partitioning, would increase spike dry weight at anthesis (Slafer et al. 2001; Whitechurch et al. 2007; González et al. 2011) and number of fertile florets (González-Navarro et al. 2015). However, in this study, the duration of the rapid spike growth phase for the subset of 27 lines was negatively associated with grain number and fruiting efficiency.

Another aspect that has not been discussed is that understanding source and sink limitation in crops is complicated by the fact that wheat cultivars show significant G×E interaction due to year effects, even within a relatively stable environment. Bread wheat is generally more stable over years than durum wheat or triticale (Reynolds et al., 2005). In this study, while the year effect was significant for some sink and source traits, it is importance to study the fluctuations of temperature and other environmental factors to understand GxE.

Genetic variation, which is vital for genetically improving a trait, was observed for most of the source and sink traits evaluated during three years in a high-yielding environment with in general good heritability estimates. These results break the paradigm that genetic variation for yield potential related traits in elite wheat lines is scarce (Able et al. 2007), as has been demonstrated by previous genetic studies with CIMMYT elite material (Dreisigacker et al., 2004). In addition, the large genetic range observed among the traits evaluated highlights the utility of the CIMCOG panel in practical breeding programs aiming to achieve yield potential combining different approaches.

### **Conclusions**

- This study suggests that it is possible to increase yield potential using the current elite material from breeding programs as genetic variation was obtained for agronomic, sink, and source traits.
- The CIMCOG panel used in this study represents this genetic variation and a large set of physiological traits were evaluated to further assist breeding strategies.

- These results may be useful for breeders for: i) selecting potential parents for strategic crosses and ii) the indirect selection of grain yield during the early segregating generations when yield tests are not yet being conducted.

## References

- Able, J.A., Langridge, P., and Milligan, A.S., 2007. Capturing Diversity in the Cereals: Many Options but Little Promiscuity. *Trends in Plant Science* 12: 71–79.
- Acreche, M.M., Briceño-Félix, G., Martín Sánchez, J. A., and Slafer, G.A., 2009. Radiation interception and use efficiency as affected by breeding in Mediterranean wheat. *Field Crops Research* 110: 91–97.
- Aisawi, K.A.B., 2011. *Physiological processes associated with genetic progress in yield potential of wheat (Triticum aestivum L.)*. PhD Thesis, University of Nottingham School of Biosciences, Sutton Bonington Campus, Leicestershire, UK.
- Aisawi, K.A.B., Reynolds, M.P., Singh, R.P., and Foulkes, M.J., 2015. The Physiological Basis of the Genetic Progress in Yield Potential of CIMMYT Spring Wheat Cultivars from 1966 to 2009. *Crop Science* 55: 1749–1764.
- Calderini, D.F., Dreccer, M.F., and Slafer, G.A., 1995. Genetic improvement in wheat yield and associated traits: A re-examination of previous results and the latest trends. *Plant Breeding* 114: 108–112.
- Dreisigacker, S., Zhang, P., Warburton, M.L., Van Ginkel, M., Hoisington, D., Bohn, M., and Melchinger, A. E., 2004. SSR and pedigree analyses of genetic diversity among CIMMYT wheat lines targeted to different megaenvironments. *Crop Science* 44: 381–388.
- Evans, L.T., 1993. *Crop evolution, adaptation and yield*. Cambridge University Press, Cambridge, UK, pp. 1–500.
- Fischer, R.A., 2007. Understanding the physiological basis of yield potential in wheat. *Journal of Agricultural Science* 145: 99–113.
- Fischer, R.A., 2008. The importance of grain or kernel number in wheat: A reply to Sinclair and Jamieson. *Field Crops Research* 105: 15–21.
- Fischer, R.A., Byerlee, D., Edmeades, G., 2014. Crop yields and global food security: will yield increase continue to feed the world?
- Fischer, R.A., Rees, D., Sayre, K.D., Lu, Z-M., Condon, A.G., and Saavedra, A.L., 1998. Wheat yield progress associated with higher stomatal conductance and photosynthesis rate, and cooler canopies. *Crop Science* 38: 1467–1475.
- Fischer, R.A. and Stockman, Y., 1986. Increase kernel number in Norin 10-derived dwarf wheat: evaluation of the cause. *Australian Journal of Plant Physiology* 13: 767–784.

- Foulkes, M., Reynolds, M.P., and Sylvester-Bradley, R., 2009. *Genetic improvement of grain crops: yield potential*. In: Calderini, D. (Ed.). *Crop Physiology: Applications for Genetic Improvement and Agronomy*. Academic Press, 581.
- Foulkes, M.J., Slafer, G.A., Davies, W.J., Berry, P.M., Sylvester-Bradley, R., Martre, P., Calderini, D.F., Griffiths, S., and Reynolds, M.P., 2011. Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. *Journal of Experimental Botany* 62: 469–86.
- Ghanem, M.E., Marrou, H., and Sinclair, T.R., 2014. Physiological phenotyping of plants for crop improvement. *Trends in Plant Science*: 20.
- González, F.G., Miralles, D.J., and Slafer, G.A. 2011. Wheat floret survival as related to pre-anthesis spike growth. *Journal of Experimental Botany* 62: 4889–901.
- González-Navarro, O.E., Griffiths, S., Molero, G., Reynolds, M.P., and Slafer, G.A., 2015. Dynamics of floret development determining differences in spike fertility in an elite population of wheat. *Field Crops Research* 172: 21–31.
- Hamblin, J., Stefanova, K., and Angessa, T.T., 2014. Variation in chlorophyll content per unit leaf area in spring wheat and implications for selection in segregating material. *PLoS One* 9: e92529.
- Miralles, D., Katz, S., Colloca, A., and Slafer, G., 1998. Floret development in near isogenic wheat lines differing in plant height. *Field Crops Research*: 21–30.
- Miralles, D. and Slafer, G., 1995. Individual grain weight response to genetic reduction in culm length in wheat as affected by source-sink manipulations. *Field Crops Research* 43: 55–66.
- Parry, M.A.J., Reynolds, M.P., Salvucci, M.E., Raines, C., Andralojc, P.J., Zhu, X-G., Price, G.D., Condon, A.G., and Furbank, R.T., 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. *Journal of Experimental Botany* 62: 453–67.
- Reynolds, M.P., Bonnett, D., Chapman, S.C., Furbank, R.T., Manès, Y., Mather, D.E., and Parry, M.A.J., 2011. Raising yield potential of wheat. I. Overview of a consortium approach and breeding strategies. *Journal of Experimental Botany* 62: 439–52.
- Reynolds, M.P., Foulkes, J., Furbank, R., Griffiths, S., King, J., Murchie, E., Parry, M.A.J., and Slafer, G.A., 2012. Achieving yield gains in wheat. *Plant, Cell & environment* 35: 1799–823.
- Reynolds, M.P., van Ginkel, M., and Ribaut, J.M., 2000. Avenues for genetic modification of radiation use efficiency in wheat. *Journal of Experimental Botany* 51 Spec No, 459–73.
- Reynolds, M.P., Manes, Y., Izanloo, A., and Langridge, P., 2009. Phenotyping approaches for physiological breeding and gene discovery in wheat. *Annals of Applied Biology* 155: 309–320.
- Reynolds, M.P., Mujeeb-Kazi, A., and Sawkins, M., 2005. Prospects for utilising plant-adaptive mechanisms to improve wheat and other crops in drought- and salinity-prone environments. *Annals of Applied Biology* 146: 239–259.

- Sadras, V.O. and Lawson, C., 2011. Genetic gain in yield and associated changes in phenotype, trait plasticity and competitive ability of South Australian wheat varieties released between 1958 and 2007. *Crop and Pasture Science* 62: 533–549.
- Sayre, K.D., Rajaram, S., and Fischer, R.A., 1997. Yield Potential Progress in Short Bread Wheats in Northwest Mexico. *Crop Science* 37: 36–42.
- Shearman, V.J., Scott, R.K., and Foulkes, M.J., 2005. Physiological Processes Associated with Wheat Yield Progress in the UK. *Crop Science* 45: 175–185.
- Siddique, K., Kirby, E., and Perry, M., 1989. Ear:stem ratio in old and modern wheat varieties; relationship with improvement in number of grains per ear and yield. *Field Crops Research* 21: 59–78.
- Slafer, G., Abeledo, L., Miralles, D., Gonzalez, F., and Whitechurch, E., 2001. Photoperiod sensitivity during stem elongation as an avenue to raise potential yield in wheat. *Euphytica* 119: 191–197.
- Slafer, G.A., Satorre, E.H., and Andrade, F.H., 1994. *Increases in grain yield in bread wheat from breeding and associated physiological changes*. In: Slafer, G.A. (Ed.) *Genetic improvement of field crops*. Marcel Dekker, New York, pp.1–68.
- Slafer, G.A., Savin, R., and Sadras, V.O., 2014. Coarse and fine regulation of wheat yield components in response to genotype and environment. *Field Crops Research* 157: 71–83.
- Whitechurch, E.M., Slafer, G.A., and Miralles, D.J., 2007. Variability in the Duration of Stem Elongation in Wheat and Barley Genotypes. *Journal of Agronomy and Crop Science* 193: 138–145.

## **State of the art and current situation of wheat production in Mexico**

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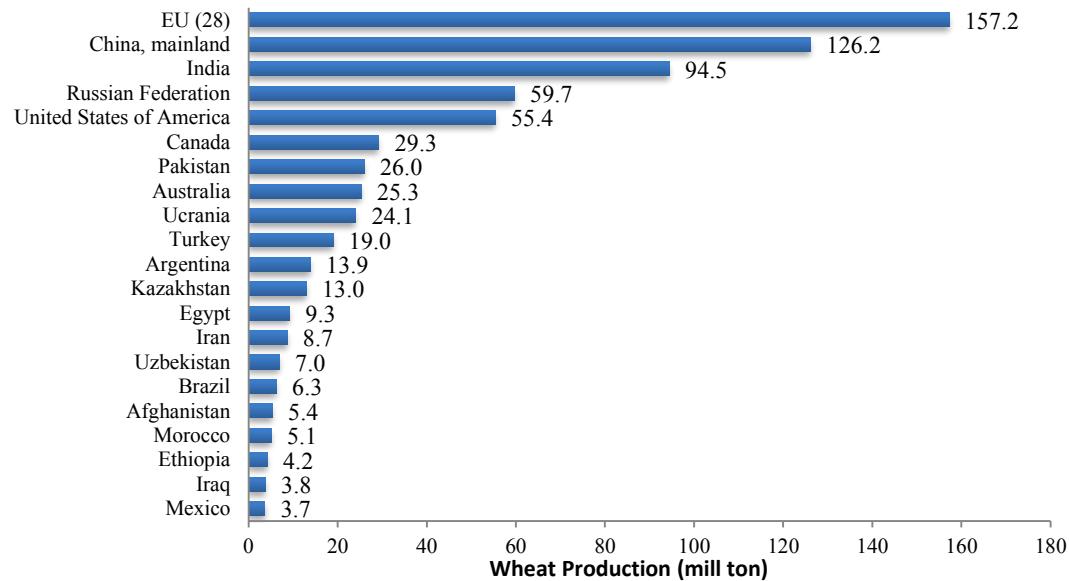
### **Abstract**

Wheat consumption in Mexico is likely to increase in the coming years due to the growth of Mexico's population and the increase in the popularity and convenience of wheat product consumption. However, Mexican wheat supply does not meet national requirements and there is a strong dependency on bread wheat imports from USA and Canada to fulfill demand. If this trend continues, and considering the weakening pesos versus the US dollar, this dependency might seriously affect Mexican food security. To enhance national wheat supply, it is therefore necessary to identify the strengths and weakness of the Mexican wheat value chain to apply research strategies to ensure that future Mexican wheat needs are met in quantity and quality.

### **Introduction**

Wheat is one of the most important staple food crops grown on the largest area with more than 220 million hectares worldwide and wheat is the most important internationally traded staple. Global wheat production grew by an average of 1.96% per year from 311 MT in 1970 to 728 MT in 2014. This increase was mainly due to unprecedented increase in wheat yields worldwide ranging from an average of 1.6 ton/ha in 1970 to 3.3 ton/ha in 2014 (FAOSTAT 2014). Production of wheat is expected to continue increasing worldwide by 12% by 2024 (from the base period 2012-2014, *OECD-FAO Agricultural Outlook 2015*).

Mexico is situated as the 21<sup>st</sup> world wheat producer with an average of 3.7 MT produced in 2014 (Fig. 1). Wheat is the second most important caloric source from plants for the Mexicans (after maize), with a total of 40% household expenditure in cereals, according to the National Mexican Millers Association (CANIMOLT, INEGI 2014). Due to its importance as a low-cost nutrient source it is necessary to promote national wheat production to ensure food security. However, while Mexico is an exporter of durum wheat, Mexico has a bread wheat deficit, which makes it necessary to import more than 50% of the total consumption to meet demand (FAOSTAT 2014). Wheat imports accounted for 4.4 MT in 2014 and have increased by 59% since 2000, mainly due to an increase of 21% in Mexican wheat consumption in the last 15 years (FAOSTAT 2014). It is predicted that bread wheat consumption in Mexico will increase by 9% by 2024 (USDA 2015), therefore it is necessary to increase national bread wheat production in the main wheat-producing areas of Mexico to reduce the dependence on imported wheat and ensuring food security. In this sense, it is important to identify the main bottlenecks of bread wheat production in Mexico to ensure a national high quality wheat supply that is locally sourced.



**Figure 1. Main wheat producers in 2014 worldwide (FAOSTAT 2014).**

## Methods

An extensive database analysis was carried out using various available online data sources (FAOSTAT, SIAP, OECD and USDA). Results were analyzed and plotted as graphs and tables. In order to have an additional source of data and information, various meetings were carried out with teams from CIMMYT's Global Wheat Program, wheat producer associations, wheat exporters, farmers and milling companies to gain some first-hand data about the situation of the wheat research strategies, wheat value chain status in Mexico, areas susceptible to improvements, and to identify strengths and weaknesses of Mexican wheat.

The annual growth in a period of one year was calculated using the formula:

$$AGR (\%) = \left[ \frac{GDP_t - GDP_0}{GDP_0} \right] \times 100$$

where:

**GDP<sub>t</sub>** is the level of activity (production, population, harvest area,...) in the later period (t)

**GDP<sub>0</sub>** is the level of activity in the earlier period (0).

And the mean annual growth rate of an activity over a specified period of time longer than one year, also known as compound annual growth rate (CAGR), was calculated using the formula:

$$CAGR (\%) = \left[ \left( \frac{GDP_t}{GDP_0} \right)^{1/n} - 1 \right] \times 100$$

where:

**n** is the number of periods between the earlier period and the later period ( t – 0 )

## Results/Discussion

In the agricultural year 2013/14, 3.7 MT of wheat were produced in Mexico; 2.0 MT (56%) was durum wheat (*Triticum durum*, used for pasta manufacturing), with bread wheat (*Triticum aestivum*, hard and soft wheat used for bread, cookies, tortillas, cakes, and pastries) making up the remaining 44% (SIAP, 2015). In the last 30 years, yields have increased by 0.7% annually (from 1985 to 2014, not considering 2015, which was the warmest crop cycle registered in history), while the area destined to wheat production decreased by 40% since 1985 (Table 1) (SIAP, 2015). Wheat yields in Mexico are above the world average (5.2 t/ha and 3.16 t/ha, respectively) (FAOSTAT, 2014) and there is high potential for future yield improvement as an outcome of research carried out by CIMMYT in close collaboration with INIFAP and other national partners. Very limited investment is made by the private sector in research and development efforts. The increase of wheat domestic use was approximately 1% per year, not matching the slight reduction of 0.3% annually of total production (considering 1990 to 2014) and, therefore, imports increased seven-fold in the last 30 years. If Mexico continues with recent trends without increasing national wheat production, imports would have to increase to 75% to meet Mexican demand, risking food security in Mexico and increasing the dependence with United States and Canada, the two main wheat suppliers of imported wheat (CANIMOLT, 2014).

**Table 1. Mexico wheat production, supply and demand from 1980 to 2015.**

Year	Wheat production in Mexico						Total domestic use <sup>2</sup> (x10 <sup>3</sup> tons)
	Area <sup>1</sup> (x10 <sup>3</sup> ha)		Yield <sup>1</sup> (ton/ha)	Production <sup>1</sup> (x10 <sup>3</sup> ton)	Imports <sup>2</sup> (x10 <sup>3</sup> tons)	Exports <sup>2</sup> (x10 <sup>3</sup> tons)	
	Seeded	Harvested					
<b>1985</b>	1,273	1,217	4.3	5,214	560	0	5,563
<b>1990</b>	959	933	4.2	3,931	339	2	4,286
<b>1995</b>	969	929	3.7	3,468	1,223	432	4,796
<b>2000</b>	730	708	4.9	3,493	2,794	548	5,650
<b>2005</b>	654	634	4.8	3,015	3,718	395	6,178
<b>2006</b>	667	646	5.2	3,378	3,447	536	6,100
<b>2007</b>	706	692	5.1	3,515	3,253	569	5,944
<b>2008</b>	845	829	5.1	4,213	3,217	1,398	5,469
<b>2009</b>	866	828	5	4,116	2,777	1,136	5,369
<b>2010</b>	701	678	5.4	3,677	3,495	437	6,329
<b>2011</b>	715	662	5.5	3,627	4,048	836	6,390
<b>2012</b>	589	579	5.7	3,274	4,642	612	6,839
<b>2013</b>	683	634	5.3	3,357	4,167	733	6,348
<b>2014</b>	713	706	5.2	3,670	4,446 <sup>3</sup>	1,104 <sup>3</sup>	6,850 <sup>3</sup>
<b>2015*</b>	844	811	4.6	3,730	4,400 <sup>3</sup>	1,100 <sup>3</sup>	7,000 <sup>3</sup>

<sup>1</sup>Data obtained from SIAP-SAGAPA (<http://infosiap.siap.gob.mx/>, consulted in January 2016)

<sup>2</sup>Data obtained from FAOSTAT ([http://faostat3.fao.org/download/Q/\\*/E](http://faostat3.fao.org/download/Q/*/E), consulted in February 2016)

<sup>3</sup> Data obtained from USDA Foreign Agricultural Service (<http://www.fas.usda.gov/data>, consulted in February 2016, preliminary and estimated results).

\* Preliminary results.

In addition, the predictions about the effects of climate change on the agriculture indicate that food production in Mexico might be reduced by 26%, Mexico being the second country in the world (after India) that would be mostly affected for climate change (Scientific American, 2010). This situation makes it essential to increase wheat yield in Mexico through understanding production constraints under climate change (especially drought and heat stress). To meet this goal, investment in research to develop climate resilient cultivars that meet the quality standards under sustainable production systems are essential. Besides, it is essential that these new varieties are available to wheat producers on time and in enough quantity through an efficient and transparent seed system.

#### *Wheat production in Mexico*

Primary wheat production occurs in more than 20 Mexican states. The vast majority of Mexican wheat is produced in northwest Mexico (Sonora, Baja California, and Sinaloa) and the center occident area, also defined as Bajío (Guanajuato, Michoacan, and Jalisco), accounting for 72% and 16% of the total national wheat production, respectively. The north of the country, comprised by the states of Chihuahua, Coahuila, and Nuevo Leon, accounts for 7% of total wheat production (Table 2). Another important producing area due to its proximity to processing and consumption areas is the Altiplano (highland) region, constituted by the Mexico State, Tlaxcala, Puebla, and Hidalgo, and represents only 3% of the total wheat production (SIAP 2014).

**Table 2. Wheat production in Mexico per state in 2014.**

State	Production <sup>1</sup>		Yield <sup>1</sup> (t/ha)
	Thousand of tons	Participation	
Sonora	1,827	49.8%	6.02
Baja California	520	14.2%	6.37
Guanajuato	285	7.8%	5.18
Sinaloa	279	7.6%	4.46
Michoacan	155	4.2%	5.02
Chihuahua	146	4.0%	5.89
Jalisco	132	3.6%	5.65
Tlaxcala	87	2.4%	2.60
Nuevo Leon	84	2.3%	3.37
Zacatecas	37	1.0%	2.17
Coahuila	29	0.8%	3.45
Mexico State	19	0.5%	2.01
Puebla	7	0.2%	1.95
Hidalgo	4	0.1%	2.06
Rest of the country	59	1.6%	2.23
<b>Total National</b>	<b>3,670</b>	<b>100.0%</b>	<b>5.19</b>

<sup>1</sup> Data obtained from SIAP-SAGAPA (<http://infosiap.siap.gob.mx/>, consulted in January 2016)

Local yields in the main producing areas in northwest and Bajío are above the world average. The autumn-winter season accounts for more than 95% of total national wheat production and 93% is produced under irrigated conditions (SIAP 2014). Higher yields are registered in irrigated areas (Sonora, Baja California, Guanajuato, Sinaloa, Chihuahua, Michoacan, and Jalisco), compared with rainfed areas (Puebla, Tlaxcala, Hidalgo, and Mexico State) (Table 2). The ideal sowing season starts in mid-

November, with most of the plantings concluding in December. The bulk of the annual harvest is concentrated during May and June.

As previously mentioned, the most common wheat type produced in Mexico is durum wheat (*Triticum durum*) with a 56% of the total production in 2014 (SIAP 2014). The production of durum wheat occurs mainly in the states of Sonora and Baja California and meets the demand of the Mexican pasta manufacturers and is even exported to more than seven countries. In 2014, from the 2.0 MT of durum wheat produced, 1.1 MT was exported. However, there is a deficiency of the wheat necessary for bread making (*Triticum aestivum*), as the national production does not meet demand. In 2014, the national production of bread wheat was 1.6 MT, which made necessary to import extra 4.5 MT of bread wheat to cover the Mexican industry demand (**Table 1**). One reason for this imbalance between wheat produced and wheat needs is that Sonora farmers, the main wheat producers with almost 50% of the total national production (**Table 2**), get better prices in the international market and lower prices are paid for soft wheat. In addition, farmers situated in Sonora perceive that durum wheat varieties used currently are more resistant to karnal bunt (a seed borne quarantine disease) infections and provide higher yields compared to bread wheat varieties. In this sense, bread wheat production is not adapted to the varieties required for the local consumption, creating a strong dependency with imported wheat to supply the quality and the quantity required by the processing wheat industry.

It is important to mention that in Mexico, current wheat production is better understood in terms of the agronomy (yield potential and water constraints) than in terms of the needs from users, including millers and consumption localization. Actual milling capacity in Mexico includes 84 mills which cover the national demand and have capacity to process an extra 20% (CANIMOLT 2014). As mentioned previously, wheat is mainly produced in northwest Mexico, where high technology and water are available, however 53% of the milling processing factories are situated in the center and the metropolitan area (**Table 3**), away from the main production areas, but where the major wheat processing and consumption takes place.

**Table 3. Wheat production per area compared to millers' location and wheat consumption.**

Region	Wheat production (%)	Milling capacity (%)	Wheat products consumption (%)
<b>North-west</b>	72%	14%	11%
<b>North</b>	7%	15%	13%
<b>Center-occident</b>	16%	23%	27%
<b>Metropolitan area</b>	3%	30%	26%
<b>South, Southeast</b>	1%	18%	23%
<b>TOTAL</b>	<b>100%</b>	<b>100%</b>	<b>100%</b>

Transport of wheat from the northern producing areas to the center is more expensive than the transport costs of imported wheat from US Gulf to the center, due to high national transportation costs compared with the shipping costs (CANIMOLT 2014). This fact might explain why producers in the northwest are more focused in the production of durum wheat for exporting than in meeting the bread wheat required

for the center and the metropolitan area. Reducing transportation costs from producers to consumers would be particularly beneficial and might stimulate the production of bread wheat.

Currently wheat varieties used in Mexico have varietal replacement of only 0.9% (calculated value according to the information published in the Mexican Seed Register and Certification System, SNICS). While in the north of Mexico the replacement is high, some areas are still using varieties that were released more than 20 years ago that do not have the quality standards required by the milling industry. Therefore, there is a necessity not only to increase bread wheat production, but also to ensure that the varieties used by the producers satisfy the quality industrial requirements, and that seed are available on time and in sufficient amount for producers. CIMMYT wants to mediate and facilitate this process by establishing a common working plan involving the main actors of the wheat value chain to ensure that new varieties reach the farmers on time.

In summary, research to create climate resilient wheat cultivars along with sustainable high yielding production systems can boost a key sector of the Mexican agricultural economy. Research should focus on wheat yield potential and end use quality, along with resilience to high temperature and water scarcity, and resistance to new races of pathogen and pests. Improvements in dissemination of new technologies to producers – including seed multiplication – are also key pieces to be able to meet bread wheat demand to ensure food security in Mexico in the coming years.

## **Conclusions**

To satisfy Mexican bread wheat demand and ensure food security in Mexico in the coming years it is necessary to:

- Boost the development of improved bread and durum wheat varieties in the main wheat producing areas of Mexico that meet industrial quality requirements, as well as encompassing resilience to environmental effects caused by climate change (mainly high temperatures and water scarcity), and changing patterns of disease incidence.
- To develop a common business plan to align wheat research and genetic breeding with the main actors involved in the wheat value chain, ensuring that sufficient amounts of improved seed is being multiplied and is readily accessible to the farmers.
- To improve the seed system and access to improved cultivars that meets industrial requirements for all wheat producers so that increased consumer demand for wheat products can be met and Mexican food security is underpinned.

## **References**

- CANIMOLT, 2014. Reporte estadístico, <http://www.canimolt.org/> (accessed January 2016).
- FAOSTAT, 2011. [http://faostat3.fao.org/browse/FB/\\*/E](http://faostat3.fao.org/browse/FB/*/E) (accessed February 2016).
- OECD/Food and Agriculture Organization of the United Nations, 2015. OECD-FAO Agricultural Outlook 2015, OECD Publishing, Paris. [http://dx.doi.org/10.1787/agr\\_outlook-2015-en](http://dx.doi.org/10.1787/agr_outlook-2015-en).
- OECD/Food and Agriculture Organization of the United Nations, 2015. Overview of the OECD-FAO Agricultural Outlook 2015-2024. In: OECD-FAO Agricultural Outlook 2015, OECD Publishing, Paris.

SIAP-SAGAPA, Servicio de Información Agroalimentaria y Pesquera – Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación de México, [http://infosiap\\_siap.gob.mx/](http://infosiap_siap.gob.mx/) (accessed January 2016).

USDA, 2015. International Long-Term projections to 2024.

## **Wheat breeding in Mexico: Delivering newly developed advanced lines**

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### **Abstract**

Over the past 70 years, Mexican wheat breeding has delivered varieties with higher yields and traits with genetic disease resistance to producers in the various wheat-growing areas. Co-evolution of hosts and pathogens (mainly rusts), sparks a need for the constant development of new sources of resistance and long-term strategies like durable resistance. Annually, CIMMYT's bread wheat breeding program tests thousands of newly developed advanced lines for yield, disease resistance, tolerance to heat and drought, and other important traits. In the summer of 2015, 1460 advanced lines were selected, based on yield potential, from 9100 lines and were evaluated for resistance to leaf and yellow rust, Septoria and Fusarium head blight, at CIMMYT's Toluca and El Batán experimental stations. Wheat industrial processing quality of the selected lines was also evaluated. More than 88% of the lines were resistant to yellow rust (less than 5% of infection), based on prevalent races in Mexico. For Septoria, 426 lines showed 50% infection or less and were selected for a second year of testing. Twenty percent of the lines showed no more than 5% infection for Fusarium head blight and some lines were better than the resistant checks. A large number of lines also demonstrated good industrial processing quality. Pyramiding different traits into a single line requires a constant breeding process to make improved varieties available for wheat producers in Mexico and elsewhere.

### **Introduction**

In Mexico, wheat is the fifth most cultivated crop (after maize, dry beans, sorghum, and oats), based on area planted. In terms of production, it is the third most important; production peaked in 1985 (5 million tons harvested) but since then, production has steadily declined and imports are increasing every year (4.2 million tons in 2014). The area planted to wheat has declined due to water scarcity, high production costs, diseases (Karnal bunt in northwest Mexico, yellow rust in highlands and Bajío area), and varieties not accepted by the industry. Mexico imports bread wheat with bread making quality parameters at a cost close to a billion dollars (Canimolt 2014). The FAO and other international organizations forecast higher wheat prices, and increased imports for Mexico.

Until the summer of 2002, the varieties recommended by Mexico's National Institute for Forestry, Agriculture and Livestock Research (INIFAP) for planting under irrigated or rainfed conditions had good resistance to yellow rust, except for Temporalera M87 which was moderately susceptible (Villaseñor et al., 2007). However, in the summer of 2003, a new yellow rust race was identified (MEX03.37; 219MEX0), which infected wheat heads on many of the resistant varieties (Rodríguez et al. 2008). In the summer of 2004, this new race overcame the resistance of varieties like Zacatecas VT74, Salamanca S75, Pavón F76, Saturno S86, Gálvez M87, Cortázar S94, Batán F96, and the newly released variety Bárcenas S2002. Farmers experienced yield losses of up to 75% due to the new yellow rust race. In 2010, a new race affected some of the new varieties released in 2007, such as Monarca F2007, Norteña F2007, and Josecha F2007. These three varieties showed 40-80% yield losses in Valle de Santiago, Salamanca, and

Celaya in the state of Guanajuato. Rust control was suggested by applying fungicide, increasing production costs and, if seed was available, replacement with resistant varieties. During the winter cycle of 2013-14, a new race overcame the resistance of Nana F2007, Luminaria F2012, and Don Carlos M2015, which was in the process of being released. The reaction to the pathogen in these varieties was susceptible, moderately susceptible, and moderately resistant, respectively. Don Carlos had more than 50% of infection.

Rodríguez et al. (2010) reported up to 30 physiological races of yellow rust in highlands of Mexico, and eight in the Bajío area. Virulence for genes Yr1, 2, 6, 7, 17, 27, 31 and Polly A were reported in most of the wheat growing areas in central Mexico in irrigated and rainfed areas (Rodríguez et al. 2008).

Fusarium head blight (FHB) is a devastating disease of wheat and other small grains. It is mainly caused by *Giberella zeae* (Schwein.) Petch [anamorph: *Fusarium graminearum* Schwabe] and has the potential to adversely affect yield only a few weeks before harvest. In addition to direct yield losses, FHB infection leads to a deterioration in grain quality (Bai and Shaner 1994) and contamination with mycotoxins such as nivalenol (NIV), deoxynivalenol (DON), and zearalenone (Buerstmayr et al. 2012), making the products unfit for consumption as food or feed. Changes in agricultural practices, including more acreage to maize-wheat rotation and adoption of conservation agriculture, in addition to climate change, have contributed to an enhanced incidence and severity of FHB (He et al. 2013). The disease often results in 30-40% yield losses, which may increase up to 70% for susceptible cultivars under severe epidemics. Host resistance is considered an economically and environmentally friendly means of managing FHB. Thus, breeding for improved FHB resistance is an important objective for numerous cereal breeding programs (Buerstmayr et al. 2009). Response to FHB involves different mechanisms and can be assessed in a number of ways. Mesterházy (1995) described five types of resistance, of which Type I (resistance to initial infection by the pathogen), Type II (resistance to fungal spread within an infected spike), and Type III (resistance to the accumulation of toxins in the grain) have been given more attention, whereas resistance to kernel infection (Type IV), and resistance to yield loss (Type V) are less studied.

The development and release of new varieties takes at least eight years in northwestern Mexico and longer in some other areas. Wheat breeding is a continuous process and genetic diversity for disease resistance and other important traits for farmers and industry are constantly incorporated into the gene pool. The threat of the appearance of new races is a permanent challenge for wheat breeding programs. Evaluation of thousands of advanced lines is a normal process in CIMMYT's wheat breeding programs aiming at new potential varieties to be released for the different wheat growing areas of Mexico and many countries worldwide. CIMMYT's breeding program has been constant for the last 70 years, with two cycles per year as part of the shuttle breeding scheme.

## Methods

During the wheat growing cycle of 2014-15, 1460 advanced lines were selected from 9100 new lines that were tested in 325 yield trials in the Yaqui Valley, Cd. Obregón, Sonora, Mexico. Selection was based on yield potential compared to the recently released Borlaug 100 F2104 and the experimental line KACHU #1. Four sets of this new nursery were prepared for planting. One set was planted at CIMMYT's experimental station in Toluca (latitude 19°22' N, longitude 99°55' W, 2582 masl and annual precipitation of 1000 mm), and three sets at El Batán (latitude 19°53' N, longitude 98°84' W, altitude of 2270 masl, and annual precipitation of 625 mm). Both locations are in the state of Mexico. In Toluca, experimental

lines were artificially inoculated with yellow or stripe rust and *Septoria tritici Blotch*. In El Batán, lines were artificially inoculated with leaf rust and Fusarium Head Blight, and an additional set was used for seed increase.

#### *Evaluation of resistance against yellow and leaf rust*

Lines were planted in a double row of 0.70 m and susceptible spreaders were also planted flanking the nursery. Around 45 days after planting, spreader rows were inoculated. Susceptible rows were inoculated with a suspension of uredinospores of yellow or leaf rust. Spores were suspended in mineral oil (Soltrol®170) with a concentration of 2-3 mg/ml (Herrera-Foessel et al. 2003) and sprayed into the susceptible row. Artificial inoculation was repeated every other day four times. Uredinospores of leaf rust were from races MBJ/SP, MCJ/SP, and a mixture of other races. For yellow rust, isolate 96.11 (virulent to Yr27), Pastor's race (virulent to Yr31), and the new race Mex14.141 (virulent to Yr27, Yr31, Yr8, and Tatara's gene) were used. Level of infection was measured two times using the modified Cobb scale and the level of infection. Disease reaction was taken 70 to 80 days after planting and repeated a week after.

#### *Fusarium head blight (FHB)*

For FHB, the spray inoculated field experiments were conducted in the FHB nursery at the El Batán experimental station, during the summer season (May to September). Germplasm was planted on 20th May, 2015, in 1 m double rows. A mixture of five aggressive *F. graminearum* isolates, identified based on molecular and pathogenicity analysis, were used for field inoculation. Spray inoculation was targeted to each line's anthesis stage using an inoculum of 55,000 spores/ml and was repeated two days later. To enhance FHB disease development, from anthesis to the early dough stage of the tested germplasm, the nursery was misted for 10 minutes each hour, from 9am to 8pm, to create a humid environment favorable for FHB development. Additionally, the nursery was developed in fields wherein maize-wheat rotation under conservation agricultural practices was adopted to enhance FHB disease development. Total numbers of infected spikes and spikelets of each spike were counted to calculate the FHB index using the formula *FHB index (%) = Severity x Incidence*, where *Severity* is the averaged percentage of diseased spikelets, and *Incidence* is the percentage of symptomatic spikes. Based on the FHB index, 453 of 2,460 of the C50IBWSN lines, and 40 of 284 of the C7HPAN lines, were selected for further evaluation in 2016.

#### *Septoria tritici blotch (STB)*

STB was evaluated at the Toluca station during the summer cycle (May to September). Materials were sown in 0.75 m double rows. To evaluate wheat genotypes for STB resistance, a mixture of six virulent isolates {St1 (B1), St2 (P8), St5 (OT), St6 (KK), 64 (St 81.1) and 86 (St133.4)} of *Z. tritici*, collected from naturally infected wheat fields in Mexico, was used to prepare the spore inoculum, which was produced on yeast malt agar medium. Spore suspension from the six isolates was mixed and adjusted to 1 x 10<sup>7</sup> spores/ml for field application. The first inoculation of the germplasm was conducted between 28 and 30 days after planting i.e. four to five leaf stage. The inoculation was continued every week with a total of three applications. Approximately four weeks after the last inoculation, disease severity was visually scored for each plot, using the double-digit scale (00–99), wherein the first digit (D1) indicates disease progress in canopy height from the ground level, and the second digit (D2) refers to severity measured based on diseased leaf area. Both D1 and D2 were scored on a scale of 1 to 9. Five disease

evaluations were conducted on August 20th and 27th, and September 1st, 8th, and 15th. For each evaluation, percentage disease severity was estimated based on the following formula:

$$\% \text{ severity} = (\text{D1}/9) \times (\text{D2}/9) \times 100$$

The area under disease progress curve (AUDPC) was subsequently calculated using the formula:

$$\text{AUDPC} = \sum_{i=1}^n \left[ \frac{(Y_i + Y_{(i+1)})}{2} \times (t_{(i+1)} - t_i) \right]$$

Where  $Y_i$  = Septoria tritici blotch severity at time  $t_i$ ;  $t_{(i+1)} - t_i$  = time interval (days) between two disease scores; and  $n$  = number of times when Septoria tritici blotch was recorded.

#### *Other variables measured*

Days to heading and plant height were also measured. Days to heading was counted from day of planting or irrigation to the day that 50% of the spikes were above the flag leaf. Plant height was measured from the top of the bed to the tip of the spike, not including beards.

#### *Industrial processing quality analysis*

##### *a. Grain and flour parameters*

Grain morphological characteristics were evaluated using the digital image system SeedCount SC5000 (Next Instruments, Australia). Thousand kernel weight (TKW, g) and test weight (TW, kg/hl) were obtained. Grain hardness (GRNHRD), grain protein (GRNPRO), flour protein (FLRPRO), and moisture content were determined by near-infrared spectroscopy (NIRS) using the instrument NIR Systems 6500 (Foss, Denmark) according to official methods AACC 39-70A and 39-10 (AACC 2010). NIRS instrument was calibrated based on AACC methods (AACC 2010) for particle size index (AACC Method 55-30), moisture (AACC Method 44-15A), and protein (AACC Method 46-11A). Lower hardness index (% of flour particles not passing through the sieve) values correspond to harder cultivars. GRNPRO values were reported at 12.5% moisture basis. Grain samples previously conditioned at 14-16% of moisture were milled into flour using Brabender Quadrumat Jr (C. W. Brabender OHG, Germany) and flour yield was calculated (FLRYLD, %).

##### *b. Rheological and baking tests*

Dough development properties were determined by a Mixograph of Swanson (National Mfg., U.S.A.) using 35g of flour (AACC method 54-40A). Two parameters were obtained: dough development time (MixT) and %Torque\*min (TQ). The Chopin Alveograph (Trippette & Renaud, French) was used to determine dough strength (ALVW) and tenacity/extensibility ratio (ALVPL) (AACC 54-30A) using 60g of flour. The bread-making process was conducted using the direct dough method with 100g of flour (AACC method 10-09) and bread loaf volume (LOFVOL) was determined by rapeseed displacement using a volumeter.

##### *c. Classification of lines based on the use type*

Lines can be classified according to potential industrial use using variables measured in flour and dough properties (Table 1).

**Table 1. Classification of wheat according to the final use and protein content.**

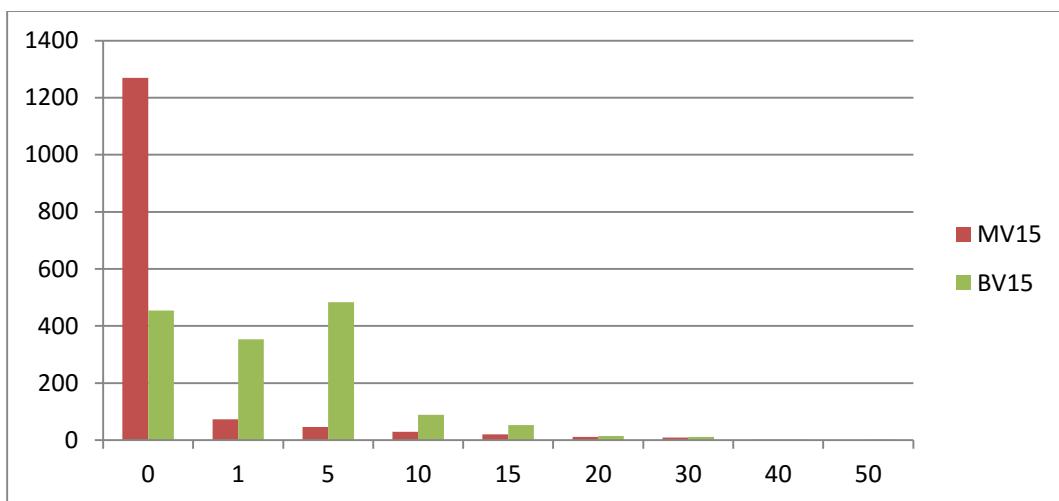
Final use	Type*
<b>Hard wheat</b>	
Mechanized industry	1a, 1b
Semi-mechanized Industry (loafs using yeast)	2a
Steamed breads (North of China), Wheat flour tortilla, Chapati	2b
Dense breads and wheat flour tortilla	3a, 3b
<b>Soft wheat</b>	
Steamed breads (South of China), Chinese noodle	4a
Cookies, cakes and other	4b
<b>Tenacious wheat</b>	
Not fit for mechanized or semi-mechanized processes	5
For dense breads or mixtures with other types of wheat.	

\*Based on kernel hardness, grain color, dough development properties, strength and extensibility of gluten harvested in Ciudad Obregon under the experimental conditions and yield of 7 to 9 ton/ha. Letters "a" and "b" refer to grain protein according to type: 1a >12.5%; 2a y 3a >11.5% y 4a > 11.0%. Lines from 1 to 4 must have medium to high levels of extensibility.

## Results/Discussion

### *Yellow rust (*Puccinia striiformis*).*

Yellow rust infection in susceptible spreaders was very good and worked as a source of spores for all advanced lines tested in Toluca and El Batán. At El Batán, yellow rust infection was natural and was established in spreader rows before the inoculation of leaf rust. Leaf rust infection was very low and was not scored. Resistance in experimental lines for yellow rust was scored in August in both locations. Overall disease reaction for both stations is shown in Figure 1. At Toluca, 89% of lines showed no yellow rust infection and 6% showed 1-5% disease severity. At El Batán, 33% of lines did not show yellow rust infection and 55% of lines were between 1 and 5%. Less than 2% of the lines tested had disease severity of 20% or higher. The cause of higher levels of infection at El Batán was due to an earlier presence of the disease. Nevertheless, more than 88% of lines showed high levels of resistance under natural or artificial inoculations. Susceptible spreaders had 100% of disease severity when first scores were taken. All lines, during segregating phases, are selected under artificial inoculations for at least two cycles, plus an additional cycle at the F6 or F7 level before yield selection in the Yaqui Valley.



**Figure 1. Infection levels of the 1460 lines screened at Toluca and El Batán stations during the summer of 2015.**

#### *Leaf rust (*Puccinia recondita*)*

Artificial inoculations were done according to the original plan. Three days after the first disease symptoms were detected, a 4- minute hail storm affected wheat lines and disease presence. No scores were taken for leaf rust but natural infection of yellow rust was good. Disease resistance will be evaluated on the selected lines from the 1460 original lines during the winter cycle of 2015-16 in the Yaqui Valley.

#### *Fusarium (FHB)*

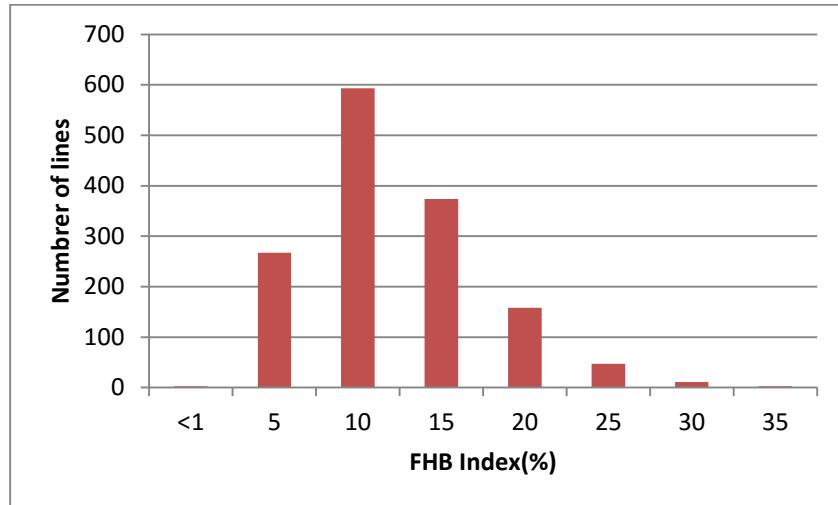
Screening for resistance to FHB was done in El Batán. The nursery included five checks (two resistant, two with intermediate resistance, and one susceptible) repeated five times. Scores for intermediate and susceptible checks showed high values so disease inoculation was considered reliable. Resistant checks showed low infection levels. Average infection for checks is shown in Table 2.

**Table 2. Average infection of checks used in the evaluation of 1460 advanced lines for FHB resistance.**

Genotype	% FHB	
SUMAI #3	1.16	Resistant
GAMENYA	74.16	Susceptible
FALCIN/AE.SQUARROSA (312)/3/THB/CEP7780//SHA4/LIRA	36.80	Intermediate
OCORONI F 86	31.59	Intermediate
HEILO	1.91	Resistant

Advanced lines screened for FHB resistance showed infection levels from less than 1% to 35% of FHB index. Figure 2 shows the results of screening 1460 lines. Around 20% of the lines show infection levels of 5% or less, with some lines below the best resistant check. 460 lines were considered for a second year of testing. In high rainfall areas, released varieties must have acceptable levels of resistance to avoid or reduce disease development and the presence of mycotoxins caused by the pathogen. During the second year of testing, mycotoxin (DON) evaluation is performed together with FHB infection rate. According to previous results, few CIMMYT lines have as good levels of resistance as SUMAI 3, considered as good

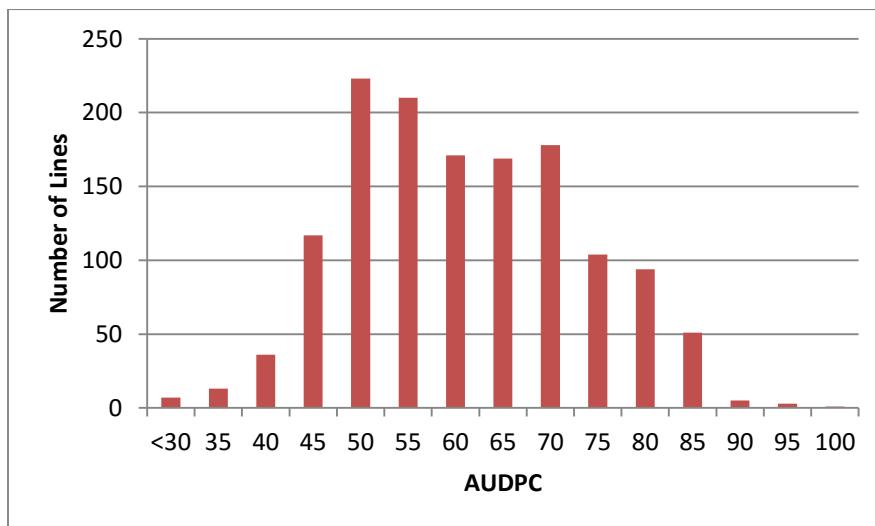
type II resistance. To have better resistance for FHB in CIMMYT lines, it is important to introgress *Fhb1* and/or *Fhb2* genes to improve type II resistance. The frequency of *Sr2* gene in CIMMYT lines is high and this gene is closely linked to *Fhb1* locus but in repulsion. Newly developed lines have both genes in coupling and several crosses have been made to increase the frequency of FHB resistant gene.



**Figure 2. FHB index measured in 1460 lines and group according to the level of infection.**

#### *Septoria tritici Blotch (STB)*

Screening for resistance to STB was done at Toluca. Disease measurements show that 27.5% of the lines have 50% or less disease severity (Fig. 3). In these lines, the AUDPC is low and it is related a lower development of the disease. Based on the disease reaction, 425 lines were selected for a second year of evaluation. STB resistance helps the plant to have a good grain filling process and is recommended for high rainfall and rainfed areas, like some of the highland areas of central Mexico.



**Figure 3. Screening for resistance to *Septoria tritici Blotch*, based on AUDPC and grouped by range of infection.**

#### *Other traits measured*

Lines had days to heading ranging from 52 to 83 days at El Batán, and 62 to 95 days at Toluca, with averages of 64 and 70 days, respectively. Temperatures were lower in Toluca than El Batán. The cycle length is generally shorter in El Batán. Plant height ranged from 65-113 cm at El Batán and 75-110 cm at Toluca, with averages of 88 and 92 cm, respectively. This set contains diversity for the two traits in order to fit the needs of different wheat growing areas of Mexico.

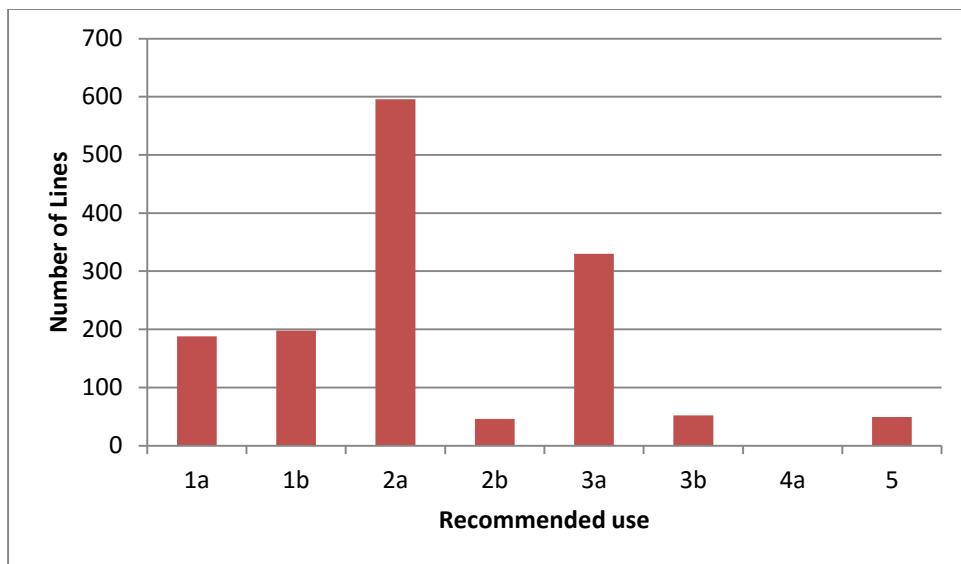
#### *Industrial processing quality analysis*

Twelve traits were measured in the 1460 lines evaluated during the summer of 2015 (Table 3). Desired levels of each trait are dependent on the variable, for example, low values of grain hardness are required for hard wheat types, whereas for soft types, the ideal is to have low levels of grain protein. Grain protein can be affected by levels of grain yield, but yield can also be affected by the temperature in the wheat growing cycle.

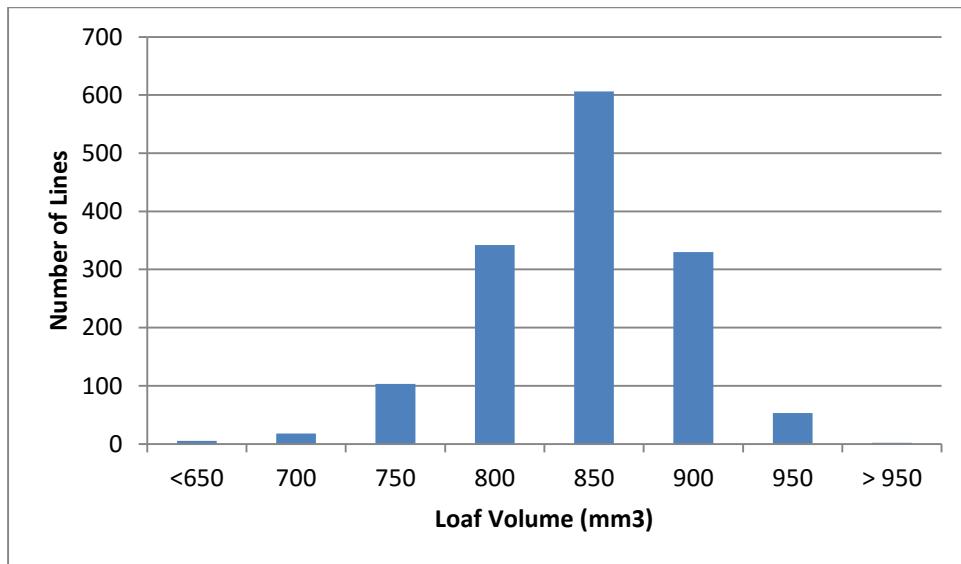
**Table 3. Means and ranges of quality variables measured in the 1460 lines evaluated and harvested in Ciudad Obregón during the 2014-2015 cycle.**

	<b>Mean</b>	<b>Min</b>	<b>Max</b>
Test Weight (kg/l)	80.8	77.7	83.9
Thousand Kernel Weight (gr)	44.6	34.9	57.7
Grain Hardness	45.9	38.3	58.0
Flour Yield (%)	70.9	62.8	76.4
Grain Protein (%, 12.5% MB)	12.5	10.5	14.5
Flour Protein (%, 14% MB)	11.0	9.0	13.1
Sedimentation (mm)	14.8	9.5	21.5
Mixing Time	3.3	1.4	6.9
Torque * min	126.9	53.5	260.8
Alveograph W	263.7	85.0	631.0
Alveograph P/L	0.7	0.3	2.2
Loaf Volume (mm <sup>3</sup> )	821.6	605.0	960.0

Using some variables and in accordance with Table 1, lines were grouped into their recommended uses. Only 3.4% of lines were considered tenacious (Fig. 4). Other lines were assigned to groups 1, 2, and 3, with 26%, 44%, and 26% of the total, respectively. Seventy percent of the lines can be used in the mechanized and semi-mechanized industry. Figure 5 shows the results from the bread making test having 68% with loaf volumes greater than 800 mm<sup>3</sup>, recommended for the mechanized and semi-mechanized industry.



**Figure 4.** Quality use suggested based on the evaluation of 1460 lines for 12 traits.



**Figure 5.** Bread making quality test for the 1460 advanced lines.

CIMMYT's bread wheat breeding program aims to have lines with yield potential and yield stability, durable rust resistance to the three rusts, tolerance to heat and drought stress, and industrial processing quality. Depending on the target region, resistance to other diseases is also included, like Septoria and FHB for high rainfall and rainfed conditions, or Karnal bunt resistance for northwest Mexico. As more traits are included in a single line, the probability of finding a single genotype with all traits is reduced, so many more lines have to be tested or screened. Wheat breeding needs to be an ongoing, continuous process as better varieties have to be released to improve yield or to deploy new resistant genes for different diseases or better adapted genotypes. Every year, a similar number of lines are evaluated after the first year of yield testing. The 2015 evaluation showed that 255 lines have three of the four traits evaluated (without considering leaf rust resistance), but only 41 (3%) have all four traits.

Further, 1092 selected lines from this nursery were planted in Obregon under five different environments: two with irrigation, one with mild water stress, one with severe water stress, and one for heat stress, and will be scored for leaf rust and Karnal bunt resistance. The best performing lines will be distributed in five international nurseries in the summer of 2017. These lines were delivered to INIFAP for further testing in the Bajío and central Mexican highlands.

## Conclusions

- Rusts (stem rust, leaf rust and yellow rust) are the main disease affecting wheat worldwide, and new pathogen races are rendering many resistance genes ineffective.
- Wheat production systems have been improving in Mexico and the newest varieties have higher yield potential. The wheat industry is demanding better quality wheats and climate change and the outbreak of new pathogen races are constant challenges for breeding programs.
- New tools have to be included as part of the breeding process in order to have genetic improvement at faster rates than before.
- The delivery of new varietal releases to the farmers must be fast and efficient for the different wheat growing areas of Mexico.

## References

- American Association of Cereal Chemists. 2000. Approved methods of the AACC. St.Paul, MN, USA.
- American Association of Cereal Chemists. 2010. Approved methods of the AACC. St.Paul, MN, USA.
- Bai G. and Shaner, G., 1994. Scab of wheat: Prospects for control. Plant Disease 78:760-766.
- Buerstmayr H., Ban, T., and Anderson, J.A., 2009. QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breeding 128:1-26.
- Buerstmayr H., Adam, G., and Lemmens, M., 2012. *Resistance to head blight caused by Fusarium spp. in wheat*. In: Sharma, I. (Ed) Disease resistance in wheat. CABI, Wallingford, Oxfordshire, UK; Cambridge, MA, pp 236-276.
- Canimolt, 2014. Reporte estadístico 2014. Cámara Nacional de la Industria Molinera de Trigo. pp. 34.
- Diario Oficial de la Federación. 2007. Ley Federal de producción, certificación y comercio de semillas. DOF 1506207, pp. 59-71.
- He X., Singh, P.K., Duveiller, E., Schlang, N., Dreisigacker, S., and Singh, R.P., 2013. Identification and characterization of international Fusarium head blight screening nurseries of wheat at CIMMYT, Mexico. European Journal of Plant Pathology 136:123-134.
- Herrera-Foessel, S.A., Singh, R.P., Huerta-Espino, J., Yuen, J., and Djurle. A., 2003. *Diversity of resistance to leaf rust in five CIMMYT germoplasm derived durum wheats*. In: Proceedings of the Tenth International Wheat Genetics Symposium 1-6 September 2003. Paesetum, Italy. Volume 1: 361-363.

- Mesterhazy A. 1995. Types and components of resistance to Fusarium head blight of wheat. *Plant Breeding* 114:377-386.
- Peña, R.J., Amaya, A., Rajaram, S., and Mujeeb-Kazi, A., 1990. Variation in quality characteristics associated with some spring 1B/1R translocation wheats. *Journal of Cereal Science* 12: 105-112.
- Rodríguez-García, M.F., Huerta-Espino, J., Villaseñor-Mir, H.E., Sandoval Islas, J.S., and Singh, R.P., 2010. Análisis de virulencia de la roya amarilla (*Puccinia striiformis f.sp. tritici*) del trigo (*Triticum aestivum* L.) en los valles altos de México. *Agrociencia* 44: 491-502.
- Rodríguez G. M. F., Huerta-Espino, J., and Villaseñor-Mir, H.E., 2008. *Virulencia de Puccinia striiformis f. sp. tritici en los Valles Altos de México en 2005*. In: XXIII Congreso Nacional y II Internacional de Fitogenética. Chapino, México. pp: 217.
- Villaseñor-Mir., H. E., Huerta-Espino, J., María A. R., Espitia E.R., Limón, A. O., and Rodríguez, M.F.G., 2007. Variedades de trigo para siembras de temporal en el estado de Tlaxcala. INIFAP-CIRCE. S. E. Tlaxcala. Folleto Técnico Núm, pp. 30-33.

## PARTITIONING

### Key molecular and physiological traits/mechanisms affecting the setting of potential grain weight in two contrasting spring wheat cultivars

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#### Abstract

It is clear that if the grain yield potential of wheat is to be improved, grain weight must also be increased. Breeding programs have conducted various evaluations in contrasting high yielding environments, but the associated traits and physiological mechanisms determining potential and actual grain weight are only partially understood. This field study – conducted under optimal agronomical management at the Austral Agricultural Experimental Station of the Universidad Austral de Chile, Valdivia – aimed to identify key physiological and molecular traits/mechanisms controlling the setting of potential grain weight in two spring wheat cultivars with contrasting grain weights. At the booting stage, control and thinning treatments were applied, with the latter used to improve the environmental growing conditions for plants. Carpel weight, grain weight, grain length, and water content were recorded. Furthermore, the relative expression of TaExpA6, XTH2, and XTH5 proteins, as well as the glucose, fructose, and sucrose content and concentration in floral carpels, were evaluated. Significant different between treatments, and between cultivars, were found in distinct molecular and physiological traits. Likewise, possible associations of grain length and water content were found in relation to the different genes.

#### Introduction

Grain weight (GW) is a key component of grain yield of wheat, yet the traits and mechanisms determining potential grain weight (PGW) and actual GW are only partially understood. The need to increase food security is driving efforts to better understand both these relationships and the negative association between GW and grain number (GN) often reported for wheat. Studying molecular and physiological traits/mechanisms in pre- and post-anthesis stages can provide important information to help build a physiological-genetic framework.

The expansive growth of plant organs is often determined by the growth of the outer tissues (Kutschera and Niklas 2007); these tissues are formed by the plant cell wall and are key determinants of cell expansion, shape, and volume. Cellulose-hemicellulose networks of the plant cell wall determine the extensibility of cell walls, but the proteins that act on this network control the process of cell growth (Lizana et al. 2010). The proteins that help to regulate cell expansion are called expansins (McQueen-Mason et al. 1992). The action of cell wall expansins (EXP) in the pericarp cells is a new topic that could explain the limitations in obtaining higher grain volumes and GW in wheat. Although the expansins are probably expressing in all grain tissues, a specific action in the pericarp cells is hypothesized, which can then be a target for increasing grain volume, in the sense that GW is associated with grain length (GL; Lizana et al. 2010; Quintero et al. 2014) and carpel weight (Scott et al. 1983; Yang et al. 2009; Hasan et al. 2011).

Another protein family has also been shown to play an important role in cell wall growth: Xyloglucan endotransglucosylase/hydrolases (XTHs) are a class of wall enzymes that cleave xyloglucan chains endolytically and then rejoin the newly generated free ends to other xyloglucan free ends or to H<sub>2</sub>O (Rose et al. 2002). This provides a potential molecular mechanism for controlled turgor-driven wall expansion (Muñoz and Calderini 2015), though direct evidence is still lacking. On the other hand, in cereals, water soluble carbohydrates (WSC) have been acknowledged for contributing to maintaining grain filling rate (Dreccer et al. 2009), since it is largely dependent on assimilate supply and metabolic regulation (Weber et al. 2005). The importance of the period immediately before anthesis in determining PGW has been disregarded (Calderini et al. 1999), but studies in other crops report a relationship between carpel weight (CW) at anthesis and GW at harvest (Scott et al. 1983; Yang et al. 2009; Hasan et al. 2011). The floral carpel (grain pericarp after pollination) is formed during pre-anthesis phases, thus WSC accumulation from emergence to anthesis could compromise GN determination and possibly GW. The study of carbohydrates in floral carpels therefore represents a potential means for improving yield.

This study aimed to identify key molecular and physiological traits/mechanisms controlling the setting of PGW in two wheat cultivars with contrasting GW, taking into account previous studies highlighting the importance of GL, water content, expansin protein (TaExpA6), and XTHs genes in grain, as well as WSCs in the carpels for determining wheat PGW (Lizana et al. 2010; Hasan et al. 2011; Muñoz and Calderini 2015).

## Methods

Two wheat cultivars with contrasting GW were selected from the CIMMYT Core Germplasm (CIMCOG) panel and assessed at the Austral Agricultural Experimental Station (39° 47' 18"S, 73° 14' 5"O), Universidad Austral de Chile, Valdivia, Chile, during the 2014-15 growing season. The set was sown on 27th August 2014 under two treatments (control, SR, and rows thinned in booting, CR). Plots were arranged in a completely randomized design with three replicates. For SR, genotypes were sown in plots 2.5 m long and 1.35 m wide, compared to dimensions of 3.5 m long and 1.35 m wide for the CR plots. Both treatments had a seeding rate of 333 seeds/m<sup>2</sup>. CR was conducted at booting to increase the availability of resources to the remaining plants (Fig. 1) and was carried out by removing all plants from the two closest rows. Plots were irrigated when necessary, weeds were periodically removed by hand, and recommended doses of pesticides were used to prevent and control insects and diseases.



**Figure 1. Control (SR) and thinning (CR) treatments.**

Phenological stages were recorded in accordance with Zadoks et al. (1974). The timing of physiological maturity was estimated when grain growth stopped, as per Hasan et al. (2011). At anthesis, 22 main-shoot spikes of a similar size and development were chosen in each plot to follow grain growth (fresh and dry weight) of grain position 2 (the second grain from the rachis: G2). After anthesis, two main-shoot spikes were harvested 11 times (A+5, A+10, A+15, A+20, A+25, A+30, A+35, A+42, A+48, A+54, and A+60). With the same frequency, grain samples of G2 were harvested for molecular analyses (qPCR of TaExpA6, XTH2, and XTH5). Individual GW and grain dimensions of G2 were measured in the central spikelets of the spike. Using the Waddington et al. (1983) scale, floral carpel in position 2 (C2) between W9.5 and W10 were collected for analyzing the concentration of different carbohydrates (glucose, fructose, and sucrose), as well as individual CW. Data were subjected to analysis of variance (ANOVA), and mean comparison by least significant difference procedures.

## Results

GW was significantly affected by both genotype ( $P<0.001$ ) and thinning ( $P<0.01$ ) treatments (Table 1). Conversely, CW did not show significant differences, likely due to the high coefficient of variation for this trait. As expected, genotypes demonstrated different GL ( $P<0.001$ ) and there was a positive association between GW and GL ( $R^2=0.95$ ;  $P<0.05$ ). GL and water content dynamics showed higher values under the thinning treatment (Fig. 2), but this difference was not found when the stabilized L was contrasted between SR and CR (Table 1). In previous studies, contrasting CW, GL, and water content were reported for genotypes displaying different GW (Calderini and Reynolds 2000; Lizana et al. 2010; Hasan et al. 2011). Interestingly, in this study, improved growing conditions (thinning) favored GL and water content dynamics of grains (Fig. 2).

The GW of cultivar ENT1 under both CR and SR treatments stabilized around 42 days after anthesis (DAA; Fig. 2a), while for ENT2 this trait under both treatments stabilized around 48 DAA (Fig. 2). GL stabilized by 16 DAA in ENT 1 and at 20 DAA in ENT 2 (Fig. 2). Taking into account grain filling duration, ENT 2 reached the highest GW in both thinning treatments due to a higher grain growth rate.

Table 1. Carpel weight, grain weight, and grain length of two contrasting cultivars under thinning (CR) and control (SR) treatments.

CULTIVARS	Floral Carpels "C2"			Grain "G2"								
	SR	CR	% difference	Significance	SR	CR	% difference	Significance	SR	CR	% difference	Significance
ENT1	0.82	0.80	2	ns	50.47	60.42	20	<0.01	7.90	8.22	4	ns
ENT2	0.81	1.04	28	ns	72.50	85.97	19	<0.01	9.40	9.80	4	ns
Average	0.81	0.92			61.48	73.20			8.65	9.01		
CV	20.17	22.75			4.55	4.85			3.34	3.50		
P-value(ENT)	ns	ns			<0.001	<0.001			<0.001	<0.001		
LSD (0.05)	0.23	0.29			3.88	4.93			0.13	0.14		

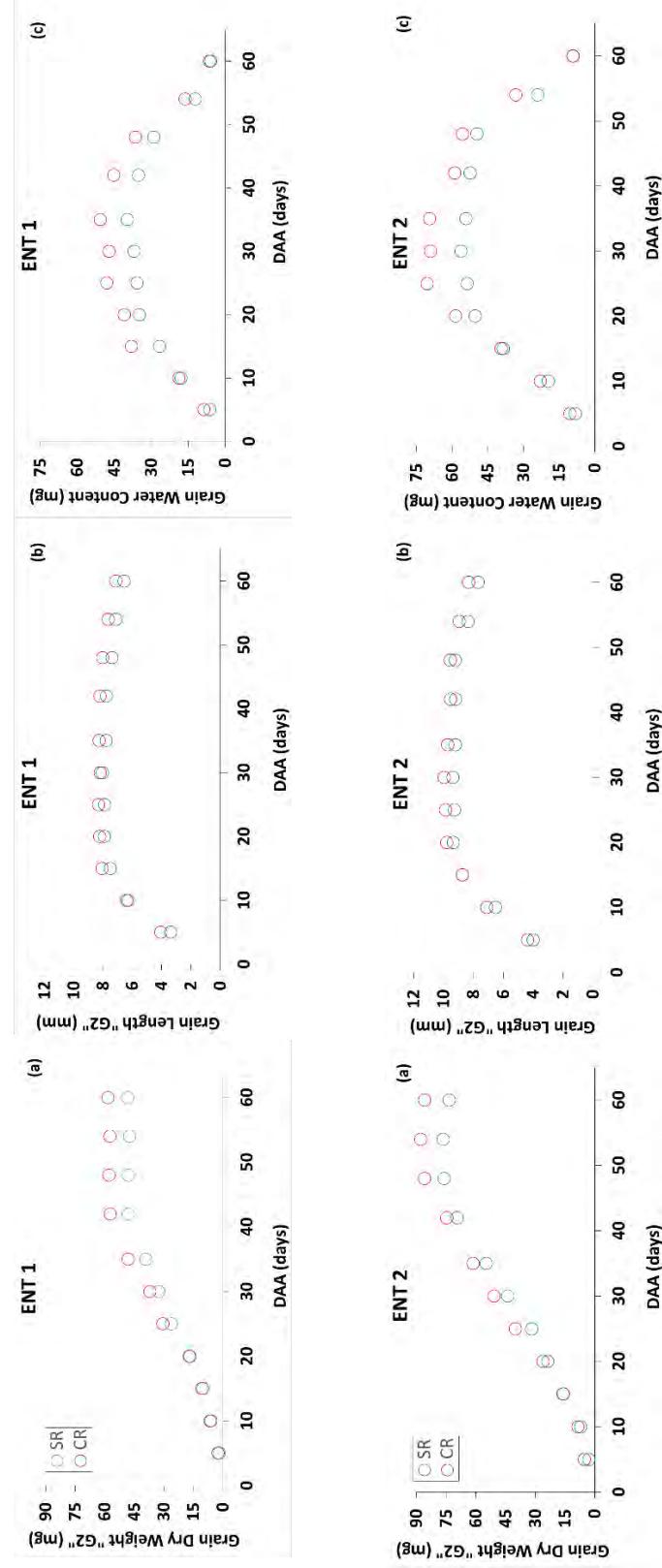


Figure 2. Dynamics of: a) weight, b) length, c) and water content of grain in "ENT1" and "ENT2" of control (SR) and thinning (CR) treatments during grain filling.

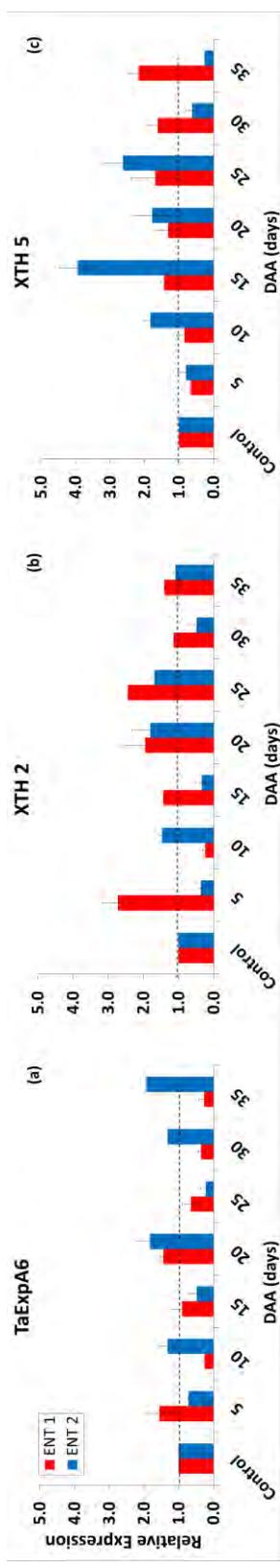
Compared to the control, the heavier genotype ENT2 showed a higher relative expression of TaExpA6 at A+10, A+20, A+30, and A+35; while in ENT1, higher expression was recorded at A+5 and A+20 (Fig. 3a). The relative expression of XTH2 in both cultivars was higher between A+20 and A+25 (Fig. 3b), though ENT1 had a high expression at the beginning of grain filling. The relative expression of XTH5 was detected from A+10 to A+35; ENT2 showed higher expression during early grain filling, while ENT1 was higher at the end of grain filling (Fig. 3c).

The expression of TaExpA6 has been associated with the period when the grain is enlarging until GL it is levelled off (Lizana et al. 2010), however expression of TaExpA6 after GL levelled off could be due to endosperm cells, which are still expanding when GL levelled off. It is important to highlight that the molecular evaluation was carried out on whole grains, instead of pericarps as in Lizana et al. (2010). Therefore, the later expression of TaExpA6 at 30 and 35 DAA in the cultivar ENT2 may be associated with processes other than GL or setting of PGW. The same could be true for the expression of XTH5, which has been correlated with the increment of water content (Muñoz and Calderini 2015) in the pericarp of grain. In this experiment, the expression of TaXT5 in both cultivars continued throughout the evaluated period, i.e. until 35 DAA (Fig. 4c). Therefore, the expression of XTH5 would be involved in several processes of grain growth further in addition to the water content of the grain pericarp. Nevertheless, it is remarkable that ENT2 showed more expression of TaExpA6 and TaXT5 between 10 and 20 DAA in association with the higher dry weight, length, and water dynamics of G2 under the thinning treatment. Furthermore, water content of grains reached the stabilized plateau by 25 DAA, in agreement with the expression of TaXTH5. Little information is available in the literature regarding the expression of these genes in growing grains of wheat and, to the best of our knowledge, this is the first study reporting the expression under source-sink treatments.

Signalling involving monosaccharides could be proposed for growing florets of wheat, as was hypothesized for cell expansion by Wang and Ruan (2013). This study evaluated content and concentration of sucrose (Suc), glucose (Glu), and fructose (Fru) in carpels of flowers. The content and concentration of Suc did not show differences between treatments ( $P>0.05$ ), and it was not affected by either genotypes or thinning (Table 2; Table 3). However, treatments did affect Glu and/or Fru content and concentration (Tables 2; Table 3). Glu and Fru content was more affected by the treatments than their concentrations. Thus, Glu content of floral carpels was higher in the CR treatment for both cultivars, and difference between cultivars ( $P<0.05$ ) was only found in the CR treatment (Table 2). The higher content of Fru was recorded in the CR treatment. Differences of content of Fru between treatments were obtained in both cultivars, but only the SR treatment showed differences between cultivars (Table 2). In the light of these results, the more consistent effect of treatments was found in thinning, which increased Glu and Fru content of carpels and the higher concentration of Glu and Fru found in ENT2 and ENT1, respectively, when growing conditions were improved by removing border plants.

## Conclusions

In this study, GW was affected by genotype and by improving the growing conditions of plants through the removal of border plants (thinning treatment). Treatments increased water content of grains and a linear association was found between GW and GL. The expression of TaExpA6 and TaXTH5 genes between 10 and 20 DAA could be involved in the growth of wheat grains. Finally, Glu and Fru concentrations in carpel flowers are proposed as signals for grain weight potential.



**Figure 3. Relative expression of thinning treatment to the control of:** a) TaExpA6, b) XTH2, and c) XTH5, in G2 of cultivars “ENT1” (red bars) and “ENT2” (blue bars). The black lines on the bars show the standard error of the means.

**Table 2. Content of glucose (Glu), fructose (Fru) and sucrose (Suc) in the floral carpel “C2” of two contrasting cultivars under thinning (CR) and control (SR) treatments.**

CULTIVARS	Floral Carpels "C2"				Suc (μg)
	SR	CR	% difference	Significance	
ENT 1	3.15	4.17	32	<0.05	0.19
ENT 2	3.02	6.19	105	<0.01	3.91
Average	3.08	5.18			2.05
CV	5.00	5.91			34.75
P-value(ENT)	ns	<0.05			<0.01
LSD (0.05)	0.28	0.56			1.55
					1.60
					0.14
					0.89

Fru (μg)

Glu (μg)

Suc (μg)

**Table 3. Concentration of glucose (Glu), fructose (Fru) and sucrose (Suc) in the floral carpel “C2” of two contrasting cultivars under thinning (CR) and control (SR) treatments.**

CULTIVARS	Floral Carpels "C2"				Suc (μg)/CW (mg)
	SR	CR	% difference	Significance	
ENT 1	3.54	5.14	45	ns	0.24
ENT 2	3.72	5.14	38	<0.01	4.77
Average	3.63	5.14			2.51
CV	12.01	4.54			12.31
P-value(ENT)	ns	ns			<0.001
LSD (0.05)	1.33	0.71			0.67
					1.29
					0.07
					0.62

## References

- Calderini, D.F. and Reynolds, M.P., 2000. Changes in grain weight as a consequence of de-graining treatments at pre- and post-anthesis in synthetic hexaploid lines of wheat (*Triticum durum* x *T. tauschii*). *Australian Journal of Plant Physiology* 27: 183-191.
- Calderini, D. F., Abeledo, L. G., Savin, R., and Slafer, G. A., 1999. Effect of temperature and carpel size during pre-anthesis on potential grain weight in wheat. *Journal of Agricultural Science Cambridge* 132: 453–459.
- Dreccer, M. F., Herwaarden, A. F. Van, and Chapman, S. C., 2009. Grain number and grain weight in wheat lines contrasting for stem water soluble carbohydrate concentration. *Field Crops Research* 112: 43–54. <http://doi.org/10.1016/j.fcr.2009.02.006>
- Hasan, A. K., Herrera, J., Lizana, C., and Calderini, D. F., 2011. Carpel weight, grain length and stabilized grain water content are physiological drivers of grain weight determination of wheat. *Field Crops Research* 123(3): 241–247. <http://doi.org/10.1016/j.fcr.2011.05.019>
- Kutschera, U. and Niklas, K. J., 2007. The epidermal-growth-control theory of stem elongation: An old and a new perspective. *Journal of Plant Physiology* 164(11): 1395–1409.
- Lizana, X. C., Riegel, R., Gomez, L. D., Herrera, J., Isla, A., McQueen-Mason, S. J., and Calderini, D. F., 2010. Expansins expression is associated with grain size dynamics in wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* 61(4): 1147–1157. <http://doi.org/10.1093/jxb/erp380>
- McQueen-Mason, S., Durachko, D. M., and Cosgrove, D. J., 1992. Two endogenous proteins that induce cell wall extension in plants. *The Plant Cell* 4(November): 1425–1433.
- Muñoz, M. and Calderini, D. F., 2015. Volume, water content, epidermal cell area, and XTH5 expression in growing grains of wheat across ploidy levels. *Field Crops Research* 173: 30–40.
- Quintero, A., Molero, G., Reynolds, M. P., Le Gouis, J., and Calderini, D. F., 2014. Trade-off between grain weight and grain number and key traits for increasing potential grain weight in CIMCOG population. In: Reynolds, M.P., Molero, G., Quilligan, E., Listman, M., and Braun, H.J. (Eds.), *Proceedings of the 4th International Workshop of the Wheat Yield Consortium*. CENEZ, CIMMYT, Cd. Obregón, Sonora, México, pp. 114-121.
- Rose, J. K. C., Braam, J., Fry, S. C., and Nishitani, K., 2002. The XTH family of enzymes involved in xyloglucan endotransglucosylation and endohydrolysis: Current perspectives and a new unifying nomenclature. *Plant and Cell Physiology* 43(12): 1421–1435. <http://doi.org/10.1093/pcp/pcf171>
- Scott, W. R., Appleyard, M., Fellowes, G., and Kirby, E. J. M., 1983. Effect of genotype and position in the ear on carpel and grain growth and mature grain weight of spring barley. *The Journal of Agricultural Science* 100(02): 383. <http://doi.org/10.1017/S0021859600033530>.
- Waddington, S. R., Cartwright, P. M., and Wall, P. C., 1983. A quantitative scale of spike initial and pistil development in barley and wheat. *Annals of Botany* 51(1947): 119–130. Retrieved from <http://aob.oxfordjournals.org/content/51/1/119.short>.
- Wang, L. and Ruan, Y.-L., 2013. Regulation of cell division and expansion by sugar and auxin signaling. *Frontiers in Plant Science* 4(May): 163. <http://doi.org/10.3389/fpls.2013.00163>.
- Weber, H., Borisjuk, L., and Wobus, U., 2005. Molecular physiology of legume seed development. *Annual Review of Plant Biology* 56: 253–279.
- Yang, Z., Van Oosterom, E. J., Jordan, D. R., and Hammer, G. L., 2009. Pre-anthesis ovary development determines genotypic differences in potential kernel weight in sorghum. *Journal of Experimental Botany* 60(4): 1399–1408. <http://doi.org/10.1093/jxb/erp019>.

# **Wheat spike fertility and fertile tillers number are enhanced by exogenous application of Cytokinin and Auxin**

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## **Abstract**

Four elite wheat genotypes from a CIMMYT Core Germplasm (CIMCOG) trial were selected for an investigation of the effects of externally applied hormones on spike fertility and grain yield. Hormones were applied at the 1<sup>st</sup> node stage and at the booting stage. Three hormones were tested: an auxin, a gibberellin (via the use of a gibberellin inhibitor), and a cytokinin under field conditions at three different concentrations under irrigated conditions. Results showed that spike fertility increased with a high concentration of cytokinin (100 ppm) applied at the 1<sup>st</sup> node stage and low/moderate concentration (25 ppm) applied at the booting stage. The auxin treatment increased the number of fertile tillers per square meter. However, only for one genotype did yield increase significantly under hormone treatment, compared to the control plants (where no hormone was applied).

## **Introduction**

Wheat is one of the most important crops in the world, feeding more than half of the global population. The pressure of the growing population and the resulting increase in food demand under climate change increases the need to enhance crop production. In the future, greater wheat production per unit land area will increasingly rely on genetic improvements (Slafer et al. 1996). However, grain yield is a complex trait resulting from a compromise between grain weight, number of tillers per unit area, and number of grain per spike. A better understanding of the physiology of crop yield might help to increase the efficiency in breeding selection (Slafer, 2005). Numerous studies have shown that the negative relationship between number of grains and average individual grain weight is a common phenomenon. Furthermore, grain weight and number is unequally distributed in the spike, depending on spatial position of the kernel (Slafer et al. 1996; Calderini and Reynolds 2000), and yield per spike decreases from early to late tillers (Zhang et al. 2010). Several studies have also demonstrated the important role of plant hormones in regulating auxiliary bud growth: Auxin indole-3-acetic acid inhibits the growth of axillary buds (Leopold 1949), and the external application of naphthal acetic acid (a synthetic auxin) completely inhibits tiller bud growth in rice (Liu et al. 2011). In contrast to auxin, cytokinins (CKs) stimulate lateral bud outgrowth (Langer et al. 1973), releasing lateral buds as a result of inhibition of auxin-induced apical dominance, and also promote axillary bud outgrowth (Chatfield et al. 2000)

Many studies have shown that plant hormones are involved in determining grain set in cereal crops. Most hormonal studies have investigated grain development after anthesis (Miralles et al. 1998), with only a few focusing on the pre-anthesis hormonal regulation of floret development in wheat. Colombo and Favret (1996) showed that the sensitive period for male sterility caused by GA3 application in wheat was from glume differentiation to pre-meiotic interphase in the oldest florets of the spike. Thus the role of hormones in the regulation of floret development at different floral stages is still not clear, especially for the promotion of floret development. Cai et al. (2013) studied endogenous hormones and determined that a change of hormone titre is dependent on stage of development during floret development. This study

therefore aimed to explore the regulatory effects of exogenous auxin (AUX), anti-gibberellin (AGA), and CK on spikes, with hormones applied at 1<sup>st</sup> node and at booting stage. This information will be helpful for elucidating the roles of endogenous plant hormones in regulating floret development and therefore spike fertility in wheat plants.

## Methods

### *Plant material and experimental design*

Field experiments were conducted under irrigated conditions from the end of May until the end of September 2013, at the CIMMYT station, El Batán, Texcoco, Mexico. A complete randomized design was used consisting of three replications for each concentration and control. Plot size was 10 m x 0.75 m x 3 beds, with 2 rows per bed. Each row was subdivided into 7 uniform sub-plots of 1 m length for each application of products and control (no product application); a border of 0.5 m was left at the beginning and end of each bed. Between two sub-plots, 0.25m of non-treated material was left as a border to avoid contamination due to applications and/or concentration differences.

Four contrasting genotypes of bread wheat were selected from a CIMMYT Core Germplasm (CIMCOG) trial to test the effect of different concentrations of different hormones on yield and yield components. Selection was based on yield resilience under heat stress; compared to the overall population, one genotype (G3) was resilient, two genotypes (G1 and G2) had low resilience, and one (G4) showed high susceptibility (Table 1). These genotypes were classified using a novel stress index (Thiry et al. *in preparation*) developed as part of an extended study to relate productivity and stress resilience of wheat genotypes to key hormone signaling pathways (Thiry, *in preparation*)

**Table 1. List of genotypes**

Entry	CID	SID	GID	Cross-name
1	521017	12	6179185	OASIS/SKAUZ//4*BCN/3/2*PASTOR/5/FRET2*2/4/SNI/T RAP#1/3/KAUZ*2/TRAP//KAUZ/7/CAL/NH//H567.71/3/S ERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR
2	6831	33	775	SIETE CERROS T66 (Hist 1)
3	510201	41	5993957	TRCH/SRTU/5/KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4 /HUITES
4	516689	38	6176346	WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/ AE.SQUARROSA (213)//PGO/4/HUITES

### *Hormone products*

The three hormones – AUX, CK, and gibberellin – were tested using indol-3 butyric acid (Commercial name: Rooter Plus-3000), 6-Benzylaminopurine (6-BAP; Pure concentrate state 99.9%), and 1,1-dimethylpiperidinium chloride (Mepiquat chloride, an inhibitor of Gibberellin (AGA - Commercial name: Regulex)), respectively. Concentrations were based on the recommended doses stated by the manufacturer, except for 6-BAP. In total, three concentrations were applied for each product; C1 represents the maximum dose recommended (Table 2).

**Table 2. Hormone concentrations applied according to treatments.**

Hormone	C1	C2	C3
Auxin (AUX)	600 ppm	300 ppm	150 ppm
Anti-Gibberellin (AGA)	420 ppm	105 ppm	26.25 ppm
Cytokinin (CK)	100 ppm	25 ppm	12.5 ppm

#### *Field methodology*

Hormones were applied at 3:00 pm at two phenological stages for each concentration. The first stage was the beginning of jointing stage, 1st node (1N; mid-July), and the second was at booting (B; end July). For each stage, 12 sub-plots were sprayed with each product at each concentration (four genotypes x 3 repetitions = 12 sub-plots). The application at booting stage was performed in two steps as G2 and G3 reached booting stage two days before G1 and G4.

Three conventional sprayers (4 liters capacity) were used to apply the concentrations. The discharge of each sprayer was initially calculated (50 ml/15 seconds) to estimate the volume released during the application process.

Each application was protected by a “U” shape screen so that treatments could be applied to just 1 m of crop without contaminating other sub-plots. The screen acted as a windbreak and helped avoid problems of drift. After each application of each product the screen was cleaned with water and dried with paper, to avoid contamination by product remaining on the screen.

Considering the aim of this experiment, preventative fungicide and pesticides were not applied; the active agent in some fungicides and herbicides is a plant growth regulator and could consequently affect the result of the study. However, G2 showed a high sensitivity to yellow rust and it proved necessary to apply a fungicide to these plants at around heading stage. The yield of G2 may therefore have been affected by the rust/fungicide. Others genotypes did not develop any disease symptoms. Data for G2 are therefore not discussed here due to the cumulative effect of the biotic stress and the exogenous hormones.

#### *Measurements*

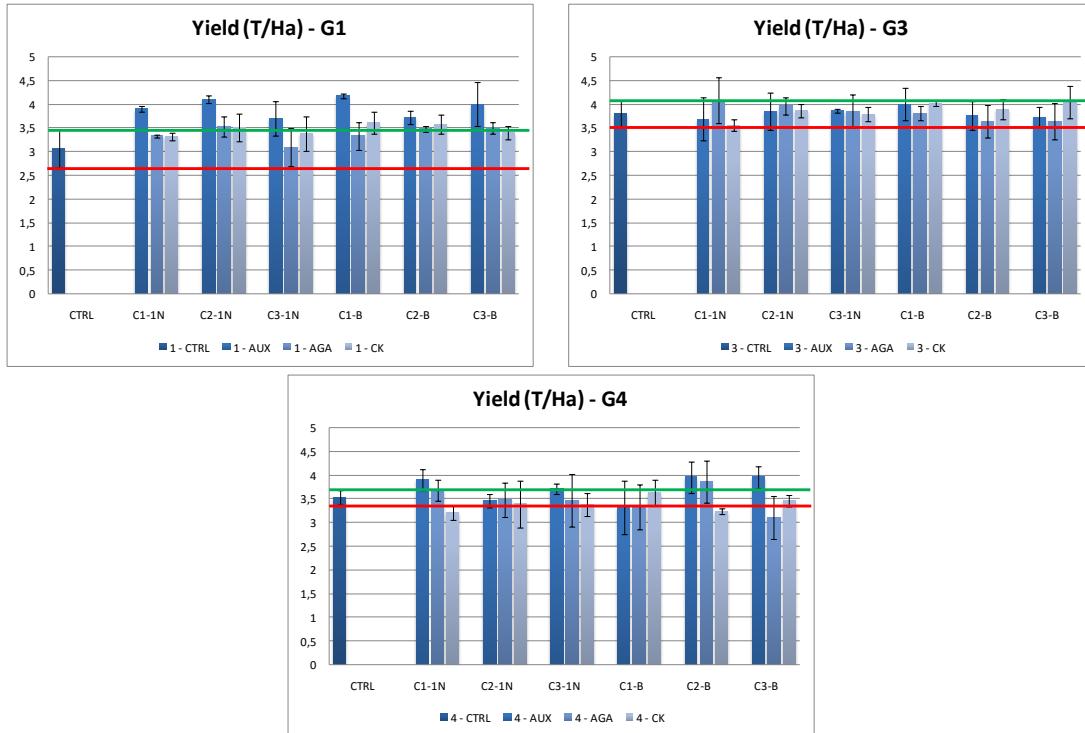
After heading, each stem was counted twice in each sub-plot (1 m<sup>2</sup>) to quantify the total number of tillers/m<sup>2</sup> so that tiller number could be compared with the number of fertile tillers. Fertile tillers and other yield components were calculated following the protocols outlined in Pask et al. (2012).

At early maturity, six spikes were randomly chosen to measure spike length and count the total spikelet number, sterile spikelet, and fertile spikelet per spike. These measurements were made for each sub-plot and repetition. At maturity, 50 spikes were cut randomly and the rest of the 1 m sub-plot was hand harvested. The 50 spikes were threshed separately from the rest of the harvest to determine the yield components and spike index. Total yield was composed of remaining spikes + 50 spikes.

#### **Results/Discussion**

##### *Yield*

Only G1 differed significantly from the control yield when hormones were applied. In this case, yield was increased significantly by AUX-C1-1N, AUX-C2-1N, AUX-C1-B, and AUX-C2-B. G3 did not show specific changes in yield, and while the yield of G4 tended to increase in response to AUX-C1-1N, AUX-C2-B, and AUX-C3-B, these responses are not significant (Figure 1).

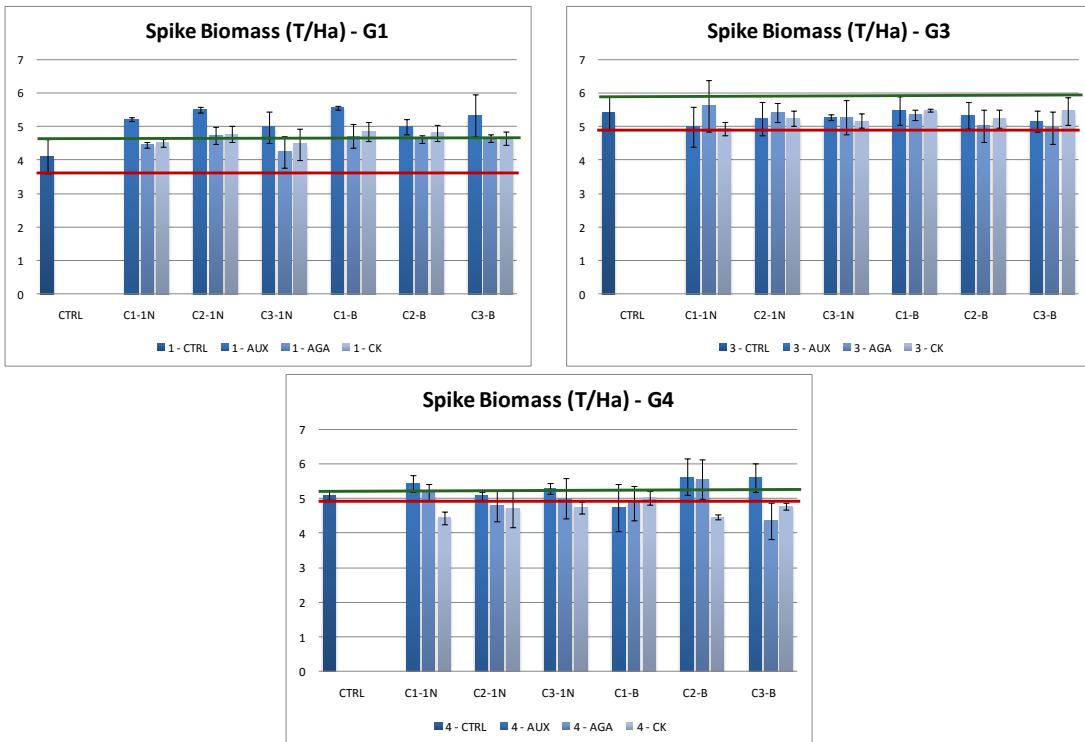


**Figure 1. Different genetic yield responses of three genotypes (G1, G3, G4) treated with three hormones (AUX, AGA, CK) at three concentrations (c1, c2, c3) at two phenological stages (1N, B). The green line corresponds to the upper limit of the standard error of the control plants and the red line to the lower limit.**

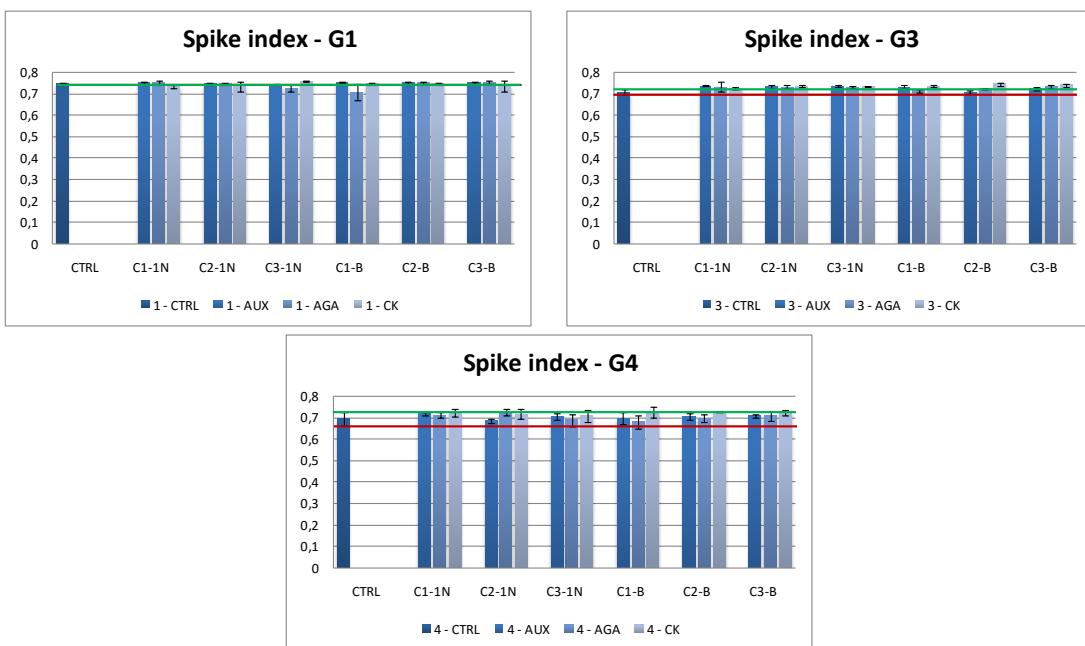
#### Spike biomass and spike index

For G1, spike biomass was significantly increased by AUX-C1-1N, AUX-C2-1N, and AUX-C1-B. The spike biomass for G4 tended to be increased by AUX-C1-1N, AUX-C2-B, and AUX-C3-B, but was significantly reduced by CK-C1-1N and CK-C2-B (Figure 2).

No changes were observed in spike index (all genotypes; Figure 3).



**Figure 2.** Different genetic responses in the spike biomass of three genotypes (G1, G3, G4) treated with three hormones (AUX, AGA, CK) at three concentrations (c1, c2, c3) at two phenological stages (1N, B). The green line corresponds to the upper limit of the standard error of the control plants and the red line to the lower limit.

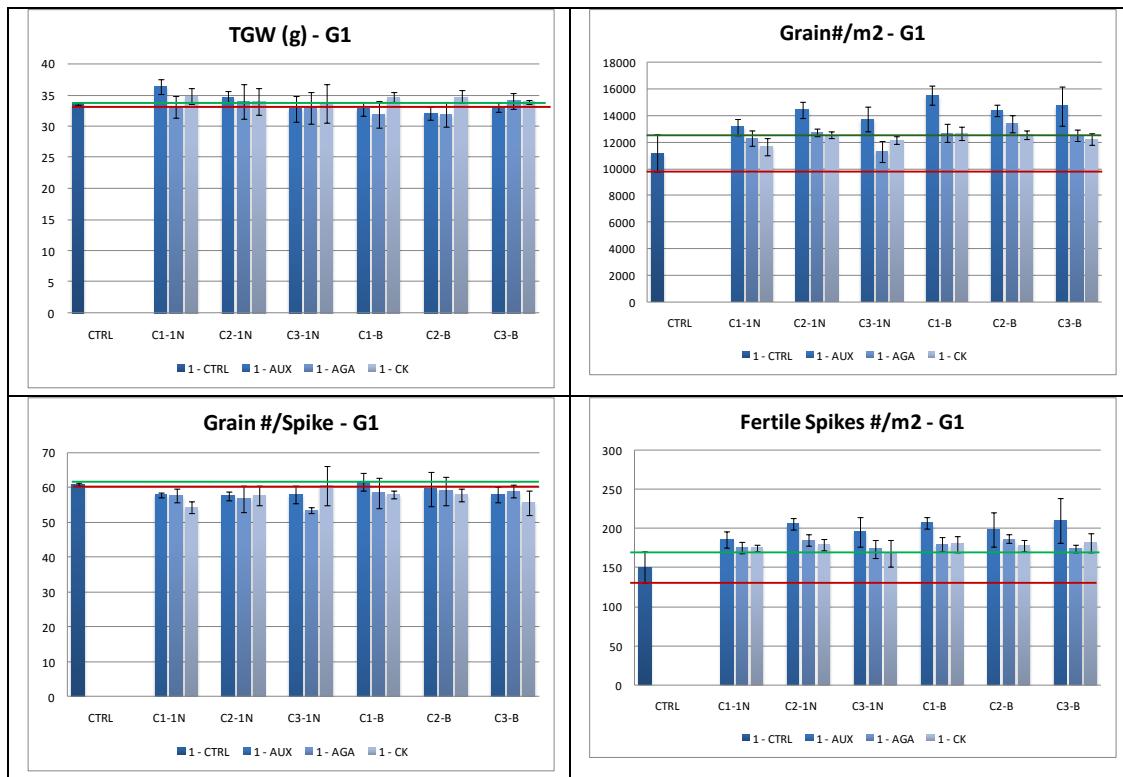


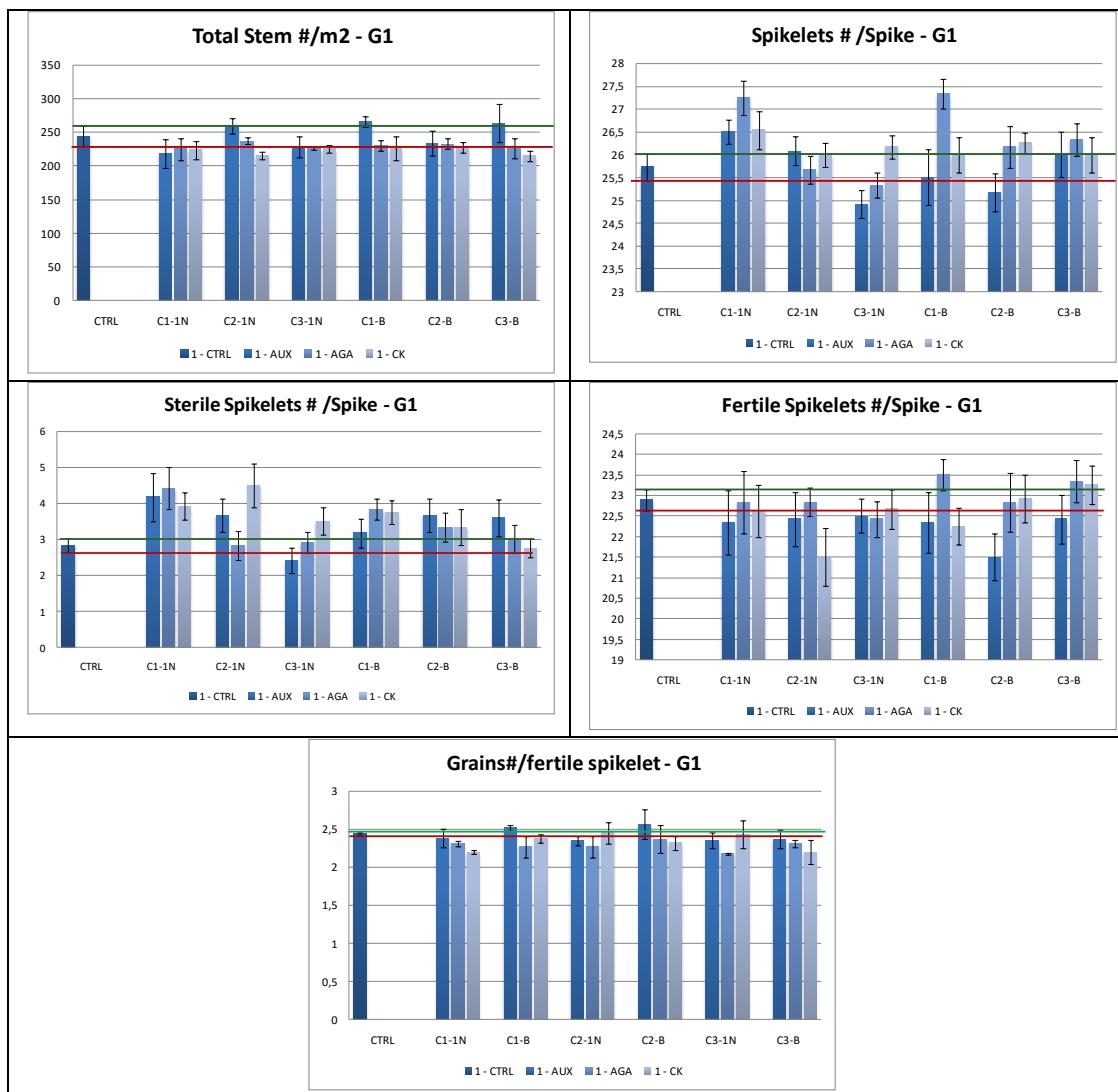
**Figure 3.** Different genetic responses in spike index of three genotypes (G1, G3, G4) treated with three hormones (AUX, AGA, CK) at three concentrations (c1, c2, c3) at two phenological stage (1N, B). The green line corresponds to the upper limit of the standard error of the control plants and the red line to the lower limit.

## *Yield components*

### a. Genotype I (G1)

Thousand grain weight (TGW) was significantly increased at jointing stage by a high concentration of auxin (AUX-C1-1N; Fig. 4a). Number of grains per square meter was significantly increased by AUX-C2-1N, AUX-C1-B, AUX-C2-B, and AUX-C2-B, with the greatest increase observed in response to the highest concentration of auxin at booting stage (AUX-C1-B; Fig. 4b). Number of grains per spike tended to reduce with the majority of the treatments; the reduction was significant at jointing stage for treatments AUX-C1-1N, AGA-C1-1N, CK-C1-N1, and AUX-C2-1N, with the greatest reduction observed with AGA-C3-1N (Fig. 4c). In contrast, number of fertile spikes tended to increase with all treatments, and was significant for all auxin treatments (Fig. 4d), while the total number of stems did not change (Fig. 4e). Total number of spikelets per spike was increased by treatment with a high concentration of AGA at jointing and booting stages (AGA-C1-1N and AGA-C1-B) and also by a high concentration of AUX and CK at jointing stage (Fig. 4f). However, the increased number of spikelets/spike was compensated by an increase in the number of sterile spikelets (Fig. 4g, h, i).





**Figure 4. Effect of three hormones (AUX, AGA, CK) on yield components at three different concentrations (c1, c2, c3) and at two phenological stages (1N, B) on G1:** a) TGW (top, left), b) grains#/m<sup>2</sup> (top, right), c) grains#/spike (middle top, left), d) fertile spike#/m<sup>2</sup> (middle top, right), e) total stems#/m<sup>2</sup> (middle bottom, right), f) spikelets#/spike (middle bottom, left), g) sterile spikelets#/spike (bottom, left), h) fertile spikelet#/spike (bottom, right), i) grain#/fertile spikelet (bottom). The green line corresponds to the upper limit of the standard error of the control plants and the red line to the lower limit.

Yield of G1 increased with AUX application due to an increased number of grains/m<sup>2</sup>, caused by an increase of the number of fertile stems/ m<sup>2</sup>. In this case, the total number of tillers/m<sup>2</sup> was not significantly modified by either AUX or CK. CK tends to reduce total stem number/m<sup>2</sup>, but it is not significant. This result for CK seems to contradict conventional physiological studies where CK is usually considered to promote growth of the auxiliary bud and AUX to inhibit it (Leopold 1949; Liu et al. 2011). However, the mechanism of auxiliary bud outgrowth depends on the ratio of these two hormones, rather than the absolute level of either hormone (Shimizu-Sato and Mori 2001), and thus endogenous hormone assessment (see later studies) should help interpret the results reported here.

Application of AUX significantly increased the number of fertile tillers/m<sup>2</sup>. This result could be associated with the ability of endogenous AUX to reduce male sterility induced by heat stress (Sakata et al. 2010). This hypothesis needs to be tested in irrigated heat stress trials and drought heat stress trials where AUX is both applied and measured endogenously, allowing hormone balances (CK/Auxin and ABA/Eth) to be measured.

*b. Genotypes 3 and 4 (G3 and G4) – a brief overview*

Final yields of G3 and G4 were not significantly modified by the hormone treatments. However, this does not mean that no changes were observed in the plant at deeper level, and the study of the yield components showed several compensated modifications. G4 showed an increase in TGW associated with a high AUX concentration at jointing stage (AUX-C1-1N); G1 and G3 also showed a tendency to increase TGW at the same concentration, but this response was not significant. TGW is usually associated with the grain filling period after anthesis. However, our hypothesis to explain the increased TGW is based on the possibility that the wheat plant will be able to take up more nitrogen at an early stage due to the AUX signal and enhanced root development. This hypothesis is based on the research of Kiba et al. (2011), who demonstrated an impact of AUX on nitrogen uptake as a result of the development of additional lateral roots, and by the research of Langer and Liew (1973) who showed that single grain weight increases when wheat plants receive more nitrogen at the early stages (double ridge to floret initiation), opposed to no changes (compared to control) if the nitrogen supply comes after heading stage.

For G3 and G4, spike fertility was improved at jointing and booting stage, by high and medium concentrations of CK (G3: CK-C2-B; G4: CK-C1-1N and CK-C2-B). Effectively, CK increased the number of grains per spike by enhancing the number fertile spikelets per spike (G3), or the number of grains per spikelet (i.e. more fertile florets per spike; G4). However, this action was compensated, in both cases, by a reduction in the number of total stems per square meter (a result also observed with G1) and the number of fertile spikes per square meter. The results obtained with CK for G3 are in agreement with the results obtained in barley by Ahokas (1982), where CK demonstrated some ability to restore male fertility, and for G4 with those presented by Ashikari et al. (2005) in rice, where the CK accumulation in inflorescence meristems increases the number of reproductive organs. G3 seems to be insensitive to the exogenous hormonal concentrations used in this study as, independently of the changes at the spikelet level, no changes were observed in other yield components.

## **Conclusions**

Yield components of a susceptible genotype (G4) and a genotype with low resilience to heat stress (G1) show more sensitivity to externally applied hormones at jointing stage but also at booting stage, compared to a stress tolerant genotype (G3). G3 showed no response to variation of exogenous hormones at the concentrations used in this study, with the only exception to this being an effect on spikelet development.

The external application of plant hormones generates responses indicating that plant hormones have an important role in regulating auxiliary bud growth. The results obtained for the relationship between tiller development and CK were unexpected, compared to the results of previous studies where the total number of spikes per square meter was reduced with CK treatments. To interpret this we need to assess endogenous hormone accumulations and hormone balances after each application if we are to properly

understand the factors controlling the growth of auxiliary buds. This approach is addressed in later chapters of Thiry (*in preparation*).

Spike fertility was enhanced by high concentration of CK (C1-1N) for G3 and G4, and at booting stage in the case of G4 (C2-B). However, this change in spike fertility has two different bases: for G3, the number of spikelets per spike was increased, whereas for G4, the number of grains per fertile spikelet was increased and this consequently improved floret fertility. For G1, AUX treatments increased the number of fertile tillers per unit area without changing the total number of spikes per square meter.

This study has shown that not all genotypes react in a similar way to exogenous hormone application, and a similar effect on one or another yield component does not automatically come from a common modification in plant biology. Additionally, it was observed that fertility can be improved at different levels by CK, but we need to understand how to reduce the compensatory mechanisms inherent in the plant in order to increase yield. This experiment should be repeated under heat + irrigation and under yield potential conditions, but potentially indicates that stress susceptibility of certain genotypes may be linked to low levels of key hormones.

### Acknowledgements

We are grateful for financial support from CIMMYT WHEAT CRP and to Elizabeth Morales Eliosa for her help in collecting field data.

### References

- Ahokas, H., 1982. Cytoplasmic male sterility in barley : Evidence for the involvement of cytokinins in fertility restoration. *Proceedings of the National Academy of Sciences* 79(December): 7605–7608.
- Ashikari, M., Sakakibara, H., Lin, S., Yamamoto, T., Takashi, T., Nishimura, A., Angeles, E.R., Qian, Q., Kitano, H., and Matsuoka, M., 2005. Cytokinin oxidase regulates rice grain production. *Science* 309(5735): 741-745.
- Cai, T., Xu, H., Peng, D., Yin, Y., Yang, W., Ni, Y., Chen, X., Xu, C., Yang, D., Cui, Z., and Wang, Z., 2014. Exogenous hormonal application improves grain yield of wheat by optimizing tiller productivity. *Field Crops Research* 155: 172-183.
- Calderini, D. F. and Reynolds, M. P., 2000. Changes in grain weight as a consequence of de-graining treatments at pre- and post-anthesis in synthetic hexaploid lines of wheat. *Australian Journal of Agricultural Research* 27: 187–191.
- Chatfield, S. P., Stirnberg, P., Forde, B. G., and Leyser, O., 2000. The hormonal regulation of axillary bud growth in *Arabidopsis*. *The Plant Journal : For Cell and Molecular Biology* 24(2): 159–69.
- Colombo, N. and Favret, E. A., 1996. The effect of gibberellic acid on male fertility in bread wheat. *Euphytica* 91(3): 297–303.
- Kiba, T., Kudo, T., Kojima, M., and Sakakibara, H., 2011. Hormonal control of nitrogen acquisition: Roles of auxin, abscisic acid, and cytokinin. *Journal of Experimental Botany* 62(4): 1399–1409.

- Langer, R. H. M. and Liew, F. K. Y., 1973. Effects of varying nitrogen supply at different stages of the reproductive phase on spikelet and grain production and on grain nitrogen in wheat. *Australian Journal of Agricultural Research* 24: 647–56.
- Langer, R. H. M., Prasad, P. C., and Laude, H. M., 1973. Effects of kinetin on tiller bud elongation in wheat (*Triticum aestivum* L.). *Annals of Botany* 37(3): 565–571.
- Leopold, A. C., 1949. The Control of Tillering in Grasses by Auxin. *American Journal of Botany* 36(6): 437–440.
- Liu, Y., Ding, Y.-F., Wang, Q.-S., Li, G.-H., Xu, J.-X., Liu, Z.-H., & Wang, S.-H., 2011. Effect of Plant Growth Regulators on Growth of Rice Tiller Bud and Changes of Endogenous Hormones. *Acta Agronomica Sinica* 37(4): 670–676.
- Miralles, D. J., Katz, S. D., Colloca, A., and Slafer, G. A., 1998. Floret development in near isogenic wheat lines differing in plant height. *Field Crops Research* 59(1): 21–30.
- Pask, A., Pietragalla, J., Mullan, D., and Reynolds, M. P., 2012. *Physiological Breeding II: A Field Guide to Wheat Phenotyping*. CIMMYT.
- Sakata, T., Oshino, T., Miura, S., Tomabechi, M., Tsunaga, Y., Higashitani, N., Miyazawa, Y., Takahashi, H., Watanabe, M. and Higashitani, A., 2010. Auxins reverse plant male sterility caused by high temperatures. *Proceedings of the National Academy of Sciences* 107(19): 8569–8574.
- Shimizu-Sato, S. and Mori, H., 2001. Control of outgrowth and dormancy in axillary buds. *Plant Physiology* 127(4): 1405–1413.
- Slafer, G. A., 2005. Physiology of determination of major wheat yield components. In Buck, H.T., Nisi J.E., and Salomón, N. (Eds.), *Environments Proceedings of the 7th International Wheat Conference, 27 November - 2 December 2005, Mar del Plata, Argentina (Developments in Plant Breeding)* (Springer., pp. 557–565). Springer.
- Slafer, G. A., Calderini, D. F., and Miralles, D. J., 1996. Yield Components and Compensation in Wheat: Opportunities for Further Increasing Yield Potential. In *Increasing Yield Potential in Wheat: Breaking the Barriers*.
- Yin, C., Park, J.-J., Gang, D. R., and Hulbert, S. H., 2014. Characterization of a tryptophan 2-monoxygenase gene from *Puccinia graminis* f. sp. *tritici* involved in auxin biosynthesis and rust pathogenicity. *Molecular Plant-Microbe Interactions : MPMI* 27(3): 227–35.
- Zhang, J., Wang, J., Dang, J., and Zhang, D., 2010. Differences in grain yield and quality between the main stem and the tillers of wheat. *Journal of Triticeae Crops* 30(3): 526–528.

# Achieving increases in spike growth, fruiting efficiency, and harvest index in high biomass wheat cultivars

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## Abstract

While allocation of carbon to the developing wheat spike determines grain sink strength, concurrent growth of other plant organs (structural and non-structural dry matter; DM) competes for carbon. This study observed genetic variation in structural and non-structural DM partitioning to the true stem, leaf sheath and spike, and associations with spike growth, harvest index (HI), and grain yield in 26 CIMMYT cultivars and advanced lines under high radiation, irrigated conditions in northwestern Mexico during 2011-12 and 2012-13. Structural DM partitioning was assessed in the true-stem internodes and amongst the spike morphological components (rachis, glume, palea, lemma, awn) at harvest. Results showed a positive association between spike partitioning index (spike DM/above ground DM at anthesis + 7 days; SPI) and HI ( $R^2 = 0.16$ ,  $P < 0.05$ ). Lower true-stem structural DM partitioning was associated with higher SPI and spike DM growth per unit area at anthesis + 7 days. There were negative associations between SPI and spike DM per unit area and true-stem internode 2 and 3 DM partitioning ( $P < 0.05$ ), but no associations with other internodes. A positive linear association was determined between each of rachis specific weight (rachis DM per unit length) and DM partitioning to the palea and the fruiting efficiency (grains per unit spike DM at anthesis + 7 days). A Genome Wide Association Study (GWAS) is ongoing to identify genetic markers for these key target traits to enhance HI in the High Biomass Association panel at the field site in northwestern Mexico during 2015-16, as part of the PhD of Aleyda Sierra-Gonzalez.

## Introduction

Progress in yield potential of CIMMYT spring wheat in the Yaqui Valley, northwestern Mexico, from 1990 to 2009 is associated with greater above-ground biomass and grain weight, and decreases in harvest index (HI) (Aisawi et al. 2015). Biomass is deemed to be an important determinant of genetic gains in yield potential, but it will be crucial to also identify traits and markers that enable breeders to maximize assimilate partitioning to the grains. In this context, identifying traits to increase spike growth at anthesis and fruiting efficiency (ratio grain number to spike dry matter [DM] at anthesis; FE) in new current high biomass cultivars will be crucial to fully exploit beneficial impacts in the area of photosynthesis research.

One avenue to increase spike DM partitioning and spike growth at anthesis is to decrease true-stem DM partitioning, particularly structural true-stem DM (Foulkes et al. 2013). Strategies to boost spike growth by reducing assimilate partitioning to true-stem structural DM are complementary to those increasing the FE (Foulkes et al. 2011; Lázaro and Abbate 2011), for which there is clear variability among modern cultivars (González et al. 2011; Lázaro and Abbate 2011). Although a trade-off between FE and spike DM partitioning index is sometimes observed (Gonzalez et al. 2011; Lázaro and Abbate, 2011), some work has demonstrated the possibility to identify genotypes combining high SPI with high FE (Lázaro

and Abbate, 2011; Bustos et al. 2013). This investigation aims to identify: (i) genetic variation in assimilate partitioning to increase spike fertility and HI in high biomass backgrounds by screening diverse germplasm, and (ii) traits and molecular markers for improved assimilate partitioning, spike fertility, and HI for application in wheat breeding.

## Methods

A set of 26 CIMMYT elite spring wheat (*T. aestivum* L.) cultivars and advanced lines were selected from the CIMMYT Mexico Core Germplasm (CIMCOG) panel and grown during 2011-12 and 2012-13 at the CIMMYT experimental station near Ciudad Obregon, northwestern Mexico. The experimental design was an alpha-lattice with three replications under raised beds; plots were irrigated using a gravity-based system with 4–6 flood irrigations during the crop cycle.

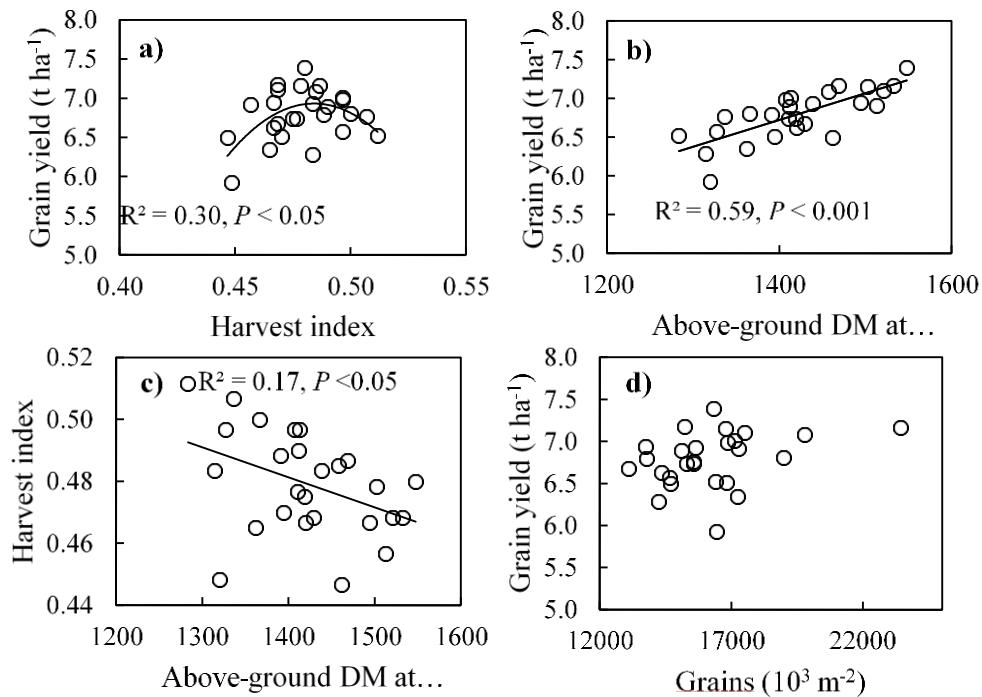
The 26 cultivars were evaluated for above-ground dry-matter (AGDM), DM partitioning, and spike fertility (SPI and FE) at anthesis (GS65) + 7 days, and for HI at harvest from growth analysis of 0.8 m<sup>2</sup> quadrat samples at GS65+7d and 100 fertile shoot samples at harvest. Grain yield was measured in a plot area of 5 m<sup>2</sup> at harvest. For a subset of nine cultivars with a restricted range of anthesis date but representing the full range of stem partitioning at anthesis, further DM partitioning analyses were carried out of: (i) AGDM and DM partitioning at initiation of booting (GS41) and (ii) true-stem internode DM partitioning (peduncle, internode 2, internode 3, and internode 4+) at GS65+7d. In addition, for seven of the nine cultivar subset, spike DM morphological DM partitioning (glume, palea, lemma, rachis and lemma) were also assessed at harvest in 2011-12.

## Results/Discussion

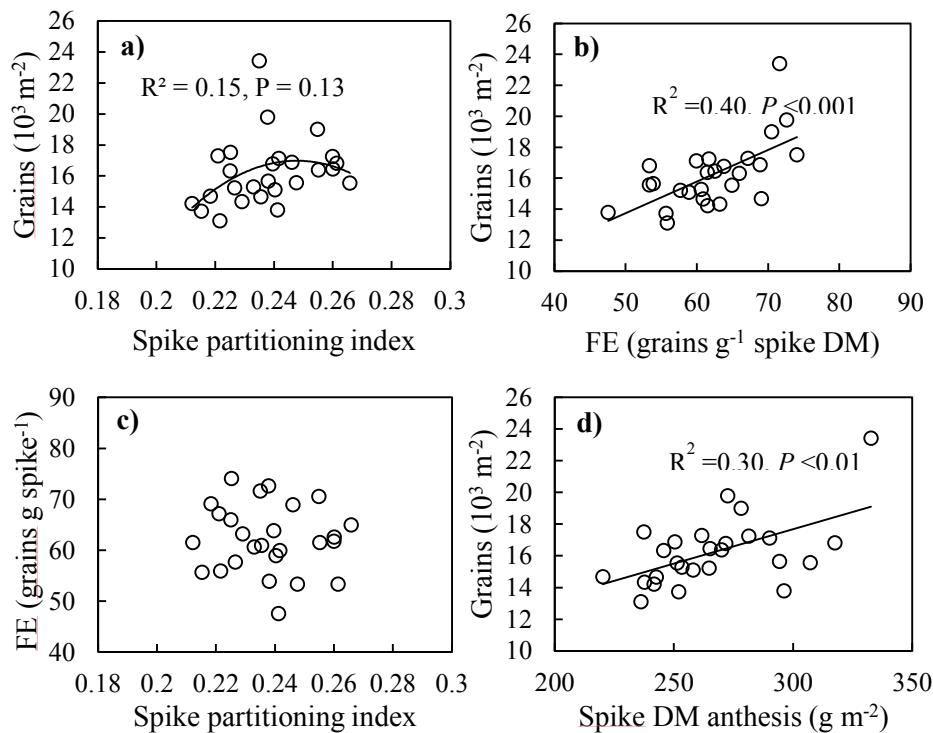
### *Relationships between yield potential and yield components*

Results showed that HI was positively associated with grain yield ( $R^2 = 0.30$ ;  $P < 0.001$ ; Fig. 1a), with yield increasing up to *ca.* 0.49 and then levelling off. Overall HI among the 26 genotypes ranged from 0.45-0.51 ( $P < 0.001$ ), indicating significant scope for raising HI towards the theoretical maximum of *ca.* 0.65 (Austin 1982; Foulkes et al. 2011). Although grain yield was strongly related to above-ground biomass among the 26 cultivars ( $R^2 = 0.59$ ,  $P < 0.001$ ; Fig. 1b), this was not observed when only the 10 cultivars with the highest biomass values were considered. Moreover, among the 26 cultivars, there was a negative linear relationship between HI and above-ground biomass ( $R^2 = 0.17$ ;  $P < 0.05$ ; Fig. 1c). These findings indicate that the highest biomass cultivars were less efficient at partitioning assimilate to grain and that novel traits to maximize partitioning to grain must therefore be identified.

The results showed a tendency for a positive non-linear (quadratic) association between SPI at GS65+7d and grains m<sup>-2</sup> ( $R^2 = 0.16$ ,  $P = 0.13$ ; Fig. 2a), but there was a stronger positive linear association between FE and grains m<sup>-2</sup> ( $R^2 = 0.40$ ,  $P < 0.001$ ; Fig. 2b). Plant height ranged from 84-116 cm ( $P < 0.001$ ) and showed a trend for a negative association with HI ( $R^2 = 0.12$ ,  $P = 0.09$ ; data not shown), but was not associated with grain yield or AGDM.



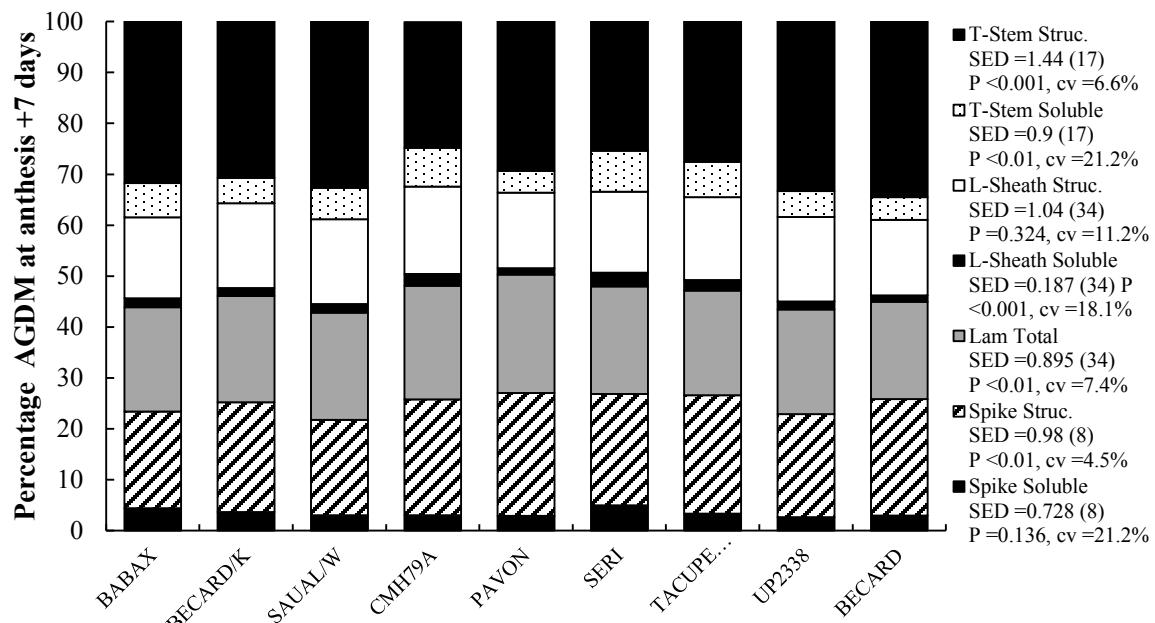
**Figure 1.** Regressions of a) grain yield (100% DM) on HI; b) grain yield on above-ground DM; c) HI on AGDM at harvest; and d) grain yield on grains  $\text{m}^{-2}$  for 26 CIMCOG panel cultivars. Values represent means in 2011-12 and 2012-13.



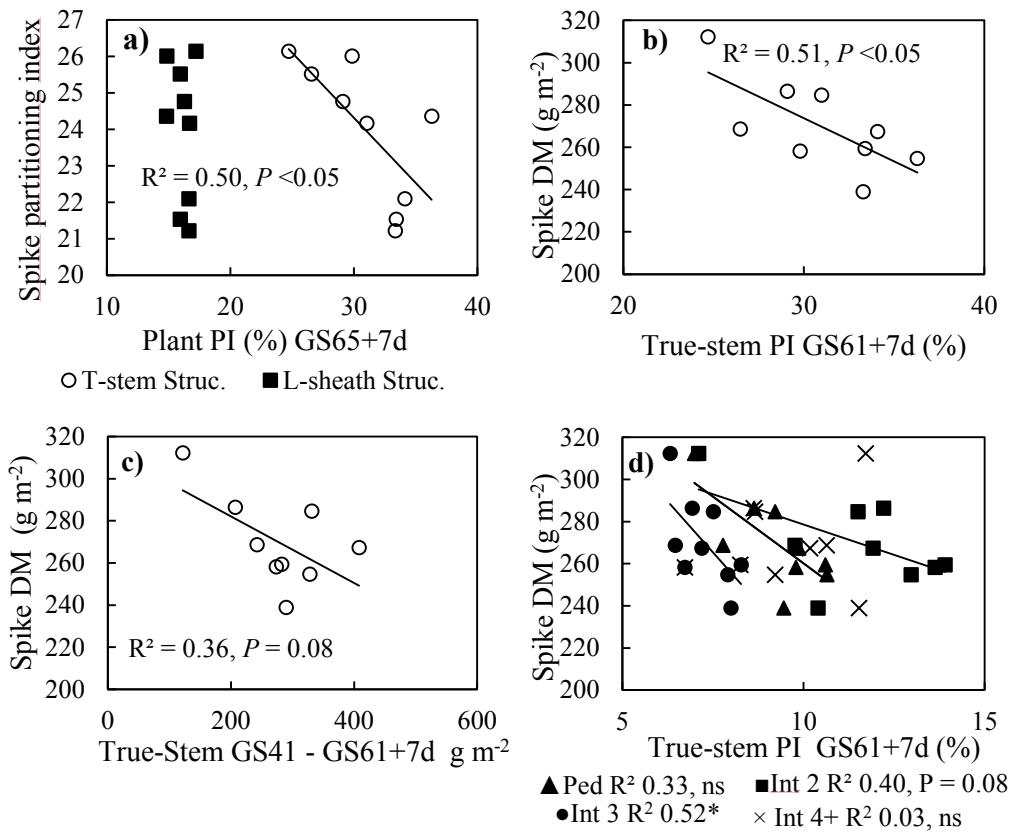
**Figure 2.** Regression of a) grains  $\text{m}^{-2}$  on SPI; b) grains  $\text{m}^{-2}$  on FE; c) FE on SPI; and d) grains  $\text{m}^{-2}$  on spike DM ( $\text{g m}^{-2}$ ) for 26 CIMCOG panel cultivars. Values represent means in 2011-12 and 2012-13.

### *Genetic variation in structural DM in plant components in a subset of nine cultivars*

Genetic variation in structural and soluble DM partitioning at GS65+7d is shown for the leaf lamina, leaf sheath, true-stem, and spike (Fig. 3). There was significant genetic variation for DM partitioning to all plant components, except for the leaf-sheath structural and the spike soluble DM components. True-stem structural partitioning ranged from 24.7 to 36.3% ( $P < 0.01$ ). SPI was negatively correlated with true-stem DM partitioning at anthesis + 7 days ( $R^2 = 0.50, P < 0.05$ ), but was not associated with leaf sheath structural DM partitioning (Fig. 4). True-stem DM partitioning was also negatively correlated with spike DM per unit area at anthesis + 7 days ( $R^2 = 0.51, P < 0.05$ ). Moreover, the amount of structural true-stem DM accumulated from initiation of booting (GS41) to anthesis + 7 days was linearly and negatively associated with the amount of spike DM amount at anthesis + 7 days ( $R^2 = 0.36, P = 0.08$ ; Fig. 4), as well as with SPI (data not shown). These results indicated that, in modern high yield potential CIMMYT cultivars, the structural true-stem component is the main target for decreasing DM partitioning to enhance spike growth.

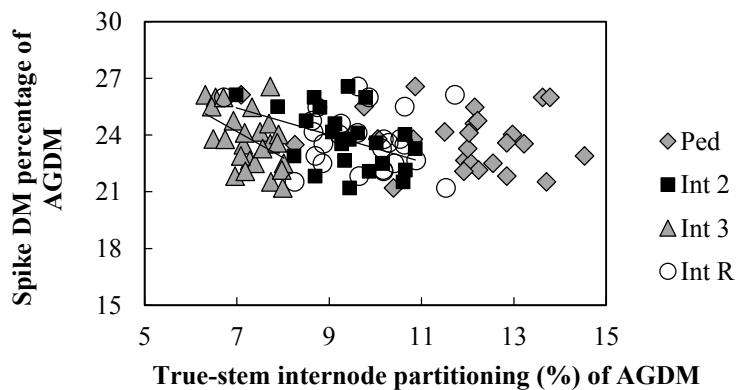


**Figure 3. Soluble and structural DM as a proportion of total AGDM for each plant component and cultivar at anthesis + 7 days (GS65+7d) for the subset of nine cultivars. Values represent means in 2011-12 and 2012-13.**



**Figure 4.** Regression of SPI on true-stem PI and leaf-sheath PI at GS65+7d, b) spike DM per unit area at GS65+7d on true-stem PI at GS65+7d, and c) True-stem DM accumulated from GS41 to GS65+7d amongst the subset of nine cultivars. Values represent means in 2012 and 2013.

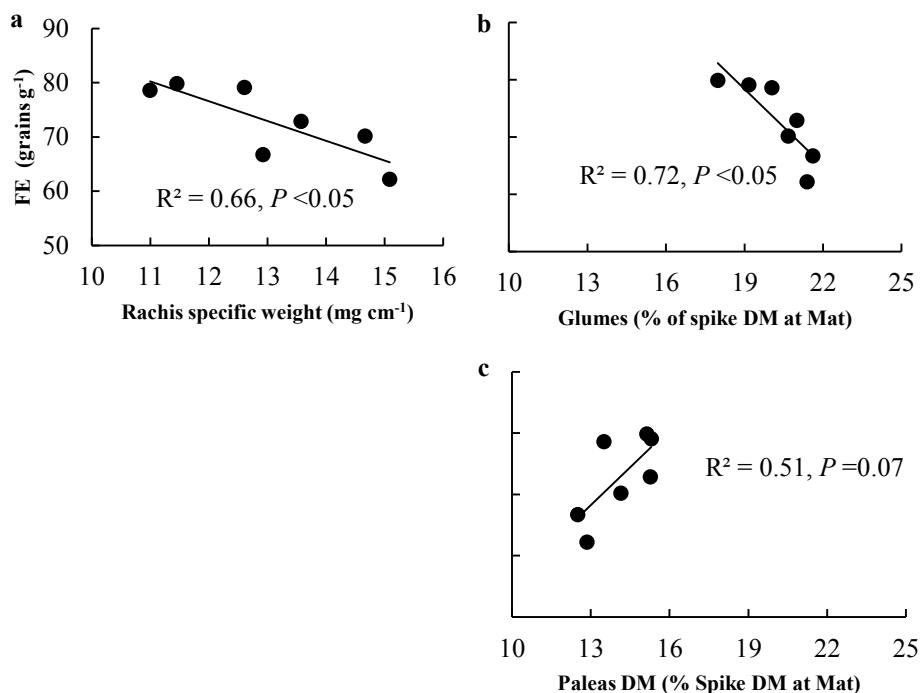
In order to reduce true-stem DM partitioning in the most effective way to benefit spike growth, it is necessary to identify those true-stem internodes that compete most strongly with spike growth during stem elongation. Our results showed that, for the four internode classes assessed, internode 2 and internode 3 had the strongest negative association with spike partitioning (Fig. 5) and spike DM per unit area at anthesis + 7 days.



**Figure 5.** Regression of SPI at GS65+7d on true-stem internode DM PI among 26 CIMCOG panel cultivars. Values represent means in 2012 and 2013.

### Fruiting efficiency and association with spike morphological partitioning

In 2012, spike DM at harvest (without gains) was dissected into its morphological components for seven of the nine cultivar subset; rachis length was measured and rachis specific weight (rachis DM rachis length; RSW) was calculated. Differences in RSW ranged from 11.0 to 15.1 mg cm<sup>-1</sup> ( $P < 0.01$ ). The FE (calculated based on the chaff DM at harvest) was linearly negatively associated with RSW ( $R^2 = 0.66$ ,  $P < 0.05$ ; Figure 6a) and with glumes DM (% of spike DM;  $R^2 = 0.72$ ,  $P < 0.05$ ; Figure 6b). Interestingly, a trend for a positive association between paleas DM (% of spike DM) and FE was also observed ( $R^2 = 0.51$ ,  $P = 0.07$ ).



**Figure 6.** Linear regression of fruiting efficiency on a) rachis SW; b) Glumes % of DM; and c) paleas % of DM (as a proportion of spike DM at harvest, without grains) among seven cultivars in 2012.

We calculate that HI  $> 0.6$  can be achieved in CIMMYT spring wheat by combining the biggest expression for ‘useful’ biomass traits, including decreased partitioning to the lower internodes 2 and 3 (0.206; Babax/LR42) to enhance spike growth, and decreased rachis specific weight (11.0 mg cm<sup>-1</sup>; Becard/Kachu) and increased lemma fraction (0.256; Pavon) to enhance FE. Stepwise regression analysis shows that 51% of variance in spike partitioning and 53% of variance in FE is accounted for by these traits. A Genome Wide Association Study (GWAS) is ongoing in to identify genetic markers for the key target traits, including structural stem DM partitioning to internodes 2 and 3 and rachis specific weight determining SPI and FE in the High Biomass Association panel at the Yaqui Valley field site during 2015-16, as part of the PhD of Aleyda Sierra-Gonzalez.

### Conclusions

- HI >0.6 can be achieved by combining the best expression for ‘useful’ biomass traits. Subsets of germplasm with the highest expression of favorable traits (whilst minimizing trade-offs) for SPI and FE have been identified for strategic crosses in pre-breeding at CIMMYT.
- Ongoing analysis is determining the key combination of true-stem internode morphological properties (DM, length, and specific weight), as well as trade-offs with water soluble carbohydrate accumulation and plant height, and effects of phenology, in order for these traits to be implemented in breeding programs aiming to optimize DM partitioning related traits.
- Identification of marker-trait associations for the key traits determining SPI and FE in a Genome Wide Association Study (GWAS) is ongoing in a High Biomass Association panel to develop markers for marker-assisted selection in order to increase HI in high biomass backgrounds.

## Acknowledgments

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## References

- Aisawi, K.A.B., Reynolds, M.P., Singh, R.P., and Foulkes, M.J., 2015. The Physiological Basis of the Genetic Progress in Yield Potential of CIMMYT Spring Wheat Cultivars from 1966 to 2009. *Crop Science* 55(4): 1749.
- Bustos, D.V. Hasan, A.K., Reynolds, M.P., and Calderini, D.F. 2013. Combining high grain number and weight through a DH-population to improve grain yield potential of wheat in high-yielding environments. *Field Crops Research* 145: 106-115.
- Foulkes, M.J., Rivera-Amado, C., Trujillo-Negrellos, E., Molero, G., Sylvester-Bradley, R., and Reynolds, M.P., 2013. Proceedings 3rd International Workshop Wheat Yield Consortium Cd. Obregón, Mexico 5-7 March, 2013. pp. 26-32;
- Foulkes, M.J., Slafer, G.A., Davies, W., Berry, P., Sylvester-Bradley, R., Martre, P., Calderini, D.F., and Reynolds, M.P., 2011. Raising yield potential in wheat: Optimizing partitioning. *Journal of Experimental Botany* 60: 1899-1918.
- González F.G., Terrile, I.I., and Falcón, M.O., 2011. Spike Fertility and Duration of Stem Elongation as Traits to Improve Potential Grain Number (and Yield): Variation in Argentinean Wheats. *Crop Science* 51: 1693-1702.
- Lázaro, L. and Abbate, P.E., 2011. Cultivar effects on relationship between grain number and photothermal quotient or spike dry weight in wheat. *Journal of Agricultural Science (Cambridge)* 150: 442-459.

## Avoiding lodging in wheat through the development of genetic resources and the genetic dissection of lodging proof traits

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### Abstract

A model of the lodging process has been successfully adapted for use on spring wheat by evaluating lodging-associated traits in field experiments in northwestern Mexico using spring wheat cultivars from the CIMMYT Mexico Core Germplasm (CIMCOG) panel. In order to elucidate the genetic control of these traits, we evaluated a winter wheat Avalon x spring wheat Cadenza doubled haploid population grown under UK conditions. Both spring and winter wheat data suggested that increased structural stem and root biomass will be necessary for improving lodging resistance. This will incur trade-offs with grain yield, primarily because of the overlapping development of lodging traits with grain yield formation. Genetic ranges for spring and winter wheat indicated that the most challenging dimension to achieve would be root plate spread, and new diverse germplasm must be identified to achieve this. Quantitative trait loci (QTL) related to lodging traits were identified in the Avalon x Cadenza genotypes; fine mapping and validation of these QTL will be paramount in accelerating selection for lodging resistance while also improving genetic yield potential.

### Introduction

Lodging – defined as the permanent displacement of the plant/shoots from their vertical position (Pinthus 1973; Berry et al. 2004) – reduces wheat grain quality and yield (Berry et al. 2004). During the Green Revolution, the introduction of semi-dwarf wheat varieties increased resistance to lodging and thus enabled growers to increase nitrogen fertilization, resulting in considerable increases in grain yield (Conway 1997). After the Green Revolution, plant growth regulators were used to further reduce plant height, resulting in even better lodging resistance and grain yield (Berry et al. 2004). However, lodging susceptibility has not been completely eliminated and, as yields increase, there is evidence that growers cannot continue to rely on these strategies because a minimum plant height for optimum yield (0.7 m) has already been reached (Flintham et al. 1997). Collaborative studies by physiologists, biologists, and engineers have generated a deeper understanding of lodging (through stem and anchorage failure mechanisms), and have proposed models of the lodging process, together with better crop husbandry and agronomic management strategies (Crook and Ennos 1994; Baker et al. 1998; Berry et al. 2000; Berry et al. 2003b; Sterling et al. 2003; Berry et al. 2007). Most of these studies were conducted using winter wheat and resulted in a preliminary attempt to estimate an ideotype design for lodging-resistant wheat for the UK. However, lodging is also a problem in spring wheat, particularly under irrigated conditions, and it is not yet known whether the winter wheat model is applicable for irrigated spring wheat.

This study adapted the winter wheat lodging model for use in spring wheat germplasm from the CIMMYT Mexico Core Germplasm (CIMCOG) panel and evaluated the genetic control of traits in a winter wheat x spring wheat doubled haploid population (Avalon x Cadenza). The following objectives were addressed:

- a. Estimation of the biophysical properties and structural biomass requirements of a lodging-proof plant for irrigated spring wheat grown in northwestern Mexico.
- b. Evaluation of the genetic diversity of lodging traits and their interactions and associations with other physiological traits.
- c. Assessment of possible trade-offs between lodging traits and yield and yield forming processes.
- d. Implementation of QTL analysis for lodging traits to develop lodging-related genetic markers.

## Methods

Lodging-related traits were evaluated using spring wheat cultivars from the CIMCOG panel. Field trials were conducted in the Yaqui Valley of northwestern Mexico during four seasons: 2010-11, 2011-12, 2012-13, and 2013-14 (referred to hereafter as 2011, 2012, 2013, and 2014, respectively). Phenotypic screening of the Avalon x Cadenza doubled-haploid population was carried out in UK field experiments at ADAS High Mowthorpe, North Yorkshire during 2012-13, and at Sutton Bonington, University of Nottingham farm during 2013-14 (henceforth referred as High Mowthorpe and Sutton Bonington, respectively). Detailed methods for experiments, measurements, calculations, and statistical analyses are explained in three papers generated from this investigation (Piñera-Chavez et al. 2016a; Piñera-Chavez et al. 2016b; Piñera-Chavez et al. 2016c).

## Results/Discussion

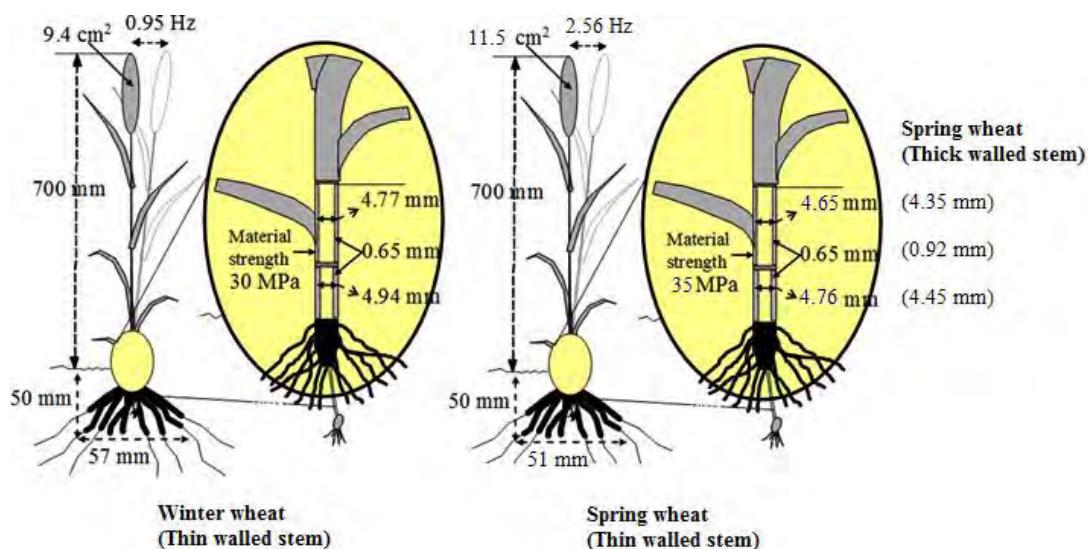
The lodging-proof plant estimated for irrigated spring wheat will require a maximum height of 0.7 m, a minimum root plate spread of 51.1 mm, and a minimum stem strength for the bottom internode of 268 N·mm (Table 1). It will have a 1 in 25 years probability of lodging and would support the typical on-farm average yield of 6 t ha<sup>-1</sup> (Fischer and Edmeades 2010) with 500 shoots m<sup>-2</sup> and 200 plants m<sup>-2</sup> for crops grown in northwestern Mexico. The target stem strength could be achieved with a diameter of 4.76 mm and a material strength of 35 MPa, or with a diameter of 4.12 mm and a material strength of 50 MPa, each with a maximum wall width of 0.65 mm. The cross-year cultivar analysis demonstrated wide genetic variation ( $P < 0.01 - 0.001$ ) and indicated that not all of the target traits could currently be achieved by a single cultivar. For instance, the maximum stem strength found in the CIMCOG panel (252 N·mm) was 6% below the target requirement (268 N·mm), and the best root plate spread observed (42.2 mm) was 17% less than the target (51.1 mm) (Table 1). Coincidentally, the best values observed in winter wheat cultivars were also lower than the target stem strength and root plate spread of the estimated lodging-proof winter wheat in a UK environment (Berry et al. 2007). All traits (except for root plate spread) had good heritability, which is desirable for breeding selection.

**Table 1. Ideotype target dimensions, genetic range, heritability ( $h^2$ ), and best value observed for the key lodging traits of spring wheat (CIMCOG panel) for 27 wheat cultivars. Values represent means of 2011-13.**

Trait	Ideotype target	Genetic range*	$h^2$	Best observed value
Stem diameter (mm)	4.12 – 6.03	3.35 – 4.47	0.93	4.47
Stem wall width (mm)	0.65	0.64 – 0.92	0.76	0.64
Stem strength (N mm)	268	134 – 252	0.78	252
Stem material strength (MPa)	20 – 50	27.4 – 59.4	0.72	59.4
Root plate spread (mm)	51.1	33.7 – 42.2	0.11	42.2
Height (m)	0.70	0.73 – 1.07	0.96	0.73
Structural stem biomass (t ha <sup>-1</sup> ) <sup>a</sup>	3.93 <sup>a</sup>	4.77 – 6.32 <sup>b</sup>	–	6.32
Root biomass (t ha <sup>-1</sup> ) <sup>c</sup>	1.10	0.9 – 0.69	–	0.69

\*All with significant differences ( $P < 0.01 - 0.001$ ); <sup>a</sup> included only lignin and structural carbohydrates; <sup>b</sup> included total non-grain above biomass; <sup>c</sup> root biomass at the top 10 cm of soil.

The first design of a lodging-proof plant was developed on winter wheat under UK growing conditions (Berry et al. 2007). One of the approaches proposed by Berry et al. (2007) indicates that the minimum investment of biomass to increase lodging resistance could be achieved through raising stem strength by increasing stem material strength or stem diameter, rather than stem wall width (based on engineering principles). In this regard, for winter wheat the best combination of traits indicated dimensions of 30 MPa stem material strength, a 4.94 mm bottom internode diameter, and a minimum 0.65 mm stem wall width (i.e. thin walled stem). For spring wheat with a thin walled stem, the best combination of traits indicated dimensions of 35 MPa stem material strength and a 4.76 mm bottom internode diameter (Fig. 1). However, spring wheat showed a strong positive correlation between stem strength and stem wall width that will be difficult to break (Table 2), thus breeders must consider a thicker walled stem for spring wheat. This is likely to represent a greater trade-off in spring wheat between grain yield and lodging traits to achieve a lodging-proof crop design, compared to winter wheat.



**Figure 1. Dimensions of a lodging-proof plant for UK winter wheat and for northwestern Mexico spring wheat (modified from Berry et al. 2007).**

Genetic and phenotypic correlations among lodging-related traits and grain yield have indicated that achieving a lodging-resistant ideotype crop is feasible. In fact, most of these associations are favorable for combining the lodging-proof dimensions in a single wheat genotype (Berry et al. 2003b; Berry et al. 2007; Berry and Berry 2015).

**Table 2. Phenotypic (upper diagonal) and genetic (lower diagonal) correlations between cross-year means of lodging, agronomic, and physiological traits of 27 spring wheat cultivars grown the Yaqui Valley during 2011, 2012, and 2013.**

Traits	1	2	3	4	5	6	7	8	9
1. Grain yield		-0.12	-0.09	0.19	0.37	0.24	0.18	-0.32	0.14
2. Plant height		-0.16		-0.06	-0.09	0.33	0.12	-0.09	0.00
3. Root plate spread		0.04		-0.12		<b>0.70</b>	0.23	0.09	0.23
4. Root dry weight		<b>0.50</b>		-0.07	<b>0.94</b>		<b>0.48</b>	0.30	0.35
5. Stem diameter		<b>0.41</b>		0.34	0.35	<b>0.71</b>		0.31	0.27
6. Stem wall width		0.37		0.16	<b>-0.44</b>	0.23	0.32		<b>0.59</b>
7. Stem dry weight per length		<b>0.54</b>		-0.17	—	<b>0.70</b>	<b>0.48</b>	<b>0.90</b>	
8. Stem material strength		-0.36		-0.01	-0.33	<b>-0.58</b>	<b>-0.86</b>	0.30	-0.11
9. Stem strength		0.24		0.37	0.07	<b>0.44</b>	<b>0.46</b>	<b>0.97</b>	<b>0.78</b>

Coefficients in bold indicate significant correlations ( $P < 0.05 - 0.001$ )

Regions on chromosomes 3B and 5B have indicated the presence of major QTL affecting key stem strength and anchorage traits (root plate spread) (Table 3). Despite the G×E interaction identified for these traits, these QTL were not affected and were identified whether the QTL analysis was done by using combined means or experiment means.

**Table 3. Summary of the QTL detected for cross-experiment means.**

Trait	Chr	QTL	Position (cM)	CI (cM)	LOD	PVE (%)	Additive effect <sup>a</sup>	Peak marker
SS	1D	<i>qSSID</i>	37.1	33.8 – 38.5	3.33	6.39	-7.80	<i>cf19</i>
	3B	<i>qSS3B</i>	284	282 – 285	15.6	42.3	-19.5	<i>wPt-4412</i>
SMS	2D	<i>qSMS2D</i>	55.2	48.7 – 56.0	7.44	12.8	-3.25	<i>BS00022730</i>
	3B	<i>qSMS3B</i>	282	282 – 284	19.2	48.7	-6.05	<i>BS00003884</i>
SID	2D	<i>qSID2D</i>	55.2	49.3 – 56.7	11.7	23.8	0.16	<i>BS00022730</i>
	3B	<i>qSID3B</i>	285	283 – 287	13.6	30.4	0.18	<i>cos4Gb</i>
	4B	<i>qSID4B-1</i>	51.6	50.0 – 52.9	4.02	6.88	0.086	<i>BS000022972</i>
	4B	<i>qSID4B-2</i>	57.9	54.3 – 60.3	4.56	7.45	0.090	<i>BS00009272</i>
SIWW	3B	<i>qSIWW3B</i>	279	277 – 280	34.5	81.7	-0.17	<i>BS00022401</i>
	4B	<i>qSIWW4B</i>	156	147 – 161	4.38	3.82	0.032	<i>BS00022847</i>
	4D	<i>qSIWW4D-1</i>	72.2	63.2 – 76.8	3.81	3.26	0.033	<i>BS00021924</i>
	4D	<i>qSIWW4D-2</i>	83.4	72.4 – 103	3.76	3.22	0.032	<i>BS00064001</i>
RPS	5B	<i>qRPSSB</i>	180	180 – 186	10.1	32.6	-1.20	<i>BS00023078</i>
PH	2D	<i>qPH2D</i>	35.4	28.1 – 46.9	10.2	21.9	-5.55	<i>cos2Q</i>
	3A	<i>qPH3A</i>	145	143 – 153	8.38	16.1	-4.70	<i>wPt-9215</i>
	3B	<i>qPH3B</i>	282	271 – 285	3.57	5.89	2.87	<i>BS00088726</i>
	4D	<i>qPH4D</i>	63.3	59.3 – 67.3	10.6	23.3	-5.65	<i>RhtMrkDI</i>
GY	2D	<i>qGY2D</i>	55.2	47.8 – 74.1	8.41	27.6	-0.27	<i>BS00022730</i>

Chr, chromosome or linkage group; CI, confidence interval (LOD  $\pm 1$ ); LOD, logarithm of odds; PVE, phenotypic variation explained; <sup>a</sup>, additive effect with negative sign indicates that the positive alleles come from parent Cadenza, while a positive sign indicates that positive alleles come from Avalon; SS, stem strength; SMS, stem material strength; SID, stem internode diameter; SIWW, stem internode wall width; RPS, root plate spread; PH, plant height; GY, grain yield.

Confirmation of these results comes from the literature. For example, QTL affecting stem strength and stem diameter on chromosome 3B were also located in CA9613 x H1488 DH population under Chinese growing conditions (Hai et al. 2005; Hai 2006). QTL affecting root plate spread were identified on chromosome 5B and have also been located in a Solstice x Xi19 DH population evaluated in the UK

(Berry et al. 2008; Berry and Berry 2015). All chromosomes with QTL affecting plant height identified for Avalon x Cadenza in this study can be matched with the chromosomes for plant height meta-QTL from four doubled haploid populations reported by (Griffiths et al. (2012).

## Conclusions

- A spring wheat ideotype susceptible to lodging only 1 in 25 years has been identified, with the following characteristics: grain yield  $6 \text{ t ha}^{-1}$ , plant height 0.7 m, 200 plant  $\text{m}^{-2}$ , 500 shoots  $\text{m}^{-2}$ , stem strength 268 N·mm, and root plate spread 51.1 mm.
- The cost of improving lodging resistance in terms of stem structural biomass indicated possible trade-offs with yield. Therefore, plant breeders must improve overall above-ground plant biomass in order to improve both grain yield and lodging resistance.
- The elite spring wheat evaluated in this study demonstrated broad genetic ranges for most of the key lodging resistance traits (with good heritability in all cases, except for root plate spread). However, not all the ideotype dimensions were achieved in the germplasm phenotyped in this study (e.g. stem strength and particularly root plate spread).
- Correlations among key lodging traits indicate that it is feasible to combine all the ideotype dimensions in a single genotype. Nevertheless, it will be difficult to optimize structural biomass in order to diminish trade-offs with yield (e.g. due to strong positive correlation between stem strength and wall width).
- Rapid methods to assess lodging can be developed in order to accelerate breeding for lodging resistance. In this regard, developing lodging resistance genetic markers seems to be the most sensible strategy. Major QTL on chromosomes 3B (*qSS3B*) and 5B (*qRPS5B*) may be developed into reliable markers.

## References

- Baker, C. J., Berry, P. M., Spink, J. H., Sylvester-Bradley, R., Griffin, J. M., Scott, R. K., and Clare, R. W., 1998. A method for the assessment of the risk of wheat lodging. Journal of theoretical biology 194(4): 587-603.
- Berry, P.M. and Berry, S.T., 2015. Understanding the genetic control of lodging-associated plant characters in winter wheat (*Triticum aestivum* L.). Euphytica 205(3): 671-689.
- Berry, P.M., Berry, S.T., and Spink, J.H., 2008. *Identification of genetic markers for lodging resistance in wheat*. Home-Grown Cereals Authority, Research Project No 441, pp. 14.
- Berry, P. M., Griffin, J. M., Sylvester-Bradley, R., Scott, R. K., Spink, J. H., Baker, C. J., and Clare, R. W., 2000. Controlling plant form through husbandry to minimise lodging in wheat. Field Crops Research 67(1): 59-81.
- Berry, P. M., Spink, J. H., Gay, A. P., and Craigon, J., 2003a. A comparison of root and stem lodging risks among winter wheat cultivars. The Journal of Agricultural Science 141(02): 191-202.
- Berry, P. M., Sterling, M., Baker, C. J., Spink, J., and Sparkes, D. L., 2003b. A calibrated model of wheat lodging compared with field measurements. Agricultural and Forest Meteorology 119(3): 167-180.
- Berry, P.M., Sterling, M., Spink, J.H., Baker, C.J., Sylvester-Bradley, R., Mooney, S.J., Tams, A.R. and Ennos, A.R., 2004. Understanding and reducing lodging in cereals. Advances in Agronomy 84:

217-271.

Berry, P.M., Sylvester-Bradley, R., and Berry, S., 2007. Ideotype design for lodging-resistant wheat. *Euphytica* 154:165–179.

Conway, G., 1997. *The Doubly Green Revolution. Food for all in the 21st century*. Penguin Group, London, England

Crook, M. J. and Ennos, A. R., 1994. Stem and root characteristics associated with lodging resistance in four winter wheat cultivars. *The Journal of Agricultural Science* 123(02): 167-174.

Fischer, R.A. and Edmeades, G.O., 2010. Breeding and Cereal Yield Progress. *Crop Science* 50 (Supplement\_1):S–85.

Flintham, J. E., Börner, A., Worland, A. J., and Gale, M. D., 1997. Optimizing wheat grain yield: effects of Rht (gibberellin-insensitive) dwarfing genes. *The Journal of Agricultural Science* 128(01): 11-25.

Griffiths, S., Simmonds, J., Leverington, M., Wang, Y., Fish, L., Sayers, L., Alibert, L., Orford, S., Wingen, L. and Snape, J., 2012. Meta-QTL analysis of the genetic control of crop height in elite European winter wheat germplasm. *Molecular Breeding* 29(1): 159-171.

Hai, L., 2006. *Analysis of genetic diversity among current spring wheat varieties and breeding for improved yield stability of wheat (Triticum aestivum L.)*. Ph. D. Thesis. Justus-Liebig-University Giessen, Giessen, pp. 100.

Hai, L., Guo, H., Xiao, S., Jiang, G., Zhang, X., Yan, C., Xin, Z. and Jia, J., 2005. Quantitative trait loci (QTL) of stem strength and related traits in a doubled-haploid population of wheat (*Triticum aestivum L.*). *Euphytica* 141(1-2): 1-9.

Piñera-Chavez, F.J., Berry, P.M., Foulkes, M.J., et al., 2016a. Avoiding lodging in irrigated spring wheat I. Stem and root structural requirements. *Field Crops Research*, Under review.

Piñera-Chavez, F.J., Berry, P.M., Foulkes, M.J., et al., 2016b. Avoiding lodging in irrigated spring wheat II. Genetic variation of stem and root structural properties. *Field Crops Research*, Under review.

Piñera-Chavez, F.J., Berry, P.M., Foulkes, M.J., et al., 2016c. Quantitative trait loci analysis for lodging resistance traits in wheat. Drafted.

Pinthus, M.J., 1973. Lodging in wheat, barley, and oats: the phenomenon, its causes, and preventive measures. *Advances in Agronomy* 25:209–263.

Sterling, M., Baker, C.J., Berry, P.M., and Wade, A., 2003. An experimental investigation of the lodging of wheat. *Agricultural and Forest Meteorology* 119:149–165.

## **Variation in developmental patterns among elite wheat lines and relationship with spike fertility**

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### **Abstract**

Developmental patterns play an important role in spike fertility and grain number, which is primarily determined during the stem elongation period (i.e. time between terminal spikelet phase and anthesis). It has been proposed that the length of the stem elongation phase may – to an extent – affect grain number; thus it would be beneficial to identify genetic variation for the duration of this phase in elite germplasm. Variation in developmental patterns was studied using 27 elite wheat lines in four experiments across three growing seasons. The results showed a larger than expected variation in time to anthesis. The length of the stem elongation phase was (i) only slightly related to that of the period from seedling emergence to terminal spikelet, and (ii) relevant for determining time to anthesis. Thus the phenological phases were largely independent and a certain time to anthesis may be reached with different combinations of its component phases. Yield components were largely explained by the fruiting efficiency of the elite lines: the relationships were strongly positive and strongly negative with grain number and with grain weight, respectively. Although fruiting efficiency showed only a positive trend with the duration of stem elongation, the relationship became strongly positive when using a frontier analysis. This suggests that the length of this phase may impose an upper threshold for fruiting efficiency and grain number, and that maximum values of fruiting efficiency may require a relatively long stem elongation phase.

### **Introduction**

Grain number in wheat is largely determined during the stem elongation phase (Fischer 1985; Slafer and Rawson 1994) when the juvenile spikes grow while floret developmental processes determine the survival of floret primordia (Kirby 1988). A longer phase of floret development may influence the likelihood of a floret primordium becoming a fertile floret and setting a grain (Miralles and Slafer 2007; Gonzalez-Navarro et al. 2015). This in turn may be due to two alternative, non-exclusive possible mechanisms: a longer period of stem elongation may (i) cause an increase in accumulated growth, thus improving resource availability for the juvenile spike where florets are developing (and then the increase in fertility would be associated with increases in spike dry weight at anthesis), or (ii) enable the successful production of a fertile floret from a floret primordia that would not normally progress to produce one (and thus the increase in fertility would be associated with increases in fruiting efficiency).

This study aimed to determine the degree of variation in patterns of phenological development within the elite germplasm of the CIMCOG population, ascertaining whether the differences were related to traits determining spike fertility within the population.

### **Methods**

Four field experiments were conducted under optimal conditions (fertilized and irrigated with prevention/control of diseases, pests, and weeds) at the Mexican Phenotyping Platform (MEXPLAT) near Ciudad Obregon, Mexico, during the 2010-11, 2011-12, and 2012-13 seasons. Each experiment was comprised of a subset of 27 genotypes from the CIMCOG panel (22 elite lines, 4 historic lines, and 1 durum wheat line). The original panel of 60 genotypes was only grown and measured in experiments 1 and 2. All four experiments were designed in randomized complete blocks with two replicates of experiment 2 and three replicates of experiments 1, 3, and 4.

Plots were periodically inspected after sowing to score the developmental phases of seedling emergence, anthesis, and maturity. Up to three times per week, one plant per plot was sampled to determine terminal spikelet phase using a binocular microscope (Carl Zeiss, Germany) to detect the developmental stage of the apex. At maturity, yield was determined by harvesting the plot (excluding the extreme 50 cm to avoid border effects) using standard protocols (Pask et al. 2012). Prior to harvesting, 100 fertile culms were sampled, dried, weighed, and threshed to allow calculation of yield components.

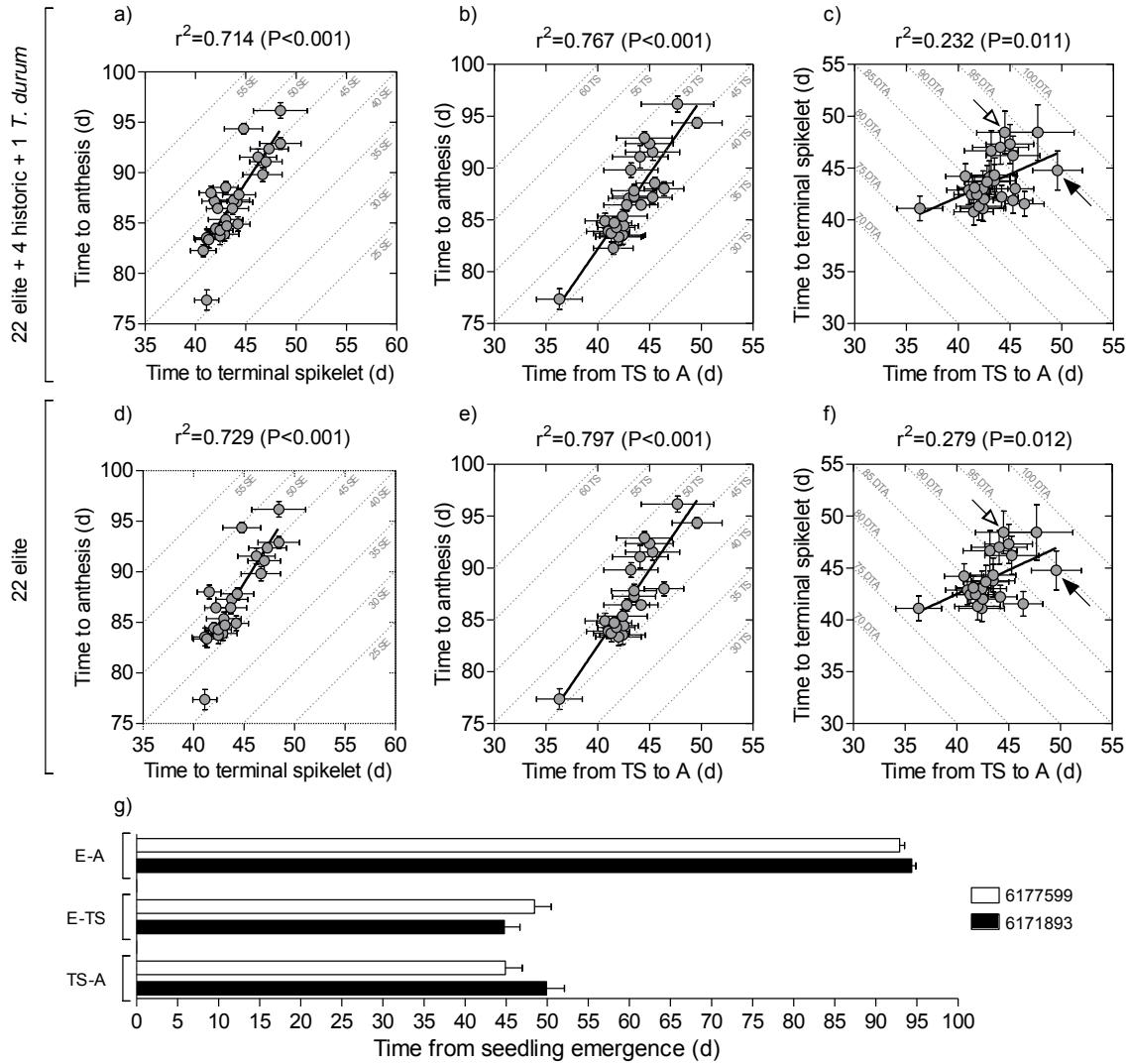
## Results/Discussion

In analysis of the 27 line subset, or only the 22 elite hexaploid lines (disregarding the four historic cultivars and *T. durum*), the variation found in phenology and the relationships between the durations of different phases were quite similar (both in terms of ranges explored and in degree of association between phases in the regressions) (Figure 1).

Time from seedling emergence to anthesis was similarly and highly correlated with the duration of its two component phases: time from emergence to terminal spikelet (Figure 1a,d) and time from terminal spikelet to anthesis (Figure 1b,e). However, the duration of the late reproductive phase was more relevant than that of the period from emergence to terminal spikelet in determining variation in total time to anthesis. This was not only because the coefficients of determination were slightly higher for the relationship with the duration of the late reproductive phase ( $r^2=0.77-0.80$ ) than with the time to terminal spikelet ( $r^2=0.71-0.73$ ), but also because the range of variation in the former (abscissa in Figure 1b,e) was noticeably larger than the latter (abscissa in Figure 1a,b).

More importantly, the length of the two phases constituting time to anthesis were largely independent; despite that they were significantly positively related, the proportion of the duration of time to terminal spikelet related to the duration of the late reproductive phase was only c. 25% (Figure 1c,f), which indicates that cultivars may combine contrasting durations of these two phases. This shows that even in a restricted range of well adapted elite lines, there may be a large number of possible combinations for reaching a particular aim regarding phenology. For instance, a particular duration of the stem elongation phase (any of the isolines in Figure 1a,c) could be combined with different durations of the phase to terminal spikelet; changes in time to anthesis may therefore be achieved by modifying exclusively the duration of phenological phases when leaf and spikelet primordia are being formed. The contrary is also true and a particular duration of the period to terminal spikelet (any of the isolines in Figure 1b,e) could be combined with different durations of the late reproductive phase. Changes in time to anthesis may therefore be achieved by modifying exclusively the duration of phenological phases when floret primordia are being formed. Or a similar time to anthesis (isolines in Figure 1c,e) may well be achieved by combining a relatively short phase to terminal spikelet and a relatively long stem elongation phase, or *vice-versa*. Pairs of genotypes with the same duration to anthesis but differing in how this developmental

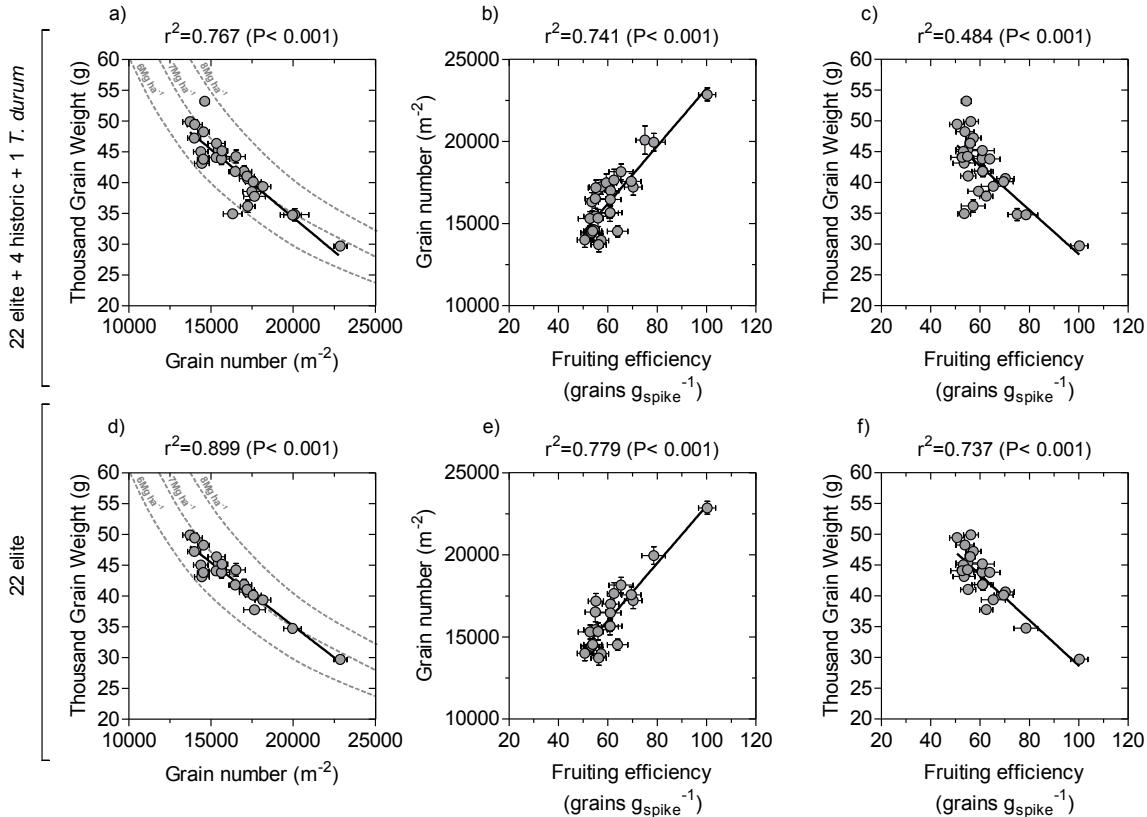
time is partitioned between phases occurring before or after the initiation of the terminal spikelet can easily be identified (Figure 1c,f).



**Figure 5. Relationships between time to anthesis and either time to terminal spikelet (a,d), or time from terminal spikelet to anthesis (b,e), and the relationship between these two component phases (c,f). All analyses considered the whole subset of 27 genotypes (top panels) or only the 22 elite hexaploid genotypes (bottom panels). Within each of the panels, isolines for the same duration of complementary phases were drawn. They were the stem elongation period (SE) in panels a and d; time to terminal spikelet (TS) in panels b and e; and time to anthesis (DTA) in panels c and f. Each data-point is the average across the four environments and segments stand for the standard error of the means (not seen when smaller than the size of the symbol). The arrows in panels c and f indicate genotypes 6177599 (open arrow head) and 6171893 (closed arrow head), illustrating a pair of genotypes with similar time to anthesis but different developmental partitioning.**

Essentially it could be seen that – within the CIMCOG panel – yield differences between genotypes were determined by particular combinations of grain number and grain weight of the different genotypes and

that (i) yield was not particularly strongly related to any of them and (ii) there was a clear negative relationship between these components (Figure 2a,d). This negative relationship was more acute when analyzing the 22 elite lines (Figure 2d) than when the whole subset was considered (Figure 2a). Yield within the 22 elite lines yield was maximized when cultivars presented intermediate values of grains per unit land area (when data-points crossed over the curves representing iso-yields; Figure 2d). Compared to lines with the lowest number of grains, these intermediate lines had smaller grains but not small enough to compensate for the increase in grain number. For genotypes with even greater grain number, the reduction in grain size more than compensated for the increase in grain number (Figure 2d).

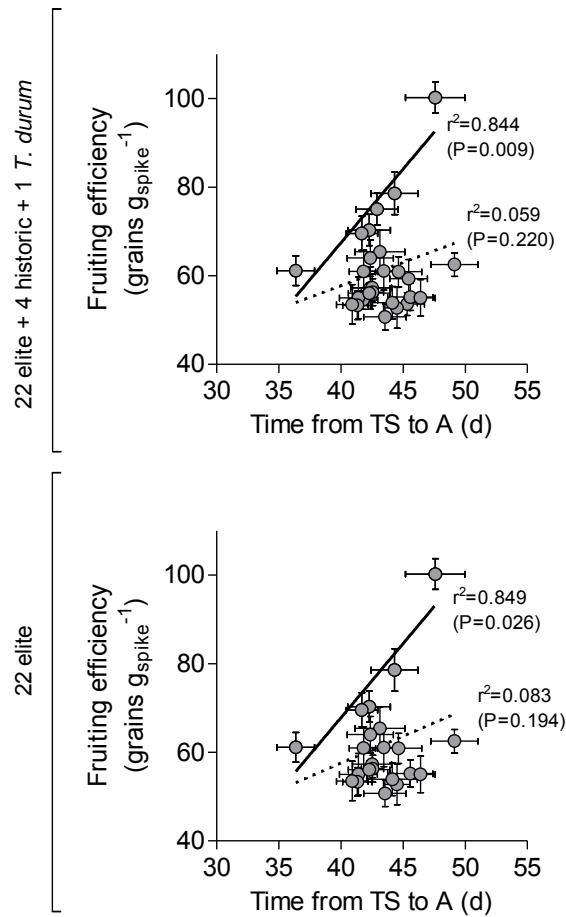


**Figure 6. Relationships between the two major yield components (a,d) and between each of them (grains per unit land area [b,e]; average weight of grains estimated as thousand grain weight [c,f]) and fruiting efficiency, considering the whole subset of 27 genotypes (top panels) or only the 22 elite hexaploid genotypes (bottom panels). Within panels a and d, isolines for the yields of 6, 7 and 8 Mg ha<sup>-1</sup> were drawn. Each datapoint is the average across the four environments and segments stand for the standard error of the means (not seen when smaller than the size of the symbol).**

Fruiting efficiency was the trait that best explained both yield components; the relationship was positive with grain number (Figure 2b,e) and negative with grain weight (Figure 2c,f), which would be the functional cause of the partial compensation between both yield components within this panel (Figure 2a,d). The relationships mentioned between yield components and fruiting efficiency held for both the whole subset of 27 genotypes (Figure 2b,c) and for the analysis restricted to the 22 elite hexaploid lines (Figure 2e,f), but the latter was more acute. Even though both fruiting efficiency and grain number had a highly significant negative correlation towards grain weight, this association was weaker for fruiting

efficiency. Similar results from González et al. (2014) provide some reassurance on using fruiting efficiency as a tool for the potential improvement of grain yield, though there are potential drawbacks (Slafer et al. 2015).

Analyzing the relationship between fruiting efficiency and the length of the late reproductive phase produced a positive, though not significant, trend (Figure 3). In part the relationship was only subtle because of the unexpected variation within the panel on time to anthesis. It would be likely that in another panel varying less in time to anthesis differences in duration of stem elongation phase may appear more evident. As the likely effect would be subtle it was not expected to find a highly significant degree of association between them. However, using a frontier analysis for the relationship there was a rather strong positive relationship both for both the whole subset and the 22 elite hexaploid genotypes (Figure 3), implying that the length of the late reproductive phase might set an upper threshold for fruiting efficiency.



**Figure 7. Relationships between fruiting efficiency and the duration of the late reproductive phase from terminal spikelet (TS) and anthesis (A) considering the whole subset of 27 genotypes (top panel) or only the 22 elite hexaploid genotypes (bottom panel). Each datapoint is the average across the four environments and segments stand for the standard error of the means. The solid line shows the linear regression using the French and Schultz (1984) frontier concept. The dashed line represents the linear regression for all datapoints.**

## Conclusions

- Variation in time to anthesis was much greater than normally expected in a panel of elite material.
- The length of the stem elongation phase was (i) only slightly related to that of the period from seedling emergence to terminal spikelet, and (ii) relevant for determining time to anthesis.
- Fruiting efficiency was critical for determining grain number but also induced a negative relationship between grain number and grain weight.
- Although fruiting efficiency was not strongly related to the duration of stem elongation, the length of this phase seemed to impose an upper threshold for fruiting efficiency and grain number.

## References

- Fischer, R.A., 1985. Number of kernels in wheat crops and the influence of solar radiation and temperature. *The Journal of Agricultural Science* 105: 447-461.
- French, R. and Schultz, J., 1984. Water use efficiency of wheat in a Mediterranean-type environment. I. The relation between yield, water use and climate. *Crop and Pasture Science* 35: 743-764.
- González, F.G., Miralles, D.J., Slafer, and G.A., 2011. Wheat floret survival as related to pre-anthesis spike growth. *Journal of Experimental Botany* 62: 4889-4901.
- González-Navarro, O.E., Griffiths, S., Molero, G., Reynolds, M.P., and Slafer, G.A., 2015. Dynamics of floret development determining differences in spike fertility in an elite population of wheat. *Field Crops Research* 172: 21-31.
- Kirby, E.J.M., 1988. Analysis of leaf, stem and ear growth in wheat from terminal spikelet stage to anthesis. *Field Crops Research* 18: 127-140.
- Miralles, D.J. and Slafer, G.A., 2007. Sink limitations to yield in wheat: how could it be reduced? *The Journal of Agricultural Science* 145: 139-149.
- Pask, A., Pietragalla, J., Mullan, D., and Reynolds, M., 2012. *Physiological breeding II: a field guide to wheat phenotyping*. CIMMYT.
- Slafer, G.A. and Rawson, H.M., 1994. Sensitivity of Wheat Phasic Development to Major Environmental Factors: a Re-Examination of Some Assumptions Made by Physiologists and Modellers. *Australian Journal of Plant Physiology* 21: 393-393.
- Slafer, G.A., Elia, M., Savin, R., García, G.A., Terrile, I.I., Ferrante, A., Miralles, D.J., and González, F.G., 2015. Fruiting efficiency: an alternative trait to further rise wheat yield. *Food and Energy Security* 4: 92-109.

# PHOTOSYNTHESIS

## Potential to include spike photosynthesis in breeding programs: Genetic variation for spike photosynthesis and identification of molecular markers

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### Abstract

Spike photosynthesis (SP) can contribute from 10 to almost 80% of final grain yield, and its importance has been recognized for more than 50 years. In fact, high SP has been suggested as an important trait in the conceptual model for yield potential, heat, and drought, yet breeding programs have made no systematic attempts to improve it. The main limitation has been the difficulty to measure SP and the lack of phenotyping tools available for field conditions. The development of new tools at CIMMYT (i.e. a spike illumination chamber), together with surrogates to estimate SP contribution to grain filling (based on photosynthesis inhibition treatments and the use of stable isotopes) has led to the identification of genetic variation for this trait and the selection of lines of interest into elite material. The differences among the genotypes in photosynthetic rate ranged from 8.4-23.4  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  and from 10-42% in spike contribution to grain weight. On average, SP contributed 30% to grain weight, even in elite material. The technique developed to measure SP contribution to yield was tested in a mapping population of 95 recombinant inbred lines (RIL) developed from a cross between *Triticum turgidum* L. ssp. *durum* and *T. dicoccum*, selected for their contrasting SP contribution to grain yield under yield potential and heat stress conditions for two years. Three QTLs on chromosomes 1A, 3B, and 5B were detected using composite interval mapping; among them, the QTL on chromosome 5B was detected under both yield potential and heat stress conditions and explained 21-41% variation in the trait. The QTLs detected on chromosomes in the present study are candidates for further validation. The development of these new tools, together with the novel identification of molecular markers associated with SP, open the possibility to genetically improve SP in breeding programs using marker assisted selection, and will also be valuable in gene discovery and screening of genetic resources.

### Introduction

It is predicted that future increases in yield potential will rely largely on improved radiation use efficiency through improved photosynthesis. Whereas emphasis has traditionally been given to the leaves, other photosynthetic organs (such as spikes) play an important role at least during specific stages of different crops. The importance of spike photosynthesis (SP) contribution to grain yield has been recognized for more than 50 years, with estimates from 10 to almost 80% of the assimilates deposited in grains, depending on cultivar, growing conditions, and even on the methodology used to estimate the contribution (Kriedemann 1966; Evans and Rawson 1970; Araus et al. 1993; Maydup et al. 2010; Molero et al. 2014; Sanchez-Bragado et al. 2014b). Even considering the minimum value reported, SP contribution is of great importance, particularly when final yield is considered in economic terms. However, to date, no cereal breeding program has made a systematic attempt to improve SP or to use this trait as a selection criterion, despite preliminary evidence for genetic variation for this trait in wheat (Abbad et al. 2004; Maydup et al. 2012; Molero et al. 2014). This is primarily because SP is difficult to

measure. The development of surrogates to estimate spike photosynthetic rate and SP contribution to grain yield is therefore of great importance and has been addressed recently by the authors (Molero et al. 2013, 2014). The ability to accurately measure these effects is a prerequisite to being able to combine their favorable expression in breeding platforms.

The development of new tools in CIMMYT (i.e. a spike illumination chamber), together with surrogates to estimate spike photosynthesis contribution to grain filling (based on photosynthesis inhibition treatments and the use of stable isotopes), has led to the identification of genetic variation for this trait and the selection of lines of interest into elite material with differences in photosynthetic rate (Molero et al. 2013, 2014; Sanchez-Bragado et al. 2014a,b). The estimation of spike photosynthesis contribution (SPC) through different surrogates resulted in acceptable values of coefficient of variation and repeatability values. In this sense, the use of textile to estimate SPC to grain yield seems a feasible approach to identify genetic variation for this trait under field conditions. This approach was used to identify genetic loci for spike photosynthesis in wheat from a mapping population of 95 recombinant inbred lines (RIL) developed from a cross of *Triticum turgidum* L. ssp. *durum* (Atil) × *T. dicoccum*, evaluated under yield potential and heat stress conditions during two years. To our knowledge, no attempt to identify molecular markers associated with spike photosynthesis has been performed to date. Therefore, this is the first approach in wheat (and in any other species) aiming to identify QTL associated with spike contribution to grain filling.

## Methods

Four different panels were evaluated during two years at CENEB in the Yaqui Valley, northwest México: PADs POP (parental lines of mapping populations), CIMCOG I (CIMMYT Core Germplasm I, elite lines), CIMCOG II (CIMMYT Core Germplasm II, elite lines), and RILs Atil/dicoccum (mapping population of 95 recombinant inbred lines (RIL developed from a cross of *Triticum turgidum* L. ssp. *durum* (Atil) × *T. dicoccum*). Spike photosynthetic rate (expressed as spike area or dry matter basis) and/or SPC to grain yield (estimated using photosynthesis inhibition treatments, see below) were evaluated during two years in each of the panels, as described in Table 1.

**Table 1. Number of lines evaluated for SP in each panel under yield potential (YP) or heat stress conditions during two years in northwest Mexico. SPC was measured using the light inhibition treatments. Spike photosynthetic rate (SPR) was measured with the spike illumination chamber connected to Li-6400XT. Env indicates the environment of evaluation, n indicates the total number of lines in each panel, and the numbers in SPR and SPC indicates the number of genotypes evaluated.**

	Years*	Env	n	SPR	SPC
PADs POP	2012 & 2013	YP	12	12	12
CIMCOG I-Subset	2012 & 2013	YP	30	15	30
CIMCOG II	2014 & 2015	YP	60	16	60
RILs Atil/dicoccum-YP	2012 & 2014	YP	95	2 (parents)	95
RILs Atil/dicoccum-Heat	2013 & 2014	Heat	95	-	95

\*2012, 2013, 2014 and 2015 refers to 2011-2012, 2012-2013, 2013-2014 and 2014-2015 growing cycles respectively

Spike photosynthetic rates (SPR) were measured using a specially designed chamber connected to LI-6400XT, as described in Molero et al. (2013). An external light source was placed around the chamber during the measurement, achieving saturating PPFD of approximately  $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$  inside the chamber. Once the measurements were finished, the spikes were harvested to measure the projected area with a leaf area meter (LI3050A/4; LICOR, Lincoln, NE).

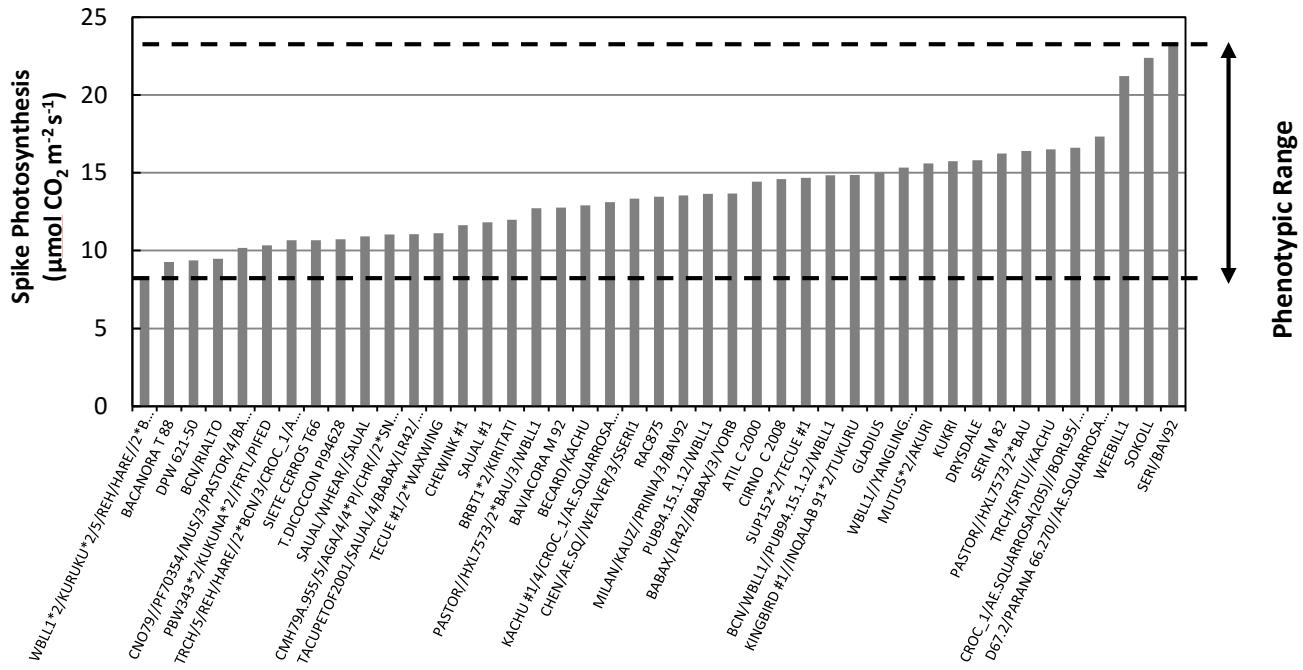
SPC was estimated by applying light inhibition treatments using a textile shading the spikes 10 days after anthesis for yield potential experiments and 10 days after heading for heat stress experiments. At physiological maturity, treated and control plants were harvested and spike dry weight, number of grains per spike, and thousand grain weight were measured. SP contribution to grain filling was calculated as described in Molero et al. (2014).

A population of 95 RILs (ATIL/Dicoccum) was developed from a cross between durum wheat (*Triticum turgidum* L. ssp. *durum*, ATIL C 2000) and *T. Dicoccum* (PI94628). The two parents contrasted in terms of their relative contribution of SP to grain filling (Molero et al. 2013, 2014). In addition, Atil is a high yielding drought tolerant durum and *T. dicoccum* has acceptable agronomic type and good performance under hot irrigated conditions. SPC was evaluated in the population using spike photosynthesis inhibition treatments during two years under irrigated conditions and heat stress. This phenotypic information was used to identify molecular markers associated with SP.

## Results/Discussion

The direct methodology to measure SPR using a hand-made chamber connected to a gas exchange instrument (LI-6400XT) was successfully applied under field conditions in previous studies (Molero et al. 2013, 2014). In this study, spike photosynthetic CO<sub>2</sub> assimilation rate (SPR) in the 43 lines evaluated varied from  $8.4-23.4 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (Fig. 1), indicating a large phenotypic variation among genotypes, even larger than the phenotypic variation observed for the flag leaf under irrigated conditions (Driever et al. 2014).

Some of the observed differences could be associated with differences at the time when the measurements were performed as SPR were performed after anthesis with some days of variation. This is consistent with a previous study conducted under controlled conditions, where SP contributed a greater proportion to total grain requirements during early growth than during the period of most rapid grain growth in grain filling (Evans and Rawson 1970). As measurements were performed after anthesis, differences among genotypes can also be associated with grain respiration rates (Araus et al. 1993) or with differences in CO<sub>2</sub> refixation rates (Bort et al. 1996), even though to date, no study has evaluated genotypic differences associated with these traits. Even the influence of these factors to obtain a net value of SPR, difference among genotypes were noticeable and supported by additional studies using stable isotopes (Sanchez-Bragado et al. 2014a,b).



**Figure 1. Spike Photosynthetic rate (SPR) screening for 43 wheat lines conducted after anthesis. Values are the mean of two replicates, each one is the average of at least two measurements per plot and the values are the adjusted means from a combined analysis. The genotypes are the lines from the four different panels, as described in Table 1.**

To our knowledge, these are the first studies to screen a large set of genotypes for SPR, despite preliminary evidence for genetic variation for this trait (Abbad et al. 2004). The difficulty in measuring SPR is linked to a number of factors, including the complex and genetically diverse geometry of spikes, which makes standardization of photosynthetic measurement a challenge. Another more serious issue is the fact that the metabolism of the spike itself, together with the respiration of growing kernels associated with the conversion of carbon assimilates to starch in the endosperm, confounds any straightforward measurement of carbon fluxes that would indicate a net photosynthetic rate. The development of surrogates to estimate SP contribution to grain filling is therefore of great importance and has been recently addressed by the authors (Molero et al. 2013; Sanchez-Bragado et al. 2014b). These surrogates are based on the use of photosynthesis inhibition treatments (by masking the spikes with a specially designed textile) and the use of stable isotopes ( $\delta^{13}\text{C}$  of water soluble carbohydrates in specific organs compared with  $\delta^{13}\text{C}$  of grains). In this study, the use of photosynthesis inhibition treatments enabled the identification of large genetic variations for SPC to grain yield, with values ranging from 10-45% (Table 2). On average, SP contributed 30% to the assimilates deposited in the grains, and the values were similar in the two panels of elite material studied (CIMCOG I and CIMCOG II), whose breeders would realistically use in their strategic crosses aiming to further raising yield potential.

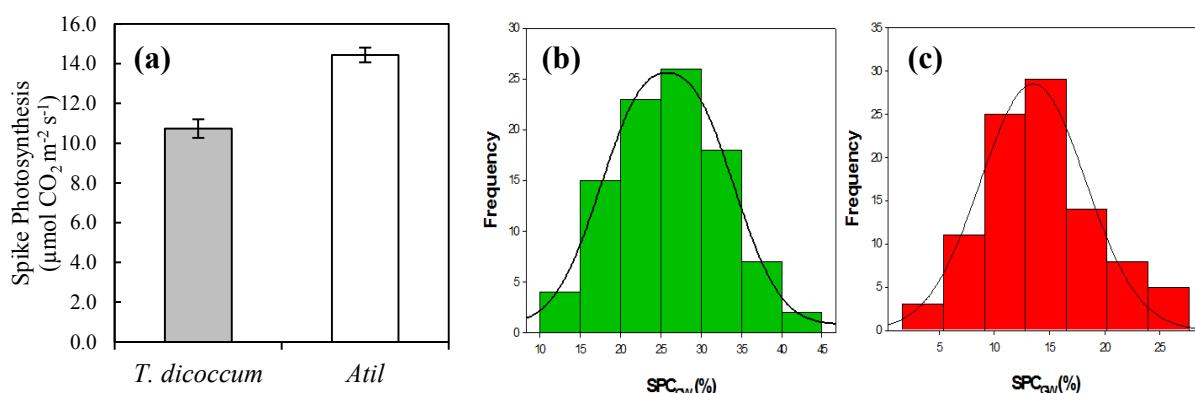
**Table 2. Combined analysis of SPC to grain weight (%) evaluated during two growing cycles on MEXPLAT in parental lines population (PADS POP, n=12 genotypes), CIMCOG I-Subset (n=30 genotypes), CIMCOG II (n=60 genotypes), and the RILs ATIL/Dicoccum mapping population (n=95 genotypes).**

	Years*	n	SPC Mean	SPC Min	SPC Max
PADs POP	2012 & 2013	12	34.6	25.2	41.2
CIMCOG I-Subset	2012 & 2013	30	28.2	16.1	41.6
CIMCOG II	2014 & 2015	60	28.5	12	45.2
RILs Atil/dicoccum	2012 & 2014	95	26	10.1	41.8

\*2012, 2013, 2014, and 2015 refers to 2011-2012, 2012-2013, 2013-2014, and 2014-2015 growing cycles, respectively

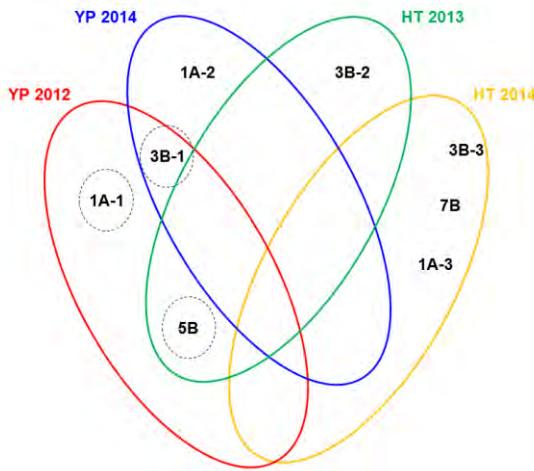
Together with the important contribution to grain weight, wheat spikes are the first organ intercepting a significant proportion of incident radiation; values typically range from 25-40%, depending on spike architecture (López-Castañeda et al. 2015), for almost half the crop's duration, indicating highly significant genetic effects in the potential of spikes to contribute to total canopy photosynthesis (Lopez-Castañeda et al., 2014). That given, any strategy to improve radiation use efficiency of wheat should also consider genetic variation for SP. These results show a high phenotypic range for SPR (Fig. 1) and SPC (Table 2), making this trait attractive for breeding programs. This is also supported by the relatively high heritability estimates for SPR and SPC from previous studies (Molero et al. 2014) suggesting that sustainable genetic gains can be achieved by including SP as a selection trait.

Identifying the QTLs controlling SP is an important step towards molecular breeding for yield improvement. In this study, a wheat RIL population developed from a cross between a durum wheat line *Atil C 2000* (*Triticum turgidum* L. ssp. *durum*) and an emmer wheat line *PI94628* (*T. dicoccum*) was evaluated using SP inhibition treatments. The parents were contrasting in SPR (Fig. 2a) and SPC to grain yield, and this effect was consistent under different environmental conditions. SPC was evaluated during two years under yield potential and heat conditions (Table 2) showing a large variation among lines (Figs. 2b,c).



**Figure 2. SPR of parental lines (*T. dicoccum* and the durum *Atil*) measured with Li-COR 6400XT during grain filling and express according area (a), and SPC of the 95 RILs evaluated under yield potential (b) and heat stress conditions (c).**

Three QTL on chromosomes 1A, 3B, and 5B were detected using composite interval mapping and are candidates for further validation. Among them, the QTL on chromosome 5B was detected under both yield potential and heat stress conditions and explained 21-41% variation in the trait (Fig. 3). The development of these new tools, together with the novel identification of molecular markers associated with SP, open the possibility to genetically improve SP in breeding programs using marker assisted selection, and will also be valuable in gene discovery and screening of genetic resources.



**Figure 3. Venn diagram illustrating different QTL detected for SPC to grain yield under yield potential and heat stress conditions based on individual environment QTL analysis. More than one QTL detected on the same chromosome are shown with different numbers as suffix. Dotted circles indicate QTLs detected in the combined analysis of YP environments. Combined analysis of the four environments resulted in only one QTL (1A-1) but on individual analysis it was present only in YP 2012.**

## Conclusions

- This study highlights the importance of SP to final grain yield, indicating phenotypic variation for SPR and SPC to grain yield.
- On average, 30% of the assimilated deposited in the grains is associated with SP in elite lines.
- Three QTL were detected on chromosomes 1A, 3B, and 5B. The QTL on chromosome 5B was detected under both yield potential and heat stress conditions and explained 21-41% of variation in the trait.

## References

- Abbad, H., Jaafari, S.E., Bort, J., and Araus, J.L., 2004. Comparison of flag leaf and ear photosynthesis with biomass and grain yield of durum wheat under various water conditions and genotypes. *Agronomie* 24: 19–28.
- Araus, J.L., Brown, H., Febrero, A., Bort, J., and Serret, M., 1993. Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory CO<sub>2</sub> to differences in grain mass in durum wheat. *Plant, Cell & Environment* 16: 383–392.
- Bort, J., Brown, H., and Araus, J.L., 1996. Refixation of respiratory CO<sub>2</sub> in the ears of C3 cereals. *Journal of Experimental Botany* 47: 1567–1575.

- Driever, S.M., Lawson, T., Andralojc, P.J., Raines, C.A., and Parry, M.A.J., 2014. Natural variation in photosynthetic capacity, growth, and yield in 64 field-grown wheat genotypes. *Journal of Experimental Botany* 65: 4959–73.
- Evans, L. and Rawson, H., 1970. Photosynthesis and respiration by the flag leaf and components of the ear during grain development in wheat. *Australian Journal of Biological Sciences* 23: 245–254.
- Kriedemann, P., 1966. The photosynthetic activity of the wheat ear. *Annals of Botany* 30: 349–363.
- Maydup, M.L., Antonietta, M., Guiamet, J.J., Graciano, C., López, J.R., and Tambussi, E.A., 2010. The contribution of ear photosynthesis to grain filling in bread wheat (*Triticum aestivum* L.). *Field Crops Research* 119: 48–58.
- Maydup, M.L., Antonietta, M., Guiamet, J.J., and Tambussi, E.A., 2012. The contribution of green parts of the ear to grain filling in old and modern cultivars of bread wheat (*Triticum aestivum* L.): Evidence for genetic gains over the past century. *Field Crops Research* 134: 208–215.
- Molero, G., Sanchez-Bragado, R., Araus, J.L., and Reynolds, M.P., 2013. *Phenotypic selection for spike photosynthesis*. In: Reynolds, M.P. et al. (Eds.), Proceedings of the 3rd International Workshop of the Wheat Yield Consortium. CENEB, CIMMYT, Cd. Obregón, Sonora, Mexico: CIMMYT, 9–11.
- Molero, G., Sukumaran, S., and Reynolds, M.P. 2014. *Spike photosynthesis contribution to grain yield and identification of molecular markers: A potential trait for breeding programs?* In: Reynolds, M.P. et al. (Eds.), Proceedings of the 4th International Workshop of the Wheat Yield Consortium. CENEB, CIMMYT, Cd. Obregón, Sonora, Mexico: CIMMYT, 175–186.
- Parry, M.A.J., Reynolds, M.P., Salvucci, M.E., Raines, C., Andralojc, P.J., Zhu, X-G., Price, G.D., Condon, A.G., and Furbank, R.T., 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. *Journal of Experimental Botany* 62: 453–67.
- Sanchez-Bragado, R., Elazab, A., Zhou, B., Serret, M.D., Bort, J., Nieto-Taladriz, M.T., and Araus, J.L., 2014a. Contribution of the ear and the flag leaf to grain filling in durum wheat inferred from the carbon isotope signature: Genotypic and growing conditions effects. *Journal of Integrative Plant Biology* 56: 444–54.
- Sanchez-Bragado, R., Molero, G., Reynolds, M.P., and Araus, J.L. 2014b. Relative contribution of shoot and ear photosynthesis to grain filling in wheat under good agronomical conditions assessed by differential organ  $\delta^{13}\text{C}$ . *Journal of Experimental Botany* 65: 5401–13.

# Predicting physiological traits in wheat landraces using hyperspectral reflectance

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## Abstract

This study investigates if the models predicting  $V_{cmax}$ ,  $V_{cmax25}$ ,  $J$ ,  $N_{area}$ , SPAD, LMA, and  $V_{cmax25}/N_{area}$  with hyperspectral reflectance and partial least square regression (PLSR) can be used in wheat landraces other than those used to train the model. The set of genotypes consisted of 230 wheat landraces and 5 elite wheat genotypes. Reflectance and SPAD were measured in the first survey. From this, 21 wheat landraces and 2 elite wheat genotypes were measured for reflectance, SPAD,  $J_{800}$ , LMA, and  $N_{area}$  in a second survey. Previously constructed models using hyperspectral reflectance were able to make useful predictions for  $J_{800}$  and more accurate predictions for SPAD, LMA, and  $N_{area}$ .

## Introduction

In previous experiments using elite wheat germplasm (data not shown), hyperspectral reflectance was calibrated to predict Rubisco activity ( $V_{cmax25}$ ), electron transport rate ( $J$ ), leaf nitrogen ( $N_{area}$ ), leaf chlorophyll (SPAD), leaf dry mass per area (LMA), and  $V_{cmax25}/N_{area}$ . Other studies have successfully predicted photosynthetic parameters in tropical trees, aspen, cotton, and soybean (Doughty et al. 2011; Serbin et al. 2012; Ainsworth et al. 2014), and nitrogen content and LMA in wheat (Ecarnot et al. 2013), and thus we expect that the model generated previously by our team using elite wheat germplasm can be applied to diverse wheat genotypes measured in the field. In this study, we explore whether the models generated from elite wheat germplasm work with wheat landraces that did not contribute to the development of the model. We also trialed using hyperspectral reflectance to rapidly screen plants in order to select a subset of wheat genotypes for more detailed gas exchange analysis.

## Methods

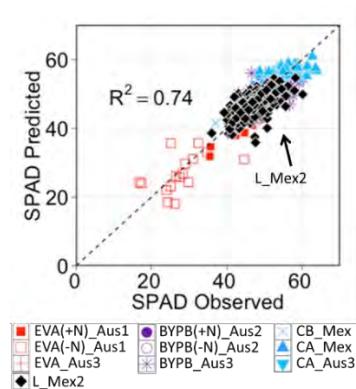
Wheat landraces (**L**) were selected from CIMMYT's Spike Germplasm Bank and 230 wheat landraces plus five elite wheat genotypes (235 wheat genotypes in total) were grown in the field at the Norman E. Borlaug Experimental Center (CENEB), located in the Yaqui Valley, Sonora, Mexico over a winter-spring cycle. Measurements were done in two main steps:

- 1) **Survey: L** flag leaves were measured for reflectance and SPAD on all plots including repetitions and checks. **L** plants ( $n = 270$ ) were 110 to 111 DAE, which for 22 genotypes was the period from 7 days before anthesis to anthesis, with the remainder of the genotypes being from 1 to 36 days after anthesis.
- 2) **Second measurement (S):** After the survey, 23 L genotypes were selected and measured a second time (details of the selection is described under results). Measurements were performed on different plants of the same genotype. In the second measurement, **LS** was 117 DAE for reflectance, SPAD and photosynthetic rate at 400 and 800 inlet  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ . **LS** genotypes were also sampled to

calculate leaf mass area (LMA) and leaf nitrogen content per unit leaf area ( $N_{\text{area}}$ ). The additional ‘S’ to L refers to the second time of measurements.

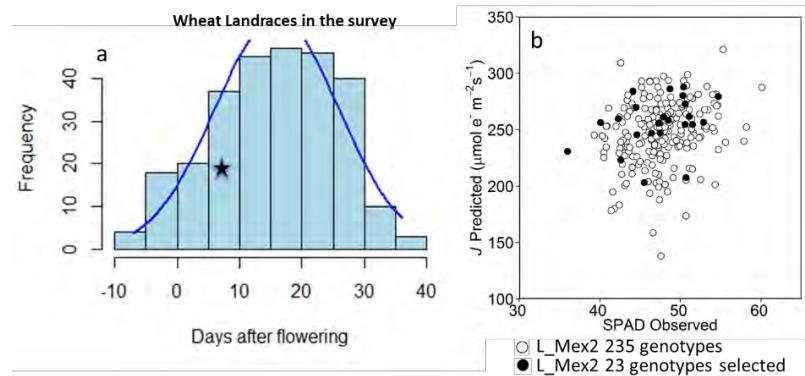
## Results/Discussion

First, 235 **L\_Mex2** wheat genotypes were screened in a survey for reflectance and SPAD. Observed SPAD values fell within the range previously observed for elite genotypes (Fig. 1). SPAD could be well predicted from hyperspectral reflectance with predictions falling within the calibration range. The correlation including **L\_Mex2** was  $R^2 = 0.74$ .



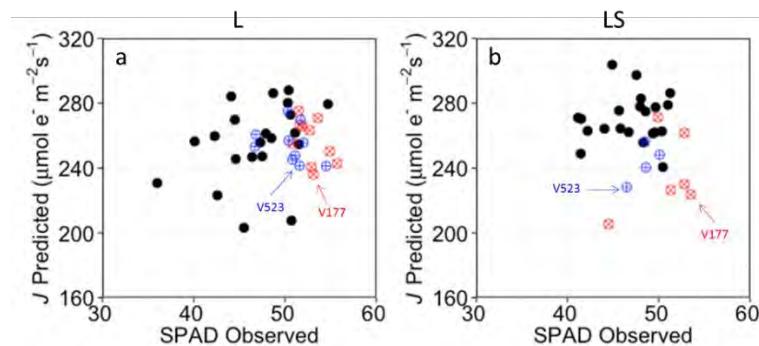
**Figure 1 Comparison of SPAD predicted from reflectance using the model developed previously and actual SPAD measurements for the wheat landraces set (L\_Mex2).**  $R^2$  includes all the points of the plot. The dashed line represents the 1:1.

When the reflectance survey of 235 wheat landraces was conducted, their phenological development ranged from 7 days before to 36 days after flowering (Fig. 2a). Consequently, plants between six and nine days after flowering were selected because the spike reaches its maximum dry weight around seven days after anthesis, grain weight is insignificant, and water soluble carbohydrate reserves in stem are at their peak (Pask 2012). The SPAD reading taken during the survey was compared to the  $J$  predicted from reflectance model. Twenty-nine wheat genotypes between six and nine days after anthesis were dispersed within the whole range of measurements. From these, 21 wheat landraces and two elite wheats (checks) were chosen for more detailed measurements (Fig. 2b).



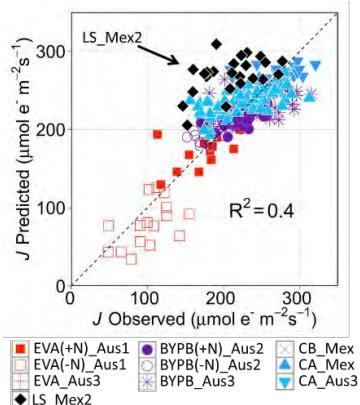
**Figure 2.** a) Histogram of the days after flowering (DAF) when the landraces were surveyed for reflectance, the star marks 5-10 DAF. b) The rate of electron transport,  $J$  predicted from the model derived previously with elite wheat shown in relation to observed SPAD. A subset of 23 wheat genotypes were selected based on their flowering time (6-9 DAF) from 235 wheat genotypes measured in the first survey.

The plot of predicted  $J$  versus observed SPAD for the subset of **L\_Mex2** genotypes measured in the survey and again six days after the survey (**LS\_Mex2**) are shown in Figure 3. In general, SPAD and  $J$  increased in the second measurement (compared to the first) for landrace wheats, while the elite genotypes maintained SPAD values and reduced  $J$  (V523 and V177). The changes between the first and the second measurement show that trait values for genotypes can vary a lot in a few days. The spread in values for each check genotype indicates the number of replicates needed to achieve a given precision. It is also interesting that the correlation between  $J$  and SPAD is not high, indicating that these traits could vary independently of one another.

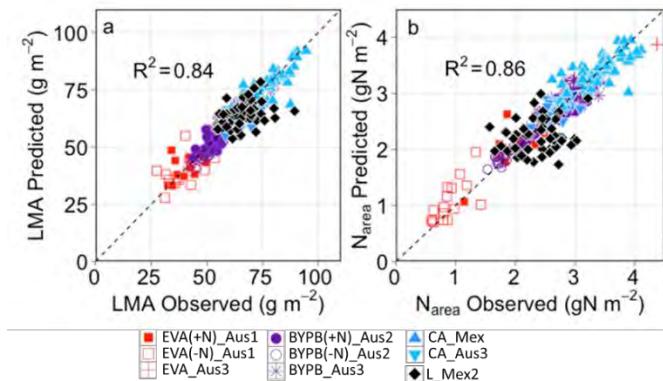


**Figure 3.** Relationship between predicted  $J$  and SPAD for a subset of 21 wheat landraces and 2 elite genotypes: a) measured in the first survey (L), each dot is one repetition; genotypes V177 and V523 are elite genotypes with 10 repetitions each; b) six days after the survey (LS), black dots represent the mean of two repetitions while 6 and 4 repetitions are shown for V177 and V523, respectively.

Values of  $J$  observed for landraces fell within the range previously observed for fertilized elite genotypes (Fig. 4). There was a tendency for predicted  $J$  to exceed observed  $J$ . Excellent predictions of LMA and  $N_{area}$  for landraces were made from reflectance models developed from elite genotypes (Fig. 5).



**Figure 4.**  $J$  predicted from reflectance measurements as function of  $J$  observed, which was calculated from gas exchange measurements. Black diamonds represent LS\_Mex2 superimposed over the data used to validate the model.



**Figure 5.** Validation of reflectance models using landrace measurements: a) LMA; b) N<sub>area</sub> (21 genotypes with two repetitions, check V177 with six repetitions and V523 with four repetitions for LS). There were 295 observations in total for LMA and N<sub>area</sub>.

Models derived from a combination of several sets of wheat genotypes were tested on genotypes that had not been used to develop the original model in the partial least square regression. Results revealed that it is possible to screen for  $J$ , LMA, N<sub>area</sub>, and SPAD using reflectance, and we expect that the technique can also be used for  $V_{cmax25}$ . The best predictions were obtained for LMA and N<sub>area</sub>. Interestingly, models derived from aspen and cotton were able to predict leaf nitrogen concentration and LMA from reflectance measured on soybean (Ainsworth et al. 2014). It would be useful to compare the models derived here for wheat with those derived from aspen and cotton.

It is still difficult to define the size and composition of the germplasm training set required to build a robust model with 2000 wavelengths while balancing good prediction against ‘over fitting’. In this study, 282 measurements were used to build models to predict LMA, N<sub>area</sub>, and SPAD, and 262 measurements to predict  $V_{cmax25}$ ,  $J$ , and  $V_{cmax25}/N_{area}$ . These models performed well at predicting traits in 223 novel elite wheat genotypes and 235 novel wheat landraces. In another experiment with wheat, Ecarnot et al. (2013) used reflectance to predict LMA and N<sub>area</sub> using a calibration obtained from a diverse collection of wheats measured under multiple conditions and environments. In contrast, it seems that the calibration for aspen

required fewer observations (Serbin et al. 2012). In this study, a strong driver of variation was environmental treatment rather than genetic variation thus further analysis comparing different sizes of training sets is required to construct the models.

In the second measurement, gas exchange was used to validate predictions of  $J$ . The correlation between observed  $J$  and predicted  $J$  in **Mex2** was relatively poor with  $R^2 = 0.4\text{--}0.5$  (Fig. 4). During the second measurement, mean leaf temperature was 32 °C and many plants showed low stomatal conductance (average of 0.23 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), suggesting that plants were stressed on the day of measurements. Both of these factors could influence calculations of  $J$  with gas exchange. There is likely to be genetic variation for mesophyll conductance ( $g_m$ ) between elite wheats and wheat landraces, as found by Jahan et al. (2014), or between leaves with different photosynthetic capacity (von Caemmerer and Evans 1991). We assumed a constant  $g_m$  of 0.55 mol m<sup>-2</sup> s<sup>-1</sup> bar<sup>-1</sup> for all wheat genotypes in these surveys, and we anticipate that the error introduced by this assumption would be small because the measurements were made at high ambient CO<sub>2</sub> concentrations (800 ppm). While  $J$  could also be estimated from chlorophyll fluorescence, the need to surround the leaf with a high CO<sub>2</sub> concentration makes each measurement more complicated and time consuming. Wheat landraces are a source of diversity that needs to be explored more intensively in the future.

We are currently satisfied with the calibration of the models, which provide adequate estimates for six different traits from a single hyperspectral reflectance measurement. Other instruments target only one trait, such as SPAD for chlorophylls (Konica Minolta 2009–2013), or FluorPen to estimate electron transport rate from chlorophyll fluorescence. Selecting the best method for screening genotypes for photosynthetic traits will depend on the objectives of the experiment, and whether the data is used simply to rank genotypes or to provide more precise quantitative data.

## Conclusions

- Hyperspectral reflectance modeling of wheat leaves accurately predicted  $J$ , LMA, N<sub>area</sub>, and SPAD in previously uncharacterized wheat germplasm.
- This method predicts multiple variables related to photosynthetic performance with one rapid measurement, giving high accuracy for SPAD, LMA, and N<sub>area</sub>.
- This method is faster and cheaper than sending samples for biochemical analysis in laboratories, and shows promise for future application in crop breeding, agronomy, and crop physiology.

## References

- Ainsworth, E.A., Serbin, S.P., Skoneczka, J.A., and Townsend, P.A., 2014. Using leaf optical properties to detect ozone effects on foliar biochemistry. *Photosynthesis Research* 119: 65–76.
- Doughty, C.E., Asner, G.P., and Martin, R.E., 2011. Predicting tropical plant physiology from leaf and canopy spectroscopy. *Oecologia* 165: 289–299.
- Ecarnot, M., Compan, F., and Roumet, P., 2013. Assessing leaf nitrogen content and leaf mass per unit area of wheat in the field throughout plant cycle with a portable spectrometer. *Field Crops Research* 140: 44–50.
- Konica Minolta I. 2009–2013. Chlorophyl Meter SPAD-502Plus. Instruction Manual.

- Pask, A.J.D., 2012. Determining key developmental stages. In: Pask, A.J.D., Pietragalla, J., Mullan, D.M., and Reynolds, M.P. (Eds.), *Physiological Breeding II: A Field Guide to Wheat Phenotyping*. Mexico City: CIMMYT, pp. 72-77.
- Serbin, S.P., Dillaway, D.N., Kruger, E.L., and Townsend, P.A., 2012. Leaf optical properties reflect variation in photosynthetic metabolism and its sensitivity to temperature. *Journal of Experimental Botany* 63: 489-502.
- von Caemmerer, S. and Evans, J.R., 1991. Determination of the average partial-pressure of CO<sub>2</sub> in chloroplasts from leaves of several C<sub>3</sub> plants. *Australian Journal of Plant Physiology* 18: 287-305.

# **Partitioning photosynthetic limitation and yield diversity in wheat cultivars grown at CIMMYT**

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## **Abstract**

Sustainable intensification of agriculture considers the increasing of yields for the most important staple crops as a central point in the strategy to alleviate food security issues. However, in many parts of the world there has been a plateau in yields, which shows that it is necessary to develop new agronomical and technological strategies to fulfill future demands. Carbon assimilation is the main determinant of crop biomass and a key trait for improving wheat genetic yield potential. CO<sub>2</sub> diffusion from the atmosphere to the chloroplast stroma can be divided into two steps, stomatal conductance ( $g_s$ ) describes the gas phase pathway for CO<sub>2</sub> from the atmosphere to the intercellular airspace, while mesophyll conductance ( $g_m$ ) refers to the intracellular pathway to Rubisco. In this work, 13 wheat genotypes were grown in Yaqui Valley, northwest Mexico to determine yield performance, photosynthetic performance (including  $g_s$  and  $g_m$ ) and leaf anatomy traits. It was shown that these genotypes are very diverse for most of the traits analyzed. Based on yield performance, two groups were identified, either high yielding genotypes or low yielding genotypes. Data shows that yield was positively correlated with A,  $g_s$  and  $g_m$ , but negative correlations were observed between yield and SLA and between  $g_m$  and LAI. All these results suggest that leaf anatomy plays a central role in determining the photosynthetic performance of genotypes and contributes to final grain yield.

## **Introduction**

The physiological breeding of crops is used to strategically combine traits that result in productivity boosting. Particularly, traits affecting grain production and biomass increase, which necessarily relate to photosynthetic efficiency, should also be included in breeding platforms (Reynolds et al. 2012). The efficiency of carbon gain during photosynthesis determines the relative production of biomass. Stomatal conductance ( $g_s$ ) and the mesophyll conductance ( $g_m$ ) describe the travel of CO<sub>2</sub> from the atmosphere, via substomatal cavity, to the chloroplast, where carboxylation takes place. The mesophyll pathway is formed by a series of barriers to CO<sub>2</sub> diffusion: air, cell wall, lipid membranes, cytoplasm and stroma. The differences in physical characteristics and in the size of these barriers among leaves are responsible for the large variation in  $g_m$  among species, making this parameter species- and variety-specific (Flexas et al. 2012).

A slow diffusion of CO<sub>2</sub> to the site of carboxylation in chloroplasts can significantly limit photosynthesis (Flexas et al. 2007). Conversely, a higher  $g_m$  value will result in increased CO<sub>2</sub> levels near Rubisco and increased photosynthesis. Zhu et al. (2010) simulated a doubled  $g_m$  for a typical C<sub>3</sub> crop, which resulted in an increase of photosynthetic capacity of nearly 20%, and the water use efficiency similarly increased. The goal of this project is to identify variations in wheat grain yield, photosynthesis performance

(including  $g_s$  and  $g_m$ ) and leaf anatomy of 13 wheat genotypes and establish the correlations between these traits and final grain yield.

## Methods

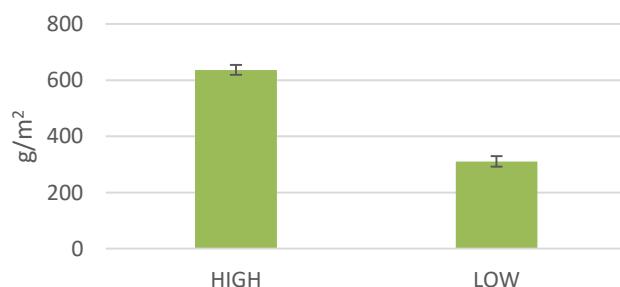
Thirteen contrasting genotypes from CIMMYT collections, were grown during 2013-2014 season in the CENEB experimental station in Yaqui Valley, NW-Mexico, and measurements made at early booting stage (Zadoks 4.3). Leaf photosynthesis, stomatal conductance, and leaf fluorescence measurements were made simultaneously using a portable leaf gas exchange and fluorescence system (LI-6400XT; LI-COR, Lincoln, NE, USA) as described by Driever et al. (2014). CO<sub>2</sub> response curves were measured at a leaf temperature of 25°C and a saturating quantum flux density of 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The CO<sub>2</sub> concentration in the cuvette (Ca) was lowered stepwise from 400 to 100  $\mu\text{mol CO}_2 \text{ mol air}^{-1}$ , then raised again to 400  $\mu\text{mol CO}_2 \text{ mol air}^{-1}$  and then raised stepwise to 1200  $\mu\text{mol CO}_2 \text{ mol air}^{-1}$ . After a first CO<sub>2</sub> response curve at ambient oxygen concentration (21%), a tank containing 2% of oxygen was connected to the air inlet of the LI-COR system and a second CO<sub>2</sub> response curve was measured.

The CO<sub>2</sub> concentration in the substomatal cavity (Ci), minus the CO<sub>2</sub> concentration at the sites of carboxylation in the chloroplasts (Cc), is used to calculate  $g_m$ . Cc can be estimated by measuring the electron transport rate (J). The more extensively used method to calculate J uses data produced from chlorophyll fluorescence measurements. In this study, an approach developed by Bellasio and Griffiths (2013) was followed that derives J from gas-exchange measurements at low and ambient O<sub>2</sub> concentrations, combined with fluorescence measurements made simultaneously. J is calibrated by analyzing response curves of photosynthetic rates to CO<sub>2</sub> (A/Ci) under non-photorespiratory conditions, typically low (1-2%) oxygen (Yin and Struik 2009).

Some agronomic parameters like yield, harvest index (HI), and number of grains per square meter were measured when plants reached physiological maturity stage. Also the aboveground biomass production, leaf area index (LAI) and specific leaf area (SLA) were estimated seven days after anthesis.

## Results/Discussion

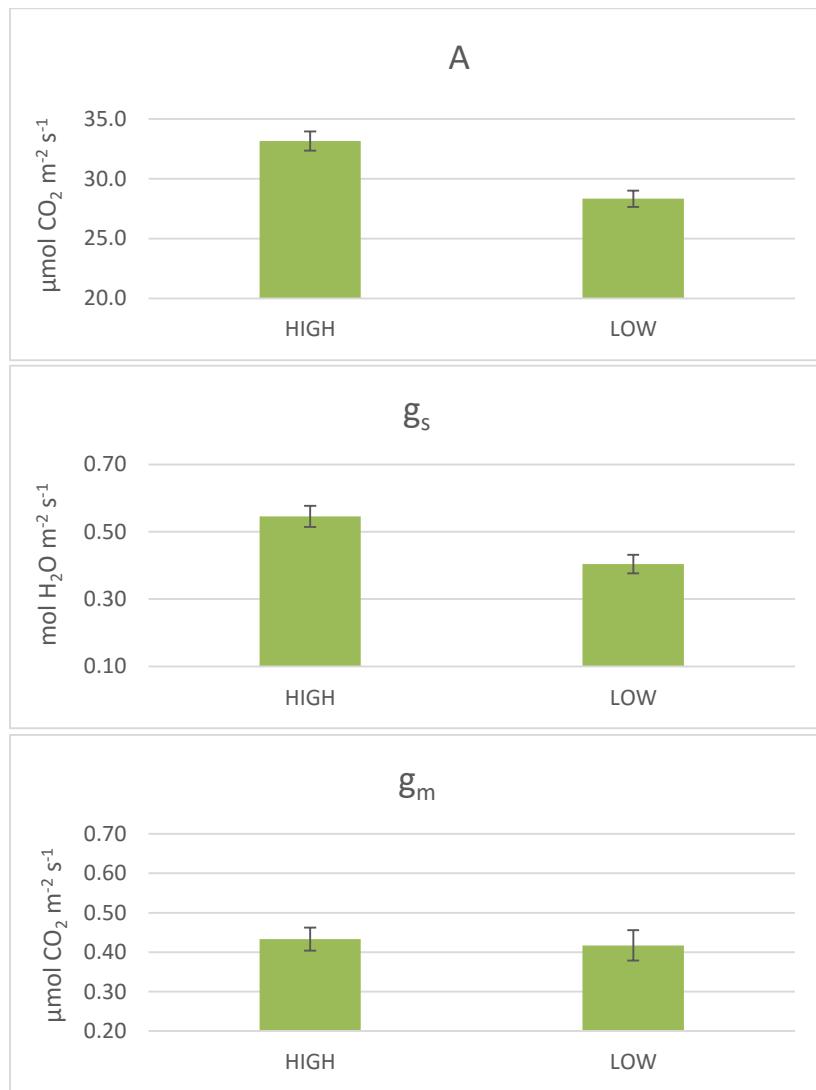
When the grain yield was measured at the end of the field season, two different groups of genotypes were identified. The first group included high yielding genotypes and in the second, the genotypes had a low yielding performance. Figure 1 shows the average value of each of these groups, which are clearly different, as were individual genotypes. For comparative purposes, the remaining traits were plotted for each of the yield categories.



**Figure 1. Yield categories for 13 genotypes of wheat under field conditions. Data plotted as mean grain yield values (g/m<sup>2</sup>) for high and low yield categories.**

In the case of HI values, the coincidence with yield categories was complete, all genotypes with high HI were in the high yielding group and genotypes with low HI, were all included in the low yielding set (data not shown).

Photosynthetic traits, A and  $g_s$  were variable in the low yielding group of genotypes, while the variation is less evident in the high yielding group. Both traits are consistently higher in the high performance group and lower in the low performance group. Average values for both sets of genotypes were consistent within each grouping (Fig. 2).



**Figure 2. Average values for carbon assimilation (A), stomatal conductance ( $g_s$ ) and mesophyll conductance ( $g_m$ ) for 13 wheat cultivars grown in Obregon, Mexico and organized into high and low yielding groups.**

For  $g_m$ , it was shown that there was a high variation between all wheat genotypes analyzed in this study with a phenotypic range of 0.305 to 0.55  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . The average values between high and low yielding groups were very similar, but there were systematic variations between individual genotypes (data not shown).

It has been demonstrated for several crops that  $g_m$  is a trait species-specific, this work confirms that  $g_m$  is also a genotype-specific character, and because of that, is probably related with other plant traits, such as leaf anatomy or Rubisco content.

Correlations between the traits, measured for individual genotypes, showed that grain yield was positively correlated with A and with  $g_s$ . Biomass at seven days after anthesis had also positive correlation with A and  $g_s$  but there was no correlation with  $g_m$ . Similarly, assimilation had a positive correlation with  $g_s$ , however, A and  $g_m$  showed no correlation across genotypes. Spike index (SPI) was positively related with A and yield (data not shown).

When yield was compared with  $g_m$ , there was a positive relation ( $r=0.48$ ,  $p=0.01$ ). Conversely, yield was negatively related with SLA and a negative correlation was also found between  $g_m$  and LAI. This could indicate that the travel of CO<sub>2</sub> from the substomatal cavities to the vicinity of Rubisco is regulated by the anatomical characteristics of the leaves, i.e. cell wall thickness, porosity and heterogeneity, chloroplast arrangement and cells to airspace ratio. This should be investigated in a more detailed way in the future comparing genotypes with differences in morphological traits.

These observations suggest that photosynthetic parameters (both, final assimilation and CO<sub>2</sub> conductance) have a role to determine the productivity of wheat. On the other hand, differences in anatomical traits have an influence on the photosynthetic performance of genotypes. The information from these two sets of results, shows that in general, smaller and thinner leaves have the highest photosynthetic and yield performances.

## Conclusions

Increasing yield potential of crops is a key step to achieve sustainable intensification of agriculture. In this work, several physiological and anatomical traits of wheat were evaluated to determine differences in yield potential and to establish the main sources of that variation. Thirteen wheat genotypes were grown and evaluated in northwestern Mexico, and the screening showed that:

- There is a considerable diversity in terms of yield performance, photosynthetic performance and leaf anatomical traits.
- Photosynthetic traits are positively related with yield and anatomical characters are negatively correlated with both, yield and mesophyll conductance.
- This suggests that photosynthesis and leaf anatomy of wheat should be considered in the design of breeding programs that aim to increase yield potential of this crop.
- These results may indicate that  $g_m$  has a more crucial role in the final photosynthetic capacity of wheat genotypes, than has been considered in the past.

## References

Bellasio C. and Griffiths H., 2013. The operation of two decarboxylases (NADPME and PEPCK), transamination and partitioning of C<sub>4</sub> metabolic processes between mesophyll and bundle sheath cells allows light capture to be balanced for the maize C<sub>4</sub> pathway. Plant Physiology (Nov): 115.

- Driever, S.M., Lawson, T., Andralojc, P.J., Raines, C.A., and Parry, M.A.J., 2014. Natural variation in photosynthetic capacity, growth, and yield in 64 field-grown wheat genotypes. *Journal of Experimental Botany* 65: 4959–73.
- Flexas, J., Diaz-Espejo, A., Galmés, J., Kaldenhoff, R., Medrano, H., and Ribas-Carbó, M., 2007. Rapid variations of mesophyll conductance in response to changes in CO<sub>2</sub> concentration around leaves. *Plant, Cell and Environment* 30:1284–1298.
- Flexas J., Barbour M.M., Brendel O., Cabrera H.M., Carriquí M., Díaz-Espejo A., Douthe C., Dreyer E., Ferrio J.P., Gago J., Galle A., Galmés J., Kodama N., Medrano H., Niinemets U., Peguero-Pina J.J., Pou A., Ribas-Carbo M., Tomas M., Tosens T., Warren C.R., 2012. Mesophyll diffusion conductance to CO<sub>2</sub>: an unappreciated central player in photosynthesis. *Plant Science* 193-194:70-84.
- Reynolds, M., Foulkes, J., Furbank, R., Griffiths, S., King, J., Murchie, E., Parry, M., and Slafer, G., 2012. Achieving yield gains in wheat. *Plant, Cell and Environment* 35: 1799-1823.
- Yin, X. and Struik, P.C. 2009. Theoretical reconsiderations when estimating the mesophyll conductance to CO<sub>2</sub> diffusion in leaves of C<sub>3</sub> plants by analysis of combined gas exchange and chlorophyll fluorescence measurements. *Plant, Cell and Environment* 32:1513–1524.
- Zhu, X., Long, S.P., and Ort, D.R., 2010. Improving photosynthetic efficiency for greater yield. *Annual Review of Plant Biology* 61:235–261.

## Nitrogen contribution of the ear to grain filling in wheat

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### Abstract

Several studies have analyzed the photosynthetic contribution of the ear to grain filling. However, its contribution in terms on nitrogen supply remains far less investigated. The nitrogen accumulated in the developing grains is supplied by remobilization of proteins from different organs of the plant. The aim of this work was to study the effect of growing conditions (optimal agronomic conditions and different levels of water and nitrogen deficit) on the potential nitrogen contribution of the spike and the different vegetative organs of the plant to the grains. To that end, natural abundance of  $\delta^{15}\text{N}$  and total N concentration in the flag leaf blade, peduncle, and different tissues of the spike (glumes and awns) were compared to values of  $\delta^{15}\text{N}$  in mature grains as well as in the total N content of grains per spike, in order to trace the origin of the nitrogen redistributed into the grains. In overall terms,  $\delta^{15}\text{N}$  and the total N content of the flag leaf blade, peduncle, glumes and awns, correlated positively with the  $\delta^{15}\text{N}$  and the total N content of the mature kernels per spike, suggesting that all organs could be potentially exporting part of its N to the grains. In spite of that, flag leaf blade under good agronomic conditions (and thus high grain yield) was the main organ which potentially contribute to grain N, whereas under water stress conditions (low GY) were the glumes and the penduncle. Such findings indicate that growing conditions may affect the relative potential N contribution of the different plant parts to grain N.

### Introduction

Ear photosynthesis is believed to be an important source of assimilates to grain filling in wheat and other cereals (Araus et al. 1993; Bort et al. 1994; Tambussi et al. 2007; Sanchez-Bragado et al. 2014a; Sanchez-Bragado et al. 2014b) especially under drought conditions (Tambussi et al. 2005). So far, several studies have analyzed the photosynthetic contribution of the ear to grain filling (Maydup et al. 2010; Maydup et al. 2012; Maydup et al. 2014; Tambussi et al. 2007). However its contribution in terms on nitrogen supply remains still unclear in spite the studies that emphasize their importance (Simpson et al. 1983; Lopes et al. 2006).

Under low soil nitrogen conditions, the nitrogen requirement of the developing grains is supplied by mobilization of proteins coming from different organs of the plant (Dalling et al. 1976; Simpson et al. 1983). Therefore, before ear emergence, cereals may accumulate most part of its nitrogen in vegetative organs and redistribute it during grain development (Dalling et al. 1976). It has been reported that vegetative organs in wheat exhibit different nitrogen isotope composition ( $\delta^{15}\text{N}$ ) (Lopes et al. 2006; Yousfi et al. 2009; Yousfi et al. 2013). Providing nitrogen isotope fractionation from the vegetative organs to the growing grains is negligible (or constant)  $\delta^{15}\text{N}$  could be used as an indicator of the nitrogen source from the growing grains.

The aim of this work is to study the effect of growing conditions (optimal agronomic conditions and different levels of water and nitrogen deficit) on the nitrogen contribution of the spike and the different vegetative organs of the plant to the grains. To this end, natural abundance of  $\delta^{15}\text{N}$  and total N

concentration in the flag leaf blade, peduncle and different tissues of the spike (glumes and awns) were compared to values of  $\delta^{15}\text{N}$  (and total N concentration) in mature grains in order to trace the origin of the nitrogen redistributed into the grains.

## Methods

### *Germplasm used and experimental conditions*

#### *a. CIMMYT's Norman E. Borlaug Experimental Station (CENEB)*

Six advanced bread wheat (*Triticum aestivum* L.) lines were selected on the basis of their similar phenology, high grain yield and biomass, from the CIMMYT Mexico Core Germplasm (CIMCOG) population. The field experiment was conducted during the spring growing season of 2012 at MEXPLAT (Mexican Phenotyping Platform) situated at CENEB in the Yaqui Valley, near Ciudad Obregón, Sonora, México (27°24' N, 109°56' W, 38 m asl), under fully irrigated conditions as explained elsewhere (Sanchez-Bragado et al. 2014). Sampling was performed around mid-grain filling. Different plant material was sampled 17 days after anthesis (DAA). Ten representative ears, flag leaves and peduncles per plot were harvested, cleaned, and immediately frozen with liquid nitrogen. The samples were stored at -20 °C and then lyophilized for 48h on 2012.

#### *b. INIA's Experimental Station, Aranjuez*

Ten durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.) genotypes were sown: five historical Spanish landraces (*Blanqueta*, *Griego de Baleares*, *Negro*, *Jerez 37* and *Forment de Artes*) and five modern Spanish commercial varieties delivered after 1990 (*Anton*, *Bolo*, *Don Pedro*, *Regallo* and *Sula*). Field experiments were conducted during the 2010 and 2011 growing seasons at the experimental station of the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) of Aranjuez (40°03'N, 3°31'E, 500 m asl). Two water treatments (support irrigation, SI, and rain-fed, RF) combined with two nitrogen regimes (fertilized, HN, and non-fertilized, LN) were assayed, as explained elsewhere (Sanchez-Bragado et al. 2014a). Sampling was performed around two weeks after anthesis (18<sup>th</sup> April) in 2011. Also, in 2011 all five landraces under support irrigation were discarded due to lodging. Thereafter, five representative flag leaves and ears were collected per plot, oven dried at 70 °C for 48 hours, weighed, and finely ground for nitrogen isotope analyses (on total dry matter).

### *Nitrogen concentration and stable isotope composition*

The total N concentration and stable nitrogen isotope composition ( $\delta^{15}\text{N}$ ) in the dry matter of glumes, awns, flag leaves and peduncles, roots and mature kernels was analyzed. For  $\delta^{15}\text{N}$  analysis of the dry matter, approximately 1 mg of each sample was weighed into tin capsules and measured with an elemental analyzer (Flash 1112 EA; Thermo-Finnigan, Bremen, Germany) coupled with an Isotope Ratio Mass Spectrometer (Delta C IRMS, ThermoFinnigan, Bremen, Germany) operating in continuous flow mode in order to determine the stable nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) isotope ratios. The ( $^{15}\text{N}/^{14}\text{N}$ ) ratios of plant material were expressed in  $\delta$  notation (Coplen, 2008):  $\delta^{15}\text{N} = (^{15}\text{N}/^{14}\text{N})_{\text{sample}} / (^{15}\text{N}/^{14}\text{N})_{\text{standard}} - 1$ , where 'sample' refers to plant material and 'standard'  $\text{N}_2$  in air.

### *Statistical analysis*

Data were subjected to one-way analyses of variance (ANOVA) using the general linear model in order to calculate the effects of genotype and organ on the studied parameters. Means were compared by Tukey's

HSD test. A bivariate correlation procedure was constructed to analyze the relationships between the measured traits. Statistical analyses were performed using the SPSS 18.0 statistical package (SPSS Inc., Chicago, IL, USA). Figures were created using the Sigma-Plot 10.0 program (SPSS Inc.).

## Results/Discussion

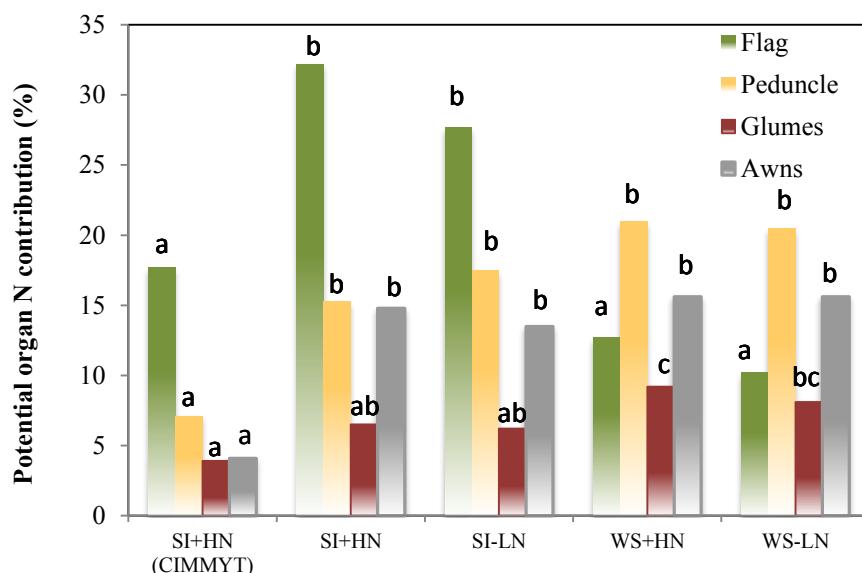
The highest average grain yield (GY) was observed either in CIMMYT ( $6.77 \text{ Mg}\cdot\text{ha}^{-1}$ ) and Aranjuez under supplemental irrigation conditions associated to fertilized (SI+HN) and non-fertilized (SI-LN) plots ( $4.37 \text{ Mg}\cdot\text{ha}^{-1}$  and  $4.63 \text{ Mg}\cdot\text{ha}^{-1}$ , respectively) (Table 1). Similarly, flag leaf blade, peduncle, glumes and awns together with to mature grains exhibited higher nitrogen concentration (N concentration) and total nitrogen content per organ (Total organ N) under support irrigation compared with rainfed conditions (Table 1). Moreover, the highest total N content per organ was observed in the flag leaf blade under irrigation conditions in CIMMYT (10.97 mg) and Aranjuez (5.80 mg and 6.53 mg for SI+HN and SI-LN conditions, respectively), whereas under rainfed conditions the peduncle (2.30 mg) and the awns (1.80 mg) exhibited the highest contents (Table 1). Regardless of the growing conditions total grain N content per spike (Grain N·spike $^{-1}$ ) was much higher than the total N content of any of the different organs analyzed, especially in CIMMYT.

**Table 1. Mean values of nitrogen isotope composition ( $\delta^{15}\text{N}$ ) in the water-soluble fraction (WSF) and dry matter (DM) of the flag leaf blade, peduncle, glumes, awns as well as in mature kernels (grains), nitrogen concentration (N concentration), total nitrogen content per organ (Total organ N), grain yield (GY) and total nitrogen of mature kernels per spike (Grain N·spike $^{-1}$ ). Nine durum wheat genotypes and three replicates per genotype (108 plots) were considered under rainfed N fertilized (RF+HN) and non-fertilized conditions (RF-LN) and supplemental irrigation under N fertilized (SI+HN) and non-fertilized conditions (SI-LN). The experiment was performed under field conditions during the crop season 2011-2012 at the INIA's Experimental Station, Aranjuez, Spain. Six bread wheat genotypes and three replicates per genotype (18 plots) were considered under good agronomical conditions. The experiment was performed in field conditions at the CIMMYT's Experimental Station, Norman E. Borlaug (CENEB), under fully irrigated conditions. Mean values across plant tissues with different letters are significantly different according to the Tukey's honestly significant difference test ( $P<0.05$ ).**

	SI+HN	INIA's Aranjuez SI-LN	RF+HN	RF -LN	CIMMYT SI+HN
<b>Flag leaf</b>					
N concentration (%DM)	3.78a	3.40c	2.66b	2.08a	3.83d
Total organ N (mg)	5.80b	6.53b	1.40a	1.46a	10.97c
$\delta^{15}\text{N}$ DM (‰)	3.70c	6.34d	-1.10a	4.52c	-1.23b
$\delta^{15}\text{N}$ WSF (‰)	3.55b	2.66b	-0.16b	1.74b	-2.05a
<b>Peduncle</b>					
N concentration (%DM)	1.77c	1.59b	1.50b	1.33a	1.64bc
Total organ N (mg)	3.47b	3.13b	2.57a	2.23a	4.43c
$\delta^{15}\text{N}$ DM (‰)	4.07b	6.98c	-0.12a	4.29b	-1.60a
$\delta^{15}\text{N}$ WSF (‰)	3.57b	2.48b	-0.79a	1.46b	-0.02a
<b>Glumes</b>					

N concentration (%DM)	1.63d	1.39bc	1.53cd	1.23a	1.35b
Total organ N (mg)	1.13a	1.20a	1.28a	1.41a	2.52b
$\delta^{15}\text{N}$ DM (‰)	3.22b	5.83c	-0.03a	4.12b	-1.31a
$\delta^{15}\text{N}$ WSF (‰)	4.14b	3.25b	0.94b	2.17b	-1.42a
<b>Awns</b>					
N concentration (%DM)	2.17c	1.88b	1.41a	1.24a	1.25a
Total organ N (mg)	3.20b	2.93b	1.77a	1.90b	2.61ab
$\delta^{15}\text{N}$ DM (‰)	3.09c	5.91d	0.35a	4.37c	-1.33b
$\delta^{15}\text{N}$ WSF (‰)	5.1c	3.47b	0.71b	1.83b	-2.56a
<b>Grains</b>					
N concentration (%DM)	2.48b	2.07a	2.39ab	2.21a	2.59b
Grain N·spike <sup>-1</sup>	20.75ab	21.94b	11.49a	13.92ab	62.82d
$\delta^{15}\text{N}$ DM (‰)	4.27c	6.6e	1.9b	5.53d	-0.25a
GY (Mg·ha <sup>-1</sup> )	4.37b	4.63b	1.68a	1.66a	6.77c

However, the relative (i.e. compared with the other plant parts studied) potential contribution of the flag leaf as a source of N to grain nitrogen (grain N) increased with better growing conditions (Fig. 1) whereas the opposite occurred for the peduncle, and glumes and awns. The flag leaf blade has been reported to be an important nitrogen exporter to other organs in bread wheat (Simpson et al. 1983). However, whereas its relative importance as a source of N, compared with other upper plant parts, may increase as the agronomical conditions improve, its absolute potential contribution may decrease under the high yielding conditions and improved varieties of CIMMYT trial (Fig. 1).



**Figure 1.** Potential relative contribution of the flag leaf blade, peduncle, glumes and awns to the nitrogen accumulated in the grains. Values were calculated as the product of nitrogen content of the different organs multiplied by their respective dry weight and standardized by the total nitrogen content of mature kernels

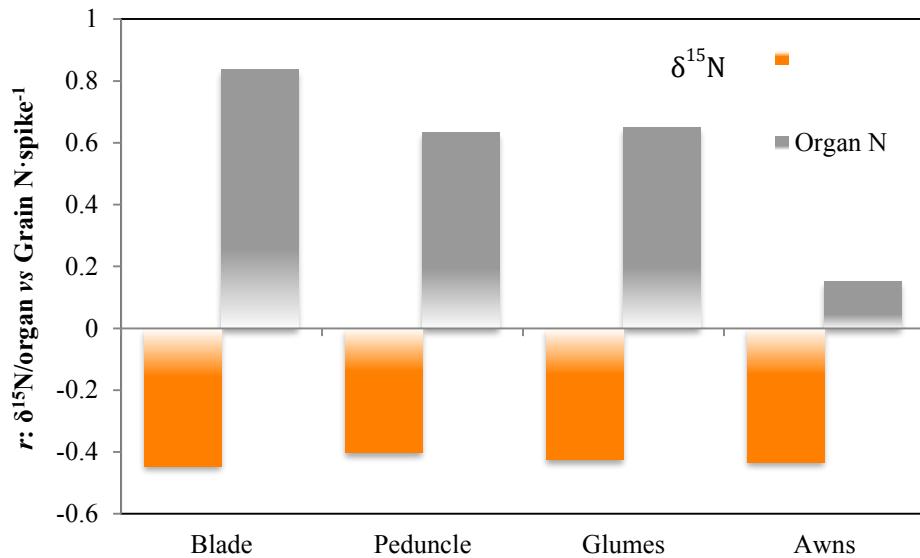
**per spike. Nine durum wheat genotypes and three replicates per genotype (108 plots) were considered under rainfed N fertilized (RF+HN) and non-fertilized conditions (RF-LN) and supplemental irrigation under N fertilized (SI+HN) and non-fertilized conditions (SI-LN). The experiment was performed under field conditions at the INIA's Experimental Station, Aranjuez, Spain during the 2011-2012 growing season. In addition, six bread wheat genotypes and three replicates per genotype (18 plots) were considered under good agronomical conditions. The experiment was performed in field conditions at the CIMMYT's Experimental Station, Norman E. Borlaug (CENEB), under fully irrigated conditions. Mean values across organs and different growing conditions are significantly different according to the Tukey's honestly significant difference test ( $P<0.05$ ).**

In spite of the high contribution traditionally assigned to the flag leaf blade as a source of N to the growing grains (Evans 1983; Araus and Tapia 1997), in our study, and regardless the growing conditions, potential contribution of the flag leaf blade was less than 30% (Fig. 1). Moreover it has been reported that less than the half of the nitrogen retranslocated from the leaves arrives to the grain because more than the half (54%) is translocated to the roots (Simpson et al. 1983). The relatively low potential N contribution of the flag leaf blade to grain N may also be indicating that besides other parts (such as the sheath) of this leaf, other organs of the plant are potentially contributing to the N accumulated in the grains such as the peduncle (and eventually lower leaves) and spike tissues (awns and glumes) (Fig. 1). This view is supported by the strong linear correlations in Aranjuez (including all growing conditions) and CIMMYT observed between the  $\delta^{15}\text{N}$  in the mature grains and the  $\delta^{15}\text{N}$  of the dry matter of all studied plant organs ( $P<0.001$ ) (Table 2). Nevertheless, the strongest correlations against the  $\delta^{15}\text{N}$  of the grains was achieved by the flag leaf blade ( $r=0.932$ ) (Table 2). The correlations of  $\delta^{15}\text{N}$  and the total N content per organ against the total N content per spike also support a slightly higher role of the flag leaf compared with the other plant parts. Thus the correlations were positive and significant for all the plant parts studied, but those of the flag leaf blade were slightly higher (Fig. 2). Lower leaves were not considered in this study as were assumed to be mainly translocate carbohydrates to the roots (Wardlaw 1968), suggesting that its contribution to grain N accumulation may be minor. However, in our study the material from CIMMYT does not support this assumption. Moreover, the role of the flag leaf sheath supplying N to the growing grains should be not neglected as a potential source of N (Araus and Tapia 1987).

**Table 2. Linear regression of the relationship between the nitrogen isotope composition ( $\delta^{15}\text{N}$ ) in the mature grains ( $\delta^{15}\text{N}$  grain DM) against the  $\delta^{15}\text{N}$  in the dry matter (DM) and the water-soluble fraction (WSF) of the flag leaf blade, peduncle, glumes and awns. Nine durum wheat genotypes and three replicates per genotype (108 plots) were considered under rainfed N fertilized (RF+HN) and non-fertilized conditions (RF-LN) and supplemental irrigation under N fertilized (SI+HN) and non-fertilized conditions (SI-LN). The experiment was performed under field conditions at the INIA's Experimental Station, Aranjuez, Spain. In addition, six bread wheat genotypes and three replicates per genotype (18 plots) were considered under good agronomical conditions. The experiment was performed in field conditions at the CIMMYT's Experimental Station, Norman E. Borlaug (CENEB), under fully irrigated conditions. Level of significance: \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; ns,  $P > 0.05$ .**

$\delta^{15}\text{N}$ grain DM					
INIA's Aranjuez			CIMMYT		
SI+HN	SI-LN	WS+HN	WS-LN	Global	SI+HN
<b>Flag leaf</b>					

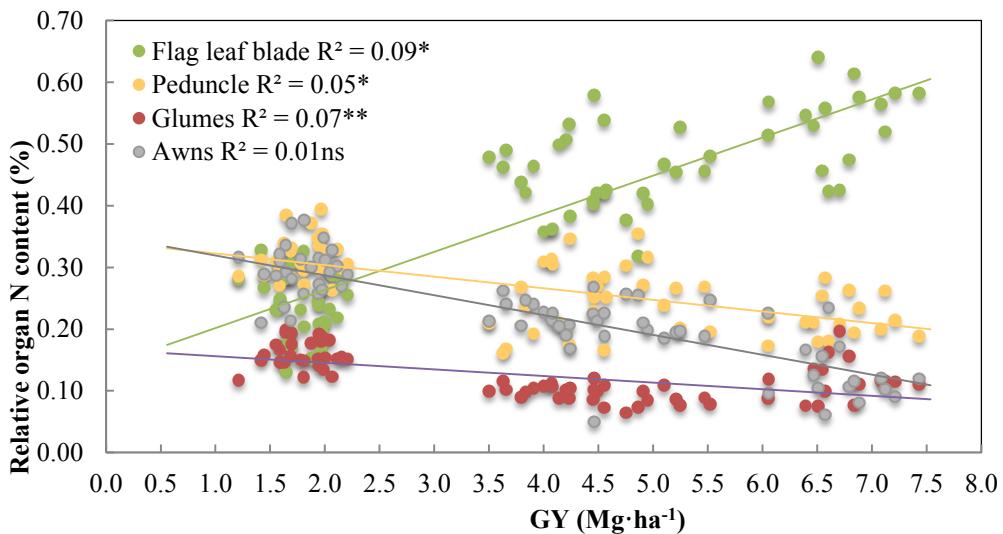
$\delta^{15}\text{N}$ DM (‰)	0.043 ns	<b>0.761</b> **	<b>0.533</b> *	<b>0.609</b> *	<b>0.932</b> ***	<b>0.909</b> ***
$\delta^{15}\text{N}$ WSF (‰)	0.198 ns	<b>0.803</b> ***	0.002 ns	<b>-0.562</b> **	<b>0.397</b> ***	<b>0.597</b> **
<b>Peduncle</b>						
$\delta^{15}\text{N}$ DM (‰)	0.364 ns	<b>0.857</b> ***	<b>0.610</b> ***	0.212 ns	<b>0.869</b> ***	<b>0.842</b> ***
$\delta^{15}\text{N}$ WSF (‰)	0.156 ns	0.441 Ns	-0.034 ns	-0.562 **	<b>0.453</b> ***	0.14 ns
<b>Glumes</b>						
$\delta^{15}\text{N}$ DM (‰)	0.377 ns	<b>0.860</b> ***	<b>0.541</b> **	0.508 **	<b>0.910</b> ***	<b>0.707</b> **
$\delta^{15}\text{N}$ WSF (‰)	0.112 ns	0.157 Ns	-0.076 ns	-0.431 *	<b>0.313</b> **	-0.01 ns
<b>Awns</b>						
$\delta^{15}\text{N}$ DM (‰)	0.165 ns	<b>0.747</b> **	0.263 ns	0.585 **	<b>0.836</b> ***	<b>0.802</b> ***
$\delta^{15}\text{N}$ WSF (‰)	-0.059 ns	0.434 ns	-0.181 ns	-0.518 **	<b>0.293</b> **	0.347 ns



**Figure 2. Correlation coefficients of the relationships of  $\delta^{15}\text{N}$  and the total N content per organ against the total N accumulated in the kernels of the spike. The different plant organs assayed were: the flag leaf blade, peduncle, glumes and awns.  $\delta^{15}\text{N}$  of the mature grains was also included in the comparison. For the calculation the 108 plots of durum wheat from the INIA experiment and the 18 plots of the CIMMYT trials were included. Growing conditions as detailed in the legend of Figure 1.**

Besides the flag leaf, the role of the ear as a supplier of N should be taken into account. Indeed accumulation of nitrogen in the grains has been observed to be closely dependent on N mobilization coming from the glumes (23% of N contribution to the grain) under absence of exogenous supply of nitrogen (Simpson et al. 1983). Moreover, our study shows that the potential N contribution of the awns and glumes as well as the peduncle with respect to the flag leaf blade increased under water stress conditions. Indeed, the sum of the potential N contribution of awns and glumes (30% approximately) under rainfed conditions was comparable to that of the flag leaf blade (30% approximately) under supplemental irrigation conditions (Fig. 1). Moreover, the negative correlation between the relative N content of glumes and the peduncle against GY (Fig. 3) supports the important role of the spike and the

shoot contributing to the N accumulated in the grains, specially under water stress conditions and thus low GY.



**Figure 3.** Pearson correlations between the grain yield (GY) and the relative organ N content of the flag leaf blade, peduncle, glumes and awns across a wide range of environmental conditions. The relative contribution of the different organs was calculated as the value for the specific organ divided by the sum of all organs (flag leaf blade, peduncle, glumes and awns). 15 genotypes (9 durum wheats and 6 bread wheats) and three replicates per genotype (126 plots) were considered, including the four growing conditions tested at the INIA's Experimental Station of Aranjuez, Spain in 2011-2012 (RF+HN, RF-LN, SI+HN and SI-LN) and the good agronomical conditions at the CIMMYT's Experimental Station, Norman E. Borlaug, Cd. Obregon (Mexico).

Besides the relative amount of N in each plant part with regard the total N accumulated in grains (or relative to the all the N available in tissues other than the grains), growing conditions may also affect the efficiency of transfer to the grains. For example part of the N accumulated in the flag leaf and other leaves may be exported back to the roots, particularly under stress (drought, low fertility) to promote root development (Palta et al. 1997). Conversely, during rapid grain filling glumes may only retranslocate N to the grains, increasing its role under water stress conditions (Waters et al. 1980).

Summarizing, the significant correlations between both the  $\delta^{15}\text{N}$  and the total N content of the different plant organs studied (flag leaf blade, peduncle, glumes and awns) against either the  $\delta^{15}\text{N}$  of mature kernels or the total N content of the grains per spike, suggest that all these organs can be potentially exporting part of its N to the grains. Moreover, the high amount of N accumulated in the whole grains of the spike, together with the relative low amount of N available in the different organs support the concept that N imported to the grains cannot be only sustained by a specific organ, rather different organs maybe simultaneously exporting nitrogen to the grains. In spite of that, the role of flag leaf blade as potential supplier of N to grains increases, as compared with the other upper parts of the plant, under good agronomic conditions (and thus high GY), whereas the relative importance of the ear and peduncle increases under water stress conditions (low GY). Such findings indicate that growing conditions may affect the relative potential contribution of the different plant parts to grain N.

## Conclusions

$\delta^{15}\text{N}$  abundance studies indicate that while all organs potentially export part of their N to the grain, growing conditions affect the relative N contribution. Under favorable conditions, the flag leaf blade was the main organ contributing to grain N, whereas under water stress conditions the glumes and the penduncles make a more significant contribution.

## References

- Araus J.L. and Tapia L., 1987. Photosynthetic gas exchange characteristics of wheat flag leaf blades and sheaths during grain filling: The case of a spring crop grown under mediterranean climate conditions. *Plant physiology* 85: 667–673.
- Araus J.L., Brown H.R., Febrero A., Bort J., and Serret M.D., 1993. Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory CO<sub>2</sub> to differences in grain mass in durum wheat. *Plant, Cell and Environment* 16: 383–392.
- Bort J., Febrero A., Amaro T., and Araus J., 1994. Role of awns in ear water-use efficiency and grain weight in barley. *Agronomy* 14: 133–139.
- Dalling M., Boland G., and Wilson J., 1976. Relation between acid proteinase activity and redistribution of nitrogen during grain development in wheat. *Australian Journal of Plant Physiology* 3: 721- 730.
- Evans J.R., 1983. Nitrogen and Photosynthesis in the Flag Leaf of Wheat (*Triticum aestivum* L.). *Plant Physiology* 72: 297–302.
- Lopes M.S., Cortadellas N., Kichey T., Dubois F., Habash D. Z., and Araus J. L., 2006. Wheat nitrogen metabolism during grain filling: comparative role of glumes and the flag leaf. *Planta* 225: 165–181.
- Martin P., 1982. Stem xylem as a possible pathway for mineral retranslocation from senescing leaves to the ear in wheat. *Australian Journal of Plant Physiology* 9: 197-207.
- Maydup M.L., Antonietta M., Guiamet J. J., Graciano C., López J. R., and Tambussi E. A., 2010. The contribution of ear photosynthesis to grain filling in bread wheat (*Triticum aestivum* L.). *Field Crops Research* 119: 48–58.
- Maydup M.L., Antonietta M., Guiamet J.J., and Tambussi, E.A. 2012. The contribution of green parts of the ear to grain filling in old and modern cultivars of bread wheat (*Triticum aestivum* L.): Evidence for genetic gains over the past century. *Field Crops Research* 134: p.208–215.
- Maydup M.L., Antonietta M., Graciano C., Guiamet J.J., and Tambussi E.A., 2014. The contribution of the awns of bread wheat (*Triticum aestivum* L.) to grain filling: Responses to water deficit and the effects of awns on ear temperature and hydraulic conductance. *Field Crops Research* 167: 102–111.
- Palta J.A. and Gregory P.J. 1997. Drought affects the fluxes of carbon to roots and soil in <sup>13</sup>C pulse-labelled plants of wheat. *Soil Biology and Biochemistry* 29: 1395–1403.
- Sanchez-Bragado R., Elazab A., Zhou B., Serret M. D., Bort J., Nieto-Taladriz M. T., and Araus J. L., 2014a. Contribution of the ear and the flag leaf to grain filling in durum wheat inferred from the

- carbon isotope signature: genotypic and growing conditions effects. *Journal of Integrative Plant Biology* 56: 444–554.
- Sanchez-Bragado R., Molero G., Reynolds M.P., and Araus JL., 2014b. Relative contribution of shoot and ear photosynthesis to grain filling in wheat under good agronomical conditions assessed by differential organ  $\delta^{13}\text{C}$ . *Journal of Experimental Botany* 65: 5401-5413.
- Simpson R.J., Lambers H., and Dalling M.J. 1982. Translocation of nitrogen in a vegetative wheat plant (*Triticum aestivum*). *Physiologia Plantarum* 56: 11–17.
- Simpson R.J., Lambers H., and Dalling M.J. 1983. Nitrogen redistribution during grain growth in wheat (*Triticum aestivum L.*) : IV. Development of a quantitative model of the translocation of nitrogen to the grain. *Plant Physiology* 71: 7–14.
- Tambussi E.A., Bort J., Guiamet J.J., Nogués S., and Araus J.L., 2007. The photosynthetic role of ears in C3 cereals: metabolism, water use efficiency and contribution to grain yield. *Critical Reviews in Plant Sciences* 26: 1–16.
- Tambussi E.A., Nogués S., and Araus J.L., 2005. Ear of durum wheat under water stress: water relations and photosynthetic metabolism. *Planta* 22: 446–458.
- Wardlaw I.F. 1968. The control and pattern of movement of carbohydrates in plants. *The Botanical Review* 34: 79–105.
- Waters S.P., Peoples M.B., Simpson R.J., and Dalling M.J. 1980. Nitrogen redistribution during grain growth in wheat (*Triticum aestivum L.*). *Planta* 148: 422–428.
- Yousfi S., Serret M.D., and Araus J.L. 2009. Shoot  $\delta^{15}\text{N}$  gives a better indication than ion concentration or  $\Delta^{13}\text{C}$  of genotypic differences in the response of durum wheat to salinity. *Functional Plant Biology* 36: 144-155.
- Yousfi S., Serret M.D., and Araus J.L., 2013. Comparative response of  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  in durum wheat exposed to salinity at the vegetative and reproductive stages. *Plant, Cell and Environment* 36: 1214–1227.



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