

Ready for phenotyping: The wheat reference samples

S. Dreisigacker¹, J. Franco², T. Payne¹, M. Zaharieva¹, F. Balfourier³, X. Zhang⁴, M. Nachit⁵, M. Warburton⁶

¹ CIMMYT, Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico

² Facultad de Agronomía, Universidad de la República, Ave. Garzón 780, Montevideo, Uruguay

³ INRA, UMR1095 Amélioration et Santé des Plantes, 234, avenue du Brézat, 63100 Clermont-Ferrand

⁴ Institute of Crop Breeding and Cultivation, Chinese Academy of Agricultural Sciences, 30 Baishiqiao Road, Beijing, China 77205

⁵ ICARDA, CYMMYT/ICARDA, P.O. Box 5466, Aleppo, Syria

⁶ University of Mississippi, MS, 38677, USA

Background

The characterization of global genetic resources provides a fundamental resource for varietal improvement. Core collections or reference samples help streamline the evaluation of genetic resources. Reference samples preserve as much as possible of the diversity present from original collections in a substantially reduced number of accessions. Such samples can be selected based on morphological, phenotypic, or molecular marker data, the latter having the advantage of not being heavily biased for any single trait, geographical area, or pedigree.

In the last three years, CIMMYT and partners have committed to assemble and complete the structural characterization of the diversity of a so-called “composite germplasm set” for wheat. In the first phase, 2,500 diverse wheat accessions were characterized using 50 SSR markers (“#02b Genotyping of composite germplasm set, Tier 1, wheat”). In the second phase (“# 01b: Completing genotyping of composite germplasm set of wheat”), 400 additional accessions were genotyped, bringing the total to 3,000 entries. By 2007, there were data for up to 44 SSRs on 2,932 entries. The ultimate goal is to provide access to global genetic resources via reference samples that may supply genes and alleles involved in key agricultural traits, especially stress tolerance.

Development of the Wheat Reference Samples

The D strategy developed by Franco et al., 2006 was applied to assemble reference samples for wheat based on the marker information of the composite germplasm set. The D-strategy maximizes the representativeness of the genetic diversity in subsets of entries by including “generalists” alleles. Entries are first stratified using genetic distance measures and clustering methods. Second, dependent on the mean genetic distance of each cluster, a number of entries are randomly selected and this step is iterated several hundred times. The reference sample with the highest diversity measures is chosen as the final reference sample. Selecting entries via the D-strategy has the additional advantage of minimizing for population structure, a key factor for future association analyses.

To facilitate subsequent phenotypic evaluations, separate reference samples have been assembled for spring, winter, and durum wheat. Excluded were wheat wild relatives and accessions for which there was no seed. Accessions selected each of the two phases of genotyping were kept separate, because different numbers of SSRs were applied to select them. The final reference samples comprise 373 accessions for spring wheat and 110 accessions for winter and durum wheat (Figure 1). Reference samples show levels of genetic diversity similar to those of corresponding composite germplasm sets (Table 1).

To phenotyping...

The reference samples will be useful for studies such as SNP discovery and the evaluation of phenotyping variation for agronomic traits, to analyze linkage disequilibrium and marker-trait associations for complex traits. Single-plant seed of each accession has been collected and will be deposited in the CIMMYT germplasm bank.

As part of the Generation Challenge Program (GCP) project “Precision phenotyping of the spring wheat reference sample for drought,” a slightly modified sample of spring wheat has been developed targeted for evaluating drought tolerance. Early-maturing off-types have been discarded and globally-important drought tolerant cultivars added to the original reference sample. Seed of this sample is currently being increased in Mexico (Figure 2), for phenotyping for physiological traits related to drought tolerance, high-throughput genotyping with DArT markers, and association mapping in 2009-10.

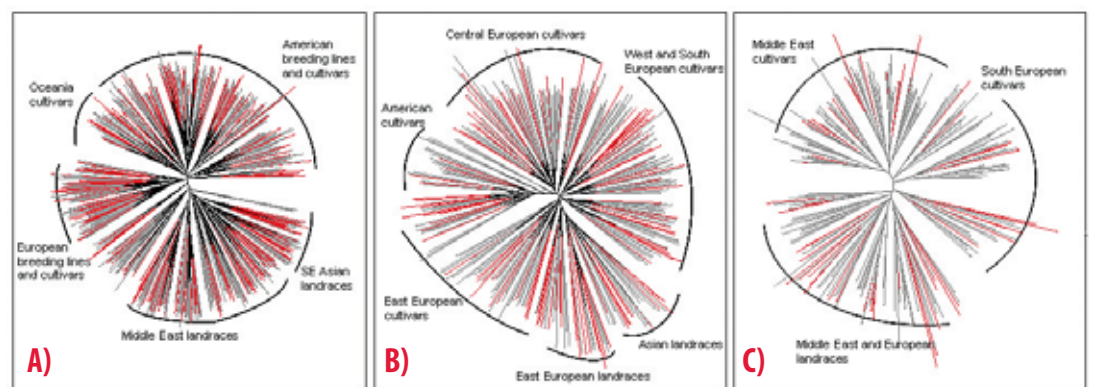


Figure 1. Associations among A) spring wheat, B) winter wheat, and C) durum wheat accessions of the composite germplasm set revealed with neighbor joining clustering based on Nei’s distance measured with 18 or 32 SSRs. Accessions in red are included in the international reference samples. Accessions analysed within the GCP project#01b are not presented.

Table 1. Genetic diversity measures of the composite germplasm set compared to the derived reference samples for spring, winter and durum wheat.

Germplasm	Sample Size	Allele No	Gene Availability	Gene Diversity	Heterozygosity	Major Allele Frequency
Spring wheat						
Composite germplasm	764	17.8	0.93	0.718	0.069	0.41
Reference set	308	14.5	0.93	0.722	0.068	0.40
Spring wheat (2nd phase)						
Composite germplasm	203	5.3	0.96	0.363	0.028	0.74
Reference set	65	4.5	0.95	0.386	0.023	0.73
Durum wheat						
Composite germplasm	246	12	0.962	0.619	0.087	0.50
Reference set	60	9.5	0.921	0.663	0.072	0.45
Durum wheat (2nd phase)						
Composite germplasm	224	9.9	0.939	0.540	0.072	0.57
Reference set	50	7.6	0.929	0.596	0.060	0.51
Winter wheat						
Composite germplasm	321	14.6	0.952	0.692	0.035	0.43
Reference set	110	11.6	0.951	0.711	0.038	0.41



Figure 2. Seed increase of the spring wheat reference sample at Cd. Obregon, Sonora, Mexico.