Research Article

Genetic structure of Argentinean hexaploid wheat germplasm

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Abstract

The identification of genetically homogeneous groups of individuals is an ancient issue in population genetics and in the case of crops like wheat, it can be valuable information for breeding programs, genetic mapping and germplasm resources. In this work we determined the genetic structure of a set of 102 Argentinean bread wheat (*Triticum aestivum* L.) elite cultivars using 38 biochemical and molecular markers (functional, closely linked to genes and neutral ones) distributed throughout 18 wheat chromosomes. Genetic relationships among these lines were examined using model-based clustering methods. In the analysis three subpopulations were identified which correspond largely to the origin of the germplasm used by the main breeding programs in Argentina.

Keywords: wheat, genetic structure, molecular markers, genetic diversity.

Introduction

Bread wheat (*Triticum aestivum* L.) is the principal winter crop grown in Argentina for both internal consumption and export. Genetically improved cultivars and agricultural practices have resulted in an increased average wheat yield during the past 41 years, from 1352 kg/ha in 1969 to more than 3500 kg/ha in 2010. To maintain this rate of wheat productivity, exploring the genetic variability at molecular levels in adaptation and yield components and integrating such information with conventional breeding methods will be critical (Chao *et al.*, 2007). The identifications of genomic regions associated with relevant agronomic traits through QTL mapping using bi-parental populations, can now be complemented with alternative genetic mapping strategies like Association Mapping, in which the accurate determination of the population genetic structure is important for the appropriate association between the genotype and the phenotype (Breseghello and Sorrells, 2006; Peng *et al.*, 2009; Le Couviour *et al.*, 2011).

In wheat, the assessment of genetic structure in different populations has been historically based on qualitative and quantitative traits (Spagnoletti-Zeuli and Qualset, 1987; Van Beuningen and Busch, 1997b) and pedigree records (Burkhamer *et al.*, 1998; Van Beuningen and Busch, 1997a; Bered *et al.*, 2002). However, pedigree records are not always available or detailed enough for this type of analysis, especially when large numbers of breeding lines or cultivars are being assessed. More recently, biochemical markers such as variation in storage protein subunits (Metakovsky and Branlard, 1998; Lerner *et al.*, 2009) and/or molecular markers like RAPDs, RFLPs, AFLPs, SSRs, DArTs and SNPs become alternative methods of obtaining a large amount of data for precisely calculating genetic relationship estimates (Mukhtar *et al.*, 2002; Parker *et al.*, 2002; Soleimani *et al.*, 2002; Neumann *et al.*, 2011; Chao *et al.*, 2010). Particularly in Argentina the levels and patterns of genetic diversity among local wheat cultivars have been investigated using SSRs (Manifesto *et al.*, 2001) and storage proteins (Lerner *et al.*, 2009).

Frequently, the biochemical and/or molecular information has been analyzed using tree-based methods that calculate genetic distance between individuals and tree construction algorithms such as UPGMA or neighbor joining to group them in clusters (Sneath and Sokal, 1973; Saitou and Nei, 1987). An alternative model-based method developed recently by Pritchard *et al.* (2000) and implemented in the software Structure aims at delineating clusters of individuals on the basis of their genotypes at multiple loci using a Bayesian approach (Breseghello and Sorrells, 2006; Chao *et al.*, 2010). However Evanno *et al.* (2005) demonstrated that in most cases the estimated "log probability of data" used in Structure fails to provide a correct estimation of the number of clusters, (K). Hence, they

developed an *ad hoc* statistic ΔK based on the rate of change in the log probability of data between successive K values, and found that Structure accurately detects the uppermost hierarchical level of structure. Based on these parameters, Earl and von Holdt (2012) developed Structure Harvester, a website and software for visualizing the output based on Evanno *et al.* (2005).

In this report, we determined the genetic structure of a set of Argentinean wheat cultivars using a model-based approach. 102 cultivars representative of the main breeding companies in Argentina were characterized using a set of 38 biochemical and molecular markers, each mapped to a single chromosome location and distributed over 18 of the 21 wheat chromosomes.

Materials and Methods

Plant material

A set of 102 bread wheat cultivars registered in Argentina was selected to determine its genetic structure based on molecular and biochemical markers. This set included old and recent commercial cultivars selected from the main wheat breeding companies in Argentina. Seed stocks were kindly provided by the Instituto Nacional de Tecnología Agropecuaria (INTA) Marcos Juárez Wheat Germplasm Bank (Marcos Juárez, Argentina).

Genotypic data

For each accession, genomic DNA was extracted from fresh leaves of single plants using a fast, small-scale DNA isolation procedure based on Helguera *et al.* (2005). Sample genotyping included: (1) functional markers *Vrn-A1* (Yan *et al.*, 2004), *Vrn-B1, Vrn-D1* (Fu *et al.*, 2005), *Ppd-D1 (*Beales *et al.*, 2007), *Rht-B1, Rht-D1* (Ellis *et al.*, 2002), *PinA-D1* (Gautier *et al.*, 1994), *Glu-A3* (Zhang *et al.*, 2004), *Wx-A1* and *Wx-B1* (McLauchlan *et al.*, 2001, Vanzetti *et al.*, 2009), *Vp1-B3* (Yang *et al.*, 2007), *Ppo-A1* and *Ppo-D1* (He *et al.*, 2007), 7oe+8* (Butow *et al.*, 2004); (2) molecular markers closely linked to genes *Lr10* (Schachermayr *et al.*, 1997), *Lr34* (Lagudah *et al.*, 2006), *Lr24* (Schachermayr *et al.*, 1995) and *Ppd-B1* (Díaz *et al.*, 2012); and (3) 17 SSR markers selected according to information available in the GrainGenes database (Matthews *et al.*, 2003) and one ISBP marker newly developed for this work. In all cases, polymerase chain reactions (PCR) were performed in 25 μL aliquots in a PTC-100 thermal cycler (MJ Research Inc., Waltham, MA, USA). The reaction contained 100 ng of genomic DNA (template), 1X *Taq* polymerase buffer (Promega Corp. Madison, WI, USA), 1 U *Taq* DNA polymerase (Promega), 0.2 mM of each deoxynucleotide, 0.2 μM of each primer, and 1.5 mM of MgCl$_2$. Primers names, sequences and cycling conditions for each molecular marker are detailed in Table S1. SSR markers were run on 6% non-denaturing polyacrylamide gels in 0.5X TBE buffer using a Mega-Gel Dual High-Throughput

Vertical Electrophoresis Unit (CBS Scientific Co, Del Mar, CA, USA), stained with ethidium bromide (0.5 g/L) and visualized under UV light. In the case of functional genes and genes closely linked to molecular markers, 10 μL of the PCR products were run on 2% agarose (Promega) gels in SB buffer (Brody and Kern, 2004) at constant power (100 V) for about 30 min. After electrophoresis, the gels were stained with ethidium bromide (0.5 g/L) and visualized under UV light.

Glutenin analysis

Glutenins were extracted from single seeds and analyzed by SDS-PAGE according to protocols described by Pflüger *et al.* (2001). Glu-A1, Glu-B1 and Glu-D1 subunits were analyzed by SDS-PAGE in 8% polyacrylamide gels (16x18 cm) in a Hoefer electrophoresis system (Hoefer Inc. Holliston, MA, USA) at 30 mA/gel for approximately 12 h. The gels were stained with 0.2% (w/v) Coomassie Blue R-250 (Promega), in 5% (v/v) ethanol and 12% (w/v) trichloroacetic acid overnight and destained in tap water for 24 h.

Allele diversity

All cultivars were treated as pure lines. A small proportion of heterozygosity was observed, and the following criteria were used to define the working allele. In the case of SSRs, where cultivars displayed two bands with different intensities, only the stronger band was considered. Yet, if the two bands showed similar intensities, then the most frequent allele was considered. If none of these options could be applied, the sample was scored as missing data. In the case of biochemical (glutenins) and functional molecular markers, samples showing heterozygous alleles were scored as missing data. Rare alleles (with frequency lower than 5%) were treated as missing data for population structure. The effective number of alleles per locus was computed on the basis of common alleles as $n_e = 1/\Sigma p_i^2$ (Hartl and Clark, 1997). The estimate n_e represents the number of equally frequent alleles that would result in the same probability observed when randomly drawing two different alleles from the population. It is a measure of variability at the locus that takes into account both allele number and frequency. The polymorphism index content (PIC), a measure of allelic diversity, was calculated according to Nei's coefficient (Nei, 1973), PIC = $1-\Sigma(p_i^2)$, where p_i is the frequency of the ith polymorphism detected in the germplasm.

Population structure

Thirty-eight unlinked or distantly linked marker loci, distributed over all the wheat chromosomes, except 6A, 6B and 7B, were used for assessment of population structure. Population structure was investigated using a Bayesian clustering approach to infer the number of clusters (populations) with the softwares *Structure* v.2.3.3 (Pritchard *et al.*, 2000) and *Structure Harvester* (Evanno *et al.*, 2005; Earl

and von Holdt, 2012). No prior information was used to define the clusters, and the number of subpopulations (K) was set from 1 to 10, without admixture and with correlated allele frequencies, burn-in phase of 10^5 iterations, and a sampling phase of 2×10^5 replicates, runs with K = 1 to 10 were repeated 10 times (Falush *et al.*, 2003; Breseghello and Sorrells, 2006). This method estimates the proportion of the genomes of each individual derived from the different clusters and assigns individuals to subpopulations based on membership probability. We used the run that assigned all the cultivars to a single cluster at a probability > 0.50. The degree of differentiation of each subpopulation was measured by a modified F_{ST} parameter (Falush *et al.*, 2003). The program *Genetix* (Belkhir *et al.*, 1996-2004) was used to compute an overall F_{ST} (Weir and Cockerham, 1984) and to conduct multiple correspondence analysis, with three dimensions.

Results

The genetic diversity of a collection of 102 bread wheat cultivars from Argentina was assessed using 35 molecular markers (13 functional markers, 4 markers closely linked to genes, 17 SSR, and 1 ISBP) and 3 storage proteins (Table 1). A total number of 124 alleles was detected in the panel, including 21 rare alleles (with frequencies lower than 5% in the panel) that were discarded in the population structure studies. The number of alleles per locus varied between two and seven, with an average of 3.26. The average numbers of common (excluding rare alleles in the analysis) and effective alleles (n_e) were 2.65 (from 1 to 6) and 2.05 (from 1.01 to 4.76), respectively. Polymorphism information content (PIC) values obtained from the 38 polymorphic markers varied between 0.076 and 0.788, with an average of 0.458. The mean frequency of missing data was 0.43%, or 1.65% when rare alleles were included (Table 1).

We explored the population genetic structure among the accessions using a model-based method. A model-based method is a cluster analysis that evaluates genetic similarity among genotypes without using prior information. After a first analysis with the *Structure* program we could not determine precisely the K number (number of subgroups or subpopulations) in the population, as the curve of the Ln probability of data [L(K)], did not generate a plateau after K = 10 (Figure 1A). Therefore, we used the output of *Structure* as input data for *Structure Harvester*, now obtaining a clear peak with the highest ΔK value at K = 3 (Figure 1B). The analysis showed that three subpopulations were optimal for assigning all except 13 cultivars into one among the three clusters, with an *a posteriori* probability > 0.80. The 13 genotypes assigned to individual clusters with an *a posteriori* probability > 0.50 (but < 0.80) are underlined in Table 2. The three subpopulations K1, K2 and K3 included 17, 48 and 37 cultivars, respectively, with F_{ST} averages slightly higher in K1 (0.1939) than in K2 and K3 (0.1279 and 0.1218, respectively), this evidencing a moderated differentiation within subgroups. Furthermore, the F_{ST} value across subpopulations was 0.1485, indicating a moderate differentiation also between subgroups.

Figure 2 shows the projection of the multiple correspondence analysis (MCA) cloud on one orthogonal plane, with different symbols identifying each subpopulation according to the classification from Structure. The cloud was continuous, with three protrusions corresponding to the three subpopulations. In agreement with F_{ST} estimates, subpopulations 2 and 3 were less dispersed than 1.

Discussion

Genetic variability of the panel

The means for allele numbers and PIC values observed across all markers used in this work were 3.26 and 0.458 respectively (Table 1). These values were considerably lower than those observed for SSR loci (9.4 and 0.720; Manifesto *et al.*, 2001) and for storage proteins (5.00 and 0.544; Lerner *et al.*, 2009) for different but overlapping collections of wheat cultivars from Argentina. The mean allele number detected herein was also significantly lower than those revealed by SSR analysis in US wheat germplasm (4.8 and 7.2 alleles), reported by Breseghello and Sorrells (2006) and Chao *et al.* (2007) respectively. It was also lower than the 6.20 and 7.49 alleles observed by Plaschke *et al.* (1995) and Le Couviour *et al.* (2011) in European wheat germplasm, and the 5.4 alleles detected by Dreisigacker *et al.* (2004) in CIMMYT germplasm. Finally, Balfourier *et al.* (2007) working with a worldwide wheat collection of 3,942 entries from 73 countries detected the very high average value of 23.9 alleles per locus. These large differences in the number of alleles detected may be due to differences in the technologies used to detect polymorphism, as well as the type of molecular markers selected for the characterization (and/or the rate and amount of the germplasm evaluated).

Markers associated with traits of agronomic interest

In our study we selected a set of markers (biochemical, functional markers and closely linked to genes markers) related with relevant traits for breeding, like growth habit and/or vernalization response *(Vrn-A1, Vrn-B1* and *Vrn-D1)*, photoperiod sensitivity *(Ppd-D1)*, plant height *(Rht-B1, Rht-D1)*, grain texture *(PinA-D1)*, starch waxy proteins variants *(Wx-A1* and *Wx-B1)*, PPO activity *(Ppo-A1, Ppo-D1)*, variants of the Viviparous-1B gene *(Vp1-B3)* associated with pre-harvest tolerance (Yang *et al.*, 2007), low molecular weight glutenins *(Glu-A3)* and high molecular weight glutenins (Glu-A1, Glu-B1, Glu-D1)

The natural variation scanned with markers based on winter/spring allelic variants from *Vrn-A1, Vrn-B1* and *Vrn-D1* loci confirmed the spring growth habit as the best adapted for the wheat production area of Argentina (91 of

Table 1 - Biochemical and molecular markers used in the study, chromosome location, number of alleles, number of common alleles, effective allele number and polymorphic information content (for details see Supplementary Tables S2 and S3).

N	Locus	Marker[1]	Chr.	AN	NCA	n_e	PIC
1	Glu-A1	B	1A	4	3	2.19	0.554
2	Glu-B1	B	1B	7	6	3.30	0.696
3	Glu-D1	B	1D	3	2	1.01	0.162
4	*Vrn-A1*	F	5A	3	3	2.18	0.541
5	*Vrn-B1*	F	5B	2	2	1.98	0.495
6	*Vrn-D1*	F	5D	2	2	1.95	0.487
7	*Ppd-D1*	F	2D	2	2	2.01	0.512
8	*Rht-B1*	F	4B	2	2	1.88	0.467
9	*Rht-D1*	F	4D	2	2	1.71	0.415
10	*PinA-D1*	F	5D	2	2	1.99	0.496
11	*Glu-A3*	F	1A	7	6	4.76	0.788
12	*Wx-A1*	F	7A	2	2	1.10	0.093
13	*Wx-B1*	F	4A	3	2	1.34	0.304
14	*Vp1-B3*	F	3B	4	4	2.94	0.555
15	*Ppo-D1*	F	2A	2	2	1.77	0.488
16	*Ppo-A1*	F	2D	2	2	1.54	0.364
17	*Lr10*	CL	1A	3	3	2.36	0.584
18	*Lr24*	CL	3D	2	2	1.17	0.144
19	*Lr34*	CL	7D	2	2	1.46	0.315
20	*Ppd-B1*	CL	2B	2	2	1.54	0.349
21	*Xgwm124*	N	1B	4	1	1.08	0.076
22	*Xgwm493*	N	3B	4	3	2.68	0.641
23	*Xgwm295*	N	7D	4	2	1.27	0.274
24	*Xgwm265*	N	2A	3	2	1.74	0.436
25	*Barc174*	N	7A	5	5	2.78	0.646
26	*Xgwm261*	N	2D	4	4	1.88	0.468
27	*Xgwm219*	N	6B	3	3	2.51	0.602
28	*Xgwm374*	N	2B	2	2	1.56	0.359
29	*Xgwm156*	N	5A	4	4	2.39	0.581
30	*Barc70*	N	7A	6	4	3.86	0.747
31	*Xwmc147*	N	1D	2	2	2.00	0.508
32	*Xgwm111*	N	7D	5	2	1.39	0.386
33	*Xgwm113*	N	4B	2	2	1.61	0.379
34	*Xgwm264*	N	3B	3	3	2.58	0.612
35	*Xgwm533*	N	3D	4	2	1.35	0.315
36	*Wms5*	N	3A	4	4	3.39	0.704
37	*Xwmc44*	N	1B	5	3	2.07	0.561
38	*Cs37*	N	4D	2	2	1.44	0.303

[1]B: Biochemical, F: Functional, CL: Closely linked to genes, N: Neutral.

the tested cultivars carried at least one spring allele, considering *Vrn-A1*, *Vrn-B1* and *Vrn-D1* loci, *vs.* 11 cultivars with the triple winter alleles combination). These data agree with previous phenotypic (Appendino *et al.*, 2003) and molecular data (Fu *et al.*, 2005). We also noticed a higher frequency of the photoperiod insensitive (PI) alleles *Ppd-D1a*

and/or *Ppd-B1a* alleles (74 cultivars) than the combination of *Ppd-D1b* and *Ppd-B1b* alleles associated with photoperiod sensitivity (PS) (28 cultivars), this suggesting a better adaptation of photoperiod insensitivity to the environmental conditions in Argentina (between 27° and 38° S). A high frequency of PI alleles was also observed in low latitude re-
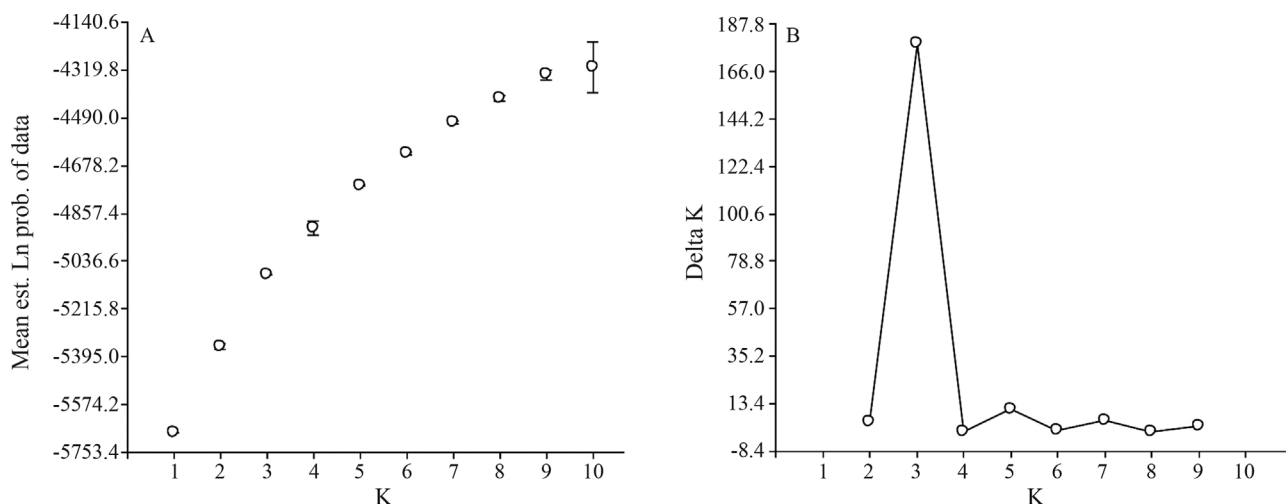
Figure 1 - Population genetic structure analysis of Argentinian wheat germplasm. (A) Mean L(K) (± SD) over 10 runs for each K value. The model considered here is a hierarchical island model using all 102 individuals per population and 38 loci. (B) ΔK calculated as $\Delta K = m|L''(K)|/s[L(K)]$. The modal value of this distribution is the true K or the uppermost level of structure, here 3 clusters.

Table 2 - Distribution of 102 Argentinean wheat cultivars in the three subpopulations detected using a model-based approach.

K	Cultivar name[*]
1	Baguette 10, Baguette 17, Baguette 18, Baguette 19, Baguette 21, Baguette 30, Baguette 31, Baguette 9, Baguette P. 11, BSYN 100, BSYN 200, BSYN 300, Klein 32, Klein Atlas, Klein Centauro, Sinvalocho, SRN Nogal
2	ACA 223, ACA 801, ACA 901, ACA 906, ACA 907, BIOINTA 1001, BIOINTA 1003, BIOINTA 1004, BIOINTA 1005, BIOINTA 1006, BIOINTA 3004, Buck AGP-FAST, Buck Huanchen, Buck Puelche, Don Mario Arex, Don Mario Atlax, Don Mario Cronox, Don Mario Onix, INIA Centinela, INIA Churrinche, INIA Torcaza, Klein Brujo, Klein Cacique, Klein Capricornio, Klein Carpincho, Klein Castor, Klein Don Enrique, Klein Escorpión, Klein Gavilán, Klein Gladiador, Klein Guerrero, Klein León, Klein Pantera, Klein Proteo, Klein Rayo, Klein Tauro, Klein Tigre, Klein Yarará, Klein Zorro, LE 2330, LE 2331, LE 2333, LE 2341, ProINTA Elite, ProINTA Guazú, ProINTA Isla Verde, ProINTA Oasis, Relmo Sirirí
3	ACA 201, ACA 202, ACA 320, ACA 903B, Barletta 77, BIOINTA 1000, BIOINTA 1002, BIOINTA 2001, BIOINTA 2004, BIOINTA 3003, BIOINTA 3005, Buck 55CL, Buck Aniversario, Buck Baqueano, Buck Bigua, Buck Brasil, Buck Chacarero, Buck Guapo, Buck Malevo, Buck Mangrullo, Buck Meteoro, Buck Napostá, Buck Norteño, Buck Pingo, Buck Pronto, Buck Ranquel, Buck Taita, Don Mario Themix, INIA Cóndor, Klein Chajá, Klein Impacto, Klein Nutria, Klein Rendidor, Marcos Juárez INTA, Olaeta Artillero, ProINTA Gaucho, ProINTA Granar

[*]Underlined cultivars were assigned to individual clusters with a p > 0.50, cultivars not underlined were assigned with a p > 0.80.

gions of Japan (36° N), associated also in this case with early flowering to avoid rains at harvest and preharvest sprouting (Seki *et al.*, 2011). Unlike this situation, Lanning *et al.* (2012) evaluated PS and PI spring near-isogenic lines (NILs) and observed better agronomic perfomance in PS NILs planted at higher latitudes (between 45° and 54° N) and considering early planting dates, as no difference between PS and PI lines occurred for the latest planting date. In the case of the dwarfing genes *Rht-B1* and *Rht-D1*, the most frequent allelic combinations were semi-dwarf phenotypes (37 *Rht-B1b*/*Rht-D1a* and 29 *Rht-B1a*/*Rht-D1b*) followed by tall phenotypes (35 *Rht-B1a*/*Rht-D1a*) and finally, only one dwarf phenotype (*Rht-B1b*/*Rht-D1b*). The reduced height alleles *Rht-B1b* and *Rht-D1b* are incorporated in breeding programs to reduce lodging (Flintham *et al.*, 1997) and increase the harvest index (Gooding *et al.*, 2012) under favorable environments, including problably, most fertile environments in Argentina.

These data would support a better adaptation of spring, photoperiod insensitive and semidwarf wheats to dominant environments in Argentina, however, a fine tuning evaluation of spring NILs carrying different combinations of vernalization, photoperiod insensitivity, as well as plant height alleles is still a pending issue.

In the case of markers closely linked to *Lr* genes, the most valuable information is perhaps, the relatively high number of cultivars that probably possess the adult plant leaf rust resistance gene *Lr34* (20 cultivars), a finding which agrees with Vanzetti *et al.* (2011). This gene has supported resistance to leaf rust in wheat for more than fifty years and is extensively used in breeding programs worldwide (Krattinger *et al.*, 2009).

Relevant information for bread-making quality can be the presence of the Glu-B1 7oe subunit associated with improved dough strength of wheat (Butow *et al.*, 2004) in ten cultivars. Valuable alleles for the development of culti-
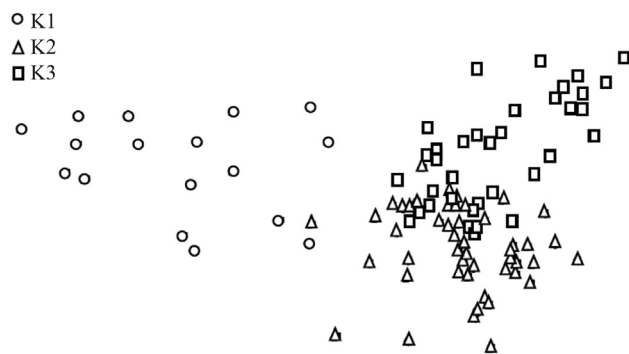
Figure 2 - Orthogonal projection of the cloud of points representing the genetic distance among cultivars, based on 38 unlinked molecular markers analyzed by multiple correspondence analysis. Subpopulations K1-K3 were inferred using Structure harvester.

vars with superior bread quality, partial waxy wheats, low PPO activity, and pre-harvest sprouting tolerance were also detected in the panel.

K = 3 is associated with the main breading programs in Argentina

In this work, using a model-based approach we detected three subpopulations in the collection of 102 Argentinean wheat cultivars. Our hypothesis is that this subpopulation division actually reflects the origin of the germplasm used by the main breeding programs in Argentina. For example, K1 is composed mainly (70.58%) by cultivars from Nidera and Syngenta breeding programs, as 100% of the cultivars tested and released by these companies were grouped only in K1. It is worthy of note that this germplasm (at least early materials released by Nidera (Bulos *et al.*, 2006), has a European origin, mainly from France, and was introduced gradually to Argentina since 1999. The K1 subpopulation also includes old cultivars from the Klein breeding program, like Klein 32 (released in 1932), Klein Atlas (1963) and Klein Centauro (1989) (Figure 3). K2 is mainly composed (60.41%) by cultivars from Klein, INIA and Don Mario breeding programs, the 72% of Klein,

87.5% of INIA and 80% of Don Mario tested cultivars were included in K2 (Figure 3). The cultivars grouped in K2 are basically (1) introductions and selections made in CIMMYT, as well as crosses made in Argentina, including CIMMYT material like Bobwhite, Kavkaz, Pastor, Seri, Veery and Weebill, (2) introductions from Brazil (some materials from Don Mario Breeding Company), and (3), to a lesser degree, materials selected from crosses including traditional germplasm from Argentina. Finally, K3 is composed mostly by cultivars belonging to the Buck Breeding Program (43.24%). INTA and ACA Breeding Programs have an even distribution of their tested cultivars between the K1 and K2 subpopulations (Figure 3). The cultivars grouped in K3 are mostly derived from traditional germplasm from Argentina and, to a lesser degree, from CIMMYT.

A similar type of grouping of cultivars by geographic origin and breeding history using a model-based approach was observed by Le Couviour *et al.* (2011) working with an elite wheat panel from Europe. They identified four subpopulations, including cultivars from UK, Germany and France divided into two subgroups and proposed that the separation between French, German and UK cultivars can be explained by the geographic origin and, in the case of France, the conformation of two subgroups as being due to the breeding history. Furthermore, Chao *et al.* (2007), when using a similar approach to analyze the genetic structure of U.S. wheat cultivars and breeding lines, found four subpopulations and suggested that the genetic diversity existing among these U.S. wheat germplasm was influenced by regional adaptation. Our data would suggest that in Argentina the most important factor explaining the genetic variability of adapted commercial bread cultivars would be the different core collections of germplasm used by the main breeding programs instead of geographic adaptation, as observed in Europe and US.

The results obtained in this paper are a very valuable source of information for breeding programs for the creation of novel combinations of alleles from genes involved
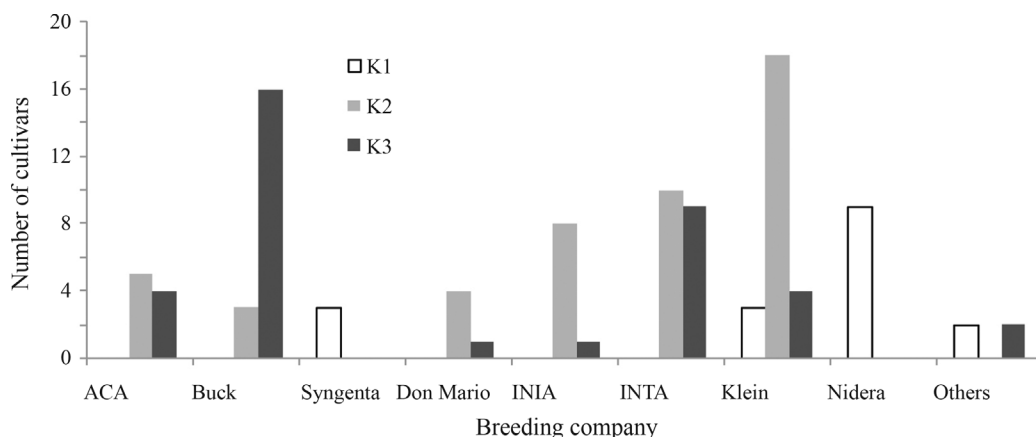

Figure 3 - Bar chart representing the frequency of cultivars belonging to each breeding company distributed in the subpopulations K1 to K3.

in adaptation, disease resistance and bread-making quality between other traits. Additionally, the genetic structure of the panel of cultivars analyzed in this study is being used as the starting point of association studies considering additional phenotypic traits of interest for the breeding like drought tolerance and yield components like kernel weight between others.

Acknowledgments

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Internet Resources

GrainGenes database, http://wheat.pw.usda.gov/GG2/index.shtml (September 15, 2012).

Structure software, http://pritch.bsd.uchicago.edu/structure.html (September 15, 2012).

Structure harvester, http://taylor0.biology.ucla.edu/structureHarvester/ (September 15, 2012).

Genetix software, http://kimura.univ-montp2.fr/genetix/ (September 15, 2012).

Supplementary Material

The following online material is available for this article:

Table S1 - Molecular markers used in the study.

Table S2 - Biochemical and functional molecular markers in Argentinean wheat cultivars.

Table S3 - Neutral molecular markers in Argentinean wheat cultivars.

This material is available as part of the online article from http://www.scielo.br/gmb.

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