

Genetic variability of Brazilian wheat germplasm obtained by high-density SNP genotyping

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Abstract: *The aim of this study was to evaluate the genetic diversity of the wheat germplasm using high-density genotyping with SNP markers. A set of 211 wheat varieties genotyped with 35,142 SNP markers were used in the experiment. Genetic distances ranged from 0.013 to 0.471, with the highest frequency of distances varying between 0.31 and 0.40. In the cluster analysis by the UPGMA method, 81% of the varieties were clustered in three groups. Genetic variability in the Brazilian wheat germplasm has remained constant for over 70 years. Mean genetic distances among the varieties developed in each decade ranged from 0.33 to 0.34. A trend of genetic distance between genotypes from different eras has been observed over time as a result of breeding. Results described in this study can help Brazilian wheat breeders to manage more adequately genetic variability in the Brazilian wheat germplasm.*

Keywords: *Triticum aestivum (L.), genetic variability, genotypes from different eras, breeding.*

INTRODUCTION

One of the features of plant breeding in Brazil is the possibility of using commercial varieties in crosses regardless of the intellectual property associated with them (Riede et al. 2001). This is the so-called breeder's right, provided for by the Plant Variety Protection (PVP) Law in Brazil (Law 9456, from 1997). This possibility allows the sharing of genetic variability between the various breeding programs. Varieties developed by a breeding program can be used as germplasm source by other breeding programs.

Genetic variability is the foundation of breeding. Breeding programs are aimed at exploiting the genetic variability of species to obtain genetic combinations of adapted, high yielding, disease-resistant, and higher-quality varieties, in addition to other characteristics. Although genetic variability can be increased through the introduction of exotic germplasm, only a fraction of this variability is useful in breeding. The most part of the exotic genome is not adapted and must be eliminated after being introduced as a source of variability, through successive breeding cycles. For this reason, in most cases, breeding programs use exclusively germplasm already improved for the generation of breeding populations and variability.

Because the genetic variability used in breeding programs is only a fraction of the variability present in the species, knowing the existing variability in the

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germplasm used by breeding programs is essential for the rational use of this germplasm. Further, it is essential to monitor variability over the years, since short-term monitoring may erroneously lead to biases in the interpretation of the data towards reduction of variability.

Molecular markers have been one of the main tools employed in studies of genetic variability (Caixeta et al. 2009, Cruz et al. 2014). Among them, Single Nucleotide Polymorphism (SNP) markers stand out for their abundance in the genome of species; their high automation capacity; and the existence of high-density SNP microarrays for the many species of interest to the breeder. Wheat germplasm evaluation with SNP markers is just beginning, and only few works have been published using this marker in wheat, e.g. Shavrukov (2014) in Kazakstan.

In view of the management and preservation of the existing genetic variability in the Brazilian wheat germplasm, the present study was conducted to analyze the genetic variability present in the Brazilian wheat germplasm by using, for the first time, high-density SNP markers. Additionally, this study examined the evolution of genetic variability in the Brazilian wheat germplasm over four decades.

MATERIAL AND METHODS

Genetic material

This work was conducted in the biotechnology laboratory of Coodetec, in Cascavel, PR, Brazil. The genetic material was composed by 185 varieties and one elite line of wheat [*Triticum aestivum* (L.)] developed in Brazil; 11 varieties from Paraguay; seven from Mexico; six from China; and one from Argentina, totaling 211 varieties (Table 1). Among the Brazilian varieties, twenty-one were developed before 1980, thirty-five were developed in the 1980s, twenty-four in the 1990s, sixty-four in the 2000s, and forty-one in the 2010s.

DNA extraction and genotyping of SNP markers

DNA was extracted according to the protocol described by Schuster et al. (2004). DNA samples were genotyped using the Axiom™ WhtBrd-1 Array kit, which contained 35,143 SNP markers, at Affymetrix Company (Santa Clara, CA, USA). All information pertaining to the SNP present in the platform can be accessed at <http://www.cerealsdb.uk.net>.

After genotyping, the obtained data were filtered in Excel sheet to discard monomorphic markers, markers that did not have one of the homozygous genotypes, markers with a call rate lower than 90% (over 10% of missing data), markers with minimum allele frequency lower than 5%, and markers with over 30% heterozygous genotypes among the 211 varieties of wheat used.

Analysis of genetic variability

Genetic distances among the wheat varieties were obtained by 1-IBS (identity by state), where IBS is defined as the probability of the alleles observed in the same locus in two individuals being the same at random. Therefore, the distance between one individual and itself is defined as 0.

This estimate is based on the following definition: for a bi-allelic locus with A and B alleles, the probability of IBS, $p_{IBS}(AA, AA) = 1$, $p_{IBS}(AA, BB) = 0$, $p_{IBS}(AB, xx) = 0.5$, where xx is any genotype other than AB . For two taxa, p_{IBS} is obtained as the average of all loci without lost data. The estimates of genetic distance among the wheat varieties were obtained using Tassel software (Bradbury et al. 2007).

Genetic distances among the groups of varieties developed in each period were obtained by Rogers' genetic distance estimator:

$$D_R = \frac{1}{m} \sum_{i=1}^m \sqrt{\frac{1}{2} \sum_{j=1}^{n_i} (p_{ij} - q_{ij})^2}$$

Where m is the number of markers; n_i is the number of alleles in marker i ; and p_{ij} and q_{ij} are the frequencies of allele j in marker i in the pairs of eras considered in each comparison of group of genotypes. Rogers' genetic distances were obtained using an Excel^(TM) spreadsheet.

Table 1. List of varieties used in the study of genetic variability with high-density SNP markers

Variety	Company	Year	Country	Variety	Company	Year	Country
Estrela Atria	Biotrigo	2013	Brazil	Ocepar 6 Flamingo	Coodetec	1983	Brazil
FPS Nitron	Biotrigo	2011	Brazil	Ocepar 7 Baturai	Coodetec	1984	Brazil
TBIO Alvorada	Biotrigo	2012	Brazil	Ocepar 8 Macuco	Coodetec	1984	Brazil
TBIO Bandeirante	Biotrigo	2012	Brazil	Ocepar 9 Perdiz	Coodetec	1984	Brazil
TBIO Iguaçú	Biotrigo	2012	Brazil	Pavão	Coodetec	1982	Brazil
TBIO Itaipu	Biotrigo	2012	Brazil	Tucano	Coodetec	1980	Brazil
TBIO Mestre	Biotrigo	2012	Brazil	JF90	DNA Melhoramento Vegetal	2012	Brazil
TBIO Pioneiro	Biotrigo	2010	Brazil	Frontana	EEF de Bagé	1940	Brazil
TBIO Seleteo	Biotrigo	2012	Brazil	Encruzilhada	EEF de Encruzilhada do Sul	1970	Brazil
TBIO Sinuelo	Biotrigo	2012	Brazil	Colonias	EEF de Veranópolis	1949	Brazil
TBIO Tibagi	Biotrigo	2010	Brazil	Vacaria	EEF de Veranópolis	1976	Brazil
TBIO Toruk	Biotrigo	2014	Brazil	BR 18 Terena	Embrapa Agropecuária Oeste	1986	Brazil
Candeias	Coodetec	1982	Brazil	BR 30 Cadiveu	Embrapa Agropecuária Oeste	1988	Brazil
CD 101	Coodetec	1997	Brazil	BRS 207	Embrapa Cerrados	1999	Brazil
CD 102	Coodetec	1996	Brazil	BRS 254	Embrapa Cerrados	2005	Brazil
CD 103	Coodetec	1998	Brazil	BRS 264	Embrapa Cerrados	2005	Brazil
CD 104	Coodetec	1999	Brazil	EMBRAPA 21	Embrapa Cerrados	1993	Brazil
CD 105	Coodetec	1999	Brazil	EMBRAPA 42	Embrapa Cerrados	1995	Brazil
CD 106	Coodetec	2000	Brazil	BRS 193	Embrapa Soja	2000	Brazil
CD 107	Coodetec	2002	Brazil	BRS 208	Embrapa Soja	2001	Brazil
CD 108	Coodetec	2003	Brazil	BRS 210	Embrapa Soja	2002	Brazil
CD 109	Coodetec	2003	Brazil	BRS 220	Embrapa Soja	2003	Brazil
CD 110	Coodetec	2003	Brazil	BRS 229	Embrapa Soja	2004	Brazil
CD 1104	Coodetec	2014	Brazil	BRS Albatroz	Embrapa Soja	2011	Brazil
CD 111	Coodetec	2003	Brazil	BRS Pardela	Embrapa Soja	2007	Brazil
CD 112	Coodetec	2004	Brazil	BRS Sabiá	Embrapa Soja	2013	Brazil
CD 113	Coodetec	2004	Brazil	BR 1	Embrapa Trigo	1979	Brazil
CD 114	Coodetec	2004	Brazil	BR 14	Embrapa Trigo	1985	Brazil
CD 1144*	Coodetec	#	Brazil	BR 15	Embrapa Trigo	1985	Brazil
CD 115	Coodetec	2005	Brazil	BR 2	Embrapa Trigo	1979	Brazil
CD 116	Coodetec	2006	Brazil	BR 23	Embrapa Trigo	1987	Brazil
CD 117	Coodetec	2007	Brazil	BR 27	Embrapa Trigo	1988	Brazil
CD 118	Coodetec	2008	Brazil	BR 28	Embrapa Trigo	1988	Brazil
CD 119	Coodetec	2009	Brazil	BR 34	Embrapa Trigo	1989	Brazil
CD 120	Coodetec	2009	Brazil	BR 37	Embrapa Trigo	1990	Brazil
CD 121	Coodetec	2010	Brazil	BR 4	Embrapa Trigo	1979	Brazil
CD 122	Coodetec	2010	Brazil	BR 43	Embrapa Trigo	1991	Brazil
CD 123	Coodetec	2010	Brazil	BR 5	Embrapa Trigo	1980	Brazil
CD 124	Coodetec	2012	Brazil	BR 7	Embrapa Trigo	1981	Brazil
CD 1252	Coodetec	2012	Brazil	BR 8	Embrapa Trigo	1983	Brazil
CD 1440	Coodetec	2013	Brazil	BRS 120	Embrapa Trigo	1997	Brazil
CD 150	Coodetec	2009	Brazil	BRS 177	Embrapa Trigo	1999	Brazil
CD 154	Coodetec	2012	Brazil	BRS 179	Embrapa Trigo	1999	Brazil
CD 1550	Coodetec	2012	Brazil	BRS 276	Embrapa Trigo	2008	Brazil
CD 1805	Coodetec	2014	Brazil	BRS 296	Embrapa Trigo	2009	Brazil
Esporão	Coodetec	2014	Brazil	BRS 327	Embrapa Trigo	2010	Brazil
Jandaia	Coodetec	1981	Brazil	BRS 328	Embrapa Trigo	2012	Brazil
Nambu	Coodetec	1979	Brazil	BRS 329	Embrapa Trigo	2012	Brazil
Ocepar 10 Garça	Coodetec	1984	Brazil	BRS 331	Embrapa Trigo	2012	Brazil
Ocepar 11 Juriti	Coodetec	1984	Brazil	BRS 49	Embrapa Trigo	1996	Brazil
Ocepar 12 Maitaca	Coodetec	1985	Brazil	BRS Angico	Embrapa Trigo	2002	Brazil
Ocepar 13 Acauã	Coodetec	1985	Brazil	BRS Camboatá	Embrapa Trigo	2003	Brazil
Ocepar 14	Coodetec	1988	Brazil	BRS Camboin	Embrapa Trigo	2004	Brazil
Ocepar 15	Coodetec	1988	Brazil	BRS Guamirim	Embrapa Trigo	2005	Brazil
Ocepar 16	Coodetec	1989	Brazil	BRS Louro	Embrapa Trigo	2003	Brazil
Ocepar 17	Coodetec	1989	Brazil	BRS Parrudo	Embrapa Trigo	2012	Brazil
Ocepar 18	Coodetec	1990	Brazil	BRS Timbaúva	Embrapa Trigo	2002	Brazil
Ocepar 19	Coodetec	1990	Brazil	BRS Umbu	Embrapa Trigo	2003	Brazil
Ocepar 20	Coodetec	1990	Brazil	CNT 1	Embrapa Trigo	1975	Brazil
Ocepar 22	Coodetec	1994	Brazil	CNT 10	Embrapa Trigo	1977	Brazil

Variety	Company	Year	Country	Variety	Company	Year	Country
CNT 5	Embrapa Trigo	1976	Brazil	Alcover	OR Sementes	2000	Brazil
CNT 6	Embrapa Trigo	1976	Brazil	Ametista	OR Sementes	2011	Brazil
CNT 7	Embrapa Trigo	1976	Brazil	Avante	OR Sementes	2001	Brazil
CNT 9	Embrapa Trigo	1977	Brazil	Campeiro	OR Sementes	2009	Brazil
MGS 1 Aliança	Epamig	1999	Brazil	Jadeite 11	OR Sementes	2012	Brazil
CEP 14 Tapes	Fundacep	1985	Brazil	Marfim	OR Sementes	2007	Brazil
CEP 29	Fundacep	2001	Brazil	Mirante	OR Sementes	2008	Brazil
CEP 72 Sulino	Fundacep	1982	Brazil	Onix	OR Sementes	2001	Brazil
Fundacep 30	Fundacep	1999	Brazil	Pampeano	OR Sementes	2003	Brazil
Fundacep 37	Fundacep	2002	Brazil	Quartz	OR Sementes	2007	Brazil
Fundacep 40	Fundacep	2002	Brazil	Rubi	OR Sementes	1998	Brazil
Fundacep 50	Fundacep	2005	Brazil	Safira	OR Sementes	2004	Brazil
Fundacep 52	Fundacep	2005	Brazil	Supera	OR Sementes	2004	Brazil
Fundacep Bravo	Fundacep	2010	Brazil	Taurum	OR Sementes	1998	Brazil
Fundacep Campo Real	Fundacep	2009	Brazil	Topázio	OR Sementes	2011	Brazil
Fundacep Cristalino	Fundacep	2006	Brazil	Turquesa	OR Sementes	2011	Brazil
Fundacep Horizonte	Fundacep	2009	Brazil	Valente	OR Sementes	2004	Brazil
Fundacep Nova Era	Fundacep	2004	Brazil	Vanguarda	OR Sementes	2008	Brazil
Fundacep Raízes	Fundacep	2006	Brazil	Vaqueano	OR Sementes	2008	Brazil
TEC Frontale	Fundacep	2012	Brazil	Bandeirantes	Unknown origin	1949	Brazil
TEC Triunfo	Fundacep	2012	Brazil	Pasuco	Introduction	#	Argentina
TEC Veloce	Fundacep	2012	Brazil	Canindé 11	IPTA	2010	Paraguay
TEC Vigore	Fundacep	2012	Brazil	Canindé 12	IPTA	2010	Paraguay
IAC 375 Parintins	IAC	2003	Brazil	Canindé 13	IPTA	2010	Paraguay
IAC 5 Maringá	IAC	1966	Brazil	Canindé 3	IPTA	2007	Paraguay
Altar	IAPAR	2000	Brazil	IAN 10	CAPECO/INBIO	#	Paraguay
IAPAR 1 Mitacoré	IAPAR	1980	Brazil	IAN15	CAPECO/INBIO	#	Paraguay
IAPAR 17 Caeté	IAPAR	1986	Brazil	Itapua 40	CAPECO/INBIO	#	Paraguay
IAPAR 28 Igapó	IAPAR	1988	Brazil	Itapua 45	CAPECO/INBIO	#	Paraguay
IAPAR 3 Aracatu	IAPAR	1981	Brazil	Itapua 50	CAPECO/INBIO	#	Paraguay
IAPAR 30 Pirata	IAPAR	1988	Brazil	Itapua 60	CAPECO/INBIO	#	Paraguay
IAPAR 6 Tapejara	IAPAR	1982	Brazil	Itapua 70	CAPECO/INBIO	#	Paraguay
IAPAR 78	IAPAR	1996	Brazil	Anahuac	Introduction/CIMMYT	1981	Mexico
IPR 128	IAPAR	2006	Brazil	Cocoraque	Introduction/CIMMYT	1981	Mexico
IPR 129	IAPAR	2006	Brazil	Jupateca 73	Introduction/CIMMYT	1978	Mexico
IPR 130	IAPAR	2007	Brazil	Kosoro	Introduction/CIMMYT	#	Mexico
IPR 136	IAPAR	2007	Brazil	Moucho	Introduction/CIMMYT	#	Mexico
IPR 144	IAPAR	2009	Brazil	Sonora 63	Introduction/CIMMYT	1972	Mexico
IPR 85	IAPAR	1999	Brazil	Sonora 64	Introduction/CIMMYT	1975	Mexico
IPR Katuara	IAPAR	2012	Brazil	Chinese Spring	Introduction	#	China
IAS 54	IAS/IPEAS	1970	Brazil	NING 7840	Introduction	#	China
IAS 58	IAS/IPEAS	1972	Brazil	Shangai 5	Introduction	#	China
IAS 64	IAS/IPEAS	1974	Brazil	Suzhoe #2	Introduction	#	China
Londrina	IAS/IPEAS	1972	Brazil	Suzhoe #6	Introduction	#	China
Confiança	IPB	1977	Brazil	Suzhoe #8	Introduction	#	China
Abalone	OR Sementes	2006	Brazil				

¹ Variety's registration year. * Elite line.

The cluster analyses between varieties and between genotypes of different eras were performed by the UPGMA hierarchical method, using JMP software (SAS Institute 2015).

RESULTS AND DISCUSSION

Of the 35,143 markers used, 10,049 met the quality requirements, accounting for 28.6% of the total number of markers present in the SNP panel. In the set of useful markers, the number of markers per chromosome ranged from

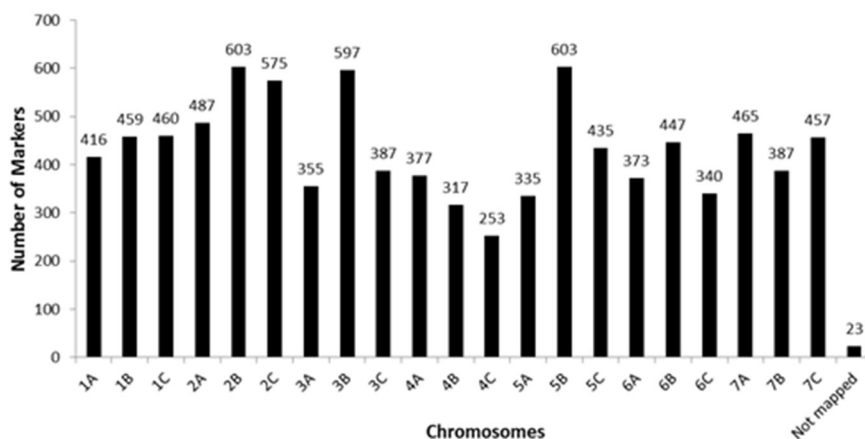


Figure 1. Distribution of the 10,049 SNP markers used to estimate the genetic distances in the 211 wheat chromosomes.

207 in chromosome 4D to 767 in chromosome 5B (Figure 1), averaging 478.5 markers per chromosome. The number of markers observed in each wheat genome was 3,429 in genome A (34%), 3,808 in genome B (38%), and 2,762 in genome D (28%). Fifty markers have not yet been mapped in the wheat genome.

Shavrukov et al. (2014) used wheat varieties from Kazakhstan and the SNP platform Infinium 9k (Illumina) for wheat and identified 46% informative markers. In their work, 49% were associated with the A genome, 46% with the B genome, and 5% with the D genome. In addition to being larger, the marker panel used in the present study is better distributed across the three wheat genomes.

The estimates of genetic distances among the 211 varieties ranged from 0.013 (between ‘BRS 193’ and ‘Tucano’) to 0.471 (between ‘Caniné 13’ and ‘BR 34’). Of all distances, 71.34% lay between 0.31 and 0.40 (Figure 2). Khan et al. (2015) evaluated the variability existing in a collection of 95 tetraploid and hexaploid varieties of wheat from India and Turkey. The genetic distances obtained among hexaploid varieties varied from 0.02 to 0.29 in India and from 0.05 to 0.58 in Turkey, which is a similar level of variability to that obtained in this study.

In a previous study, we investigated the genetic variability of a set of 36 Brazilian varieties of wheat using microsatellite markers, and identified genetic distances of 0.10 to 0.88 (Schuster et al. 2009). Bered et al. (2001) also evaluated the variability of Brazilian wheat varieties, using RAPD markers, and observed distances from zero to 0.32. In these two studies, a small number of varieties and a small number of markers was used, resulting in higher estimates of variability in one case and lower estimates in the other. Here, we used a broader coverage of the genome, obtained from the larger quantity of markers used, which made it possible to evaluate more accurately the real genetic variability situation of the wheat.

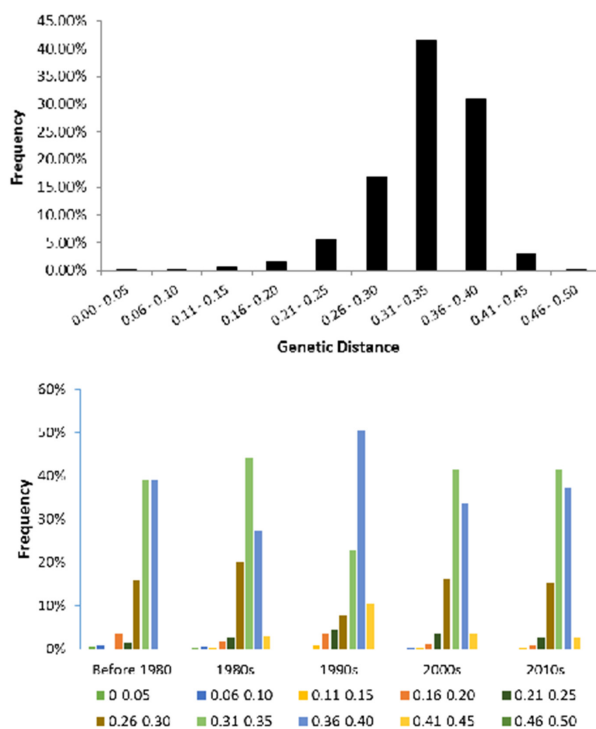
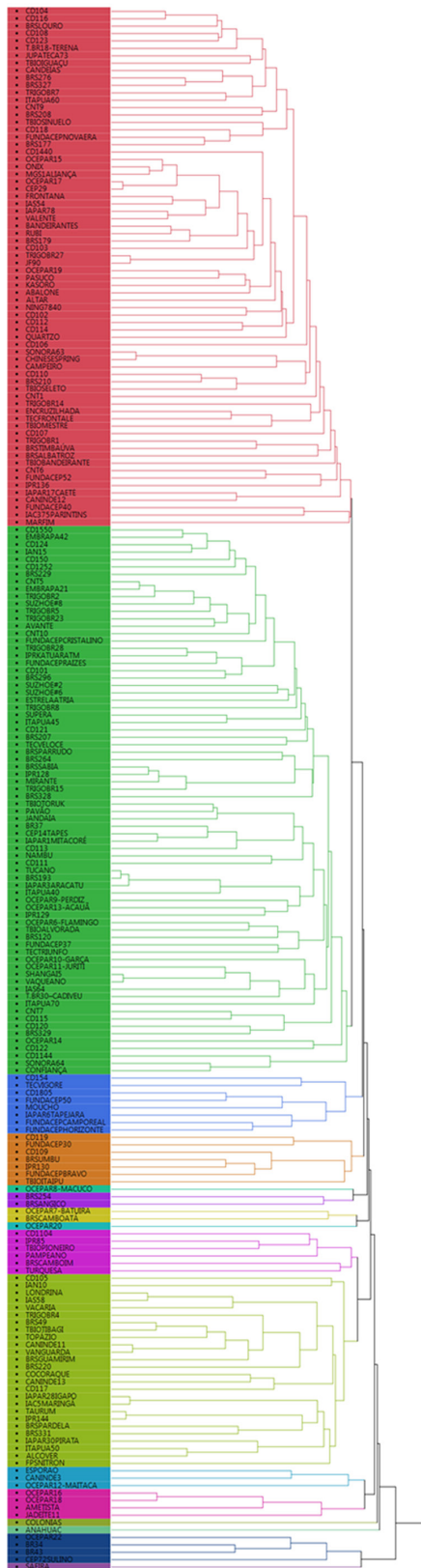


Figure 2. Frequency distribution of genetic distances among 211 wheat varieties using 10,049 SNP markers. A. Set of 211 varieties. B. Set of 185 Brazilian varieties, grouped by release decade.



Cluster analysis by the UPGMA method (Figure 3) had 12 groups formed containing more than one variety. The two largest groups contained 75 and 71 varieties, which represents 69% of the 211 varieties (Table 2). The third largest group comprised 26 varieties (group 10 in Table 2), with 172 (81%) of the set of 211 varieties clustered in these three groups. The other groups contained two to eight varieties. Five varieties did not cluster with any other (Ocepar 8 Macuco, Ocepar 20, Colonias, Anahuac, and Safira).

No relationships were observed between the groups and the breeding program of origin of the germplasm; i.e., the genetic variability observed in the wheat germplasm in Brazil is equally distributed across the local wheat breeding programs. The varieties developed by the institutions owning the largest number of wheat varieties in Brazil (Coodetec, Embrapa, OR Sementes, Fundacep, Iapar, and Biotrigo) are equivalently represented in the largest groups. This is a consequence of the so-called breeder's right, provided for in the PVP Law in Brazil (Law 9456 of 1997). Article 10 (subsection III) of that law allows breeders to use commercial varieties of any origin to perform crosses and originate new varieties. In this way, there is some sort of germplasm being shared across the many breeding programs, and the genetic base of breeding programs may be similar.

Varieties developed in Paraguay and Mexico were distributed across the groups proportionally to the size of these groups. The exception was Mexican variety Anahuac, which did not cluster with any other variety. This was an expected result, since the development of wheat varieties in Brazil involved frequent use of the germplasm developed by CIMMYT (Mexico). In Paraguay, wheat varieties are developed using both the CIMMYT germplasm and Brazilian varieties. The six varieties introduced from China were clustered in the two largest groups. This means there is not a clear distinction between the wheat germplasms from Mexico, China, and Paraguay, when compared with the Brazilian wheat germplasm.

To evaluate the evolution of genetic variability in wheat over time in Brazil, we considered only the 185 varieties developed in the country. Twenty-one varieties were developed before 1980. Genetic distances among these varieties ranged from 0.05 to 0.40, averaging 0.33. The highest frequency of genetic distances among the varieties developed in Brazil before 1980 was between 0.30 and 0.40 (Figure 2); 78% of genetic distances were above 0.30 and over 93% of them were higher than 0.25.

In the group of Brazilian varieties, 35 were developed in the 1980s. Genetic distances among these varieties varied from 0.02 to 0.45, averaging 0.33. The highest frequency of genetic distances among the wheat varieties developed in the 1980s in Brazil lay in the range of 0.31 to 0.35 (Figure 2). Over 74% of genetic distances among the varieties developed in that period were higher than 0.30 and more than 92% were higher than 0.25.

In the 24 varieties developed in the 1990s, genetic distances ranged from 0.13 to 0.45, averaging 0.35. The highest frequency of genetic distances between the varieties developed in that decade was between 0.36 and

Figure 3. Clustering of 211 wheat varieties including 190 Brazilian and 31 introduced varieties obtained by UPGMA.

Table 2. Clustering of 211 wheat varieties by UPGMA analysis based on the genetic distances obtained by high-density SNP marker data

Cluster	Variety					
1	CD 104	CD 1440	JF 90	Quartzo	TBIO Mestre	
	CD 116	Ocepar 15	Ocepar 19	Suzhoe #6	Estrela Atria	
	BRS Louro	Onix	Pasuco	CD 106	BR 1	
	CD 108	MG5 1 Aliança	Kosoro	Sonora 63	BRS Timbaúva	
	CD 123	Ocepar 17	Abalone	Chinese Spring	BRS Albatroz	
	BR 18 Terena	CEP 29	Altar	Campeiro	TBIO Bandeirante	
	Jupateca 73	Frontana	Ning 7840	CD 110	CNT 6	
	TBIO Iguaçu	IAS 54	CD 102	BRS 210	Fundacep 52	
	Candeias	Iapar 78	CD 112	TBIO Seleto	IPR 136	
	BRS 276	Valente	CD 114	BR 14	Iapar 17 Caeté	
	BRS 327	Bandeirantes	TBIO Sinuelo	BRS 328	Canindé 12	
	BR 7	Rubi	CD 118	CNT 1	CD 109	
	Itapua 60	BRS 179	Fundacep Nova Era	CD 107	BRS Umbu	
	CNT 9	CD 103	BRS 177	Encruzilhada	IPR 130	
	BRS 208	BR 27	Suzhoe #2	TEC Frontale	Fundacep Bravo	
	2	CD 1550	BR 28	BRS Parrudo	CD 111	Itapua 70
		Embrapa 42	IPR Catuara	BRS 264	Tucano	Ocepar 6 Flamingo
		CD 124	Fundacep Raízes	BRS Sabiá	BRS 193	TBIO Alvorada
		IAN 15	CD 101	IPR 128	Iapar 3 Aracatu	BRS 120
CD 150		BRS 296	Mirante	Itapua 40	Fundacep 37	
CD 1252		CNT 10	BR 15	Ocepar 9 Perdiz	TEC Triunfo	
BRS 229		CNT 7	TBIO Toruk	Ocepar 13 Acauã	Turquesa	
CNT 5		CD 5	Pavão	IPR 129	Ocepar 14	
Embrapa 21		BR 8	Jandaia	Ocepar 10 Garça	CD 122	
BR 2		BRS 207	BR 37	Ocepar 11 Juriti	CD 1144	
Suzhoe #8		TEC Veloce	CEP 14 Tapes	Shangai 5	Sonora 64	
BR 5		CD 121	Iapar 1 Mitacoré	Vaqueano	Confiança	
BR 23		Supera	CD 113	IAS 64	CD 120	
Avante		Itapua 45	Nambu	BR 30 Cadiveu	BRS 329	
Fundacep Cristalino						
3		Fundacep 40	IAC 375 Parintins	Marfim		
4	CD 154	CD 1805	Moucho	Fundacep Campo Real		
	Fundacep Horizonte	TEC Vigore	Fundacep 50	Iapar 6 Tapejara		
#	Ocepar 8 Macuco					
5	BRS 254	BRS Angico				
6	Ocepar 7 Batuíra	BRS Camboatá				
#	Ocepar 20					
7	CD 1104	IPR 85	TBIO Pioneiro	Pampeano	BRS Camboim	
	CD 105	BRS 49	BRS Guamirim	Iapar 28 Igapó	BRS 331	
	IAN 10	TBIO Tibagi	BRS 220	IAC 5 Maringá	Iapar 30 Pirata	
	Londrina	Topázio	Cocoraque	Taurum	Itapua 50	
	IAS 58	Canindé 11	Canindé 13	IPR 144	Alcover	
	Vacaria	Vanguarda	CD 117	BRS Pardela	FPS Nitron	
BR 4						
9	CD 119	Fundacep 30	TBIO Itaipu			
10	Esporão	Canindé 3	Ocepar 12 Maitaca			
11	Ocepar 16	Ocepar 18	Ametista	Jadeíte 11		
#	Colonias					
#	Anahuac					
12	Ocepar 22	BR 34	BR 43	CEP 72 Sulino		
#	Safira					

0.40 (Figure 2). More than 83% of the genetic distances exceeded 0.30, and over 90% of them were greater than 0.25.

Genetic distances among the 64 varieties developed in the 2000s varied from 0.07 to 0.45, averaging 0.33. The highest frequency of genetic distances among the varieties developed in the 2000s was between 0.30 and 0.35; however, a high frequency of distances between 0.36 and 0.40 was also observed (Figure 2). Over 78% of the varieties developed in this period exhibited genetic distances greater than 0.3, and more than 94% were above 0.25.

For the 41 varieties developed in the 2010s until the year 2014, genetic distances ranged from 0.12 to 0.43, averaging 0.34. The highest frequency of genetic distances observed in these newly developed varieties was between 0.31 and 0.35, but there was also a high frequency of distances between 0.36 and 0.40 (Figure 2). More than 81% of the genetic distances observed in this group of varieties was above 0.30, with over 96% higher than 0.25.

In the last four decades, the average genetic distances among wheat varieties in Brazil have remained between 0.33 and 0.34, and maximum distances between 0.43 and 0.45. Before the 1980s, the maximum genetic distance was 0.40. From the 1990s onwards, there has been a trend towards an increase in minimum genetic distances among the wheat varieties developed in Brazil. This means that genetic variability among the wheat varieties produced in Brazil have had a slight upward trend in variability for more than 40 years, as observed by the increasing minimum distances among the recently developed varieties.

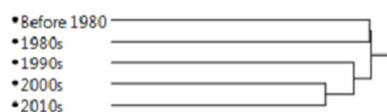
Analyses of genetic variability based on pedigree or morphological data usually demonstrate a reduction of genetic variability over time. The narrowing of genetic variability would typically occur in stages: initially, by the substitution of local varieties (landraces) for improved varieties, followed by modern breeding practices, especially through interspecific breeding of a small group of elite varieties.

Recent studies investigating genetic variability at DNA level have shown maintenance and, in some cases, increases in genetic variability with time. Manifesto et al. (2001) also observed that genetic variability was kept the same in Argentinean wheat varieties developed between the 1960s and the 1990s. In central and northern Europe, Huang et al. (2007) detected increased genetic variability among wheat varieties developed between 1950 and 1990. The same was noted by Balyan et al. (2008) in wheat varieties developed in India between 1910 and 2006; by Khlestkina et al. (2004) in Europe and Asia in wheat varieties developed from the 1920s to the 1980s (Austria), from the 1940s to the 1990s (Albania), from the 1930s to the 1970s (India), and from the 1930s to the 1970s (Nepal).

In CIMMYT, Reif et al. (2005) and Warburton et al. (2006) observed an increase in genetic variability in wheat since the 1990s. Huang et al. (2007) reported an increase in genetic variability of wheat since the 1950s in the United Kingdom, and Hysing et al. (2008) also described the same in United Kingdom since the 1970s. Prasad et al. (2009) reported an increase in genetic variability in the United States since the 1970s. Fu and Sommers (2009), on the other hand, observed a reduction in genetic variability over time in wheat varieties developed in Canada between 1845 and 2004.

Genetic variability among the genotypes of different eras represented by the varieties developed in Brazil in each of the decades mentioned here ranged from 0.06 to 0.08. In spite of the small distances, a gradual evolution can be seen in these eras. The gene sets developed in the 2000s and in the 2010s are the closest, and both are closer to the gene set developed in the 1990s. The genotypes of these three eras are slightly farther from the genotypes developed in the 1980s and before (Figure 4). This demonstrates that although genetic variability was maintained, the current group of genotypes are farther from the older group of genotypes, suggesting that the germplasm is being modified (improved) by the breeding program without having its variability reduced.

In theory, plant breeding causes narrowing of genetic



	Before 1980	1980s	1990s	2000s	2010s
Before 1980	0.00				
1980s	0.08	0.00			
1990s	0.08	0.08	0.00		
2000s	0.07	0.07	0.07	0.00	
2010s	0.08	0.08	0.07	0.06	0.00

Figure 4. Genetic distances and UPGMA cluster analysis among Brazilian wheat varieties as a function of the time they were developed.

variability, because all breeding programs select the new germplasm in the same direction; i.e., high yields, disease resistance, grain quality, narrow maturity group interval, uniform plant high, and other characteristics. Crossing few number elite lines to generate new breeding populations results in increased inbreeding, which means, in theory, a reduction of genetic variability. Genetic distances between the individuals estimated by pedigree is based on probability. The probability of similarity of each pair of sisters, for instance, is the same. This means we need to assume that all pairs of sisters have the same genetic distance, which is not true. Accessing the genetic differences between the individuals through molecular markers makes it possible to quantify the real number of differences between individuals. To accurately estimate genetic variability, molecular markers need to adequately cover the genome of the species. High-density genotyping is the best way to better cover the genome with molecular markers.

Using estimates of genetic variability based on molecular markers also allows for better exploring variability, because molecular markers can reveal variability that cannot be accessed by other ways. In this work, we demonstrate that the wheat germplasm being used in Brazil has a good level of variability, and this variability has been maintained in the last four decades. Introducing germplasm and using commercial varieties from other companies is part of the strategy used in Brazil, and this needs to be continued to avoid the narrowing of variability in Brazilian wheat.

CONCLUSION

Wheat variability in Brazil has been maintained in the last four decades. The approach used by Brazilian breeders is effective in avoiding reducing genetic variability while increasing performance. Germplasm introduction and the possibility of breeders freely using commercial varieties from other companies as a source of variability, in crosses, could be the main reasons to allow the maintenance of genetic variability.

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