

## ARTICLE

# Parental selection of wheat lines based on phenotypic characterization and genetic diversity

Alice Casassola<sup>1\*</sup>, Sandra Patussi Brammer<sup>2</sup>, Márcia Soares Chaves<sup>2</sup>, Paula Wiethölter<sup>2</sup> and Eduardo Caierão<sup>2</sup>

Received 24 April 2012

Accepted 25 September 2012

**Abstract** – Parental selection is an important step in breeding programs, and genetic variability increases the chances of obtaining variance in progenies. The objectives of this study were to phenotype 29 wheat genotypes and determine the genetic variability among them, in order to identify potential parental lines for breeding programs at Embrapa Wheat. For phenotyping, traits such as plant height, cycle and grains characteristics were assessed and the data were analyzed by the Euclidean distance. The genetic distance was estimated using 97 microsatellite molecular markers and the data were analyzed by Nei72 coefficient. The average distance observed for phenotyping was 10.1, and the genetic distance was 31 %. SSR markers were efficient for selecting genetically diverse genotypes despite their phenotypic similarity, and lines PF 9027, PF 950351, PF 030132, PF 979002, PF 040488 and IWT 04019 can be used as parental for future crosses, since they have genetic diversity and suitable agronomic traits.

**Key words:** *Triticum aestivum* L., genetic variability, microsatellite, agronomic characterization.

## INTRODUCTION

Wheat (*Triticum aestivum* L.) is a widely cultivated crop. This specie, together with rice and maize, is a strategic crop for worldwide food security. In the last five decades, the world wheat production increased from 200 to over 650 million tons, which represents about 30% of the global grain production. The major wheat producers are the European Union, China, India, the United States and Russia, and according to market projections, these countries have been responsible for most of the global wheat supply in the last years (Hubner 2008, Canziani and Guimarães 2009). Although Brazil is not among the major producers, wheat is a strategic crop for national agribusiness, being Paraná and Rio Grande do Sul States responsible for about 90% of total wheat production (MAPA 2010). Currently, about 10.5 million tons of wheat are consumed by Brazilian population, however in the 2008/2009 crop season the internal production supplied only 5.8 million tons of the total demand (CONAB 2010). From 2001 to 2007, Brazil produced only 40% of its internal demand, which required

imports, reaching an average value of about US\$ 930 million in order to guarantee the internal supply. In 2008, despite of the fact that 55 % of the internal demand was supplied by national production, the import values rose to US\$ 1.87 billion (Meziat and Vieira 2009). According to projections from the Brazilian Ministry of Agriculture and Supply, in 2019/2020, wheat consumption must reach 12.8 million tons, and the projected production is only 7.0 million tons. These projections also indicate that, from 2009/2010 to 2019/2020, the internal consumption must increase at an average rate of 1.53% per year, which will require imports of the order of almost 7.0 million tons (MAPA 2010).

Despite the significant advances achieved in wheat breeding programs worldwide, there are still many challenges to be overcome in order to increase the levels of productivity. During the first Global Conference on Agricultural Research for Development, held in 2010, genetics was recognized as the number-one technique for increasing yields, by means of new improved varieties developed whether by assisted selection, genetic engineering, or classical breeding methods

<sup>1</sup> Universidade de Passo Fundo (UPF), Rodovia BR 285, km 171, 99.052-900, Passo Fundo, RS, Brazil. \*E-mail: alicecasassola@yahoo.com.br

<sup>2</sup> Empresa Brasileira de Pesquisa Agropecuária (Embrapa Trigo), Rodovia BR 285, km 294, 99.052-900, Passo Fundo, RS, Brazil

(Butler 2010). Parental selection is an important first step in any breeding program. The ability to assess accurately genetic differences between parents and, subsequently, to predict progeny performance would enhance the efficiency of breeding programs (Burkhamer et al. 1998). The use of genotypes with appropriate agronomic traits in induced crosses increases the chances of obtaining lines with enhanced performance. On the other hand, if genotypes are genetically similar, the probability of producing progenies with higher heterosis decreases (Bertan et al. 2007). Thus, the phenotyping and determination of genetic variability between materials are critical in the selection of parental genotypes, because once the genotypes have appropriate agronomic traits and high genetic variability, appropriate crosses can be made, accelerating the process of improving and reducing costs (Bered et al. 2002, Qi-Lun et al. 2008).

The phenotyping approach allows that genetic materials are evaluated and classified based on their agronomic traits. However, the high phenotypic similarity among the cultivated genotypes hampers the selection based only on the phenotype. On the other hand, the determination of genetic variability can be made at DNA level and, since it is not influenced by the environment, this approach can be of strategic importance for genotype characterization and parental selection (Bered et al. 2002, Aliyev et al. 2007, Ribeiro et al. 2011). The use of microsatellite molecular markers can assist greatly the breeders to find out genetic variability even among genotypes with similar phenotype. The microsatellite markers or SSR ("Simple Sequence Repeat") can be applied in studies of relationship and construction of genetic maps with high accuracy (Liu et al. 2007, Chandna et al. 2010), since they have co-dominant expression, multiallelism, high polymorphism information content (PIC) and are frequent and randomly distributed.

The objectives of this study were: a) to phenotype 29 wheat genotypes developed or used in wheat breeding program of Embrapa National Wheat Research Center, and to determine the genetic variability among them by microsatellite molecular markers, and b) to compare both phenotypic and molecular characterization approaches regarding their potential for assistance to the breeders in parental selection.

## MATERIAL AND METHODS

### Plant material

Six wheat cultivars and 23 wheat lines developed in the breeding program of Embrapa National Wheat Research Center (Passo Fundo, RS, Brazil) were selected for this study (Table 1). The cultivars BRS 327, BRS Umbu e BRS Guamirim were used as standards for the phenotypic characterization. Twenty seeds of each genotype were germinated in germitest paper until the first leaf was completely expanded. Leaves of ten seedlings of each genotype were collected

**Table 1.** Genotypes used in this study with their respective genealogies

Class	Genotype	Genealogy
Cultivar	Alondra I	Unknown
	BRS Tarumã	Century/BR 35
	Toropi	Frontana/Qauderma-A/Petiblanco
	Frontana	Fronteira/Mentana
	BR 35	IAC 5*2/3/CNT 7*3/Londrina/IAC 5/Hadden
	BR 23	CC/Alondra SIB/3/IAS 54-20/COP//CNT8
Lines	PF 9027	BR14/PF 839197/3/Londrina/Coker 76-35//F 25565/F...
	PF 950351	BR32/PF 869120
	PF 970313	Century/BR 35
	PF 010069	OR1/Coker 97.33//PF 92334/PF 87451
	PF 030065	CEP 24/IPF 64758
	PF 030132	Rubi/Coker 80.33
	PF 979002	VEZC/762/VEZ/PF 8569/3/BR 34/4/Amigo/BR 4//CTY/3/PF 869120
	PF 040453	PF 940041/BRS 179
	PF 010089	OR1/Oasis
	PF 010066P	Coker 80.33/BRS 194
	PF 970339	F/F//PF 87373//Embrapa 16
	PF 980414	Coker 80.33//PF 869120//BR 18
	PF 970345	PEL 73101/BR 5/PF 79777/Oasis
	PF 940266	BR 23//CEP 19/PF 85490
	IPF 70872P	C983/4/ACP//C762/FL302/3/C762 (881404-2-5)
	PF 001178	OR1/Oasis
	PF 003295 A/B	BR 23*2/PF940382
	PF 010091	Hulha Negra/Coker 80.33//OR1
	PF 030401	Century/PF 93188/PF 89156
	PF 040488	PF 93232/LR37 (=COOK*4/VPM//)/PF 940384
IWT 04019	TNNU/Attila	
PF 960258	Unknown	
PF 93318	Unknown	
Standards	BRS 327	CEP24Sel/BRS 194
	BRS Umbu	Century/BR 35
	BRS Guamirim	Embrapa 27/Buck Nadu/PF 93159

for DNA extraction and further genetic variability analysis. The remaining ten seedlings were transferred to 10 L pots containing soil and kept in growth chamber at 22 °C, with 18 h of photoperiod, until the heading stage for the analysis of cycle and plant height. After that, pots with plants were transferred to a greenhouse and kept until the full maturity of grain, when seeds were harvested to proceed the analysis of grain traits.

### **Agronomic characterization**

The agronomic characterization was based on parameters regarding plant (height and cycle) and grain traits (color, weight, hardness and diameter). The genetic diversity among the genotypes was estimated by the Euclidean distance, and the accessions were grouped by UPGMA method (Unweighted Pair Group Method using Arithmetic Averages), developed by Sokal and Michener (1958). The software used to generate the data was the NTSys (Rohlf 1998).

#### ***Plant height***

The height of the genotypes was determined in centimeters by measuring from the base of the plant to the tip of the ear, 15 days after heading. All plants of each genotype were measured and the average height was calculated. According to cultivar descriptors, BRS 327 is a high plant (Só e Silva et al. 2010) and BRS Guamirim is a short/dwarf plant (Scheeren et al. 2007) and, because of their contrasting phenotype for this trait, they were used as standards in this study.

#### ***Cycle***

The cycle of genotypes was determined considering the number of days between some pre-determined growth stages, according to the descriptions of the scale proposed by Zadoks et al. (1974) for cereals. It was evaluated the number of days from sowing to emergence; from emergence to heading (growth stages 0 to 4); from heading to maturity (growth stages 4 to 9) and emergence to maturity (complete cycle). According to cultivar descriptors, BRS Guamirim presents early cycle (Scheeren et al. 2007) and cultivar BRS Umbu presents mid-late cycle (Del Duca et al. 2004), and they were used in this study as standards due to their contrasting phenotype for this trait.

#### ***Color of the grains***

The evaluation of the grain color was visually scored considering as standards the contrasting cultivars BRS Umbu and BRS Guamirim, which have white (Del Duca et al. 2004) and red grains (Scheeren et al. 2007), respectively, using the parameters established by the Ministry of Agriculture and Supply (MAPA 2008).

### ***Hardness, weight and diameter of the grain***

The hardness, weight and diameter of the grains were determined using the adapted method 55-31 of American Association of Cereal Chemists – AACC (2000), equipment Single Kernel Characterization System - SKCS -, model 4100 (Perten Instruments). Due to the small amount of seeds available, instead of the 300 grains recommended by the protocol, only 50 grains per genotype were used, consisting in a single repeat. The hardness of the grains was determined according to the operation manual of the SKCS, which is described as the force necessary to grind the grain. The weight and diameter of the grains were analyzed by ANOVA, and the means were compared using the Scott-Knott test ( $p = 0.05$ ) (Scott and Knott 1974). The mean separation test among genotypes were done using Genes software (Cruz 2006).

### **Genetic variability**

#### ***Extraction of DNA***

DNA was extracted from 300 mg of leaves of each genotype according to Bonato (2008) protocol and quantified by comparison with DNA  $\lambda$  in 0.8 % agarose gel.

#### ***Molecular markers and evaluations***

The DNA working solutions were standardized at the concentration of 25  $\mu\text{g } \mu\text{L}^{-1}$ . The molecular markers assessed were the microsatellite (SSR) type. The SSR reactions were prepared for a 15  $\mu\text{L}$  volume. Each reaction contained 0.2 mM of each primer (forward and reverse), 0.2 mM of each dNTP, 2.5 mM of  $\text{MgCl}_2$ , 0.75 U of Taq-DNA polymerase enzyme, Taq buffer 1X, and 100 ng of DNA. The DNA was amplified using the following program: one denaturation at 94 °C for 3 minutes; 5 cycles of 94 °C for 1 minute, 60 °C for 1 minute (decreasing 1 °C per cycle until 55 °C), 72 °C for 1 minute; 30 cycles of 94 °C for 1 minute, 55 °C for 1 minute, 72 °C for 1 minute; and an extension of 72 °C for 10 minutes. The amplified DNA fragments were separated in 2 % ultrapure agarose gel (Invitrogen), stained with ethidium bromide and visualized under ultraviolet light (GelDoc XR+ equipment, Bio-Rad). The 50 pb DNA ladder marker was used as molecular weight standard. PCR reactions and gel visualization were carried out for all individuals together for each primer. Ninety-seven primers, which were distributed on all the wheat genomes, were tested (Table 2).

The genetic diversity among the genotypes was estimated by the Nei72 coefficient (Nei 1972). The accessions were grouped by UPGMA method (Unweighted Pair Group Method using Arithmetic Averages), developed by Sokal and Michener (1958), where the genotypes were considered

**Table 2.** Microsatellite molecular markers used for wheat genotype characterization and their chromosomal location

Primer	Chromosome	Primer	Chromosome	Primer	Chromosome	Primer	Chromosome
WMS114 <sup>1</sup>	3D	WMS427 <sup>1</sup>	6AL	WMS193 <sup>1</sup>	6BS	WMS232 <sup>1</sup>	1D/5DL
WMS608 <sup>1</sup>	2DL	WMS626 <sup>1</sup>	6B	WMS499 <sup>1</sup>	5DL	WMS349 <sup>1</sup>	2DL
WMC215 <sup>2</sup>	5D/5AL/3A	WMS533 <sup>1</sup>	3BS	WMS118 <sup>3</sup>	4AL/5BL	WMS291 <sup>1</sup>	5AL
WMS344 <sup>1</sup>	7A/7BL	WMS550 <sup>1</sup>	1BS	WMS112 <sup>1</sup>	3B/4B/7B	WMS484 <sup>1</sup>	2D
WMS639 <sup>1</sup>	5AL/5BL/5D	WMS272 <sup>1</sup>	5DL	WMS18 <sup>1</sup>	1BS/4BS	WMS157 <sup>1</sup>	2DL
WMS403 <sup>1</sup>	1BL/2B/3A	WMS389 <sup>1</sup>	3BS	WMS350 <sup>1</sup>	7AS/7B/7DS/4AL	WMS52 <sup>1</sup>	3DL
WMS617 <sup>1</sup>	5A/6A	WMS169 <sup>1</sup>	6AL	WMS383 <sup>1</sup>	3D	WMS293 <sup>1</sup>	5AS
WMS46 <sup>1</sup>	7BS/7BL	WMS219 <sup>1</sup>	6B	WMS408 <sup>1</sup>	5BL	WMS233 <sup>1</sup>	7AS
WMS162 <sup>1</sup>	3A/4A	WMS644 <sup>1</sup>	1BL/3BL/3BS/6BS/7BL	WMC25 <sup>2</sup>	2BS/2DS	WMS249 <sup>1</sup>	2AS
WMS174 <sup>1</sup>	5D	WMS369 <sup>1</sup>	3AS/4BS	WMS106 <sup>1</sup>	1DS	WMS282 <sup>1</sup>	7A
WMS181 <sup>1</sup>	3BL	WMS120 <sup>1</sup>	2BL	WMC331 <sup>2</sup>	4DL	WMS148 <sup>1</sup>	2BS
WMS191 <sup>1</sup>	2BL/5B/6B/3D	WMS95 <sup>1</sup>	2AS	WMC167 <sup>2</sup>	2DL	WMS335 <sup>1</sup>	5BL
WMS264 <sup>1</sup>	1AL/1BS	WMS518 <sup>1</sup>	6BS	WMC245 <sup>2</sup>	2DL/2B	WMS334 <sup>1</sup>	6AS
WMS437 <sup>1</sup>	7D	WMS539 <sup>1</sup>	2D	WMS295 <sup>1</sup>	7DS	WMS375 <sup>3</sup>	4BL
WMS554 <sup>1</sup>	5B/7A	WMS261 <sup>1</sup>	2D	WMS642 <sup>1</sup>	1DL	WMS107 <sup>1</sup>	4BS
WMS325 <sup>1</sup>	6B/6D	WMS471 <sup>1</sup>	7A/7B	WMS136 <sup>1</sup>	1AS	WMC44 <sup>2</sup>	1BL
WMS397 <sup>1</sup>	4AL/4AS	WMS161 <sup>1</sup>	3D	WMS247 <sup>1</sup>	3BL/3A	WMS609 <sup>1</sup>	4DL
WMS604 <sup>1</sup>	1BS/5BL	WMS320 <sup>1</sup>	2DL	WMS328 <sup>1</sup>	2AL	WMS111 <sup>1</sup>	7DS
WMS610 <sup>1</sup>	4A	WMS341 <sup>1</sup>	3DS	WMS526 <sup>1</sup>	2B	WMS210 <sup>1</sup>	2BS/2DS
WMS613 <sup>1</sup>	6BS/4AS	WMS183 <sup>1</sup>	3DS	WMS294 <sup>1</sup>	2AL	WMS234 <sup>1</sup>	5BS/5AS
WMS637 <sup>1</sup>	4AL	WMS186 <sup>1</sup>	5A	WMS322 <sup>4</sup>	7AL	WMS43 <sup>1</sup>	7BS
WMS192 <sup>1</sup>	5D	WMS126 <sup>1</sup>	5AL	WMS33 <sup>1</sup>	1D	WMS153 <sup>1</sup>	1BL
WMS664 <sup>1</sup>	5DL/4B	WMS149 <sup>1</sup>	4BL	WMS99 <sup>1</sup>	1AL		
WMS508 <sup>1</sup>	6BS	WMS160 <sup>1</sup>	4A	WMS400 <sup>1</sup>	7B		
WMS361 <sup>1</sup>	6B	WMS654 <sup>1</sup>	5DL	WMS205 <sup>1</sup>	5DS		

<sup>1</sup> Röder et al. (1998); <sup>2</sup> Somers and Isaac (2004); <sup>3</sup> Korzun et al. (1997); <sup>4</sup> Sourdille et al. (2004)

operational taxonomic units (OTUs), and the bands obtained by markers, like binary characters. The software used to generate the data was the NTSys (Rohlf 1998).

The polymorphism information content was determined using the following formula:

$$PIC = 1 - \sum P_{ij}^2$$

where  $P_{ij}^2$  is the frequency of the  $j$ th allele for  $i$ th locus, covering all alleles per locus (Nei 1973).

## RESULTS AND DISCUSSION

The results obtained in the phenotyping are presented in Table 3. Cultivar BRS Tarumã and lines PF 970313, PF 030065, PF 040453, PF 010066P, PF 980414 and IPF 70872P, were not evaluated since they showed a very late cycle. Regarding the plant height, cultivar BRS 327 was used as standard and only the old cultivars Frontana, Toropi and BR 23 were considered tall. All the other genotypes showed short size and, since this trait is more suitable for

cropping systems under high technology levels due to the enhanced resistance to lodging (Cruz et al. 2001), they are promising materials for short-term breeding programs.

Considering cycle, the genotypes were grouped as early-maturing when they showed a cycle shorter than the standard cultivar BRS Guamirim (Scheeren et al. 2007), and late-maturing, when the cycle was longer than 140 days, which is observed for the standard cultivar BRS Umbu (DeL Duca et al. 2004). Those genotypes showing cycles varying from 111 to 139 days were classified in a mid-maturing group. All genotypes, with the exception of BRS Umbu, were classified in a mid-maturing group. Short-cycled cultivars with early or mid-maturity are more suitable for crop system in southern Brazil, since they allow that the successive summer crop (mainly soybean) can be established in a timely manner, and for this reason they are preferred by breeders of Embrapa National Wheat Research Center.

The color of wheat grain can range from red to white, and since the hardness is associated with the vitreousness

(Guarienti 1996), vitreous red grains are considered hard. Grain hardness is genetically controlled, but environmental factors can alter the protein content (Troccoli et al. 2000). The baking industry prefers the vitreous grains once this trait is correlated with the protein percentage, semolina yield and cooking quality. In this study vitreous red grains were observed in lines PF 9027, PF 950351, PF 030132, PF 979002, PF 010089, PF 970345, PF 040488 and IWT 04019.

Cultivar BRS Guamirim was used as standard for grain hardness, thus the genotypes that showed hardness index higher or equal to it - hard grain - were: PF 9027, PF 950351, PF 030132, PF 979002, PF 040488, IWT 04019, PF 940266, PF 003295 A/B, PF 030401 and Alondra I. These results were consistent with previous studies that reported that the hardness is related to vitreousness (Sissons et al. 2000), since the majority of the tested genotypes showing red vitreous grains also showed hard grains.

Regarding weight and diameter of the grains, PF 003295 A/B had the highest mean value for grain weight, whereas lines PF 010069, PF 030132, PF 010089 and PF 001178 had the smallest ones; PF 93318, IWT 04019, PF 003295 A/B, Alondra I and Frontana had the highest mean value for grain diameter, whereas cultivar Toropi had the smallest one. The standards BRS 327, BRS Umbu and BRS Guamirim were classified into groups “c”, “d” and “e”, for grain weight, and “a”, “c” and “b” for grain diameter, respectively (Scott-Knott  $p = 0.05$ ) (Table 3).

The data obtained from the phenotyping was analyzed to generate a dendrogram (Figure 1A). The average distance observed for this data was 10.1. From this analysis, it was possible to separate the genotypes into groups of similarity, but the diversity observed was small.

From the 97 microsatellite molecular markers used, 42 (43.3 %) showed polymorphism: WMS642, WMS136,

**Table 3.** Agronomic traits of wheat cultivar and lines

Genotype	Plant height (cm)	Cycle (days)	Color of grain <sup>1</sup>	Hardness of grain <sup>2</sup>	Weight of grain (mg) <sup>3</sup>	Diameter of grain (mm) <sup>3</sup>
Toropi	88.4	117	GVM	SD	34.07d	2.75f
PF 9027	76.7	127	GVV	D	34.42d	2.80b
PF 950351	63.7	121	GVV	MD	33.57d	2.81b
Frontana	98.5	138	GB	M	43.99b	3.06a
BR 35	74.4	131	GVM	SM	41.29c	2.22e
BR 23	84.2	121	GB	SM	39.70c	2.85b
PF 010069	69.1	131	GVM	SM	31.92e	2.56d
PF 030132	57.3	138	GVV	ED	31.38e	2.70c
PF 979002	61.3	134	GVV	D	36.20d	2.70c
PF 010089	46.8	134	GVV	SM	31.80e	2.72c
PF 970339	74.9	125	GVM	SD	34.32d	2.78b
PF 970345	69.5	138	GVV	M	39.81c	2.86b
PF 940266	62.8	125	GVM	D	34.18d	2.78b
PF 001178	45.8	138	GVM	M	31.75e	2.73c
PF 003295A/B	69.1	121	GVM	D	45.74a	2.94a
PF 010091	58.6	122	GVM	SD	34.87d	2.77b
PF 030401	50.4	131	GVM	D	33.48d	2.81b
PF 040488	64.1	125	GVV	ED	33.98d	2.75b
IWT 04019	76.5	127	GVV	D	42.64b	2.99a
PF 960258	68.7	127	GVM	SM	34.99d	2.78b
PF 93318	72.6	131	GVM	M	39.70c	2.95a
Alondra I	66.1	127	GVM	MD	42.31b	2.95a
BRS 327	56.7	121	GVM/GM	M	40.57c	2.97a
BRS Umbu	63.5	142	GB	M	34.15d	2.66c
BRS Guamirim	50.5	111	GVM	D	32.58e	2.81b

<sup>1</sup> (GVV) Vitreous red grain, (GVM) Medium red grain, (GB) White Grain, (GM) Brown Grain.

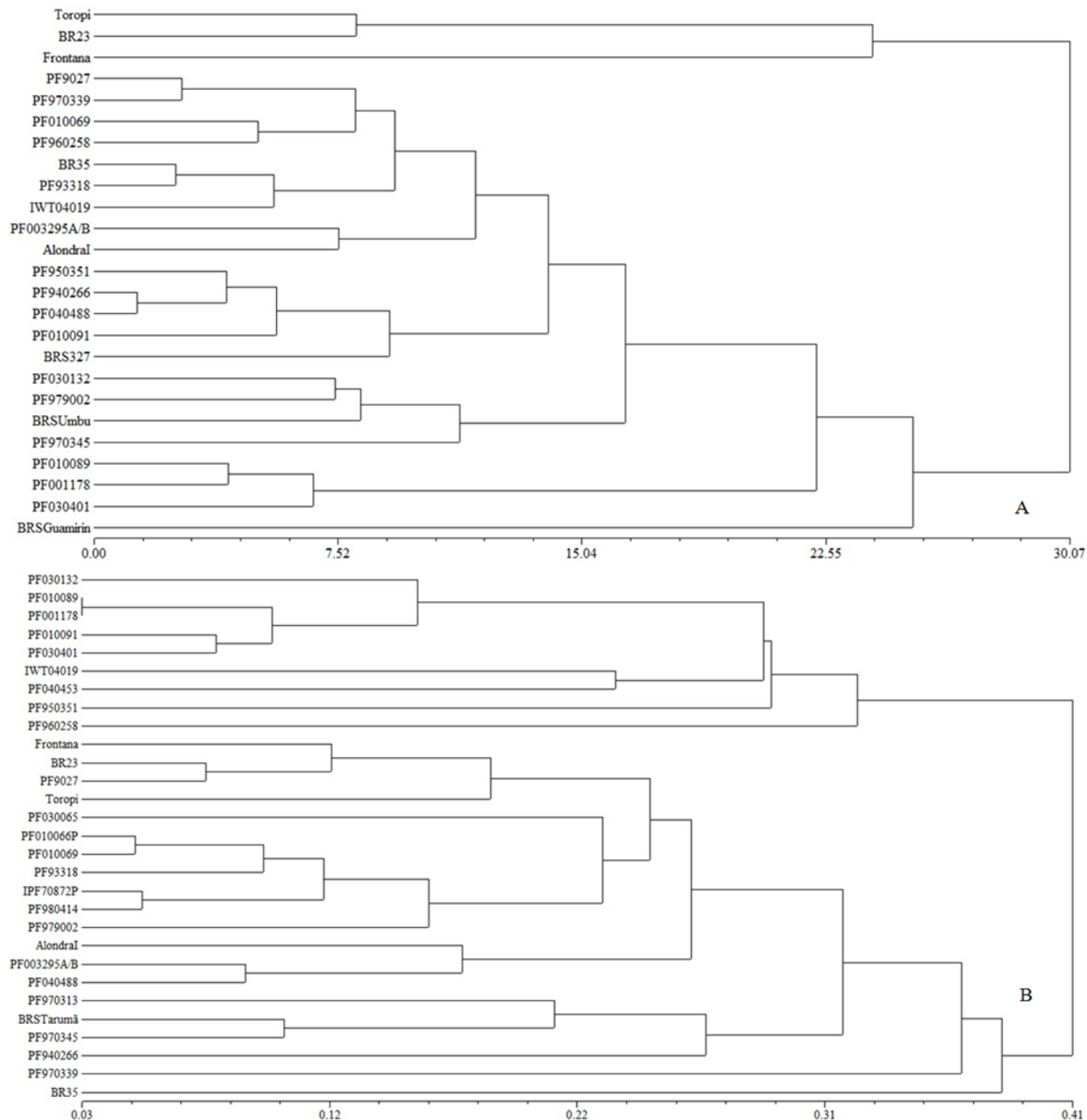
<sup>2</sup> (ED) Extra hard, (MD) Very hard, (D) Hard, (SD) Semi-hard, (SM) Semi-soft, (M) Soft, (MM) Very soft, (EM) Extra soft.

<sup>3</sup> Mean separation test between genotypes using Scott-Knott ( $p = 0.05$ ). Means followed by the same minuscule letter in a column did not differ.



WMS247, WMS99, WMS400, WMS427, WMS533, WMS160, WMS205, WMS349, WMS52, WMS148, WMS186, WMS335, WMS334, WMS294, WMS626, WMS291, WMS114, WMS344, WMS639, WMS617, WMS46, WMS181, WMS264, WMS437, WMS397, WMS604, WMS637, WMS508, WMS499, WMS261, WMS471, WMS234, WMS95, WMS518, WMS408, WMS272, WMS389, WMS219, WMS153 and WMC215.

A dendrogram generated from the molecular markers data (Figure 1B), showed a high genetic diversity of the analyzed genotypes. The average genetic distance obtained was 31 %. The number of alleles varied from one to five, and the average was 2.86 (Table 4). The highest number of polymorphic loci was found in B and A genomes, followed by D genome, and chromosome 5 was the most polymorphic. These results corroborate with previous studies such



**Figure 1.** Genetic variability among the wheat genotypes analyzed. (A) Dendrogram of genetic variability using phenotypic data and the Euclidian distance. (B) Dendrogram of genetic variability using microsatellite data and Nei72 coefficient.

as Liu et al. (2007) and Ahtar et al. (2010), who found the largest number of alleles and the greatest genetic variability in B genome. However the number of alleles is variable depending on the evaluated population (Khlestina et al. 2004, Roussel et al. 2005).

**Table 4.** Molecular markers showing polymorphic patterns, genome location, number of alleles, range of fragments and polymorphic information content (PIC)

Marker	Chromosome	Number of alleles	Range of fragments	PIC	
1	WMS99	1AL	2	100 – 150	0,43
2	WMS264	1AL/1BS	2	160 – 210	0,49
3	WMS136	1AS	5	200 – 500	0,52
4	WMS153	1BL	3	180 – 210	0,62
5	WMS604	1BS/5BL	3	110 – 130	0,64
6	WMS642	1DL	3	180 – 550	0,50
7	WMS294	2AL	4	50 – 120	0,68
8	WMS95	2AS	2	100 – 130	0,06
9	WMS148	2BS	3	100 – 170	0,52
10	WMS261	2D	3	170 – 200	0,50
11	WMS349	2DL	4	110 – 350	0,11
12	WMS181	3BL	2	140 – 160	0,32
13	WMS247	3BL/3A	2	150 – 200	0,37
14	WMS533	3BS	3	100 – 180	0,45
15	WMS389	3BS	3	130 – 180	0,61
16	WMS114	3D	4	120 – 200	0,55
17	WMS52	3DL	4	150 – 350	0,56
18	WMS160	4A	3	160 – 200	0,52
19	WMS637	4AL	2	150 – 170	0,48
20	WMS397	4AL/4AS	2	190 – 200	0,46
21	WMS186	5A	3	100 – 140	0,57
22	WMS617	5A/6A	4	100 – 190	0,73
23	WMS291	5AL	4	110 – 380	0,66
24	WMS639	5AL/5BL/5D	3	140 – 180	0,61
25	WMS335	5BL	4	180 – 260	0,63
26	WMS408	5BL	2	160 – 190	0,50
27	WMS234	5BS/5AS	3	110 – 160	0,59
28	WMC215	5D/5AL/3A	2	210 – 250	0,49
29	WMS499	5DL	2	100 – 130	0,28
30	WMS272	5DL	2	140 – 160	0,18
31	WMS205	5DS	2	140 – 170	0,50
32	WMS427	6AL	2	220 – 250	0,35
33	WMS334	6AS	2	110 – 130	0,48
34	WMS626	6B	2	100 – 130	0,23
35	WMS219	6B	3	150 – 190	0,45
36	WMS508	6BS	2	140 – 170	0,31
37	WMS518	6BS	4	190 – 250	0,69

38	WMS471	7A/7B	3	110 – 170	0,56
39	WMS344	7A/7BL	3	120 – 160	0,64
40	WMS400	7B	3	150 – 380	0,60
41	WMS46	7BS/7BL	3	160 – 200	0,65
42	WMS437	7D	3	90 – 110	0,57
Total of Alleles				120	
Genome A (exclusively)				35	
Genome B (exclusively)				40	
Genome D (exclusively)				27	

The value of polymorphism information content (PIC) ranged from 0.06 to 0.73, and the average was 0.49, confirming the high genetic diversity obtained by the Nei72 coefficient.

Considering the genealogies of the most similar genotypes, the predominant parental were OR1 (PF 010089, PF 001178, PF 010091 and PF 010069), Coker 80.33 (PF 010066P, PF 030132, PF 980414 and PF 010091), Coker 97.33 (PF 010069) and Oasis (PF 010089, PF 970345 and PF 001178). Therefore, the similarity of these materials is significantly explained by genealogy, since all of them have a common parental, which donate most of their genome even in complex crosses.

Thus, the analysis of genetic variability showed that there is high genetic diversity among genotypes, demonstrating that despite being phenotypically similar, there is diversity at the molecular level, confirming the possibility of obtaining variance in progenies using these genotypes as parental.

Concerning the desirable agronomic traits such as plant height, cycle and grain color associated with the genetic variability, the most promising lines for immediate or short-term use in the wheat breeding program of Embrapa National Wheat Research Center are: PF 9027, PF 950351, PF 030132, PF 979002, PF 040488 and IWT 04019. The other genotypes showing one or more appropriated attributes (such disease resistance, for example) also could be used as parents; however, cycles of backcrossing would be required in order to recover the desired agronomic traits from the recurrent parent.

## CONCLUSIONS

- The genotypes studied showed high genetic variability, which is essential to the breeding programs of wheat, and the use of microsatellite molecular markers allows to estimate the genetic variability even among phenotypically similar genotypes, justifying its use as a supporting methodology for parental selection;
- Lines PF 9027, PF 950351, PF 030132, PF 979002, PF 040488 and IWT 04019 can be used immediately in the improvement of wheat, due to the association of genetic variability with appropriate agronomic traits. The other lines and cultivars can be used as parental, but on the improvement of basic germplasm.

## Seleção de parentais em trigo baseado na caracterização fenotípica e diversidade genética

**Resumo** – Seleção de parentais é uma etapa importante no melhoramento e a variabilidade genética aumenta as chances de obtenção de variância nas progênes. Os objetivos deste estudo foram fenotipar 29 genótipos de trigo e determinar a variabilidade genética entre eles, visando identificar potenciais parentais para uso nos programas de melhoramento da Embrapa Trigo. Para a fenotipagem, caracteres estatura de planta, ciclo e características dos grãos foram avaliados e os dados analisados pela distância Euclidiana. A distância genética foi estimada utilizando 97 marcadores moleculares microsátélites e os dados analisados pelo coeficiente Nei72. A distância média observada pela fenotipagem foi 10.1 e a distância genética 31%. Os marcadores SSR foram eficientes na seleção de genótipos geneticamente diversos apesar da similaridade fenotípica a as linhagens PF 9027, PF 950351, PF 030132, PF 979002, PF 040488 e IWT 04019 podem ser utilizadas como parentais em cruzamentos induzidos considerando variabilidade genética associada a caracteres agrônômicos adequados.

**Palavras-chave:** *Triticum aestivum L.*, variabilidade genética, microsátélites, caracterização agrônômica.

### REFERENCES

- AACC – American Association of Cereal Chemists (2000) **Approved methods of the AACC. Methods 39-70A**. The Association, Saint Paul.
- Achtar S, Moualla MY, Kalhout A, Roder MS and Mirali N (2010) Assessment of genetic diversity among Syrian durum (*Triticum ssp. durum*) and bread wheat (*Triticum aestivum L.*) using SSR Markers. **Russian Journal of Genetics 46**: 1320-1326.
- Aliyev RT, Abbasov MA and Mammadov AC (2007) Genetic identification of diploid and tetraploid wheat species with RAPD markers. **Turkish Journal of Biology 31**: 173-180.
- Bered F, Barbosa-Neto JF, Rocha BM and Carvalho FIF (2002) Genetic variability in wheat (*Triticum aestivum L.*) germplasm revealed by RAPD markers. **Crop Breeding and Applied Biotechnology 4**: 499-506.
- Bertan I, Carvalho FIF and Oliveira AC (2007) Parental selection strategies in plant breeding programs. **Journal of Crop Sciences and Biotechnology 10**: 211-222.
- Bonato ALV (2008) **Extração de DNA genômico de cereais de inverno na Embrapa Trigo**. Embrapa Trigo, Passo Fundo, 11p.
- Burkhamer RL, Lanning SP, Martens RJ, Martin JM and Talbert LE (1998) Predicting progeny variance from parental divergence in hard red spring wheat. **Crop Science 38**: 243-248.
- Butler D (2010) Q&A: what it will take to feed the world. **Nature 464**: 969.
- Canziani JR and Guimarães VDA (2009) O trigo no Brasil e no mundo: cadeia de produção, transformação e comercialização. In Cunha GR (ed.) **Oficina sobre trigo no Brasil: bases para a construção de uma nova triticultura brasileira**. Editora Embrapa Trigo, Passo Fundo, p. 29-72.
- Chandna R, Gupta S, Ahmad A, Iqbal M and Prasad M (2010) Variability in Indian bread wheat (*Triticum aestivum L.*) varieties differing in nitrogen efficiency as assessed by microsatellite markers. **Protoplasma 242**: 55-67.
- CONAB - Companhia Nacional de Abastecimento (2010) Produtos e Serviços/Safras/Séries/históricas/trigo. Available at <<http://www.conab.gov.br/detalhe.php?c=5052&t=2>> Accessed on Jan 10, 2012.
- Cruz PJ, Carvalho FIF, Caetano VR, Silva SA, Kurek AJ and Barbieri RL (2001) Traits associated with lodging resistance in bred wheat. **Ciência Rural 31**: 563-568.
- Cruz CD (2006) **Programa Genes - estatística experimental e matrizes**. Editora UFV, Viçosa, 285p.
- Del Duca LJA, Linhares AG, Nascimento Junior A, Souza CNA, Guarienti EM, Só e Silva M, Scheeren PL, Lhamby JCB, Rodrigues O, Fontaneli RS, Pegoraro D, Rosinha RC, Almeida J and Molin R (2004) **Desempenho e características agrônômicas, fitossanitárias e industriais da cultivar de trigo BRS Umbu**. Embrapa Trigo, Passo Fundo, 18p.
- Guarienti E (1996) **Qualidade industrial do trigo**. Editora Embrapa Trigo, Passo Fundo, 36p.
- Hubner O (2008) **Análise da conjuntura agropecuária – safra 2007/2008. Trigo**. Secretaria da Agricultura e do Abastecimento do Estado do Paraná, Curitiba, 19p.
- Khlestina EK, Roder MS, Efremova TT, Borner A and Shumny VK (2004) The genetic diversity of old and modern Siberian varieties of common spring wheat as determined by microsatellite markers. **Plant Breeding 123**: 122-127.
- Korzun V, Börner A, Worland AJ, Law CN and Röder MS (1997) Application of microsatellite markers to distinguish inter-varietal chromosome substitution lines of wheat (*Triticum aestivum L.*). **Euphytica 95**: 149-155.
- Liu J, Liu L, Hou N, Zhang A and Liu C (2007) Genetic diversity of wheat gene pool of recurrent selection assessed by microsatellite markers and morphological traits. **Euphytica 155**: 249-258.
- MAPA - Ministério da Agricultura, Pecuária e Abastecimento (2010) **Projeções do agronegócio: Brasil 2009/2010 a 2019/2020**. Ministério da Agricultura, Pecuária e Abastecimento, Brasília, p. 18-20.
- MAPA - Ministério da Agricultura, Pecuária e Abastecimento (2008) **Descritores mínimos de trigo**. MAPA, Brasília, 10p.
- Meziate A and Vieira RCM (2009) Política de desenvolvimento produtivo e sua extensão à cadeia produtiva do trigo. In Cunha GR (ed.) **Oficina sobre trigo no Brasil: bases para a construção de uma nova triticultura brasileira**. Editora Embrapa Trigo, Passo Fundo, p. 15-28.



- Nei M (1972) Genetic distance between populations. **American Naturalist** **106**: 283-292.
- Nei M (1973) Analysis of gene diversity in subdivided populations. **Proceedings of the National Academy of Science of the USA** **70**: 3321-3323.
- Qi-Lun Y, Ping F, Ke-Cheng K and Guang-Tang P (2008) Genetic diversity based on SSR markers in maize (*Zea mays* L.) landraces from Wuling mountain region in China. **Journal of Genetics** **87**: 287-291.
- Ribeiro MNO, Carvalho SP, Santos JB and Antonio RP (2011) Genetic variability among cassava accessions based on SSR markers. **Crop Breeding and Applied Biotechnology** **11**: 263-269.
- Rohlf JF (1998) **NTSYS – pc. Numerical taxonomy and multivariate analysis system. Versão 2.0**. Applied Biostatistics Inc., New York.
- Roussel V, Leisova L, Exbrayat F, Stehno Z and Balfourier F (2005) SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000. **Theoretical and Applied Genetics** **111**: 162-170.
- Scheeren PL, Caierão E, Só e Silva M, Del Duca LJA, Nascimento Junior A, Linhares A and Eichelberger L (2007) BRS Guamirim: cultivar de trigo da classe pão, precoce e de baixa estatura. **Pesquisa Agropecuária Brasileira** **42**: 293-296.
- Scott AJ and Knott MA (1974) A cluster analysis method for grouping means in the analysis of variance. **Biometrics** **30**: 507-512.
- Sissons MJ, Osborne BG, Hare RA, Sissons SA and Jackson R (2000) Application of the single-kernel characterization system to durum wheat testing and quality prediction. **Cereal Chemistry** **77**: 4-10.
- Só e Silva M, Caierão E, Scheeren PL, Eichelberger L, Nascimento Junior A and Miranda MZ (2010) BRS 327 - a new bread wheat cultivar. **Crop Breeding and Applied Biotechnology** **10**: 370-373.
- Sokal RR and Michener CD (1958) A statistical method for evaluating systematic relationships. **The University of Kansas Scientific Bulletin** **38**: 1409-1438.
- Somers DJ and Isaac P (2004) **SSRs from the wheat microsatellite consortium**. Available at <wheat.pw.usda.gov/ggpages/SSR/WMC> Accessed on March 10, 2010.
- Sourdille P, Gandon B, Chiquet V, Nicot N, Somers D, Murigneux A and Bernard M (2004) **Wheat génoplante SSR mapping data release: a new set of markers and comprehensive genetic and physical mapping data**. Available at <wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical> Accessed on March 10, 2010.
- Trocilli A, Borrelli GM, De Vita P, Fares C and Di Fonzo N (2000) Durum wheat quality: o multidisciplinary concept. **Journal of Cereal Science** **32**: 99-113.
- Zadoks JC, Chang TT and Konzak CF (1974) A decimal code for the growth stages of cereals. **Weed Research** **14**: 415-421.

