Genetic diversity of Brazilian triticales evaluated with genomic wheat microsatellites

Cibele Tesser da Costa[1], Ana Christina Sagebin Albuquerque[2], Alfredo do Nascimento Junior[1], Francismar Correa Marcelino[3] and Jorge Fernando Pereira[1]

[1]Embrapa Trigo, BR 285 km 294, Caixa Postal 451, CEP 99001-970 Passo Fundo, RS, Brazil. E-mail: cibeletc@yahoo.com.br, alfredo@cnpt.embrapa.br, jorge@cnpt.embrapa.br [2]Embrapa Sede, Parque Estação Biológica s/nº, Caixa Postal 40.315, CEP 70770-901 Brasília, DF, Brazil. E-mail: ana.albuquerque@embrapa.br [3]Embrapa Soja, Rod. Carlos João Strass, Caixa Postal 231, CEP 86001-970 Londrina, PR, Brazil. E-mail: francm@cnpso.embrapa.br

Abstract – The objective of this work was to determine the genetic variability available for triticale (X *Triticosecale* Wittmack) crop improvement in Brazil. Forty-two wheat genomic microsatellites were used to estimate the molecular diversity of 54 genotypes, which constitute the base of one of the major triticale breeding programs in the country. Average heterozygosity was 0.06 and average and effective number of alleles per locus were 2.13 and 1.61, respectively, with average allelic frequency of 0.34. The set of genomic wheat microsatellites used clustered the genotypes into seven groups, even when the germplasm was originated primarily from only two triticale breeding programs, a fact reflected on the average polymorphic information content value estimated for the germplasm (0.36). The 71.42% transferability achieved for the tested microsatellites indicates the possibility of exploiting these transferable markers in further triticale genetic and breeding studies, even those mapped on the D genome of wheat, when analyzing hexaploid triticales.

Index terms: X *Triticosecale*, polymorphism information content, transferability, heterozygosity, number of alleles, frequency of alleles.

Diversidade genética de triticales brasileiros avaliada com microssatélites genômicos de trigo

Resumo – O objetivo deste trabalho foi determinar a variabilidade disponível para o melhoramento de triticale (X *Triticosecale* Wittmack) no Brasil. Quarenta e dois microssatélites de trigo foram empregados para estimar a diversidade molecular de 54 genótipos, que constituem a base de um dos principais programas de melhoramento da espécie no país. A heterozigosidade média foi 0,06, e os números médio e efetivo de alelos por lócus foram de 2,13 e 1,61, respectivamente, com freqüência alélica média de 0,34. O conjunto de microssatélites de trigo possibilitou reunir os genótipos em sete grupos, mesmo que o germoplasma utilizado seja originado de apenas duas instituições de pesquisa, o que refletiu em baixo índice de polimorfismo médio (0,36). A taxa de transferência dos marcadores testados (71,42%) indica a possibilidade de uso desses microssatélites de trigo, até mesmo os mapeados no genoma D da espécie, na análise de triticales hexaplóides em futuros trabalhos de genética e melhoramento de triticale.

Termos para indexação: X *Triticosecale*, índice de polimorfismo, transferabilidade, heterozigosidade, número efetivo de alelos, freqüência alélica.

Introduction

Triticale (X *Triticosecale* Wittmack) is a synthetic self-pollinated crop derived from wheat (*Triticum* sp., AABB or AABBDD) and rye (*Secale cereale* L., RR), crossed to bring together in a single species the technological quality and yield potential of wheat with the rye stress resistance and rusticity. Octoploid triticales, comprising 56 chromosomes, are derived by crossing hexaploid wheats (*T. aestivum* L., AABBDD) and rye, while hexaploid triticales, with 42 chromosomes, are mostly complete cariotypes, partially or totally deprived from the D genome of wheat, resulting from the cross between *Triticum durum* L. (AABB), for instance, and rye (Ammar et al., 2004; Oetler, 2005).

The combination of wheat and rye genomes allows triticale to show characteristics of good adaptability to poor or harsh environments, such as acid or waterlogged soils, metal toxicity, salinity, high elevation and adverse climatic conditions, besides greater tolerance (more than

wheat) to common wheat diseases (Horlein & Valentine, 1995). X *Triticosecale* constitutes also a valuable genetic resource for transferring genes of interest from rye into wheat, particularly those related to biotic and abiotic stresses (Vaillancourt et al., 2007).

Molecular markers such as microsatellites or SSRs (Simple Sequence Repeats) constitute an important tool for studies on genetic diversity, population structure, genetic mapping and crop breeding due to their abundance, codominance, level of polymorphism, reliability and easiness to assay (Röder et al., 1995, 1998). Additionally, these markers are not influenced by the environment or by genotype x environment interactions, contrary to what is verified for morphological and phenological characteristics. Biochemical and molecular markers most commonly used include the polymorphism of storage proteins, alozymes and DNA markers such as SSRs, RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), and others.

Microsatellites of wheat or rye have been efficiently employed in analysis of the genome of triticale (Kuleung et al., 2004, 2006; Tams et al., 2004) and the estimates revealed on the genetic diversity of triticales from distinct continents indicate that the genetic base of this cereal must be extended (Darvey, 1986, Nascimento Junior et al., 2004). Analyzing the genome of five triticales from Russia, Mexico and the United States, using 176 microsatellites from wheat (148) and rye (28), Kuleung et al. (2004) identified polymorphism in 31% of the used markers. Tams et al. (2004) determined polymorphism information content (PIC) of 0.54, indicative of moderate variability, in the evaluation of 128 triticale cultivars from five continents using three to five microsatellites per chromosome. The same value was also found by Kuleung et al. (2006), when 80 hexaploid triticales were analysed, employing 43 wheat and 14 rye microsatellites.

Until this moment, studies to estimate the genetic diversity of Brazilian triticale germplasm have not been carried out, neither on genotypes already recommended for cultivation nor on those still in the process of development and evaluation.

The objective of this work was to estimate the molecular diversity of triticale genotypes that constitute the base of one of the main triticale breeding programs in Brazil using wheat genomic microsatellites.

Material and Methods

Fifty-four triticale genotypes that formed the crossing block of the Embrapa's triticale breeding program at Embrapa Trigo, in Passo Fundo, Rio Grande do Sul (RS), Brazil, in 2005 (Table 1), were evaluated. Thirty-one genotypes were originated from the triticale breeding program conducted at the International Maize and Wheat Improvement Center (Cimmyt), in Mexico. Among these, 24 (77.42%) were developed at Embrapa's triticale breeding program; two (6.45%) at Fundação Centro de Experimentação e Pesquisa Fecotrigo (Fundacep Fecotrigo), in Cruz Alta, RS; two (6.45%) were introduced and released for commercial use in Brazil by the Instituto Agronômico (IAC), in Campinas, São Paulo; and three (9.68%) were developed at Instituto Agronômico do Paraná (Iapar) triticale improvement program, in Londrina, Paraná – one of them being released for commercial use in Brazil in conjunction with the Cooperativa Central de Pesquisa Agrícola (Coodetec), the former research department of the Sindicato e Organização das Cooperativas do Estado do Paraná (Ocepar), in Cascavel, Paraná. The other 23 analyzed accessions were Brazilian triticales derived from crosses between Brazilian wheat and rye genotypes, or among Brazilian triticales, with or without the participation of Cimmyt germplasm.

Genomic DNA was isolated from seeds (Rogers & Bendich, 1988) or seedlings (Kleinhofs et al., 1993) according to the CTAB (cetyltrimethylammonium bromide) method. A total of 42 genomic wheat microsatellites markers (Röder et al., 1998), one for each wheat chromosome arm, was used. This same set of markers (Table 2) was used by Stachel et al. (2000) to determine the genetic differentiation caused by selection for adaptation in wheat. Polymerase chain reaction (PCR), adjusted for 25 μL final volume, consisted of 1x enzyme buffer, 2 mM $MgCl_2$, 0.2 mM each dNTP, 5 μM to 10 μM primers, 1 unit *Taq* polymerase and 75 ng of genomic DNA. The PCR amplification was performed in a MJ Research PTC-100 (Programmable Thermal Controller, MJ Research INC), as follows: initial denaturation at 94°C for 3 min, followed by 35 to 45 cycles of 1 min at 94°C,

1 min at 45–60°C, 1 min at 72°C and final extension for 3 to 10 min at 72°C. The annealing temperature for each primer (Table 2) was determined according to Röder et al. (1998) and Stachel et al. (2000). Amplified DNA

Table 1. Parental genotypes used at Embrapa's triticale (X *Triticosecale* Wittmack) improvement program.

Genotype	Pedigree	Genealogy	Origin[1]	Institution[2]
BRS 148	Yogui/Tatu	CTM86B.1537-0M-0Y-0M-38RES-0B	Mexico	Embrapa
BRS 203	LT-1/Rhino	SWT2201-4Y-1M-1Y-4B-3Y-2B-0RES -10FG-03F-2F-0F	Mexico	Embrapa
BRS Minotauro	OCTO92-3(PF89358/CBR1)/HD	T0092-3-1F-1F-14F-0F	Brazil	Embrapa
CEP 23 – Tatu	BGL/3/MTZTCL/Trigo//BGL/4/Nutria	B5644-777-1Y-0M-0A	Mexico	Fundacep
CEP 28 – Guará	Daman (Tatu4/ARD1)	CTM86.293-0Y-0M-0Y-18M-9RES-0B-0A	Mexico	Fundacep
Embrapa 18	Tapir/Yogui//2*MUS (Emb18)	CTM15062-19M-23Y-0M-0Y-18M-1Y -1B-0Y-0FGS	Mexico	Embrapa
Embrapa 53	LT1117.82/Civet//Tatu(Emb53)	CTM86.1323-4M-1Y-3B-2Y-2B-2RES-0B	Mexico	Embrapa
IAC 2 – Tarasca	Tarasca	-	Mexico	IAC
IAC 3 – Bantengue	Bantengue	-	Mexico	IAC
Iapar 23 – Arapoti	CIN/CNO//BGL/3/Merino(Arapoti)	B2700- (Hare)	Mexico	Iapar
Iapar 54 – Ocepar 4	Uron	B6811-270-27Y-3Y-0M	Mexico	Iapar/Ocepar
IPR 111	ANOAS 5/STIER 13	CTB89.1174-20M-0Y-0M-2B-0Y	Mexico	Iapar
PFT 0402	LIRON_2/5/DISB5/3/SPHD/PVN//YOGUI_6/4/KER_3/6/ BULL_10/MAN_1	CTSS94Y00486T-E-1M-0Y-0B-2Y-0B	Mexico	Embrapa
PFT 0403	T1505_WG//ERIZO_10/BULL_1-1/3/ERIZO_10/BULL_1-1	CTSS94Y00715T-B-1M-0Y-0B-3Y-0B	Mexico	Embrapa
PFT 0404	ANOAS-5/STIER-13//BULL-10/MANATI-1	CTSS94Y00324S-4M-0Y-0B-1Y-0B	Mexico	Embrapa
PFT 0405	ANOAS-5/STIER-13/5/274/320//BGL/3/ MUSX/LYNX/4/RHINO_9	CTSS94Y00241S-3M-0Y-0B-2Y-0B	Mexico	Embrapa
PFT 0406	BR4/Emb53	T3925-01F-5F-0F	Brazil	Embrapa
PFT 0407	ERIZO 11*2/MILMAN*2//PICUS	CTIB93B00002F-2Y-2Y-0M-0Y-0B -3Y-0B-0F	Mexico	Embrapa
PFT 0408	ERIZO-15/FAHAD-3//POLLMER-2.1	CTSS94Y00282S-17M-0Y-0B-3Y-0B	Mexico	Embrapa
PFT 0411	FD693/2*FAHAD_4//POLLMER-4/3/POLLMER_2.1	CTSS94Y00560T-B-2M-0Y-0B-2Y-0B	Mexico	Embrapa
PFT 0413	MUSX/LYNX//STIER_12-3/3/PEURA_3/4/ASNO/3/2*MUS X/LYNX//YOGUI_1	CTSS92Y1015-A-1Y-0M-1Y-0B	Mexico	Embrapa
PFT 0415	PFT215/Emb53	T4422-01F-0F	Brazil	Embrapa
PFT 0416	PRESTO//2*TESMO_1/MUSX 603/4/ARDI_1/TOPO1419//ERIZO_9/3/SUSI_2	CTSS94Y00465T-C-2M-0Y-0B-1Y-0B	Mexico	Embrapa
PFT 0417	PRESTO//2*TESMO_1/MUSX 603/4/ARDI_1/TOPO1419//ERIZO_9/3/SUSI_2	CTSS94Y00465T-C-2M-0Y-0B-2Y-0B	Mexico	Embrapa
PFT 0420	STIER_13/FAHAD-4//MANATI_1/3/POLLMER_1.1	CTSS94Y00612T-B-1M-0Y-0B-1Y-0B	Mexico	Embrapa
PFT 0501	Arapoti/Octo65(Frontana/CBR1)	T4063-04F-06F-01F-0F	Brazil	Embrapa
PFT 0502	BR4/2*PFT8913	T2708R-0F-0F-18F-0F-5FG-0F	Brazil	Embrapa
PFT 0503	BRS148*2/Hoh85107-2-3	T3628R-00F-05F-14F-09F-0F	Brazil	Embrapa
PFT 0504	Emb18*2/Octo50(IAC5/CBR1)	T4074R-10F-03F-11F-0F	Brazil	Embrapa
PFT 0505	Emb53//PFT116/Hoh-87102-6-1	T3752R-24F-20F*-07F-0F	Brazil	Embrapa
PFT 0506	FD-693/Vicuna	SWTY91.35-12FM-0FM-35F-09F-1F-24F	Mexico	Embrapa
PFT 0507	GNU/ASAD//ARDI/3/MAN_1/4/FAHAD_5/5/BULL_10/MA N_10	CTSS94Y00575T-A-3M-0Y-0B-1Y-0B	Mexico	Embrapa
PFT 0508	Octo65(Frontana/CBR1)/BR4	TO65-1RNF-32F-12F-0	Brazil	Embrapa
PFT 0509	Octo92-3(PF89358/CBR1)/Emb53	TO9203-3RNF-13F-02F-0F	Brazil	Embrapa
PFT 0510	Octo92-3(PF89358/CBR1)/HD	TO9203-H1F-1F-07F-0F-16FG-06F-0F	Brazil	Embrapa
PFT 0511	Octo94-2(BR35/CBR1) / PFT215*2/Hoh86007-1-2	T5066-0F-0F-6Ffo-1F-0F	Brazil	Embrapa
PFT 0512	PFT116/3/Octo1303/HD//SWTY89.104	T4041R-2F-01F-1/24F-10F-0F	Brazil	Embrapa
PFT 0513	PFT215*2/Binova	T4549R-0F-0F-1M-1F-0F	Brazil	Embrapa
PFT 0514	PFT215*2/Hoh92008-3	T4548R-0F-0F-2Ffo-1F-1/6F	Brazil	Embrapa
PFT 0515	PFT701/PFT408	T4935-0F-0F-2M-1F-1/6F	Brazil	Embrapa
PFT 0516	PFT703/PFT215*2/Hoh86007-1-2	T4917-0F-0F-4Ffo-1F-0F	Brazil	Embrapa
PFT 0517	RHINO_3/BULL_1-1/3/GAUR_3/ ANOAS_2//BANT-1	CTSS93Y00105S-11Y-0Y-0B	Mexico	Embrapa
PFT 0518	T1505_WG//ERIZO_10/BULL_1-1/3/ ERIZO_10/BULL_1-1	CTSS94Y00715T-B-1M-0Y-0B-1Y-0B	Mexico	Embrapa
PFT 112	PFT512/GUARA	T4004-7F<24F	Brazil	Embrapa
PFT 116	POLLMER//2*ERIZO/BULLI	CTSS93B00541M-F-1Y-0M-0Y-0B-1Y-0B	Mexico	Embrapa
PFT 204	ERIZO/NIMIR	CTY89.72-19Y-0M-3Y-0M-3Y-0M-3B-0Y	Mexico	Embrapa
PFT 205	IGUANA//HARE/CIVET	CTY88.132-6BI-3PAP-0Y-2B-0Y<24F	Mexico	Embrapa
PFT 209	PFT215*2/TCA 3050-89	T3301R-2F-2F	Brazil	Embrapa
PFT 210	RHINO/BULL//ERIZO/YOGUI	CTSS93Y00285S-14Y-0Y-0B	Mexico	Embrapa
PFT 303	Octo65(Frontana/CBR1)/BR4	TO65-1RNF-22F<24F-0F	Brazil	Embrapa
PFT 307	PFT312/PFT511	T3992-07F-15F<24F-0F	Brazil	Embrapa
PFT 308	PFT410*2/PORT-3-6-92,F4	T3710R-8F-1F<12F-0F	Brazil	Embrapa
PFT 924	Emb18*2/HOH-85110-1-2	T2888R-3F-1F-1F-0F	Brazil	Embrapa
Triticale BR 4	BGL/CIN//MUS (BR4)	B2686-0Y-61-(25-36)FS	Mexico	Embrapa

[1]Genotypes originated from Mexico were developed at the International Maize and Wheat Research Center (Cimmyt) triticale breeding program. [2]Ocepar: Cooperativa Central de Pesquisa Agrícola (Coodetec), the former research department of Sindicato e Organização das Cooperativas do Estado do Paraná (Ocepar), in Cascavel, PR; IAC: Instituto Agronômico, in Campinas, SP; Iapar: Instituto Agronômico do Paraná, in Londrina, PR; Fundacep: Fundação Centro de Experimentação e Pesquisa da Fecotrigo, in Cruz Alta, RS; Embrapa: Empresa Brasileira de Pesquisa Agropecuária, Centro Nacional de Pesquisa do Trigo, in Passo Fundo, RS.

fragments were fractionated on 3% agarose gel stained in ethidium bromide and photographed.

DNA segments at polymorphic loci in all 54 genotypes were scored as presence (1) or absence (0) of an allele and the results analyzed with NTSYSpc, version 2.02 (Rohlf, 1989). The scores for each allele were used to construct a binary matrix, which was then transformed to genetic similarity matrix using Jaccard similarity coefficient. The genetic similarity matrix of all genotypes was analyzed using unweighted pair group method with arithmetic average (UPGMA) algorithm and the results were used to construct a dendrogram. Null alleles were omitted in calculation, and were treated as missing data.

The average gene diversity expected for each considered marker locus in this group of genotypes was estimated according to Anderson (1993): $PIC=i-\sum_{j=1}^{n} p_{ij}^{2}$, where p is the frequency of the allele j for marker i.

Additionally, the genetic diversity was described based on the number of variants determined for the germplasm, considering: 1) the proportion of polymorphic loci (P) given by $P = n_{pj}/n_{total}$, in which n_{pj} is the number of polymorphic loci and n_{total} the total number of loci; 2) average expected heterozigosity (H_e), i.e., the probability of any pair of alleles in a single locus, randomly chosen in the population, be distinct from each other, given by $H_e = S_j^L H_j/L$, where H_j is the heterozigosity per locus and L the total number of loci. Considering a locus j with i alleles, the genetic diversity in this locus is

Table 2. Wheat (*Triticum aestivum* L.) genomic microsatellites (SSR) used to amplify triticale (X *Triticosecale* Wittmack) genomic DNA via polymerase chain reaction.

SSR	Localization[1]	Forward primer sequence	Reverse primer sequence	Annealing temperature (ºC)	Number of cycles	Final extension (minutes)
WMS 135	1AL	TGT CAA CAT CGT TTT GAA AAG G	ACA CTG TCA ACC TGG CAA TG	60	45	10
WMS 136	1AS	GAC AGC ACC TTG CCC TTT G	CAT CGG CAA CAT GCT CAT C	60	45	10
WMS 294	2AL	GGA TTG GAG TTA AGA GAG AAC CG	GCA GAG TGA TCA ATG CCA GA	55	35	10
WMS 95	2AS	GAT CAA ACA CAC ACC CCT CC	AAT GCA AAG TGA AAA ACC CG	50	35	3
WMS 153	3AL	ATGAGGACTCGAAGCTTGGC	CTGAGCTTTTGCGCGTTGAC	56	40	3
WMS 369	3AS	CTG CAG GCC ATG ATG ATG	ACC GTG GGT GTT GTG AGC	60	35	20
WMS 160	4AL	TTC AAT TCA GTC TTG GCT TGG	CTG CAG GAA AAA AAG TAC ACC C	60	35	10
WMS 4	4AS	GCT GAT GCA TAT AAT GCT GT	CAC TGT CTG TAT CAC TCT GCT	50	35	3
WMS 595	5AL	GCA TAG CAT CGC ATA TGC AT	GCC ACG CTT GGA CAA GAT AT	-[2]	-	-
WMS 304	5AS	AGG AAA CAG AAA TAT CGC GG	AGG ACT GTG GGG AAT GAA TG	55	35	10
WMS 169	6AL	ACC ACT GCA GAG AAC ACA TAC	GTG CTC TGC TCT AAG TGT GGG	-	-	-
WMS 334	6AS	AAT TTC AAA AAG GAG AGA GA	AAC ATG TGT TTT TAG CTA TC	45	40	10
WMS 282	7AL	TTG GCC GTG TAA GGC AG	TCT CAT TCA CAC ACA ACA CTA GC	53	65	10
WMS 60	7AS	TGT CCT ACA CGG ACC ACG T	GCA TTG ACA GAT GCA CAC G	60	35	10
WMS 259	1BL	AGG GAA AAG ACA TCT TTT TTT TC	CGA CCG ACT TCG GGT TC	-	-	-
WMS 18	1BS	TGG CGC CAT GAT TGC ATT ATC TTC	GGT TGC TGA AGA ACC TTA TTT AGG	50	40	3
WMS 120	2BL	GAT CCA CCT TCC TCT CTC TC	GAT TAT ACT GGT GCC GAA AC	60	35	3
WMS 148	2BS	GTG AGG CAG CAA GAG AGA AA	CAA AGC TTG ACT CAG ACC AAA	50	35	3
WMS 340	3BL	GCA ATC TTT TTT CTG ACC ACG	ACA CTG TCA ACC TGG CAA TG	-	-	-
WMS 389	3BS	ATC ATG TCG ATC TCC TTG ACG	TGC CAT GCA CAT TAG CAG AT	50	40	3
WMS 375	4BL	ATT GGC GAC TCT AGC ATA TAC G	GGG ATG TCT GTT CCA TCT TAG C	55	35	3
WMS 368	4BS	CCATTTCACCTAATGCCTGC	AATAAAACCATGAGCTCACTTGC	50	40	7
WMS 408	5BL	TCG ATT TAT TTG GGC CAC TG	GTA TAA TTC GTT CAC AGC ACG C	47	40	3
WMS 234	5BS	GAG TCC TGA TGT GAA GCT GTT G	CTC ATT GGG GTG TGT ACG TG	60	35	3
WMS 219	6BL	GAT GAG CGA CAC CTA GCC TC	GGG GTC CGA GTC CAC AAC	55	35	3
WMS 518	6BS	AAT CAC AAC AAG GCG TGA CA	CAG GGT GGT GCA TGC AT	-	-	-
WMS 166	7BL	ATAAAGCTGTCTCTTTAGTTCG	GTTTTAACACATATGCATACCT	50	35	3
WMS 43	7BS	CACCGACGGTTTCCCTAGAGT	GGTGAGTGCAAATGTCATGTG	50	40	3
WMS 232	1DL	ATC TCA ACG GCA AGC CG	CTG ATG CAA GCA ATC CAC C	-	-	-
WMS 106	1DS	AAT AAG GAC ACA ATT GGG ATG G	CTG TTC TTG CGT GGC ATT AA	-	-	-
WMS 157	2DL	GTC GTC GCG GTA AGC TTG	GAG TGA ACA CAC GAG GCT TG	-	-	-
WMS 261	2DS	CTC CCT GTA CGC CTA AGG C	CTC GCG CTA CTA GCC ATT G	-	-	-
WMS 383	3DL	ACG CCA GTT GAT CCG TAA AC	GAC ATC AAT AAC CGT GGA TGG	55 - 45[3] / 45	10[3] / 30	7
WMS 161	3DS	GAT CGA GTG ATG GCA GAT GG	TGT GAA TTA CTT GGA CGT GG	-	-	-
WMS 194	4DL	GAT CTG CTC TAC TCT CCT CC	CGA CGC AGA ACT TAA ACA AG	50	40	3
WMS 608	4DS	ACA TTG TGT GTG CGG CC	GAT CCC TCT CCG CTA GAA GC	58	35	10
WMS 272	5DL	TGC TCT TTG GCG AAT ATA TGG	GTT CAA AAC AAA TTA AAA GGC CC	45	40	3
WMS 192	5DS	GGT TTT CTT TCA GAT TGC GC	CGT TGT CTA ATC TTG CCT TGC	52	35	10
WMS 325	6DL	TTT CTT CTG TCG TTC TCT TCC C	TTT TTA CGC GTC AAC GAC G	-	-	-
WMS 469	6DS	GTT GAG CTT TTC AGT TCG GC	ACT GGC ATC CAC TGA GCT G	52	35	3
WMS 437	7DL	GAT CAA GAC TTT TGT ATC TCT C	GAT GTC CAA CAG TTA GCT TA	-	-	-
WMS 44	7DS	GAT CAA GAC TTT TGT ATC TCT C	GAT GTC CAA CAG TTA GCT TA	50	35	10

[1]Wheat chromosome: 1 to 7; genome: A, B or D; and chromosome arm: long (L) or short (S). [2]These primers did not amplify triticale DNA. [3]First cycle at 55ºC, decreasing 1ºC per cycle until 45ºC.

given by $H_j = 1 - Sp_i^2$, also called PIC; 3) average observed heterozigosity (Ho), considering the proportion of heterozygote loci in all analyzed individuals; 4) the abundance of allelic variants (A); 5) the effective number of alleles (Ae) given by $A_e = 1/(1 - H_j) = 1/Sp_i^2$, where p_i is the frequency of the i-th allele in a marker locus; and 6) the average number of alleles per locus, i.e., the sum of all observed alleles in all used markers, divided by the total number of markers, a piece of information complementary to the polymorphism information: $n=(1/K)\sum_{i=1}^{K} n_i$, where, K is the number of loci and n_i is the number of alleles determined per locus.

Results and Discussion

Out of the 42 wheat genomic microsatellites tested in the parental genotypes from the Embrapa's triticale breeding program, 30 (71.42%) amplified in the genome of triticale, suggesting good transferability of these wheat markers to triticale (Table 2). Among these, 21 (70%) were polymorphic, i.e., the frequency of the most common allele was equal or superior to 0.95. About 18% of A - B wheat genome-specific microsatellite primers did not amplify in triticale (Table 2), and some of the obtained fragments may not contain SSR sequences, as suggested by Leonova et al. (2005). Kuleung et al. (2004) found 57% and 39% transferability for 148 wheat microsatellites and 28 rye microsatellites, respectively, revealing low marker transferability.

The rate of transferred markers from wheat D genome was 50%, surpassing the expected values, since the analyzed triticales are hexaploids, lacking, in general, this genome. Similarly, Tams et al. (2004) and Leonova et al. (2005) obtained amplification of triticale DNA fragments using D genome-specific microsatellite primers.

PCR amplification with microsatellites mapped in all wheat D genome chromosomes, but 1D and 2D, may be associated with the presence of wheat-rye (Lukaszewski & Gustafson, 1983) or wheat-wheat translocation (Hohmann et al., 1999; Leonova et al. 2005). However, studies have shown that alterations on the expression of certain genes in triticale compared to the parental wheat lines would result from the effect of rye chromosomes due to the change of triticale genomic composition, rather than being associated with the presence of wheat-rye translocation (Leonova et al., 2005). Both possibilities must be investigated in this group of triticales to detect the presence of translocations or recombination, employing molecular cytogenetic tools such as in situ hybridization and C-banding (Zhang et al., 2007b).

Besides, the presence of storage proteins subunits encoded by genes on the D genome must be determined in these lines, to confirm the presence of the D genome on the genomic constitution of the germplasm. Most triticale cultivars are derived from crossing triticale parents or from crossing wheat with triticale (Kuleung et al., 2004). Under the circumstances, the introgression of genes of interest encoded on the chromosome 1D can be widely exploited in breeding programs in order to improve important traits of hexaploid triticales (AABBRR), such as end-use quality.

Most genomic SSRs have neither a gene function nor a close linkage to coding regions and limited transferability among related species. An alternative for the development of triticale specific markers is the analysis and characterization of microsatellites in expressed sequence tags (EST) collections. Today, more than 1,050,000 ESTs for common wheat and 9,200 for rye are available in the public domain (National Center for Biotechonology Information, 2007) and are used to develop molecular markers such as EST-derived microsatellites. The use of this marker system would allow to detect variation in the expressed portion of the genome, which may increase the efficiency of marker-assisted selection in crop breeding (Gupta et al., 2003; Zhang et al., 2005, 2007a; Tang et al., 2006).

Considering the set of wheat microsatellites that amplified PCR products in the analyzed triticale genotypes, the proportion of polymorphic loci (P) revealed was 0.70 and the average PIC value estimated was 0.36, similar to values determined for inbred rye (Bolibok et al., 2005) and also wheat (Zhang et al., 2006, 2007a). The expected genetic diversity for this set of markers ranged between 0.24 for WMS408 and 0.88 for WMS389 (Table 3). However, it must be taken into account that these accessions come from only two breeding sources, Embrapa Trigo (43%) in Brazil and Cimmyt (57%) in Mexico (Table 1). The PIC values (0.54) revealed for triticale by Kuleung et al. (2006) and Tams et al. (2004) were obtained for 80 and 128 genotypes, respectively, representing a broad spectrum of historic and modern triticale germplasm from 17 countries, five continents

Table 3. Number and frequency of alleles, polymorphism information content (PIC) values and effective number of alleles (Ae) estimated for parental genotypes from Embrapa's triticale (X *Triticosecale* Wittmack) breeding program using 30 wheat (*T. aestivum* L.) genomic microsatellites (SSR).

SSR	Number of alleles	Frequency of alleles	PIC	Ae
WMS4	1	1.00	0.00	1.00
WMS18	1	1.00	0.00	1.00
WMS43	1	1.00	0.00	1.00
WMS44	2	1.00	0.00	1.00
	-	1.00	0.00	1.00
WMS60	2	0.85	0.26	3.84
	-	0.15	-	-
WMS 95	3	0.31	0.63	1.59
	-	0.20	-	-
	-	0.48	-	-
WMS120	2	0.37	0.47	2.14
	-	0.63	-	-
WMS135	3	0.18	0.57	1.76
	-	0.23	-	-
	-	0.59	-	-
WMS136	3	0.19	0.61	1.63
	-	0.51	-	-
	-	0.30	-	-
WMS148	2	0.38	0.47	2.12
	-	0.62	-	-
WMS153	2	0.23	0.35	2.83
	-	0.77	-	-
WMS160	3	0.26	0.64	1.56
	-	0.47	-	-
	-	0.28	-	-
WMS166	4	0.02	0.64	1.56
	-	0.46	-	-
	-	0.18	-	-
	-	0.33	-	-
WMS192	1	1.00	0.00	1.00
WMS194	2	0.84	0.26	3.81
	-	0.16	-	-
WMS219	3	0.22	0.45	2.22
	-	0.70	-	-
	-	0.07	-	-
WMS234	3	0.34	0.64	1.57
	-	0.21	-	-
	-	0.45	-	-
WMS272	1	1.00	0.00	1.00
WMS282	3	0.28	0.63	1.58
	-	0.48	-	-
	-	0.24	-	-
WMS294	3	0.19	0.63	1.59
	-	0.46	-	-
	-	0.35	-	-
WMS304	2	0.24	0.36	2.77
	-	0.76	-	-
WMS334	2	0.48	0.50	2.00
	-	0.52	-	-
WMS368	2	0.37	0.47	2.14
	-	0.63	-	-
WMS369	4	0.15	0.58	1.72
	-	0.09	-	-
	-	0.16	-	-
	-	0.60	-	-
WMS375	2	0.74	0.38	2.60
	-	0.26	-	-
WMS383	1	1.00	0.00	1.00
WMS389	2	0.30	0.88	1.14
	-	0.18	-	-
WMS408	2	0.86	0.24	4.08
	-	0.14	-	-
WMS469	1	1.00	0.00	1.00
WMS608	1	1.00	0.00	1.00
Total	64	-	-	-
Average	2.13	0.34	0.36	1.61

and at least 27 breeding programs. At the same time, the PIC of 0.71 determined by Prasad et al. (2000) for wheat was calculated for 55 accessions from 29 countries, the PIC of 0.68 determined by Hai et al. (2007) for wheat included accessions from four distinct continents and the PIC of 0.51 registered by Landjeva et al. (2006) represented the wheat germplasm created in Bulgary over a period of almost 80 years.

In this work, 64 alleles were amplified with the 30 selected SSR markers, with the average of 2.13 alleles per locus and the allelic variants abundance per locus (A) ranging between one to four (Table 3). The effective number of alleles (Ae) for the polymorphic markers ranged between 1.14, for WMS389, and 4.08, for WMS408, both mapped on the wheat B genome, and with average value of 1.61. The number of alleles expected for each of the markers from this set of microsatellites, under the circumstances, should not be lower than 1.61 when applied to a different group of progenitors if it is to be maintained, at least, the same level of diversity verified in this work. Otherwise, the strategy of choice of progenitors should be revised.

Among the 54 accessions analyzed, 104 heterozygote loci were detected (Table 4). Three accessions presented only homozygote loci for all used SSRs and five heterozygote loci were observed in one accession, resulting in an average observed heterozygosity (Ho) of 0.06, as expected for a group of genotypes formed exclusively by fixed lines.

The 54 accessions evaluated were divided into seven main groups (Figure 1), using UPGMA dendrogram based on Jaccard's coefficient of similarity (average similarity = 0.56). Since most of the analyzed germplasm is derived from Mexican triticale, a high similarity among them was expected. The PIC (0.36) and the average number of alleles (2.13) indicate the expected low polymorphism in the 54 genotypes and the obtained average similarity value (0.56), indicative of moderate variability, may change if a larger number of markers is used to screen these genotypes, revealing a more actual value. Tams et al. (2004, 2006) used wheat and rye microsatellites to study genetic diversity of European

winter triticale and their results showed no distinct clusters of lines from the same breeding source. On the other hand, Kuleung et al. (2006) divided 80 hexaploid triticale accessions available in the world collection into five clusters (average similarity = 0.45).

Clusters obtained could not be clearly characterized and besides the pedigree and the breeding source, other data on the Embrapa's germplasm were used to discuss aspects of the resulting dendrogram. Group 1 was subdivided into four subgroups, 1-a, 1-b, 1-c and 1-d (Figure 1). Subgroup 1-a comprised four accessions created at Cimmyt, all short in height, early in maturity and susceptible to *Fusarium graminearum*, causal agent of scab. About 50% of the lines included in subgroup 1-b share the germplasm of Tatu, a cultivar created at Cimmyt and released for commercial use in Brazil. Within subgroup 1-c, around 27% of the genotypes show shorter height and earlier maturity and the same proportion of accessions did not present high susceptibility to scab. Subgroup 1-d is formed by a single cultivar, CEP 28 – Guará, created at Cimmyt and released for commercial use in Brazil. It is characterized by its intermediate cycle and height as well as good behavior against *Drechslera tritici-repentis*. The male parent of CEP 28 – Guará integrates subgroup 1-b and is present in the pedigree of 50% of the genotypes forming this subgroup. In turn, CEP 28 – Guará's female parent is present in the pedigree of only two other accessions among all 54 studied ones, both included in group 5, PFT 0416 and PFT 0417, created at Cimmyt, showing early maturity and short height.

Group 2 is represented by the only two accessions comprising late cycle in this set of triticales, both showing very good agronomic traits. All accessions included in groups 3 to 4 are very susceptible to *F. graminearum*. Group 3 was formed by a single genotype, PFT 0517, a breeding line created and introduced from Cimmyt, Mexico. PFT 0517 is the only genotype among all the analyzed accessions that has the parental GAUR_3 and BANT-1, and this might be the explanation for this separated cluster. Group 5 is formed by the Brazilian line PFT 0510 and two other sib lines, PFT 0416 and PFT 0417, mentioned before.

Most accessions adapted to warm environments are found in cluster 6, grouping accessions with good agronomic type (80%), except for the Mexican lines PFT 205 and PFT 0413 (intermediate). Finally, in group 7, characterized by clustering genotypes of intermediate maturity and height, two accessions can be found, among all the analyzed triticales, that share FD-693 in their pedigree, as well as IAC 2, the genotype with the best performance against *Magnaporthe grisea*.

The use of rye microsatellites with wheat microsatellites (genomic or derived from expressed

Table 4. Number of heterozygote loci (h) observed in triticale (X *Triticosecale* Wittmack) parental genotypes from Embrapa's improvement program analyzed with 30 wheat (*T. aestivum* L.) genomic microsatellites[(1)].

Genotype	h	Genotype	h	Genotype	h	Genotype	h
BRS 148	2	PFT 0404	3	PFT 0504	1	PFT 0518	3
BRS 203	2	PFT 0405	1	PFT 0505	1	PFT 112	1
BRS Minotauro	1	PFT 0406	2	PFT 0506	0	PFT 116	2
CEP 28 – Guará	4	PFT 0407	1	PFT 0507	0	PFT 204	2
Embrapa 18	2	PFT 0408	2	PFT 0508	2	PFT 205	2
Embrapa 53	1	PFT 0411	2	PFT 0509	4	PFT 209	3
IAC 2 – Tarasca	2	PFT 0413	1	PFT 0510	2	PFT 210	1
IAC 3 – Bantengue	1	PFT 0415	3	PFT 0511	2	PFT 303	2
Iapar 23 – Arapoti	3	PFT 0416	1	PFT 0512	1	PFT 307	2
CEP 23 – Tatu	5	PFT 0417	1	PFT 0513	2	PFT 308	3
Iapar 54 – Ocepar 4	2	PFT 0420	4	PFT 0514	2	PFT 924	3
IPR 111	3	PFT 0501	1	PFT 0515	2	Triticale BR 4	0
PFT 0402	1	PFT 0502	1	PFT 0516	3		
PFT 0403	3	PFT 0503	1	PFT 0517	2		

[(1)]Total heterozygote loci: 104

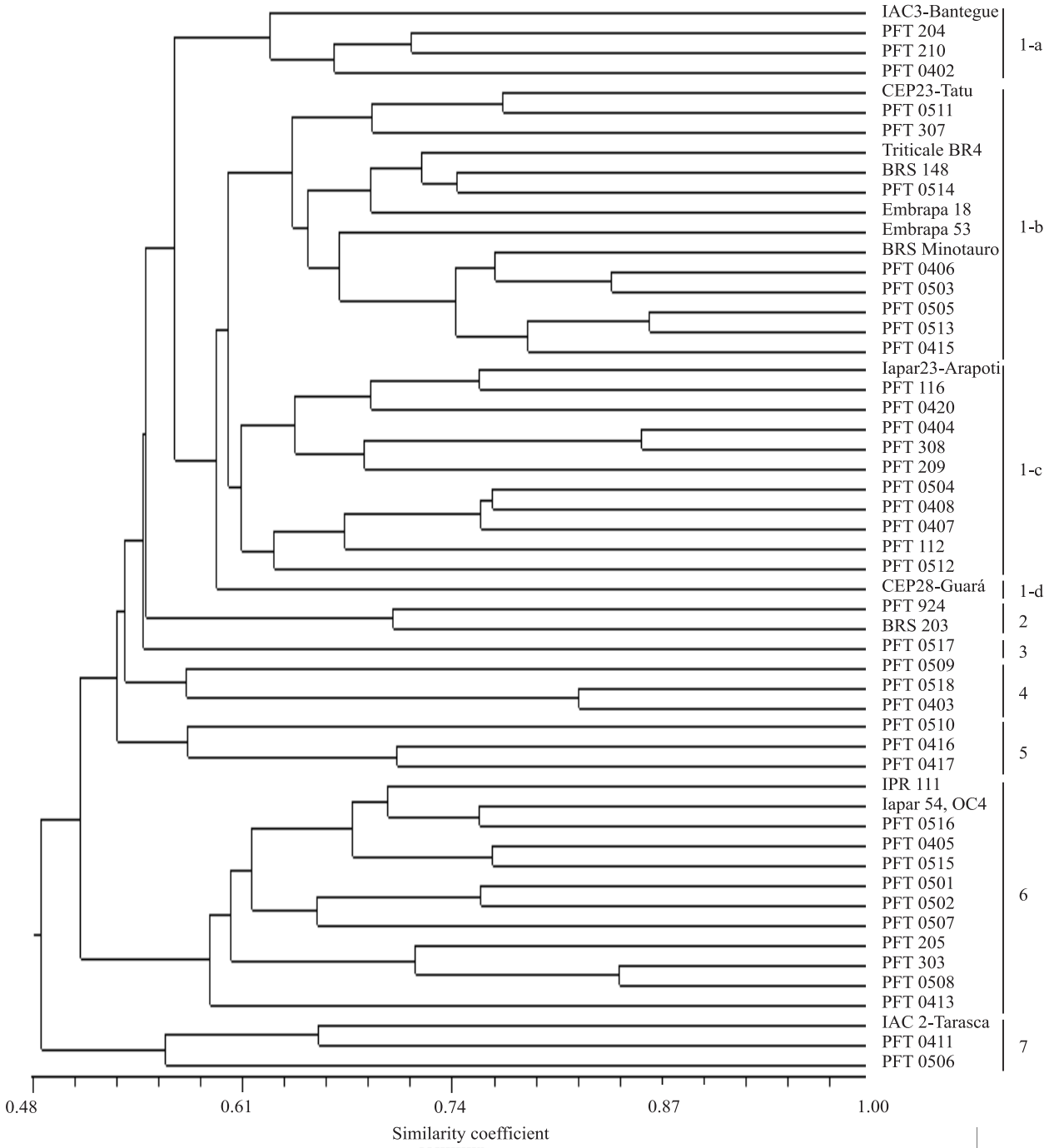

Figure 1. Dendrogram of 54 triticale genotypes estimated by Jaccard's coefficient based on 30 wheat genomic microsatellites.

sequences) must be considered in further studies with this crop, distributing at least one pair of primers per chromosome arm of all genomes. Regardless the diversity revealed by rye SSR in triticale being lower in comparison to wheat microsatellites (Kuleung et al., 2004; Tams et al., 2004), this strategy allows the balanced inclusion of all species genomes in triticale analysis. The utilization of pooled data from both species, besides data from more microsatellite markers or other marker class, including expressed regions of the genome, would provide a broader coverage of the triticale genome, leading to more complete data about the species and its diversity patterns.

Conclusions

1. The 71.42% transferability of the chosen set of wheat genomic microsatellites to triticale raises the potential of exploiting these markers in Brazilian triticale genetic and breeding studies.

2. The average number of alleles (2.13) and PIC (0.36) indicate low variability in the set of genotypes used in Embrapa's triticale breeding program.

References

AMMAR, K.; MERGOUM, M.; RAJARAM, S. The history and evolution of triticale. In: MERGOUM, M.; GOMEZ-MACPHERSON, H. (Org.). **Triticale improvement and production**. 1th. Roma: FAO, 2004. p.1-10.

ANDERSON, J.A.; CHURCHILL, G.A.; AUTRIQUE, J.E.; TANKSLEY, S.D.; SORRELS, M.E. Optimizing parental selection for genetic linkage maps. **Genome**, v.36, p.181-186, 1993.

BOLIBOK, H.; RAKOCZI-TROJANOWSKA, M.; HROMADA, A.; PIETRZYKOWSKI, R. Efficiency of different PCR-based marker systems in assessing genetic diversity among winter rye (*Secale cereale* L.) inbred lines. **Euphytica**, v.146, p.109-116, 2005.

DARVEY, N.L. Strategies for the production and utilization of triticale germoplasm. In: DARVEY, N. (Ed.). **Proceedings of the International Triticale Symposium**, 24., 1986, Sydney. p.458-564.

GUPTA, P.K.; RUSTGI, S.; SHARMA, S.; SINGH, R.; KUMAR, N.; BALYAN; H.S. Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. **Molecular Genetics and Genomics**, v.270, p.315-323, 2003.

HAI, L.; WAGNER, C.; FRIEDT, W. Quantitative structure analysis of genetic diversity among spring bread wheats (*Triticum aestivum* L.) from different geographical regions. **Genetica**, v.130, p.213-225, 2007.

HOHMANN, U.; ZOLLER, J.; HERMANN, R.G.; KAZMAN, M.E. Physical mapping and molecular-cytogenetic analysis of substitutions and translocations involving chromosome 1D in synthetic hexaploid triticale. **Theoretical and Applied Genetics**, v.98, p.647-656, 1999.

HORLEIN, A.; VALENTINE, J. Triticale (X *Triticosecale*). In: WILLIAMS, J.T. (Ed.). **Cereals and pseudocereals**. New York: Chapman and Hall, 1995. p.187-221.

KLEINHOFS, A.; KILIAN, A.; SAGHAI MAROOF, M.A.; BIYASHEV, R.M.; HAYES, P.; CHEN, F.Q.; LAPITAN, N.; FENWICK, A.; BLAKE, T.K.; KANAZIN, V.; ANANIEV, E.; DAHLEEN, L.; KUDRNA, D.; BOLLINGER, J.; KNAPP, S.J.; LIU, B.; SORRELLS, M.; HEUN, M.; FRANCKOWIAK, J.D.; HOFFMAN, D.; SKADSEN, R.; STEFFENSON, B.J. A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. **Theoretical and Applied Genetics**, v.86, p.705-712, 1993.

KULEUNG, C.; BAENZIGER, P.S.; DWEIKAT, I. Transferability of SSR markers among wheat, rye, and triticale. **Theoretical and Applied Genetics**, v.108, p.1147-1150, 2004.

KULEUNG, C.; BAENZIGER, P.S.; KACHMAN, S.D.; DWEIKAT, I. Evaluating the genetic diversity of triticale with wheat and rye SSR markers. **Crop Science**, v.46, p.1692-1700, 2006.

LANDJEVA, S.; KORZUN, V.; GANEVA, G. Evaluation of genetic diversity among bulgarian winter wheat (*Triticum aestivum* L.) varieties during the period 1925-2003 using microsatellites. **Genetic Resources and Crop Evolution**, v.53, p.1605-1614, 2006.

LEONOVA, I.N.; DOBROVOLSKAYA, O.B.; KAMINSKAYA, L.N.; ADONINA, I.G.; KOREN, L.V.; KHOTYLJOVA, L.V.; SALINA, E.A. Molecular analysis of the triticale lines with different *vrn* gene systems using microsatellite markers and hybridization in situ. **Russian Journal of Genetics**, v.41, p.1014-1020, 2005.

LUKASZEWSKI, A.J.; GUSTAFSON, J.P. Translocations and modifications of chromosomes in triticale × wheat hybrids. **Theoretical and Applied Genetics**, v.64, p.239-248, 1983.

NASCIMENTO JUNIOR, A.; BAIER, A.C.; TEIXEIRA, M.C.C.; WIETHÖLTER, S. Triticale in Brazil. In: MERGOUM, M.; GOMEZ-MACPHERSON, H. (Org.). **Triticale improvement and production**. 1th. Roma: FAO, 2004. v.1. p.93-98.

NATIONAL CENTER FOR BIOTECHONOLOGY INFORMATION. **DbEST:** database of "expressed sequence tags". Available at: http://www.ncbi.nlm.nih.gov/dbest/dbest_summary.html. Accessed on: 17 Oct. 2007.

OETLER, G. The fortune of a botanical curiosity: triticale: past, present and future**. Journal of Agricultural Science**, v.143, p.329-346, 2005.

PRASAD, M.; VARSHNEY, R.K.; ROY, J.K.; BALYAN, H.S.; GUPTA, P.K. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. **Theoretical and Applied Genetics**, v.100, p.584-592, 2000.

RÖDER, M.S.; KORZUN, V.; WENDEHAKE, K.; PLASCHKE, J.; TIXIER, M.H.; LEROY, P.; GANAL, M.W. A microsatellite map of wheat. **Genetics**, v.149, p.2007-2023, 1998.

RÖDER, M.S.; PLASCHKE, J.; KONIG, S.U.; BORNER, A.; SORRELLS, M.E.; TANKSLEY, S.D.; GANAL, M.W. Abundance, variability and chromosomal location of microsatellite wheat. **Molecular Genetics and Genomics**, v.246, p.327-333, 1995.

ROGERS, S.O.; BENDICH, A.J. Extraction of DNA from plant tissues. In.: GELVIN, S.B.; SCHILPEROORT, R.A. (Ed.). **Plant Molecular Biology Manual**. Dordrecht: Kluwer Academic Publishers, 1988. p.A6/1-A6/11.

ROHLF, F.J. **NTSYS pc:** numerical taxonomy and multivariate analysis system. New York: Exater, 1989.

STACHEL, M.; LELLEY, T.; GRAUSGRUBER, H.; VOLLMANN, J. Application of microsatellites in wheat (*Triticum aestivum* L.) for studying genetic differentiation caused by selection for adaptation and use. **Theoretical and Applied Genetics**, v.100, p.242-248, 2000.

TAMS, S.H.; BAUER, E.; OETTLER, G.; MELCHINGER, A.E. Genetic diversity in European winter triticale determined with SSR markers and coancestry coefficient. **Theoretical and Applied Genetics**, v.108, p.1385-1391, 2004.

TAMS, S.H.; BAUER, E.; OETTLER, G.; MELCHINGER, A.E.; SCHÖN, C.C. Prospects for hybrid breeding in winter triticale: II. relationship between parental genetic distance and specific combining ability. **Plant Breeding**, v.125, p.331-336, 2006.

TANG, J.; GAO, L.; CAO, Y.; JIA, J. Homologous analysis of SSR-ESTs and transferability of wheat SSR-EST markers across barley, rice and maize. **Euphytica**, v.151, p.87-93, 2006.

VAILLANCOURT, A.; NKONGOLO, K.K.; MICHAEL, P.; MEHES, M. Identification, characterization, and chromosome locations of rye and wheat specific ISSR and SCAR markers useful for breeding. **Euphytica**, 2007. Available at: http://www.springerlink.com/content/e21297314x050652/. Accessed on: 10 Oct. 2007.

ZHANG, L.Y.; BERNARD, M.; LEROY, P.; FEUILLET, C.; SOURDILLE, P. High transferability of bread wheat EST-derived SSRs to other cereals. **Theoretical and Applied Genetics**, v.111, p.677-687, 2005.

ZHANG, L.Y.; BERNARD, M.; RAVEL, C.; BALFOURIER, F.; LEROY, P.; FEUILLET, C.; SOURDILLE, P. Wheat EST-SSRs for tracing chromosome segments from a wide range of grass species. **Plant Breeding**, v.126, p.251-258, 2007a.

ZHANG, P.; DREISIGACKER, S.; BUERKERT, A.; ALKHANJARI, S.; MELCHINGER, A.E.; WARBURTON, M.L. Genetic diversity and relationships of wheat landraces from Oman investigated with SSR markers. **Genetic Resources and Crop Evolution**, v.53, p.1351-1360, 2006.

ZHANG, P.; FRIEBE, B.; GILL, B.; PARK, R.F. Cytogenetics in the age of molecular genetics. **Australian Journal of Agricultural Research**, v.58, p.498-506, 2007b.

Received on July 25, 2007 and accepted on October 9, 2007