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Genetic Diversity in Hexaploid and Tetraploid Wheat Genotypes Using Microsatellite Markers

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Abstract

The genetic diversity of a set of hexaploid and tetraploid wheat germplasm at the IPK gene bank, Gatersleben, Germany was investigated by applying 13 wheat microsatellites (WMS). The materials consisted of 63 accessions of *Triticum aestivum*, *T. dicoccon* and *T. durum* obtained from different collection missions. In total, 126 alleles were detected with an average of 9.7 alleles per locus. The average PIC values per locus varied from 0.65 for the marker Xgwm192 to 0.88 for the marker Xgwm619. All the primer pairs revealed genetic heterogeneity in more than one genotype. Genetic dissimilarity values between genotypes, calculated by the WMS derived data, were used to produce a dendrogram. In the dendrogram the genotypes were clustered in nine clear groups.

Introduction

Genetic diversity is the basis for genetic improvement. Knowledge of germplasm diversity has a significant impact on the improvement of crop plants. Due to modern breeding, it has been suggested that genetic diversity in wheat has been increasingly narrowed. Narrow genetic diversity is a problem in breeding for adaptation to biotic stresses, like diseases, and abiotic stresses, such as drought or salt tolerance. Therefore, it is necessary to investigate the genetic diversity in wheat germplasm in order to broaden the genetic variation in future breeding programme. Morphological traits can be used for assessing genetic diversity but are often influenced by the environment. The use of molecular markers for the evaluation of genetic diversity is receiving much attention. Many wheat scientists have studied genetic diversity in common wheat using different molecular markers such as RAPDs (Joshi and Nguyen 1993). RFLPs (Siedler et al. 1994, Kim and Ward 2000), AFLPs (Barrett and Kidwell 1998, Burkhamer et al. 1998), STS (Chen et al.1994) and ISSRs (Nagaoka and Ogihara 1997). However, most of these marker systems (Chao et al. 1989, Devos and Gale 1992) show a low level of polymorphism in wheat, especially among cultivated lines and/or cultivars.

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Microsatellites, also termed simple sequence repeats (SSRs), have been proposed as one of the most suitable markers for the assessment of genetic variation and diversity among wheat varieties/lines, because they are multiallelic, chromosome-specific and evenly distributed along chromosomes (Roeder et al. 1998). Microsatellite markers have been applied widely for tagging resistance genes (Peng et al. 1999, Borner et al. 2000b), identifying QTLs (Parker et al. 1998), marker-assisted selection in wheat (Korzun et al. 1998, Hung et al. 2000) and verifying the integrity and genetic stability of gene bank accessions (Boerner et al. 2000a). Such markers also revealed a high level of polymorphism among diploid species (Hammer et al. 2000) in the accessions of tetraploid wild wheat *Triticum dicoccoides* (Fahima et al. 2002) and of *Aegilops tauschii,* the D-genome donor of bread wheat (Pestsova et al. 2000), and as well as in hexaploid wheat varieties (Plaschke et al. 1995, Prasad et al. 2000, Stachel et al. 2000). The objectives of the present study were to examine the genetic factors that affected microsatellite diversity and to use wheat microsatellite markers for characterization and assessment of the genetic diversity of a set of hexaploid and tetraploid wheat germplasms from the gene bank.

Materials and Methods

Plant materials and DNA isolation: Fifty one and 12 accessions of hexaploid and tetraploid wheat, respectively were selected from the gene bank of IPK, Gatesrleben, Germany for analysis during 2003 (Table 1). These materials were obtained from several collection missions. Total genomic DNA was isolated from pooled leaf material of five to eight plants of each accession according to Plaschke et al. (1995). Chinese Spring and European commercial variety, Aztec were included as standards.

Table 1. Hexaploid and tetraploid (*) wheat accessions with their morphological group and origin extracted from the Gatersleben wheat database.

Population ID	Cultivar/ Line	Species	Morphological group	Country of origin
1	Penjamo62	*T. aestivum*	var. aestivum	Mexico
2	Penjamo62	*T. aestivum*	var. aestivum	Mexico
3	Penjamo62	*T. aestivum*	var. aestivum	Mexico
4	Penjamo62	*T. aestivum*	var. erythrospermum	USA
5	Penjamo62	*T. aestivum*	Not determined	Unknown
6	Penjamo62	*T. aestivum*	Not determined	Mexico
7	Vernal	*T. dicoccon**	var. dicoccon	USA
8	Vernal	*T. dicoccon**	var. dicoccon	USA
9	Vernal	*T. dicoccon**	var. volgense (Flaksb.) Flaksb.	USA

10	Vernal	*T. dicoccon**	var. volgense (Flaksb.) Flaksb.	USA
11	Vernal	*T. dicoccon**	Not determined	Unknown

(Contd.)

(Contd.)

Population ID	Cultivar/ Line	Species	Morphological group	Country of origin
12	Vernal	*T. dicoccon**	Not determined	Unknown
13	Vernal	*T. dicoccon**	var. farrum	USA
14	Thatcher	*T. aestivum*	var. lutescens (Alef.) Mansf.	USA
15	Thatcher	*T. aestivum*	var. lutescens (Alef.) Mansf.	USA
16	Thatcher	*T. aestivum*	var. lutescens (Alef.) Mansf.	Canada
17	Thatcher	*T. aestivum*	var. lutescens (Alef.) Mansf.	USA
18	Thatcher	*T. aestivum*	var. lutescens (Alef.) Mansf.	USA
19	Thatcher	*T. aestivum*	var. lutescens (Alef.) Mansf.	Unknown
20	2FSNossen/Orlando	*T. aestivum*	Not determined	GDR (Germany)
21	Orlando	*T. aestivum*	var. lutescens (Alef.) Mansf.	GDR (Germany)
22	Orlando	*T. aestivum*	var. lutescens (Alef.) Mansf.	GDR (Germany)
23	Orlando	*T. aestivum*	Not determined	Unknown
24	Regina	*T. durum**	Var. leucurum (Alef.) Körn	Italy
25	Regina	*T. aestivum*	var. lutescens (Alef.) Mansf.	Unknown
26	Regina	*T. durum**	var. Leucurum	Italy
27	2FSNossen/Regina	*T. aestivum*	Not determined	Czechoslovakia
36	Apollo	*T. aestivum*	var. lutescens (Alef.) Mansf.	Nederland
37	Apollo	*T. aestivum*	var. lutescens	Nederland
38	Apollo	*T. aestivum*	Not determined	Germany
39	Gabo	*T. aestivum*	var. aureum (Link) Mansf.	Australia
40	Gabo	*T. aestivum*	var. aureum (Link) Mansf.	Australia
41	Gabo	*T. aestivum*	var. aureum	Australia
42	Gabo	*T. aestivum*	var. ferrugineum	Unknown
43	Gabo	*T. aestivum*	var. albidum	Unknown
44	Gabo	*T. aestivum*	var. Albidum	Australia
45	Gabo	*T. aestivum*	var. Albidum	Australia
46	Ceres	*T. aestivum*	var. aestivum	USA
47	Ceres	*T. aestivum*	var. aestivum	USA
48	Ceres	*T. aestivum*	var. aestivum	USA
49	Ceres	*T. aestivum*	var. aestivum	USA
50	Ceres	*T. aestivum*	var. aestivum	USA
51	Ceres	*T. aestivum*	var. aestivum	USA
52	Ceres	*T. aestivum*	var. aestivum	Unknown
53	Ceres	*T. aestivum*	var. aestivum	Unknown
54	Ceres	*T. aestivum*	var. erythrospermum	USA
55	Autonomia	*T. aestivum*	var. lutescens (Alef.) Mansf.	Italy
56	Autonomia	*T. aestivum*	var. lutescens (Alef.) Mansf.	Italy
57	Autonomia	*T. aestivum*	var. milturum (Alef.) Mansf.	Italy
58	Autonomia	*T. aestivum*	var. lutescens (Alef.) Mansf.	Italy
59	Autonomia	*T. durum**	var. leucurum (Alef.) Körn	Italy
60	Autonomia	*T. aestivum*	var. aestivum	Italy

61	Autonomia	*T. aestivum*	var. aestivum	Italy
62	Autonomia	*T. aestivum*	var. lutescens (Alef.) Mansf.	Italy
63	Risciola	*T. aestivum*	var. aestivum	Italy
64	Risciola	*T. aestivum*	Not determined	Italy

Microsatellite markers: Thirteen Gatersleben wheat Microsatellites (GWM) detecting 18 loci (Table 2) were selected for genotyping (Roeder et al. 1998). The primers were described by Plaschke et al. (1995) and Roeder et al. (1995, 1998). All GWM were dinucleotide repeats.

Polymerase chain reaction and fragment analysis: PCR reactions were performed according to Roeder et al. (1998). The amplification protocol was as follows: 3 min at 94°C, followed by 45 cycles with 1 min at 94°C; 1 min either 50, 55 or 60°C. 2 min at 72°C, and a final extension step of 10 min at 72°C.

Fragment analysis was performed with automated laser fluorescence sequencers (ALF express, Amersham-Pharmacia) using short gel cassettes. Denaturing gels with 6 per cent polyacrylamide were prepared using peprogel (Amersham Biosciences). The gels were run in 0.5× TBE buffer with 900V, 50mA and 50W and a sample interval of 84 second. In each lane fragments with known size were included as standards. Fragment size was calculated using the computer programme fragment Analyser Version 1.02 (Amersham-Pharmacia) by comparison with internal size standards (Roeder et al. 1998). The wheat varieties namely, Chinese Spring and Aztec were used as references in each run to compane run-to-run variation.

Data analysis: Fragments amplified by microsatellite primers were scored for presence (1) or absence (0) and used for a binary data matrix. The binary data were used to compute a pair-wise similarity matrix using the DICE similarity index. The similarity matrix was subjected to cluster analysis using the UPGMA (Unweighted pair-group method with arithmetic average) algorithm on NTSYS-pc version 2.0 (Rohlf 1998)

Allelic polymorph information content (PIC) was calculated according to formula of Nei (1973).

PIC = $\Sigma(Pij)^2$; where pi is the frequency of the j^{th} allele for i^{th} locus summed across all alleles for the locus.

Results and Discussion

Microsatellite polymorphism: Thirteen microsatellite markers detecting 18 loci were used to characterize and evaluate the genetic diversity. In total 126 different alleles were detected with 13 WMS primer pairs. Primer pair GWM192 amplified alleles at three separate loci (Table 2). The average number of alleles per locus was 9.69. The most polymorphic marker, Xgwm389 in B genome had 16 different alleles. The least polymorphic markers, Xgwm357 in A genome and Xgwm513 in

B genome had 6 alleles each. Several authors reported that microsatellites are more variable than most of other molecular markers that are useful as tools for studying the genetic diversity of germplasm. Plaschke et al. (1995) has used wheat microsatellite for the first time for studying the genetic diversity in closely related European bread wheat varieties. The authors obtained on an average 6.2 alleles per locus by using 23 makers. They also confirmed that a small number of markers were sufficient to distinguish closely related wheat genotypes and to select genotypes for higher genetic diversity. Prassad et al. (2000) assessed of the genetic diversity of 55 genotypes of wheat obtained from 29 countries and found the average number of alleles to be 7. On the other hand, Amer et al. (2001) detected genetic diversity in 15 Libyan wheat genotypes and found the average number of 4.5 alleles per locus. The authors suggested that a relatively small number of primers could be used to distinguish all genotypes and to estimate the genetic diversity. In recent years Huang et al. (2002) investigated 998 wheat accessions of bread wheat obtained from Gatersleben Gene Bank and reported 18.1 alleles per locus.

Table 2. Microsatellite description with PIC values.

Locus	Chromo-somal location	Relative distance (%) from centromere	Range of alleles (bp)	No. of detected alleles	No. of heterogenous accessions at the locus	PIC
Xgwm95	2AS	2.09	110-130	9	4	0.79
Xgwm18	1BS	5.2	179-193	7	5	0.76
Xgwm46	7B(C)	0	141-179	13	8	0.84
Xgwm155	3AL	57.31	120-148	13	12	0.83
Xgwm160	4AL	100	164-186	9	9	0.74
Xgwm192	4AS, 4BL, 4DL	18.75, 9.3, 35.77	129-199	9	48	0.65
Xgwm357	1A (C)	0	120-130	6	3	0.74
Xgwm458	1D(C)	0	110-192	10	4	0.66
Xgwm513	4BL	12.26	138-148	6	2	0.70
Xgwm389	3BS	98.91	113-147	16	15	0.86
Xgwm619	2BL	67.79	134-172	13	8	0.88
Xgwm631	7AS	4.02	176-212	8	7	0.68
Xgwm680	6BS	9.52	107-139	7	11	0.69
Total				126		
Mean				9.69		0.76

The PIC values are informative to examine the extent of diversity. The PIC values in this study ranged from 0.65 to 0.88 with an average of 0.76. In earlier studies on wheat, the PIC values ranged from 0.21 to 0.94 (Roeder et al.1995, Plaschke et al.1995, Prassad et al. 2000, Huang et al. 2002). A higher number of alleles per locus and higher values of PIC for the markers used in these studies

may be attributed to the use of more informative markers and a relatively bigger sample with relatively more diverse genotypes. Similarly, the low values of PIC

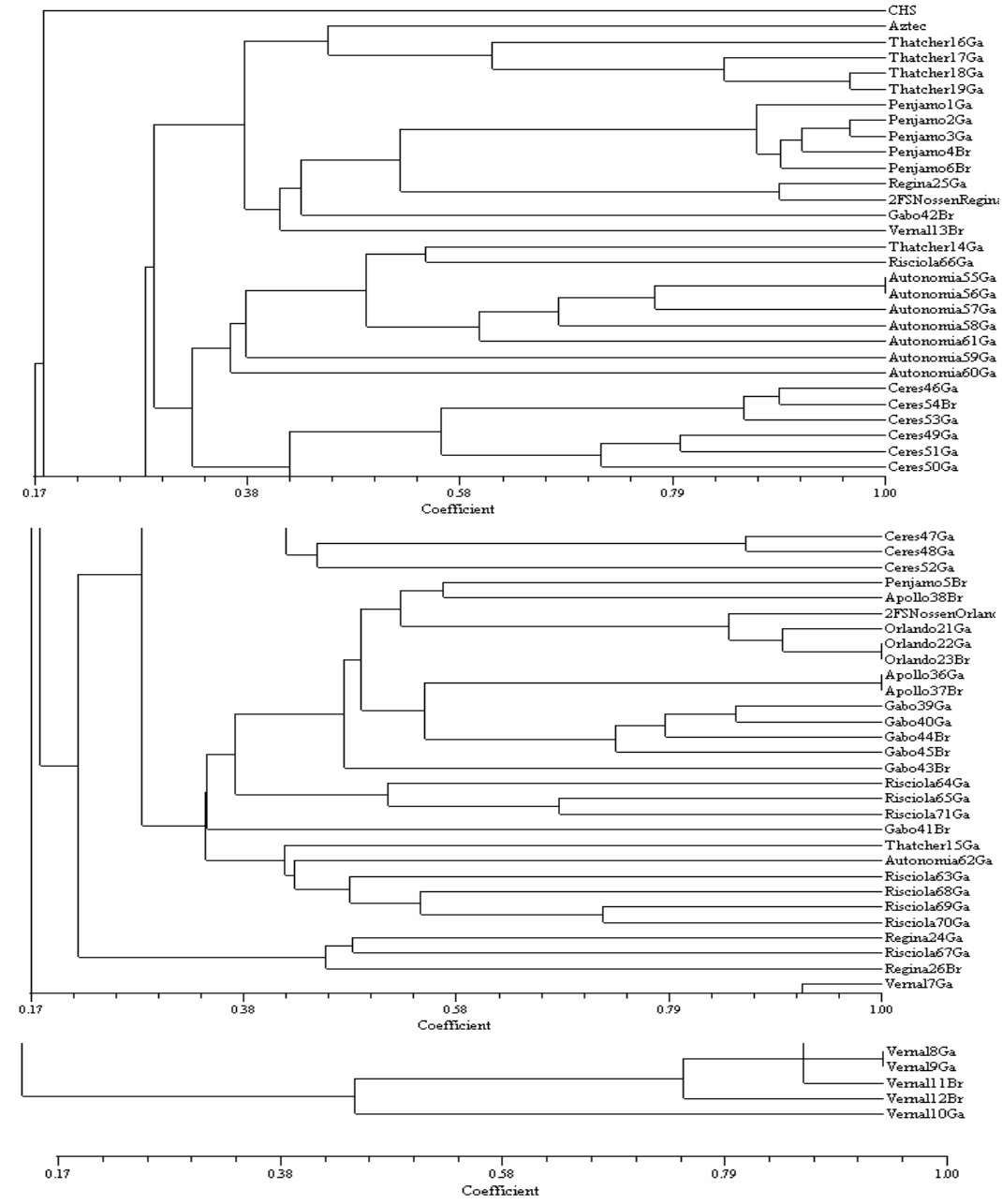

Fig. 1. Dendrogram of the 63 accessions of wheat based on genetic similarity (Ga and Br indicate wheat collection from Gatersleben and Brownshewg of Germany, respectively)

obtained in the earlier studies on wheat may be attributed either to a small population (e.g. 18 genotypes used by Roeder et al. 1995), to a set of closely related genotypes (Plaschke et al.1995) or to a set of fewer microsattellite primers (e.g. 15 markers used by Roeder et al. 1995). All primer pairs showed genetic heterogeneity in more than one accessions. The maximum heterogeneity of 48 accessions were detected at locus Xgwm192, the minimum of two accessions being at locus Xwm577.

Genetic diversity: A dendrogram derived from UPGMA cluster analysis based on the genetic similarity coefficient matrix for 63 accessions was constructed (Fig. 1). The accessions were clustered in clearly defined nine groups as follows: one group each of Penjamo 62, Vernal, Ceres, Orlando, Gabo, Thatcher, Autonomia and two groups of Risciola. In case of cultivars Ceres and Orlando, accessions included in the investigation clustered into a single group. Accessions of the cultivar Risciola formed two separate groups with genetic distance between the two at about 0.34 despite their same origin. In case of cultivars Gabo (Australian origin), Autonoma (Italian origin), Thatcher (USA and Canada origin), Penjamo (Mexican and USA origin) and Vernal (USA origin), the majority of accessions clustered in a single group with 1-3 accessions lying separately. Cultivars Appollo (Dutch and German origin) and Regina (Italian and Czechoslovakian origin) failed to form a clear group. The results of the present study were compared with those of Huang et al. (2002) and Alamerew et al. (2004). Huang et al. reported that all accessions that originated from the same geographic region did not cluster in the same group. The reason for this might be that similar genetic variation occurred independently in different geographic regions or that artificial transfer of an accession from one region to another resulted in a false determination of the geographic origin. Alamerew et al. (2004) found that the most closely related two accessions originated from different collection sites.

The results presented here confirm the utility of WMS marker for the characterization of gene bank germplasm of wheat. The WMS data can be used in selecting diverse parents in breeding improved cultivars and in appropriately maintaining genetic variation in the germplasm.

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