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Detection of genetic diversity in closely related bread wheat using microsatellite markers

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Abstract Wheat microsatellites (WMS) were used to estimate the extent of genetic diversity among 40 wheat cultivars and lines, including mainly European elite material. The 23 WMS used were located on 15 different chromosomes, and revealed a total of 142 alleles. The number of alleles ranged from 3 to 16, with an average of 6.2 alleles per WMS. The average dinucleotide repeat number ranged from 13 to 41. The correlation coefficient between the number of alleles and the average number of repeats was only slight ($r_s = 0.55$). Based on percentage difference a dendrogram is presented, calculated by the WMS-derived data. All but two of the wheat cultivars and lines could be distinguished. Some of the resulting groups are strongly related to the pedigrees of the appropriate cultivars. Values for co-ancestry (f) of 179 pairs of cultivars related by their pedigrees ($f \geqslant 0.1$) averaged 0.29. Genetic similarity (GS) based on WMS of the same pairs averaged 0.44. The rank correlation for these pairs was slight, with $r_s = 0.55$, but highly significant ($P < 0.001$). The results suggest that a relatively small number of microsatellites can be used for the estimation of genetic diversity and cultivar identification in elite material of hexaploid bread wheat.

Key words Co-ancestry · Genetic diversity · Microsatellites · Wheat

Introduction

Genetic studies in elite material of hexaploid bread wheat (*Triticum aestivum* L.) with molecular markers have been hampered by a limited number of polymorphic markers (STS, gliadine-PAGE), or by the low level of variability within this self-pollinating species (RFLP, RAPDs). Monitoring the genetic variability within the narrow pool of elite breeding material could, however, make crop improvement more efficient by the directed accumulation of favoured alleles. This is likely to speed up the breeding process and/or to decrease the amount of material which needs to be screened in such experiments. For all these approaches, markers, which are highly polymorphic and easy to use, are necessary.

Assessments of the extent of genetic variation in wheat have been carried out based on RFLP (Vaccino et al. 1993), RAPDs (He et al. 1992; Dweikat et al. 1993), specific PCR primers for low-copy sequences (Chen et al. 1994; Talbert et al. 1994), and PAGE of gliadins (Cox et al. 1985). More comprehensive studies have been carried out in varieties and cultivars of barley using RFLP markers (Graner et al. 1995; Melchinger et al. 1995), which compared to wheat, resulted in a higher level of polymorphism in this closely related crop plant.

In human and animal genetics, simple sequence repeats or microsatellites (MS) have been shown to be an easily applicable, highly informative and reliable marker system, that has not yet been extensively used in plants. The availability of sufficient MS markers for the most investigated plant genomes is mainly hampered so far by the laborious and costly process of developing suitable primer pairs. However, the data available have shown that MS are abundant and highly polymorphic in many plant species, including the *Triticeae* such as wheat (Röder et al. 1995) and barley (Saghai Maroof et al. 1994). The usefulness of MS for genotype differentiation on a larger scale has been reported for rice (Yang et al. 1994) and soybean (Rongwen et al. 1995).

In the present report, we demonstrate the application of wheat microsatellites (WMS) for the differentiation and estimation of genetic relationships between 40 wheats (mainly European cultivars), in order to show the usefulness of MS for investigations in genetically closely related material. Further, we compare the data generated to genetic similarity estimates based on pedigree records.

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Materials and methods

Plant material

Forty wheat cultivars and lines, kindly provided by Dr. Börner, wheat genetics group, Gatersleben, Germany, or Plant Breeding International (PBI) Saatzucht, Silstedt, Germany, have been used. This material consists mainly of European cultivars, which have been widely grown during the last decade. The durum cultivar 'Castelporziano', and the *Triticum aethiopicum* accession W6807 are tetraploid (AABB), whereas the remaining wheat cultivars and lines are hexaploid (AABBDD). 'Dwarf A' is a sister line of the cultivar 'Hobbit'. 'Karcagi 522 M7K' is a short-straw mutant selected from the cultivar 'Karcagi 522' after gamma-ray treatment (Viglasi 1968). The 'Marquis' selection used for developing the 'Chinese Spring'/'Marquis' substitution lines was not a true 'Marquis', but probably derived from a cross with the cultivar 'Thatcher' (Sheen and Snyder 1964; Scarth 1981; McIntosh, personal communication). Pedigree information was kindly provided by various wheat breeders or derived from Martynov et al. (1992). All wheat cultivars and lines, along with their pedigree (if known), country of origin, year of release and winter or spring type classification, are listed in Table 1.

DNA isolation

Total genomic DNA was extracted from each cultivar/line using ten half grains. Each grain was cut in two equal parts and the half without the embryo was crushed. For each line, all material was pooled and incubated in 5 ml of extraction-buffer (100 mM TrisHCl, pH 8.0; 500 mM NaCl; 50 mM Na_2EDTA; 1.25% SDS and 3.8 g/l sodium bisulfite) for 40 min at 65 °C; then an equal volume of chloroform : isoamylalcohol (24 : 1) was added and mixed well. The samples were centrifuged at 3500 g for 10 min, and the aqueous phase transferred to a new tube. The DNA was precipitated by adding two volumes of ice-cold ethanol. The pellet, recovered by centrifugation, was washed with 70% ethanol, air-dried and resuspended in 0.5 ml 1 × TE-buffer (pH 7.5) or sterile water.

The remaining half grains were stored at 4 °C. For templates which appeared to amplify more than a single allele by a microsatel-

Table 1 Wheat cultivars and lines, their pedigrees (as far as known), country of origin, year of release and winter- or spring-type classification

Cultivar/line	Country of origin/year of release	Winter (w) or spring (s) type	Pedigree
Apollo[a]	Germany/1984	w	Maris Beacon/Clement//Kronjuwel
Astron[a]	Germany/1989	w	Blaukorn × Monopol
Borenos[a]	Germany/1987	w	Alcedo//Kenya Civet/2*Dornburg 4056-67/3/2*Alcedo
Bovictus[a]	Germany/1993	w	Kenya Civet/Habicht//2*W5.25880-71/3/Hadmersleben 302
Bussard[a]	Germany/1990	w	Kranich/Huntsman//Monopol
Contra[a]	Germany/1990	w	Kronjuwel/Marksman
Greif[a]	Germany/1989	w	Maris Huntsman/Carimulti//Monopol
Herzog[a]	Germany/1986	w	Weihenstephan 616-67/Kormoran//Kronjuwel
Kanzler[a]	Germany/1980	w	Caribo/Heinrich
KogaII[a]	Germany (old cultivar)	s	Garnet/Heines Kolben//HeinesKolben/Raeckes Weisspelz
Kontrast[a]	Germany/1990	w	Lundi/Selkirk//2*Alcedo/3/Taco1.2ii00-78
Mikon[a]	Germany/1988	w	Gaines/6*Miroronovskya 808//Fakon
Obelisk[a]	Germany/1987	w	Composite cross
Orestis[a]	Germany/1988	w	Composite cross
Ramiro[a]	Germany/1989	w	Miroronovskaya 803/3/Besost.1//Erythrosp. 1526
Toronto[a]	Germany/1990	w	Disponent/Weihenstephan 616-67//Kronjuwel
Zentos[a]	Germany/1989	w	Hadmersleben 5792-71 /Alcedo//Compal
Beaver[a]	England	w	Norman/Hedgehog//Moulin
Campus[a]	England	w	Haven/Sleipner
Caprimus[a]	England/1994	w	Gaiwan/Aquila
DwarfA (Hobbit sib)[a]	England	w	Prof.Marchal//Marne/Vog 9144/3/line Hobbit1
Encore[a]	England	w	Apostle/Haven
Haven[a]	England	w	Norman/Hedgehog//Moulin
Rialto[a]	England	w	Haven (s)/Fresco(s)
Vivant[a]	England	w	Boxer/Gawain
Bersee	France/1924	w	
Renan[a]	France	w	Mironovskaya 808/Maris Huntsman/3/VPM/Moisson//Courtot
Estica[a]	Netherlands	w	Arminda/Virtue
Ritmo[a]	Netherlands/1993	w	Hobbit//1320/Wizzard/3/Marksman/4/Virtue
Ai-bian 1	China	w	
Chinese Spring (CS)	China	s	
Boheme[a]	Czech/1989	w	Caribo/Fakir
Kraka[a]	Denmark	w	Kranich/Caribo
Karcagi 522 M7K	Hungary	w	
Mara[a]	Italy	s	Autonomica/Aquila
Konsul[a]	Sweden/1990	w	Ertus/Norre//HolmeM/3/Cercosporella resistant line
Mironovskaya 808[a]	Ukraine	w	Artemovka/Artemovka
CS/Sustitution 2B Marquis		s	
Castelporziano (4x/AABB)	Italy	s	
Triticum aethiopicum W6807 (4x/AABB)	Ethiopia	s	

[a] The marked cultivars are included in the calculation of co-ancestry coefficients (f) based on their pedigrees

lite-primer-pair, DNA was extracted from the individual embryo halves, using only 1/10th of the solution amounts described above.

Isolation of microsatellites

Screening and isolation of microsatellite-containing clones, sequencing, and primer design were as described by Röder et al. (1995). In addition, two further libraries were used for MS selection (Plaschke et al. 1995).

Primer pairs and PCR amplification

Twenty-three primer pairs representing wheat microsatellites (WMS) which amplify the expected fragments (according to the sequence data) in 'Chinese Spring' ('CS') were chosen for the analyses. WMS designation, microsatellite composition, primer sequences, fragment sizes in 'CS', and chromosome-arm location of the amplified loci are presented in Table 2. For WMS2, 5, 18, 24, 43, 44 and 46 the respective data have already been published by Röder et al. (1995). The chromosomal and chromosome-arm locations were derived from 'CS' nullitetrasomic (Sears 1966) and 'CS' ditelosomic (Sears and Sears 1978) analyses, respectively.

PCR amplifications were performed as described in Röder et al. (1995) and Plaschke et al. (1995). The PCR-amplified fragments were detected on an automated laser fluorescence (A. L. F.) sequencer (Pharmacia). To allow this, one primer of each pair was labelled at the 5′ end with fluorescein. Denaturing gels (0.35 mm thick) were prepared with 6.5% Long-Ranger™ (AT Biochem), 7 M urea and 1.2 × TBE buffer, using the short gel cassettes (separation distance: 9 cm). Running conditions were 600 V, 40 mA and 50 W with a sampling interval of 0.84 s and 2 mW laser power. The running buffer was 1 × TBE. From each PCR reaction, 0.3–1.5 µl were loaded together with 2.5 µl of loading buffer (deionized formamide including 5 mg/ml dextran blue) after denaturing for 1 min at 90 °C. The gel separation was terminated after 80–90 min, allowing the detection of fragments up to approximately 300 bp. The gels were re-used up to four times. Fragment sizes were calculated in the computer program Fragment Manager (Pharmacia) by comparing with internal size standards, which were added to each lane in the loading buffer.

Statistical analyses

The average repeat number of dinucleotides within the MS over all lines and for each locus were calculated based on the sequencing data of the 'CS' allele, assuming that size differences of the fragments are only due to alterations in the repeat number of the appropriate microsatellite sequence. MS containing mononucleotide repeats (WMS194), MS not sequenced in 'CS' (WMS2) and WMS 106 were excluded from this analysis. For WMS154, it was assumed that the size differences are based on alterations of the dinucleotide repeats only.

Presence or absence of each single fragment was coded by 1 or 0, respectively and scored for a binary data matrix. Genetic distance was calculated for each pair of lines using the percentage difference in the program NCLAS of the computer package SYN-TAX IV (Podani 1990), according to the equation:

$$PD = 1 - 2N_{ij}/(N_i + N_j),$$

where N_{ij} is the number of fragments common to lines i and j, and $(N_i + N_j)$ is the total number of fragments in both lines. This value lies between 0 and 1, with a score of 0 indicating that all fragments are in common, and 1 indicating no common fragments. The average linkage (UPGMA) algorithm was chosen as a clustering method. The dendrogram was designed using DENDPLOT from the same computer package.

For correlation analysis, genetic similarity (GS), following Nei and Li (1979), was applied:

$$GS = 2N_{ij}/(N_i + N_j) = 1 - PD.$$

Coefficients of co-ancestry (f) were calculated for the 33 cultivars marked in Table 1 from the pedigree records (three to eight generations backward). A simple, linear algorithm was employed, following the assumptions of Cox et al. (1985) that: (1) ancestors are unrelated, (2) all cultivars, ancestors and parental lines are homozygous, and (3) a cultivar derived from a cross obtains one-half of its genes from each parent. A cultivar and a direct selection from that cultivar, as well as two selections from the same cultivar, were scored as $f = 1$. For example, one common parent resulted in $f = 0.5$, one common grandparent in $f = 0.25$. No common lines in the pedigree records resulted in $f = 0$. The data on the pedigrees were calculated and kindly supplied by the Saatzucht Agrar GmbH, Hadmersleben, Germany.

The co-ancestry coefficient (for $f \geq 0.1$) and the GS value for each pair of lines were used to create a scatter plot. A rank correlation (r_s) was calculated, employing all pairs of lines with a co-ancestry coefficient of $f \geq 0.1$, following the assumption of Melchinger et al. (1995), that lines with $f < 0.1$ are not related by their pedigrees. For the same pairs of lines a linear regression (GS_{reg}) of GS on f was computed, using the computer program STATVIEW.

Results

Wheat microsatellites

In total 142 alleles were detected with the 23 WMS, located on 15 different chromosomes and 19 different chromosome arms. Figure 1 shows the fragments of 20 wheat cultivars and lines amplified with WMS155. The sizes of the seven alleles observed ranged from 129 to 149 bp. Because 'CS' has 19 GA repeats and amplified a fragment of 141 bp, it is assumed that the number of repeats ranges from 13 to 23. The number of alleles per WMS ranged from 3 to 16 (Table 2). On average, 6.2 alleles were detected per locus. The average repeat number per locus ranged from 13 to 41, and was 18, 26

Fig. 1 WMS155-PCR products of 20 wheat cultivars and lines, displayed in the fluorogram-modus of the Fragment Manager program after separation on an A. L. F. sequencer

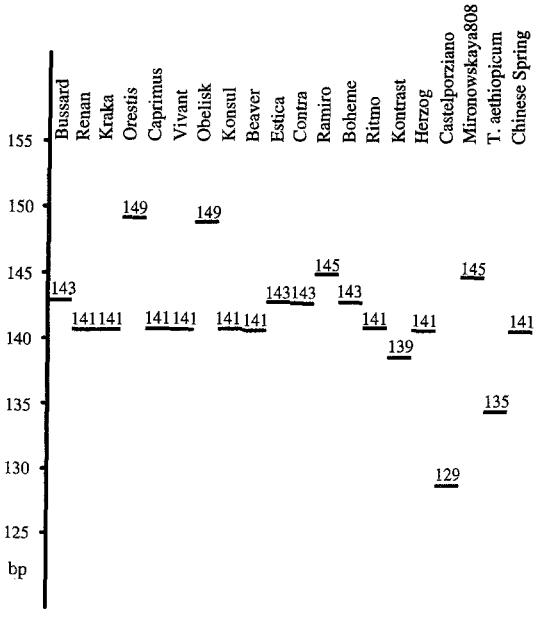

Table 2 Primer sequences, MS sequence compositions, fragment sizes, chromosomal and chromosome-arm locations and number of alleles for the wheat microsatellites employed

Designation	Primer sequences (5′→3′)	MS composition (in 'CS')	Fragment size in 'CS' (in bp)	Chromosomal location	Number of alleles
WMS2	CTGCAAGCCTGTGATCAACT CATTCTCAAATGATCGAACA	$(GA)^{a}_{18}$	132[a]	2AS	5
WMS5	GCCAGCTACCTCGATACAACTC AGAAAGGGCCAGGCTAGTAGT	$(TC)_{23}(T)_4(GT)_{12}(GA)_{10}$	172	2AS	6
WMS18	TGGCGCCATGATTGCATTATCTTC GGTTGCTGAAGAACCTTATTTAGG	$(GT)_{17}CT(AT)_4$	186	4BS	7
WMS24	CACACAAGGCACCATTGC CAATGGACATAGTTGTGTGCG	$(GT)_9GCA(TG)_8$	172	1BL	4
WMS43	CACCGACGGTTTCCCTAGAGT GGTGAGTGCAAATGTCATGTG	$(GT)_{22}$	180	7BL	6
WMS44	GTTGAGCTTTTCAGTTCGGC ACTGGCATCCACTGAGCTG	$(GA)_{28}$	182	7DS	5
WMS46	GCACGTGAATGGATTGGAC TGACCCAATAGTGGTGGTCA	$(GA)_2GC(GA)_{33}$	187	7BS	9
WMS52	CTATGAGGCGGAGGTTGAAG TGCGGTGCTCTTCCATTT	$(GT)_4AT(GT)_{20}$	150	3DL	3
WMS67	ACCACACAAACAAGGTAAGCG CAACCCTCTTAATTTTGTTGGG	$(GT)_{10}$	85	5BS	5
WMS82	ACGTTAGAAGGTGCAATGGG AGTGGATGCACCGACTTTG	$(GT)_{10}...(GT)_4T(AG)_4$	152	6AL	3
WMS88	CACTACAACTATGCGCTCGC TCCATTGGCTTCTCTCTCAA	$(GT)_{18}TT(GA)_4$	121	6BL	8
WMS95	GATCAAACACACACCCCTCC AATGCAAAGTGAAAAACCCG	$(GT)_{16}$	121	2AS	6
WMS106	CTGTTCTTGCGTGGCATTAA AATAAGGACACAATTGGGATGG	$(GA)_{24}$	139	1DS	3
WMS111	TCTGTAGGCTCTCTCCGACTG ACCTGATCAGATCCCACTCG	$(CT)_{32}(GT)_{17}$	205	7DS	7
WMS120	GATCCACCTTCCTCTCTCTC GATTATACTGGTGCCGAAAC	$(GT)_{16}(GA)_{11}$	139	2BS	7
WMS131	AATCCCCACCGATTCTTCTC AGTTCGTGGGTCTCTGATGG	$(GA)_{22}$	131	7BL	9
WMS154	TCACAGAGAGAGAGGGAGGG ATGTGTACATGTTGCCTGCA	$(GA)_4(GGGA)_4(GA)_{25}$	102	5AS	6
WMS155	CAATCATTTCCCCCTCCC AATCATTGGAAATCCATATGCC	$(GA)_{19}$	141	3AL	7
WMS159	GGGCCAACACTGGAACAC GCAGAAGCTTGTTGGTAGGC	$(GT)_{15}$	192	5DS	3
WMS174	GGGTTCCTATCTGGTAAATCCC GACACACATGTTCCTGCCAC	$(GA)_{22}$	173	5DL	16
WMS182	TGATGTAGTGAGCCCATAGGC TTGCACACAGCCAAATAAGG	$(GA)_{18}$	165	5DL	3
WMS186	GCAGAGCCTGGTTCAAAAAG CGCCTCTAGCGAGAGCTATG	$(GA)_{26}$	140	5AL	6
WMS194	GATCTGCTCTACTCTCCTCC CGACGCAGAACTTAAACAAG	$(G)_{11}(GA)_8GG(GA)_7GG$ $(GA)_5GG(GA)_7$	131	4DL	8

[a] Sequence data refer to the cultivar 'Cheyenne'

and 27 repeats for $(GT)_n$, $(GA)_n$ and compound MS, respectively. The correlation coefficient between the average number of repeats over the 40 wheat cultivars and lines for a specific WMS and the number of repeats found in 'CS' was high with $r_s = 0.84$ ($P < 0.01$). The correlation coefficient for the number of alleles versus the average number of repeats was $r_s = 0.55$ ($P < 0.05$). The sum of the average repeat numbers for each MS type divided by the total number of different alleles detected by the respective type gave 4.2, 3.3 and 4.4 repeats per allele for the six $(GT)_n$, the seven $(GA)_n$ and the six compound MS (without WMS2, 106, 154 and 194), respectively. Therefore, the $(GA)_n$ MS were more polymorphic at a given number of repeats, compared to the other two types. But, if the most-variable locus (WMS174; 16 alleles) was excluded, the advantage of $(GA)_n$ MS was reduced to a minimum.

WMS 106 amplified fragments with only 18 out of the 40 wheat templates. WMS18 failed to amplify a fragment from 'Apollo' and 'Bovictus', and WMS24 gave no amplification products with 'Apollo', 'Kanzler', 'Kontrast' and *T. aethiopicum* W6807. All these nulls were confirmed in repeated experiments and finally scored as null-alleles. WMS located on chromosomes of the D

genome failed to amplify fragments from the tetraploid lines 'Castelporziano' and *T. aethiopicum* W6807, as expected. Five line-locus combinations were treated as missing values due to ambiguous or poor amplification products. Two alleles for a single line at one locus occurred in the combination 'Mikon' – WMS5, 'Ai-bian1' – WMS18 and 'Astron' – WMS186. The analyses on single half grains detected segregation, and therefore heterozygosity, for all three loci. WMS located on the same chromosome arm gave different allele patterns across the 40 templates, and, therefore, were all informative.

Genetic diversity

The dendrogram discriminates all but two of the cultivars/lines (Fig. 2). The German cultivars 'Orestis' and 'Obelisk' could not be distinguished. There are several subgroups, related by their pedigrees. Group 1 represents a number of English cultivars with a relatively low level of variability. This group consists of 'Haven' and 'Beaver', a cultivar derived from the same cross as 'Haven', as well as cultivars with 'Haven' among their parents. The next outer branch connects this group with two cultivars having 'Hobbit' in their parentage, as all cultivars of group 1 have. The remaining two English cultivars do not carry 'Hobbit' among their parents or grandparents. Group 2 includes three German cultivars, all having 'Alcedo' among their parents. Group 3 includes the Ukrainian cultivar 'Mironovskaya 808' and all cultivars with 'Mironovskaya 808' among their parents. This group is well separated from group 4, which contains the remaining European elite cultivars only (except for 'Ai-bian1'). The smallest genetic distances were observed for 'CS' and 'CS'/'Marquis' 2B substitution. Both lines differed only for the two WMS on chromosome 2B, as expected. The two tetraploid lines were clearly separated from the hexaploid wheats. Spring and winter types were not clearly separated from each other.

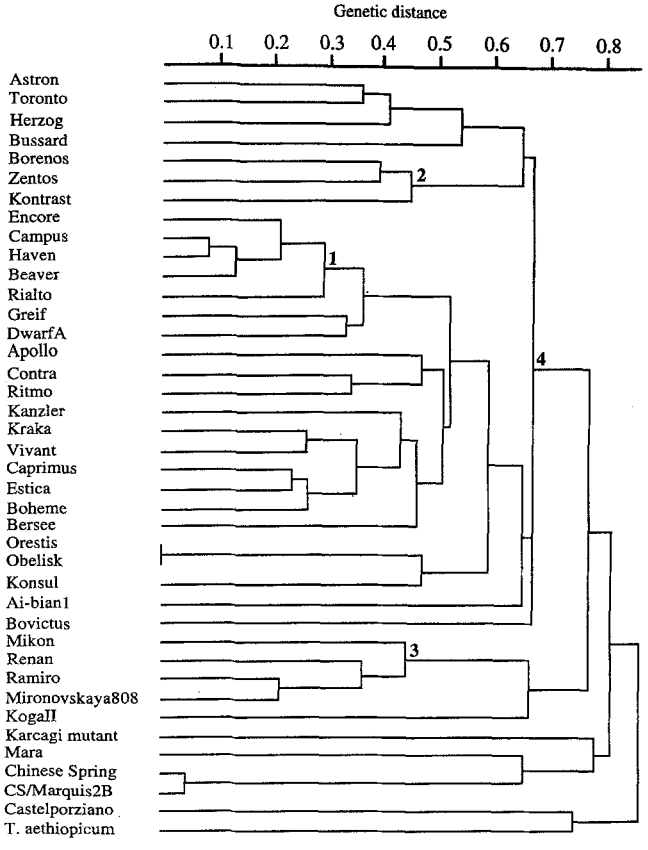

Fig. 2 Dendrogram of 40 wheat cultivars and lines, based on the percentage difference calculated from data of 23 wheat microsatellites. Marked groups are discussed in the Results section

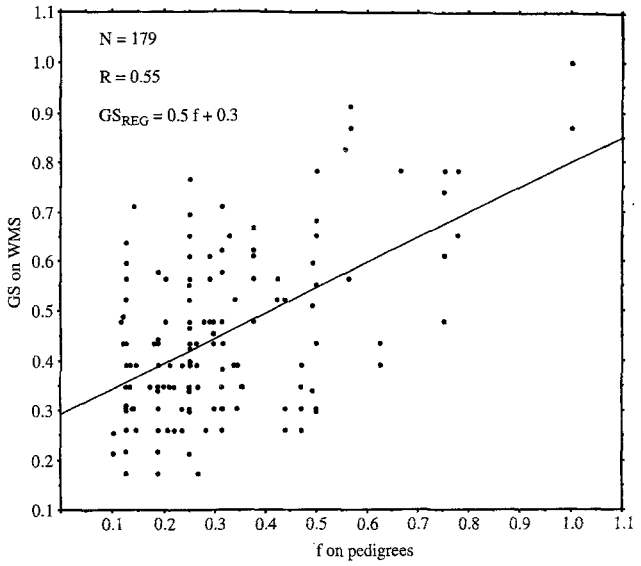

Fig. 3 Scattergram of genetic similarity (GS) based on the 23 wheat microsatellites versus co-ancestry (f) based on the pedigree records, including 179 pairs of related ($f \geq 0.1$) cultivars

The genetic similarity (GS) values for all 780 pairs ranged from 0 to 1 and averaged 0.31. For 179 pairs of cultivars, which were related by their pedigrees ($f \geq 0.1$), GS values were plotted against f values in a scattergram (Fig. 3). The values for co-ancestry of these pairs had a miximum of 1 and averaged 0.29. The average GS for these pairs was 0.44. The rank correlation between GS and f was only moderate with $r_s = 0.55$, but highly significant ($P < 0.001$).

Discussion

Wheat microsatellites

The average numbers of dinucleotide repeats for $(GA)_n$ and the compound MS are about equal and can therefore be compared. In this study compound MS had to be one-third longer than $(GA)_n$ repeats to detect the same number of different alleles. The $(GT)_n$ repeats detected less alleles per repeat, but were shorter on average. If a linear correlation between the number of repeats and

number of alleles is assumed, this would mean that $(GA)_n$ MS are more polymorphic than $(GT)_n$ MS. Data from 64 sequenced MS have shown that a selection for $(GA)_n$ repeats and compound MS may be worthwhile, because these types were, with an average of 26 and 35 repeats, respectively, longer than $(GT)_n$ with 20 repeats on average (Plaschke et al. 1995), which was exactly the same for the 241 MS sequences derived from different libraries (unpublished results). Nevertheless, both types are useful for detection of polymorphism, and a short MS (e.g. WMS67 – 13 repeats on average – 5 alleles) can be as polymorphic as one with twice as many repeats (e.g. WMS44 – 28 repeats on average – 5 alleles). This is supported by the only slight correlation between the assumed average number of repeats and number of alleles, and is in general accordance with results reported in human genetics, were the informativeness for $(GT)_n$ MS tends to increase with an increasing number of average repeats, though relatively short $(GT)_n$ MS (12 repeats) can also have a valuable information content (Weber 1990).

The missing amplification products for a number of lines with WMS18, 24 and 106 are most likely due to sequence alterations (such as point mutations, deletions, inversions) within the priming sites, as reported by Devos et al. (1995).

The detected heterozygosity in three wheat lines can be explained by residual heterozygosity, rather than by an outcross, because only 1 out of the 23 loci was affected. Another possible source could be mutations during the early stage of seed amplification. Strand slippage, as the most favoured explanation for the high level of polymorphism in MS sequences (Levinson and Gutman 1987; Murphy et al. 1989; Tautz and Schlötterer 1994), or an unequal crossover within the MS region (Wolff et al. 1989), could lead to such differences. Because among 887 WMS-line combinations only three were heterozygous, microsatellites are a reliable marker system for wheat and confirm the high level of homozygosity within wheat cultivars.

Genetic diversity

The dendrogram presented demonstrates clearly the ability of microsatellites to detect a large amount of genetic variation in genetically closely related elite material of hexaploid wheat, and to identify groups with different levels of genetic distance. Only 'Orestis' and 'Obelisk' were indistinguishable by the 23 WMS used. According to the pedigree information these two cultivars represent sister lines of a composite cross.

Permutation of the data set by leaving out the data for each single WMS in turn, resulted in slight positional changes of a few entries within the dendrogram, but the overall structure of the main groups was conserved. This indicates, that the relatively small number of WMS used in the present study was sufficient to give a good estimation of the genetic similarities within this material.

For 45 wheat accessions, representing much more diverse material than used here, a mean GS of 0.81 was derived by Chen et al. (1994), based on STS PCR markers. Cox et al. (1985) reported a mean similarity coefficient of 0.56 for 43 hard red winter wheat cultivars, based on gliadin-PAGE. This coefficient may not be directly comparable to GS, because it takes into account not only band position but also band intensity. Melchinger et al. (1995) reported a mean GS of 0.79 for unrelated pairs of barley cultivars. Graner et al. (1995) reported GS means of 0.89 for pedigree-related pairs of spring and winter barleys, based on RFLP markers. The mean values for GS based on microsatellites for all pairs (0.31), or pedigree-related pairs (0.44), in the present study are much lower than values from comparable studies, emphasising the high information content provided by WMS.

The correlation between the similarity values based on WMS and pedigrees is only moderate but highly significant. Low f values derived from pedigrees may be underestimations as the pedigree data are often limited to the most recent time, so that the assumption that the ancestors are unrelated may not be valid. Also, there are far more cultivars and breeding lines established from the limited gene pool during the last decades, than a single locus has alleles. Cox et al. (1985) reported an even weaker correlation ($r_s = 0.27$) between genetic similarity based on pedigrees and gliadin storage proteins, which may due to the poor representation of the genome by the gliadins. Similar values were obtained by Graner et al. (1995) for correlations between RFLP-derived GS estimates and co-ancestry with barley winter-($r_s = 0.21$) and spring-type ($r_s = 0.42$) cultivars. A correlation of $r = 0.61$, which is within the same range as the results reported here, was found for RAPD-based GS estimates and co-ancestry in North American barley cultivars (Tinker et al. 1993). The low or moderate correlation values between different kinds of molecular markers and co-ancestry in wheat and barley may reflect the limited value of pedigree records for the estimation of genetic similarity, since they do not take into account selection and genetic drift (Cox et al. 1985). In any case, the question of whether detailed information about genetic relationships in breeding material based on molecular markers can be applied for the accumulation or combination of agronomically important genes or gene complexes requires a marker system which is highly informative and easy to apply. Among the currently available marker systems, microsatellites can match this requirement best.

The results have shown that it is possible to distinguish closely related elite wheat breeding material, to carry out phylogenetic studies, and to select lines/cultivars for highest genetic diversity, using only a small number of microsatellites. Thus, it should be possible to establish a set of highly polymorphic WMS for cultivar identification and plant variety protection in wheat, as has been proposed for soybean (Rongwen et al. 1995). WMS markers for these purposes should be selected

carefully for amplifying only the expected fragments, in order to avoid misinterpretations and to enable detection on non-denaturing PAA or agarose gels (Plaschke et al. 1995).

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