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Genetic diversity among European cultivated spelt revealed by microsatellites

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Abstract Spelt and common wheat constitute two of the six groups of the hexaploid wheats with an AABBDD genome. Spelt culture has been progressively replaced by that of common wheat which out-yields spelt under high-input conditions. In the last decades, spelt breeders intended to introduce the yield-potential and bread-making qualities of common wheat into spelt, by frequent crossings between accessions of these two different groups. The present study aims at determining the genetic basis of modern spelt cultivars in terms of intra-group variability and inter-group (spelt vs common wheat) distances, by using microsatellite markers developed for common wheat. The allelic composition of 30 spelt and nine common wheat accessions was determined at 17 microsatellite loci. The coefficient of co-ancestry (f) and the genetic distances (1 - proportion of shared alleles) based upon allelic composition were calculated for all pairs of accessions. Two dendrograms were constructed using the UPGMA method. Amplification products were found for all loci on most accessions. A total of 113 alleles was identified, of which 60.2% were specific to spelt or common wheat. The correlation between ($1 - f$) and the genetic distance was high (0.701***). The mean pairwise genetic distance was 0.656 ± 0.181 over the 39 accessions, 0.706 ± 0.14 among common wheat and 0.573 ± 0.172 among spelt. The mean genetic distance between spelt and wheat was 0.782 ± 0.113. The two dendrograms were in accordance with each other and clearly separated the spelt from the common wheat accessions. It is concluded that microsatellites developed for common wheat and distances based on the proportion of shared alleles are powerful tools for reconstructing phylogenies in spelt, and that the genetic basis of modern spelt cultivars is narrow despite frequent crosses made with bread wheat.

Keywords Spelt · Wheat · SSR · Microsatellite · Genetic diversity

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Introduction

According to the classification of wheats (MacKey 1966), spelt [*Triticum aestivum* (L.) Thell. ssp. *spelta* (L.) Thell.] and common wheat [*Triticum aestivum* (L.) Thell. ssp. *vulgare* (Will.) MK.] represent two of the six groups constituting the hexaploid wheat species with an AABBDD genome. However, many authors retain the old terminology (*Triticum spelta* L. and *T. aestivum* L., respectively) for the sake of simplicity (Ledent 1989). Spelt was an important food grain in ancient Europe, but is now considered as a minor crop. It is mainly cultivated in marginal areas in Belgium, Germany, Switzerland and Austria. However, it has attracted an increased interest for breeding programmes in the last decades (Schmid and Winzeler 1990).

There are few, but marked, differences between spelt and common wheat (Campbell 1997). Spelt has a fragile rachis and is not free-threshing, in contrast to common wheat. The fragile rachis is also a feature of two other sub-groups of hexaploid wheat [*T. aestivum* (L.) Thell. ssp. *macha* (Dek. and Men.) MK. and *T. aestivum* (L.) Thell. ssp. *vavilovii* (Will.) MK.] but the pattern of disarticulation of the rachis in spelt is different (Cao et al. 1997). Spelt is generally taller and has longer ears (Winzeler et al. 1994). It exhibits more vigorous growth under adverse conditions (Riesen et al. 1986; Rüegger et al. 1990, 1993) and has generally a higher protein content (Schmid and Winzeler 1990). It is particularly adapted

Table 1 Pedigree and origin of spelt and common wheat accessions (cultivars, breeding lines and land races) used for microsatellite analysis. The accessions appearing in italics in the spelt section refer to bread wheats

Accession	Origin	Pedigree	Reference[a]
Spelts			
Altgold	CH	Oberkulmer 3[b] / Oberkulmer 2[b]	2
Balmegg	CH	Muri Rotkorn[b] / *Eiger* // WT50557 (Triticale)	2
Ebners Rotkorn	A	Unknown origin	
Franckenkorn	B	EL38[c] / Altgold // Altgold /3/ Rouquin	1
Hercule	B	von Rechbergs Früher Winterspelz[b] // Lignée 24 / *virtus*	1
Hubel	CH	Schwäbische Alb62A[b] / *Uniplanta80–23*	2
Lueg	CH	Ostro / *Uniplanta77–62*	2
Oberkulmer	CH	Land race	2
Ostar	CH	mutation of Ostro by gamma-radiation	2
Ostro	CH	Oberkulmer / Steiners Roter Tiroler[d]	2
Redouté	B	Wartenzee[b] / Hercule	1
Rouquin	B	Lignée 24 / Ardenne[e] // Altgold	1
Schwabenkorn	D	selection of Roter Tiroler[b]	3
Sertel	CH	Ostro / *Uniplanta80–23* // Ostro	2
Beryl	B	von Rechbergs Früher Winterspelz[b] / Ardenne[e] // Rouquin /3/ Hercule	1
Ressac	B	Rouquin / *1391.4* // Rouquin	1
95.1	B	Rouquin / *1491.2* // (Rouquin / 1430.7 (ww) // Rouquin)	1
95.2.2	B	Rouquin / *1491.2* // (Rouquin / 1430.7 (ww) // Rouquin)	1
113.1 (Cosmos)	B	57.1[f] / *Castel* // 57.1[f]	1
115.6.2	B	57.1[f] / *Rektor* // 57.1[f]	1
115.11	B	57.1[f] / *Rektor* // 57.1[f]	1
118.4.2	B	Franckenkron / *Granada* // Franckenkron	1
65313	CH	Oberkulmer / *Sardona*	2
04.87.02	D	Rouquin / Ostro	3
05.88.02	D	Rouquin / Oberkulmer	3
18.88.02	D	Roter Kolbendinkel[b] / Rouquin	3
21.87.02	D	Hercule / Oberkulmer	3
28.88.03	D	Albin[g] / von Rechbergs brauner Winterspelz[b]	3
32.87.01	D	Steiners Roter Tiroler[d] / Hercule	3
73.88.02	D	Altgold // *Maris Huntsman* / von Rechbergs brauner Winterspelz[b]	3
Common wheats			
Arina	CH	Moisson // Can3842–3663 / HeineVII	2
Chinese Spring	PRC	landrace	
Forno	CH	NR72–837 / Kormoran	2
Franco	D	Carimulti / Monopol // Monopol	4
Kanzler	D	Carstacht / Cappelle	4
Pajero	D	Ural / Caribo // Stemm / Blaukorn	4
Renan	F	Mironovskaja808 / Maris Huntsman /3/ VPM / Moisson // Courtôt	4
Rialto	GB	Haven's / Fresco	4
Tamaro	CH	Kormoran / NR72–837 // Monopol	2

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for animal feeding and for special food products. The major limitations of spelt are its lodging sensitivity and its low yield.

The genealogy analysis of the current spelts (see Table 1) suggests that their genetic basis might be very narrow : about only ten different original spelt parents represent the genetic basis of modern spelt. To our knowledge, the main spelt breeding programmes are located in Europe (Belgium, Germany and Switzerland). During the last decades, the main strategy used to improve the yield and lodging resistance of spelt, and to broaden its genetic basis, was to cross it with common wheat (see Table 1). However, several features of spelt induced its users and breeders to reconsider that approach and to look for more-typical spelt characteristics. To achieve this purpose, a better knowledge of the genetic basis of the current spelt cultivars and breeding lines is required, given the two limitations of the spelt pool: wheat introgressions and the narrowness of its genetic basis.

In common wheat, microsatellites are more convenient than RFLPs for studies of genetic diversity because they detect higher levels of polymorphism (Röder et al. 1995). The aim of the present work was to determine the usefulness of microsatellites developed for common wheat in spelt breeding applications, in order to assess the level of variability existing within the European modern spelt gene pool.

Materials and methods

Plant materials

Thirty spelt and nine wheat accessions (Table 1) were studied. The spelt accessions represent most of the available cultivars for the regions where spelt is usually grown, and most of the promising breeding lines. They were selected and provided by the Federal Research Station for Agroecology and Agriculture, Wheat and Spelt Breeding Group, Zürich, Switzerland, the University of Hohenheim, Landessaatzuchtanstalt, Hohenheim, Germany, and the Centre de Recherches agronomiques de l'Etat, Gembloux, Belgium. The wheat cultivars were provided by the Federal Research Station for Agroecology and Agriculture, and the Centre de Recherches agronomiques de l'Etat.

DNA extraction

Crude DNA was extracted from the first leaf of seedlings at the two-leaf stage. Leaves were kept overnight at –70°C, then crushed and homogenized in microtubes with liquid nitrogen. Extraction buffer (600 µl) containing 100 mM of Tris pH 8, 50 mM of EDTA pH 8, 500 mM of NaCl and 10 mM of β-mercaptoethanol was added and the mixture was incubated at 100°C for 10 min, then centrifuged at 14 000 rpm at 4°C for 15 min. The supernatant was transferred into 2 vol of absolute ethanol (–20°C) and a 1/10 vol 10 M ammonium-acetate, incubated at –70°C for 30 min and centrifuged as described above. Precipitated DNA was air-dried, then re-suspended in 50 µl of a Tris 10 mM - EDTA 1 mM-containing solution. After incubation with 2 µl of 10 mg/ml of RNAse at 37°C for 15 min, DNA was finally diluted 100-times.

Microsatellites and PCR amplification

The 23 microsatellites (WMSs) published by Plaschke et al. (1995) were analyzed on all accessions. Primers were synthesized by Eurogentec, Belgium, according to the original report. PCR amplification was performed in a volume of 10 µl containing 1 µl of DNA extract, 250 nM of each primer, 200 µM of DNTP, 0.5 U of *Taq* Polymerase (Pharmacia Biotech, Belgium) and 1 µl of reaction buffer. One primer of each primer pair was labelled at the 5' end with fluorescein. Thirty PCR cycles, each consisting of 30 s denaturation at 96°C, 30 s annealing at 50 or 60°C and 1-min polymerization at 72°C, were performed with a Techne Cyclogene Dri-Block Cycler. The first cycle was preceded by a 5-min denaturation at 96°C, and the last cycle followed by 10 min final polymerization at 72°C. All analyses were repeated independently on at least two plants.

Electrophoresis

The PCR-amplified fragments were detected on an automated laser fluorescence sequencer (A.L.F. Express, Pharmacia) using a short gel cassette. Denaturing gels were prepared with 6% readysol DNA/PAGE, 7 M urea and 0.6 × TBE. This solution was heated at 50°C for disolution and then filtered. Temed 0.088% and ammonium persulphate 0.35% were added just before filling the cassette. PCR products were denaturated at 95°C for 5 min and then kept on ice until loading. A 2.5-µl vol of PCR product was loaded with 5 µl of loading dye (containing 5 mg/ml of blue dextran and 5% formamide) and fluorescent-labelled internal markers of 60, 123, 193 and 250 bp [the 123- or 193-bp marker was omitted when it was too close to the allele of microsatellite found in Chinese spring (Plaschke et al. 1995)]. Running conditions were 0.6 × TBE running buffer, 1500 V, 50 mA, 34 W and 55°C with a sampling interval of 2 s. Running was completed after 80 min.

Data analysis

Fragment sizes were calculated using the Allelinks software, taking the sequenced allele size of Chinese Spring (Plaschke et al. 1995) as a reference. Coefficients of co-ancestry (f) were calculated on the basis of pedigree data (up to ten generations upward) for all cultivars, following the assumptions of Cox et al. (1985). Genetic distances based on microsatellite data (GD) were calculated by the different methods implemented in the Microsat software, especially designed by E. Minch for microsatellite data analysis (http://crick.stanford.edu/microsat/microsat.html): $\delta\mu^2$ (Goldstein et al. 1995a), average square (Goldstein et al. 1995b), absolute product (Shriver et al. 1995), Kinship coefficient (Cavalli-Sforza and Bodmer 1971), Nei's identity (Nei 1972), coancestry coefficient θ (Reynolds et al. 1983), 1 - proportion of shared alleles (Bowcock et al. 1994), fuzzy set similarity (Dubois and Prade 1980) and Slatkin's Rst (Slatkin 1995). A number of 10000 bootstraps was found to be appropriate. The rank correlation (Pearson) was calculated between (1 - f) and each GD formulation. Two dendrograms were constructed – the first with (1 - f) data, the second with GD data – using the unweighted pair group method average linkage (UPGMA) in the Phylip software of J. Felsenstein.

Results

Application of common wheat microsatellites to spelt and common wheat

Amplification products were found with all 23 tested WMSs. However, four of them (WMSs 24, 43, 82 and 106) failed to repeatedly provide clear amplification products with several common wheats and spelts. Also, the one-base-long repeat of WMSs 5 and 194 made the exact allele size determination difficult. These six WMSs were thus discarded.

Amplification products were routinely found in 661 out of the 663 locus × accession combinations, and never found either with WMS 111 in Forno or with WMS 186 in 21.87.02. All loci were homozygous, except for WMS 131 in Schwabenkorn. The two plants of each accession always showed the sames alleles, except in 16 out of the 661 cases (2.4%). These 16 differences concerned both spelt and common wheat, and both land races and breeding cultivars or lines.

Alleles found in spelt and common wheat

Each accession presented a unique allele composition, except for the pair Sertel – Ostar. A total of 113 different alleles was found, from which 34 were specific to one single accession (30.1%). The total number of alleles was 88 in spelt vs 70 in common wheat, from which 43 were spelt-specific (48.9%) and 25 common wheat-specific (35.7%). In total, 60.2% of the alleles were group-specific (spelt or common wheat).

The mean number of alleles per WMS was 6.65 ± 2.83 (mean ± SD) and varied from 2 to 13; the mean PIC value was 0.64 ± 0.16 and varied from 0.26 to 0.84 (Table 2). In WMSs 67, 95, 154 and 159, the PIC values were

Table 2 Number of alleles, mean number of 2-bp repeats and polymorphism information content (PIC value) of microsatellite markers. Results for spelt, common wheat, and all accessions together

Microsatellite	Number of alleles			Mean number of 2 bp repeats			PIC value		
	Spelt	Wheat	Total	Spelt	Wheat	Total	Spelt	Wheat	Total
WMS 2	4	4	5	9.8	9.8	9.8	0.62	0.52	0.61
WMS 18	6	4	8	27.6	21.9	26.2	0.76	0.72	0.81
WMS 44	5	5	7	26.2	27.4	26.4	0.65	0.71	0.7
WMS 46	6	3	8	26.2	28.8	26.8	0.56	0.59	0.63
WMS 52	5	4	5	24.3	23.9	24.2	0.7	0.67	0.74
WMS 67	2	3	3	13.1	12.9	13.1	0.23	0.59	0.43
WMS 88	5	5	6	24.7	23.4	24.4	0.74	0.72	0.75
WMS 95	3	4	6	16.7	14.2	16.2	0.18	0.67	0.49
WMS 111	8	5	9	43.1	40.5	42.6	0.75	0.69	0.77
WMS 120	6	4	7	31.9	32.7	32.1	0.74	0.67	0.77
WMS 131	9	5	9	29.5	29.1	29.4	0.72	0.77	0.78
WMS 154	4	5	7	33.4	31.6	33.0	0.24	0.77	0.54
WMS 155	3	2	4	18.0	19.2	18.3	0.38	0.35	0.39
WMS 159	2	2	2	15.0	15.6	15.2	0.06	0.49	0.26
WMS 174	9	7	13	42.9	33.3	40.7	0.77	0.82	0.84
WMS 182	3	2	3	17.8	17.1	17.6	0.63	0.2	0.59
WMS 186	8	6	11	24.5	21.8	23.9	0.67	0.77	0.78
Mean	5.18	4.12	6.65	25.0	23.7	24.7	0.55	0.63	0.64
S.E.	2.23	1.37	2.83	9.2	8.1	8.9	0.23	0.16	0.16
Total	88	70	113						

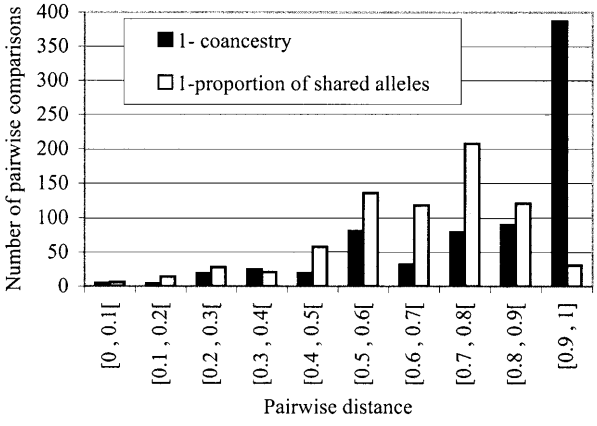

Fig. 1 Frequency distribution of genetic distances based on microsatellite analysis (1 – proportion of shared alleles) and distances based on pedigree relationships (1 – f) among 30 spelt and nine common wheat accessions

much lower in spelt than in common wheat, whereas the opposite was found for WMS 182. All spelts except 65313 showed the same allele for WMS 159. Similarly, all wheats except Chinese Spring showed the same allele for WMS 182. The mean number of repeats per WMS in spelt and in common wheat were highly correlated (0.99***) and generally close together (Table 2). However, the difference was high for some WMSs: 9.6 bp for WMS 174, 5.7 for WMS 18, and more than 2 for WMSs 46, 95, 111 and 186. Considering the PIC values and number of repeats together, differences appeared between spelt and common wheat in 10 out of the 17 studied WMSs (WMSs 18, 46, 67, 95, 111, 154, 159, 174, 182 and 186).

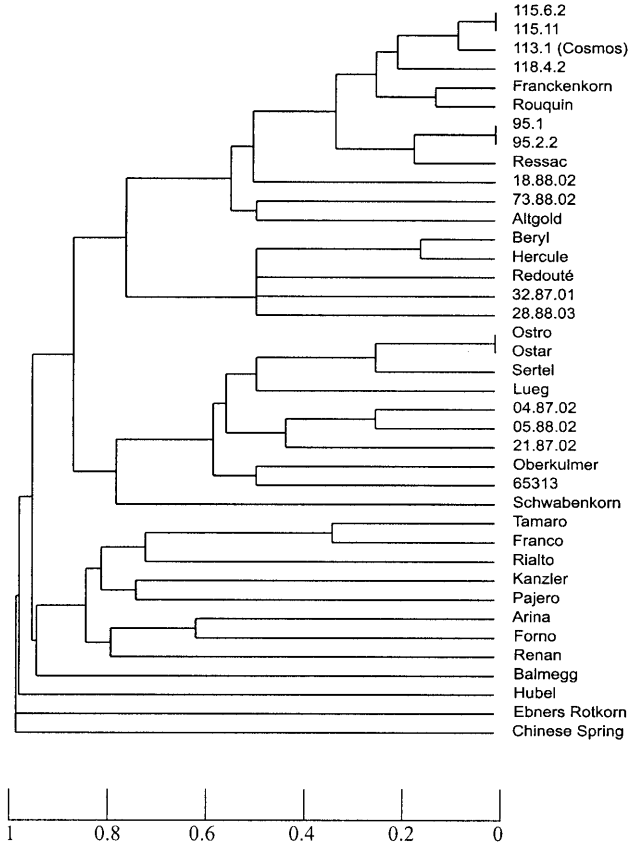

Fig. 2 Dendrogram of 39 accessions (spelts and common wheats) revealed by pedigree relationships (1 – f)

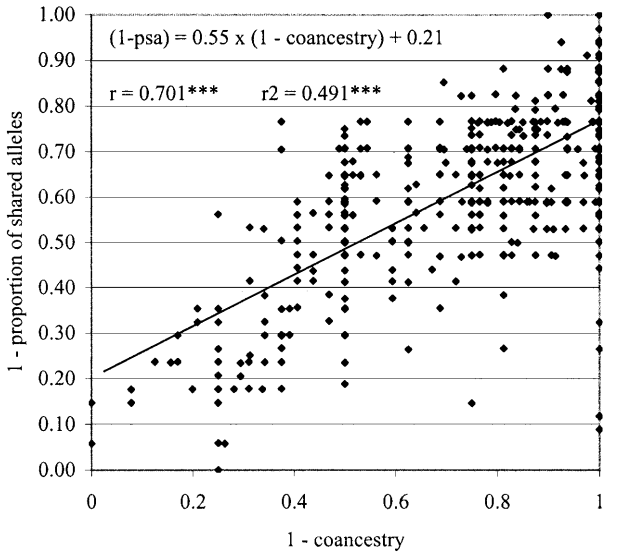

Fig. 3 Scattergram of genetic distances based on microsatellite analysis (1 – psa) vs distances based on pedigree relationships (1 – *f*) including 741 pairwise comparisons between 39 accessions. (psa = proportion of shared alleles)

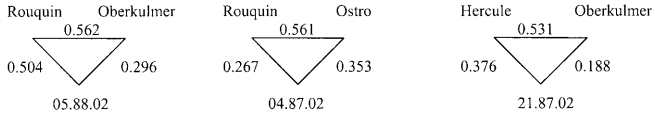

Fig. 4 Genetic distances of progeny and their progenitors based on microsatellite analysis. Progenies are on the bottom, and progenitors on the top, of each triangle

Pedigree data and their relationships with genetic distances

The value of (1 - *f*) over the 39 genotypes was 0.847 +/- 0.227 and ranged from 0 to 1. In spelt, (1 - *f*) fell to 0.77 ± 0.261, whereas it raised to 0.848 ± 0.153 in common wheat. The frequency distribution of (1 - *f*) was highly asymmetrical (Fig. 1), mainly because as many as 333 out of the 741 pairwise comparisons were equal to 1 (no known common ancestor). The dendrogram (Fig. 2) separated the common wheats from the spelts. The relative positions of the different accessions were in accordance with their pedigree relationships.

The highest correlation between (1 - *f*) and GD was 0.701*** (determination coefficient of 0.491***) and corresponded to (1 - proportion of shared alleles). This last distance was retained for further analysis. The slope of the regression of GD vs (1 - *f*) was 0.55 (Fig. 3). In simple crosses where both parents were analyzed, the progeny was never situated at the mid-distance from them (Fig. 4).

Genetic distances among and between groups

The average pairwise GD over the 39 accessions was 0.656 ± 0.181 and ranged from 0 to 1. The frequency distribution of GD was much more symmetrical than that of the coefficients of co-ancestry (Fig. 1). GDs were slightly lower among spelt (0.573 ± 0.172) than among common wheat (0.706 ± 0.14), thus showing that the genetic variability in modern spelt cultivars was quite low. The same analysis was run over the commercially available spelt cultivars only (i.e. those bearing a name instead of a number). This led to an average distance of 0.582 ± 0.179, thus showing that the low GD value among spelts was not only due to the presence of numerous sister lines and direct parents.

The average GD calculated within each spelt breeding programme revealed divergent tendencies. The distance was nearly identical within German spelts (0.59 ± 0.13), slightly lower within Swiss spelts (0.514 ± 0.213), but greatly lower within Belgian spelts (0.381 ± 0.191) than within all spelt accessions together. The GD between spelts arising from two different breeding programmes comprised between 0.559 ± 0.136 and 0.674 ± 0.083.

Pairwise comparisons between each spelt and each common wheat accession were made and led to an average GD of 0.782 ± 0.113. Thus, the average distance between both groups was higher than within each of these groups separately, attesting that, despite the numerous crosses between common wheat and spelt in breeding programmes, the spelt group remained distinct from the common wheat group.

Genetic distances between individual accessions

Chinese Spring, Tamaro and Forno were the three farthest from all other 38 accessions (respectively 0.827 ± 0.067, 0.827 ± 0.108 and 0.83 ± 0.12). Among spelt, Schwabenkorn was the most distant one (0.741 ± 0.064), followed by Balmegg (0.719 ± 0.061) and 65313 (0.7 ± 0.1).

The spelt accessions which were the most distant from common wheat had no common wheat in their known pedigree : Altgold (0.896 ± 0.077), Schwabenkorn (0.888 ± 0.048), Ebners Rotkorn (0.877 ± 0.078) and Ostro (0.86 ± 0.091). At the other extreme, the lowest average distances from common wheat corresponded to all Belgian derivatives of Rouquin (from 0.659 ± 0.129 to 0.712 ± 0.098), followed by 21.87.02 (0.718 ± 0.089) and Rouquin itself (0.719 ± 0.106).

Only five pairwise comparisons led to a GD of 1 : Forno/73.88.03, Forno/Altgold, Franco/Altgold, Tamaro/Ostro and Tamaro/Schwabenkorn, all concerning one common wheat vs one spelt accession. This result strikingly contrasted with the 333 values of (1 - *f*) that were equal to 1.

Dendrogram of genetic distances based on microsatellite data

The dendrogram based on molecular data (Fig. 5) was consistent with the GD and showed a remarkable analogy with the dendrogram based on co-ancestry (Fig. 2).

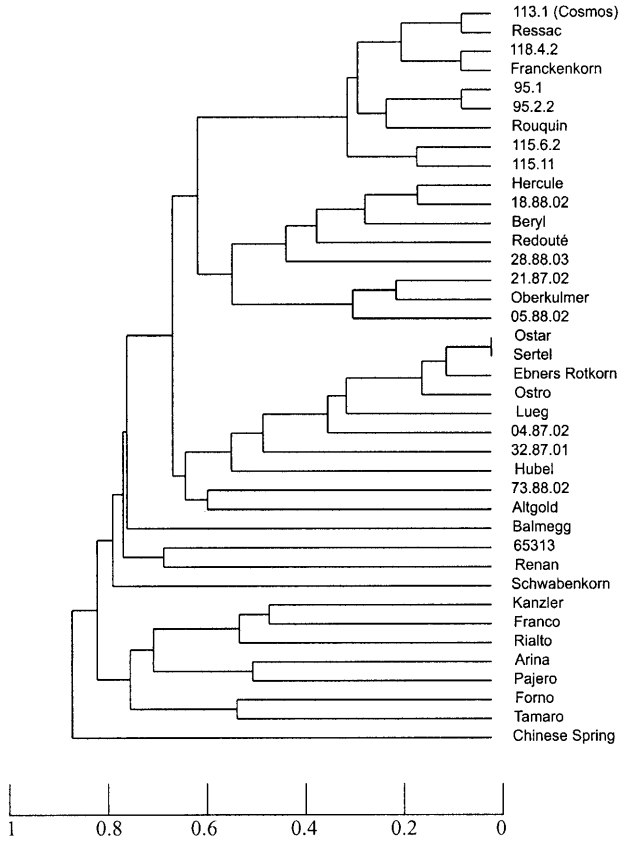

Fig. 5 Dendrogram of 39 accessions (spelts and common wheats) revealed by microsatellite analysis (1 – proportion of shared alleles)

The only important differences between the two dendrograms concerned the position of Ebners Rotkorn, Hubel and Renan, as well as that of the small clusters Altgold/73.88.02 and Oberkulmer/05.88.02/21.87.02 which switched from one spelt subcluster to another. All other changes were only limited and appeared at minor levels.

The first division in the dendrogram based on molecular data isolated Chinese Spring from all remaining accessions. The next division separated the 30 spelts plus Renan from the seven remaining common wheats. In the spelt group, Renan and the three spelts that were the most distant ones from all remaining spelts – Schwabenkorn, 65313 and Balmegg – were first isolated.

The next division in the spelts separated the Belgian accessions plus Oberkulmer from all other Swiss accessions. The German accessions were distributed among these two groups in accordance with their known pedigree, except for 04.87.02 and 32.87.01. Among the Swiss group, Altgold and its direct progeny 73.88.02 clustered separately, as well as Ostro and its progenies. The position of Ebners Rotkorn was not in accordance with the co-ancestry data and will be discussed later on. Within the Belgian group, the family of Rouquin was first isolated, each pair of sister lines clustering together, as well as Franckenkorn and its direct progeny 118.4.2. The remaining accessions were divided into two clusters. On the one hand, the family of Hercule, including 28.88.03 – a progeny of Albin, the sister line of Hercule – and surprisingly 18.88.02. On the second hand, Oberkulmer and two of its direct progenies, 05.88.02 and 21.87.02, which is in accordance with their GD compared to their other parents, as revealed in Fig. 4. The position of Oberkulmer and of two of its progenies in the dendrogram is not so surprising if one considers that Oberkulmer is an ancestor of most Belgian spelts, and that 05.88.02 and 21.87.02 are also direct progenies of Rouquin and Hercule, respectively.

Number of investigated loci

In order to test whether the number of markers was, or was not, sufficient, the analysis was run by successive elimination of the less-polymorphic WMSs. The use of only the ten most-polymorphic WMSs generated a unique allele composition for each accession except for the pair Ostar – Sertel, as previously found with 17 WMSs, and did not bring any major change to the GD nor to the generated dendrogram (data not shown). The correlation between the distances obtained in both analyses (10 and 17 WMSs) was high (0.91***).

Discussion

Potential of common wheat microsatellites for spelt genotyping

No particular problem was found when applying WMSs to spelt. The absence of amplification on some accessions with WMSs 24, 43, 82 and 106 was indeed encountered in both spelt and common wheat genotypes. The same failure was also mentioned earlier for WMSs 24 and 106 in common wheat (Plaschke et al. 1995). These results contrast with the application of common wheat microsatellites to other cereal species such as barley and rye (Röder et al. 1995), for which amplification products were scarcely obtained, even when less-stringent reaction conditions were used. These observations support the view that common wheat and spelt are two sub-groups of the same species (MacKey 1966; Campbell 1997).

Previous studies demonstrated that microsatellites revealed a higher level of polymorphism than any other marker system (Plaschke et al. 1995; Röder et al. 1995; Bryan et al. 1997). The PIC value found in the present study for the common wheats, 0.64, confirms this observation since it was even higher than those found by Röder et al. (1995) using 12 common wheat cvs, 0.54, and by Bryan et al. (1997) using ten common wheats cvs, 0.51. It was within the same range as the one found by Röder et al. (1995) using 18 common wheat cvs including six synthetic lines, 0.63.

Even with only 17 loci, the generated allele profiles allowed us to differentiate almost all 741 pairs of genotypes. The only exception was the Sertel – Ostar pair.

These two accessions, previously reported as FAP65214 and Osmut, (M. Winzeler, personal communication), were earlier found to be the closest ones using RFLP (Siedler et al. 1994). A same successful genotype identification was found using seven microsatellites in soybean (Rongwen et al. 1995), ten in rice (Garland et al. 1999) and 11 in barley (Russell et al. 1997). GD and dendrograms were generated using ten microsatellites in rice (Garland et al. 1999), 15 in barley (Struss and Plieske 1998) and 23 in common wheats (Plaschke et al. 1995). Moreover, it was shown in our case that the reduction to ten loci did not bring any significant change, either in genotype identification, or in GD, or in dendrogram construction. This result attests the reliability of the present protocol and the potentialities of microsatellite studies for genotype identification, the assessment of genetic distances, and phylogeny reconstruction.

The microsatellites allowed us to detect a low level of intra-accession variation (about 2.4%) and the presence of a very low level of heterozygousity (about 0.15%). These results confirm the potentialities of microsatellite studies in order to verify the identity of genetic stocks (Korzun et al. 1997), hence providing a useful tool for breeders.

The use of co-ancestry coefficient and genetic distances

The distance based on (1 – proportion of shared alleles) appeared to be the most reliable among the tested ones. Although not based on the stepwise mutation model (SMM) or any other evolutionary model especially designed for microsatellites, this distance and others, also based on allele frequencies shared between populations, have been shown to reconstruct phylogenies better than the SMM-based distances when using closely related genotypes (Goldstein et al. 1995a, b; Takezaki and Nei 1996).

Compared to other studies on wheat using different genetic marker systems (Plaschke et al. 1995; Barrett et al. 1998; Bohn et al. 1999), the correlation between (1 - f) and GD was high. This may be due to the large quantity of available pedigree data for most of the present accessions, and to the close relationships existing between them.

However, several limits appeared to the use of coefficients of co-ancestry as a guide to choose the progenitors in a breeding scheme. The distribution was highly asymmetrical, mainly because of the presence of 333 pairs of genotypes (44.9%) without any pedigree relationships, whereas only five pairs of accessions (0.7%) were at a GD of 1 on the basis of microsatellite data. Similar results were found among wheat cultivars from the Pacific Northwest (Barrett et al. 1998). Co-ancestry coefficients may lead to the erroneous conclusion that many accessions are genetically far removed from each other.

The lack of information and the errors in the pedigree data also severely limit the accuracy of co-ancestry coefficients. The most striking difference between the two dendrograms concerned Ebners Rotkorn, for which no pedigree data were available. Over the 17 loci, Ebners Rotkorn shared 15 alleles with Ostro and 16 with Ostar and Sertel, which gave a GD of 0.118, 0.089 and 0.089 respectively. These GD were among the seven lowest ones out of the 741 pairwise comparisons. Observations in the field revealed fairly good similarities between Ebners Rotkorn and Ostro. Such a high similarity seems contradictory to the absence of pedigree relationships. It appears reasonable to argue that they might be closely related, maybe due to uncontrolled exchanges of seeds between farmers or agronomists.

Another limitation of the use of coefficients of co-ancestry is that a given genotype is not necessarily situated at a mid-distance from its two parents. This clearly appeared in Fig. 4 and in the work of Siedler et al. (1994). Also, the coefficient of determination revealed that 49.1% of the variation in GD may be explained by the correlation with (1 - f), the remaining 50.9% being due to other sources of variation. This is obviously due to genetic drift towards the parent that shares more desirable characters as a consequence of selection.

Hence, the use of genetic distances based on molecular markers should be recommended to guide the choice of new progenitors, particularly when little is known about their origin, as is the case for land races in programmes of valorization of genetic resources.

Variability in cultivated spelt

The genetic basis of hexaploid wheats is known to be very narrow (Siedler et al. 1994). In the present study, the PIC values and the genetic distances were found to be even lower among spelt than among common wheat. The narrow sample of common wheat accessions may affect the comparison, but it has to be remembered that the spelt accessions considered in this study represent most of the existing diversity in modern spelt, which is not the case in the common wheat sample. Moreover, the PIC values and GD found in common wheat in this study are even higher than those found in other studies of common wheat (Plaschke et al. 1995; Röder et al. 1995). It is thus clear that the genetic basis in modern spelt is even more reduced than in common wheat.

However, the large distance separating some spelt land races (Schwabenkorn), or crosses between spelt land races (Altgold) and the remaining spelts, suggests that the spelt gene pool could be significantly widened by the introduction of unused spelt land races. Such land races are also available outside Northern or Central Europe, mainly in Spain, Eastern Europe and Russia. A comparison based on RAPD markers of macha wheats and spelts of diverse origins revealed that the genetic diversity was lower within spelts (Cao et al. 1998), but direct comparisons between land races and breeding lines of spelt are lacking.

The genetic basis within the Belgian and Swiss breeding programmes were found to be even narrower than

among all spelts, which justifies the existence of recent germplasm exchanges between breeders. The higher GD among German spelts is not surprising given the large genetic basis used in the crosses (Table 1).

The distance between spelt and common wheat

In the present study, all primers designed for common wheat succeeded in providing amplification products in spelt, supporting the view that the differences between these two groups are within the range of the intra-species level. However, the GD between the accessions of both groups was higher than within each individual group. Also, the dendrogram generated by the marker data clearly separated the 30 spelts from most of the nine common wheats. Although the reduced size of the common wheat sample may affect the accuracy of the inter-group distance determination, it has to be remembered that previous studies led to the same conclusion. Liu et al. (1990), Keller et al. (1999a, 1999b) and Messmer et al. (1999) found a high level of restriction fragment length polymorphism between spelt land races and common wheat. Both groups clustered separetely in the study of Dvorak et al. (1998). Differences were also found between modern spelt cultivars and common wheat using RFLP data (Siedler et al. 1994) and seed storage proteins (Harsch et al. 1997; Radic et al. 1997; Radic-Miehle et al. 1997), although some modern spelt as well as traditional ones did not show the typical spelt pattern in these three last studies. This suggests that, despite the frequent use of common wheat in spelt breeding schemes, the modern spelt cultivars remained distinct from that of common wheat.

In conclusion, the present study demonstrated the usefulness of common wheat microsatellites for genetic studies in spelt, the potentialities of genetic distances based on the proportion of shared alleles to reconstruct phylogenies in a set of closely related genotypes, and the genetic narrowness of modern spelt cultivars. This approach may be applied to the study of genetic resources in spelt land races to help the construction of a core collection and to guide the choice of spelt land races in spelt breeding programmes in order to widen the gene pool of modern spelt.

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