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H. Siedler · M. M. Messmer · G. M. Schachermayr
H. Winzeler · M. Winzeler · B. Keller

Genetic diversity in European wheat and spelt breeding material based on RFLP data

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Abstract Fifty-two winter wheat (*Triticum aestivum* L.), nine spring wheat, and 20 spelt (*Triticum spelta* L.) lines representing part of the European breeding germplasm, were assayed for RFLPs (restriction fragment length polymorphisms) with 56 wheat DNA clones and two barley cDNA clones. Objectives of this study were to (1) determine the level of variation for RFLPs in the wheat and spelt breeding lines, (2) characterize the genetic diversity within the European winter wheat germplasm, and (3) evaluate the usefulness of RFLP markers for pedigree analysis and the grouping of wheat and spelt lines of various origins. Seventy-three of the 166 RFLP loci detected with 58 probes and one restriction enzyme were polymorphic for the 81 lines. The percentage of polymorphic loci was greatest for the B genome (58%) and smallest for the D genome (21%). Among the 81 lines, 271 different RFLP bands were detected. RFLP band frequencies of the winter wheat lines differed considerably (≥0.5) from those of the spring wheat lines at five loci, and from those of the spelt lines at 17 loci. Eight cultivars that had a major impact as progenitors on the development of improved winter wheat cultivars accounted for 93% of the observed RFLP bands in winter wheat. Genetic distance (GD) estimates between two lines ranged between 0.01 and 0.21. Mean GD estimates within winter wheat (0.083), within spring wheat (0.108) and within spelt (0.096) were smaller than between spring and winter wheat (0.114), and greatest between winter wheat and spelt (0.132) and spring wheat and spelt (0.148). Principal coordinate analysis performed on GD estimates revealed a clear separation of wheat and spelt germplasm. Novel spelt lines with various proportions of wheat germplasm were positioned between wheat and traditional spelt lines. The spring wheat lines formed a distinct group at the periphery of the distribution of the winter wheat lines. Subgroupings of the winter wheat lines according to the cluster analysis were in good agreement with their origin, and lines with common ancestors were grouped together.

Key words Wheat · Spelt · RFLP · Marker
Genetic diversity

Introduction

The development and application of genetic markers in crop plants can improve practical plant breeding. The main applications of genetic markers are the characterization of breeding lines and varieties as well as marker-assisted selection. To date, about 100 protein loci have been described in wheat (Hart et al. 1993), but very few have achieved importance in wheat breeding. A substantial improvement in the number of markers that are useful is expected with the application of RFLPs (Botstein et al. 1980). However, wheat displays a low degree of restriction fragment length polymorphism (Chao et al. 1989; Kam-Morgan and Gill 1989; Gale et al. 1990; Liu et al. 1990), thus hampering the identification of markers linked to agronomically important traits as well as the differentiation of varieties and the analysis of genetic variability. Two different strategies to find more polymorphisms, and thus to improve mapping, have been used in wheat. First, new molecular techniques were developed and tested (Williams et al. 1990; Weining and Langridge 1991; Dweikat et al. 1993; Schmidt et al. 1993). Unfortunately, the efficient and successful RAPD (random amplified polymorphic DNA, Williams et al. 1990) technology was found to be ill-suited for the construction of linkage maps in wheat because a given primer can amplify a number of non-homologous sequences that may vary among varieties (Devos and Gale 1992). In a second strategy, the biological material used for mapping populations was carefully chosen to obtain the largest genetic difference possible and, thus, a high degree of polymorphism (Gill et al 1991; Liu and Tsunewaki 1991).

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H. Siedler · M. M. Messmer · G. M. Schachermayr · H. Winzeler
M. Winzeler · B. Keller (✉)
Department of Plant Breeding, Swiss Federal Research
Station for Agronomy, Zürich-Reckenholz,
Reckenholzstrasse 191,
CH-8046 Zürich, Switzerland

Among common hexaploid wheats, spelt (*Triticum spelta* L.) displays the highest amount of polymorphism relative to *Triticum aestivum* L. cv Chinese Spring (Liu et al. 1990). Spelt is a traditional crop species in Switzerland. It was the predominant bread cereal from the 5th century until the beginning of the 20th century (Winzeler and Rüegger 1990). In modern breeding programs, spelt has been crossed with wheat to improve its agronomic performance (Winzeler et al. 1991).

For breeding purposes, the practical value of RFLP markers and an RFLP-based genetic map depends on the degree of allelic variation in the breeding material. Wheat breeding programs in different European countries are characterized by the frequent usage of their own lines for further crosses (Lupton 1987). However, there is also a tradition of exchanging lines among breeders. It is not known whether these opposite tendencies have resulted in different gene pools adapted to the respective climatic conditions in Europe or if the genetic material is uniformly spread over Europe. In this study, we determined the variation within European wheat varieties and breeding lines using RFLP markers of known genetic location. Spelt breeding lines were included in this study to analyze the diversity within spelt material and to compare it with the diversity in wheat. We found that the wheat and the traditional spelt material formed two clearly distinct classes, whereas new spelt lines originating from wheat by spelt crosses showed less polymorphism than wheat. Using RFLP markers, distinct subgroups were found within the European winter wheat material.

Materials and methods

Wheat and spelt lines

A total of 52 winter wheat, nine spring wheat, and 20 spelt varieties (Table 1) were analyzed with RFLP probes. Most of the winter wheat lines originate from Germany (23) and Switzerland (17), eight lines are of French origin and the remaining lines are from the Netherlands, Great Britain, and Russia. They represent actual breeding material as well as historically important cultivars like Bezostaja, Cappelle, Carstacht, Derenburger Silber, HeineVII, Hoeser52, Probus, and Zenith, which were released between 1941 and 1969. The spelt lines originate from Switzerland with the exception of Rouquin and Hercule, which are from Belgium, and four land varieties (LV1, LV2, LV3, LV4) from southern Germany. Most of the spelt material from Switzerland is directly related to Oberkulmer, a selection of a Swiss land variety, or to Ostro, a derivative of Oberkulmer. All lines with the initials FAP were developed at our breeding station. Seeds from the other lines were obtained from the gene banks at Braunschweig, Germany, and the Station Fédérale de Recherche Agronomique de Changins, Switzerland, or else were commercial varieties.

RFLP analysis

Isolation of genomic DNA, restriction enzyme digestion, agarose gel electrophoresis, alkaline Southern blotting to nylon membranes, and hybridization methods were all performed as described by Graner et al. (1990). Probes were labelled with [^{32}P]dCTP (3,000 Ci/nmol, Amersham Buchler, Switzerland) using the random primer method (Feinberg and Vogelstein 1983). Non-incorporated nucleotides were removed by gel filtration through Sephadex G-50 (Pharmacia, Upsala, Sweden).

The 81 wheat and spelt lines were assayed with 56 wheat clones and two barley cDNA clones. The wheat clones (Xpsr probes) have been previously mapped (Hart et al. 1993) and were kindly provided by Dr. M. Gale (IPSR, Norwich, England) and Dr. P. Gay (Ciba Seeds, Basel, Switzerland). The barley clones were provided by Dr. A. Graner (BBA, Grünbach, Germany). Each RFLP probe was used in combination with one of the restriction enzymes *Eco*RI, *Hin*dIII, *Eco*RV, *Xba*I, or *Dra*I (Table 2). The RFLP probes were chosen according to the following criteria: (1) good coverage of the wheat genome, (2) good signal strength, and (3) clearly distinguishable fragments. For statistical analyses, each probe was used in combination with the most informative restriction enzyme digest.

Statistical analysis

Genetic distances (GD) between each pair of lines based on RFLP data were calculated according to the formula given by Boppenmaier et al. (1992):

$$GD_{ij} = 1 - \frac{2N_{ij}}{(N_i + N_j)}$$

where N_{ij} is the number of RFLP bands in common between lines i and j, and N_i and N_j are the total number of RFLP bands that were observed for line i and line j, respectively, across all 58 probes. Thus, GD estimates reflect the proportion of RFLP bands that differ between two lines. Standard errors of GD estimates were calculated assuming a binomial distribution of RFLP bands at a specific locus. For each RFLP band, the frequency of its occurrence was determined within the 52 winter wheat lines, the nine spring wheat lines, and the 20 spelt lines, respectively, and the absolute difference between these RFLP band frequencies was calculated.

Graphical representation of the association among the 81 lines was obtained by principal coordinate analysis based on GD estimates (Gower 1972). A dendrogram of the 52 winter wheat lines was obtained by UPGMA (unweighted pair group method average linkage) cluster analysis (Sneath and Sokal 1973). The necessary computations were performed with appropriate procedures of SAS (SAS Institute 1988) and NTSYS-pc (Rohlf 1989).

Results

Polymorphism among European wheat and spelt lines detected by RFLP markers

With the 58 probe-enzyme combinations used in this study, 271 different RFLP bands covering a minimum of 166 genetic loci were observed across the 81 wheat and spelt lines (Table 1). Most of the probes hybridized to three independent loci located on each of the homoeologous chromosomes (Table 2). Forty-five probes (78%) detected polymorphism in at least one locus with a maximum of 12 different RFLP bands per probe (Xpsr392) and four different bands per locus (Xpsr131 – chromosome 2B, Xprs1196-3A, Xpsr931-3B). Altogether, 73 loci (44%) showed polymorphism among the 81 wheat and spelt lines and 54, 53, and 50 of these loci were also polymorphic among the 52 winter wheat lines, the nine spring wheat lines, and the 20 spelt lines, respectively. Twenty-three probes (40%) revealed no polymorphism for the three genomes A, B, and D among the winter wheat lines, 33% were monomorphic among the spring wheat and 36% among the spelt lines. Considering all 81 lines, the percentage of polymorphic loci per genome was greatest for the B genome (58%), medium for the A genome (36%), and smallest for the D genome (21%). No single RFLP marker was able to differ-

Table 1A Pedigree information of the 52 winter wheat varieties and breeding lines used in the RFLP analysis

No.	Line	Origin	Pedigree[a]	Year[b]	R[c]
1	Apollo	D	Unknown	1984	
2	Ares	D	Cato×Caribo [Cato= Heine652×(HeineVII×Carstacht)]	1983	6
3	Arina	CH	Moisson×(Can3842×HeineVII)	1981	1
4	Basalt	D	Derivative of Caribo	1980	5
5	Bernina	CH	Caribo×Hoeser52	1983	1
6	Bezostaja	Russia	Lutescens17×Skorospelka2	1963	2
7	Boval	CH	(Carstacht×Hoeser48)×(Can3842×Tano^2)	1990	1
8	Bussard	D	(Kranich×Maris Huntsman)×Monopol [Kranich= (Hindukusch×Chinese)×(Thatcher×Kronen) × HeineVII×Merlin]	1963	6 3
9	Cappelle	F	Vilmorin27×Hybrid du Joncquois	1946	3
10	Caribo	D	Carstacht×Cappelle	1968	3
11	Carstacht	D	(Russian land variety×CarstenV)×(Minhardi×CarstenVI)	1952	2
12	Champlein	F	Tadepi×YGA (YGA= Vilmorin27×Red Five)	1959	4
13	Derenburger Silber	D	Svalöfs Panzer III×Peragis	1941	3
14	Disponent	D	{Carstacht×[(Thatcher cross×Walthari)×Triticale]} ×{[(Tassilo×Strubes56)×HauterII]×ToerringII}	1975	3
15	FAP62284	CH	Arminda×Roazon		1
16	FAP62420	CH	Hoeser480-73×Eiger		1
17	FAP74961	CH	{[99.229×(Probus×Nord)]×[((Probus×Nord)×Weique)×Champlein]} ×Champlein mutant		1
18	FAP75141	CH	Tabor×Yugoslavian line		1
19	FAP75245	CH	(Brigand×Arina)×(Hoeser52×Sturdy)		1
20	FAP75337	CH	Forno×(Bezostaja×Zenith)		1
21	FAP75493	CH	{Virtue×[M. Huntsman×(Bezostaja×(Mexique50×B21^2))]}×Arina		1
22	FAP75507	CH	{H79R37×[Kavkas×(Zenith×Backa)]}×[(Lichti×Probus^4) ×(Zenith×NS611)]		1
23	FAP75517	CH	Forno×Arina		1
24	FAP75527	CH	Virtue×[(Lichti×Probus^4)×(Zenith×NS611)]		1
25	Forno	CH	NR72837×Kormoran	1986	1
26	Galaxie	F	(Capite×HeineVII)×Talent	1991	1
27	Granada	D	Derivative of Jubilar (Jubilar= D. Silber×Schernauer)	1980	5
28	Greif	D	Maris Hobbit×Carimulti^2 (Carimulti= Caribo×Ibis)	1989	6
29	HeineVII	D	Hybrid à courte paille×Svalöfs Kronen	1950	3
30	Hoeser52	D	Weihenstefan477/58	1969	4
31	Iena	F	Champlein×Courtôt	1986	1
32	Kanzler	D	Derivative of Caribo	1980	5
33	Kavkas	Russia	Lutescens314H147×Bezostaja	1971	2
34	Kormoran	D	Cappelle×Carstacht×Merlin	1973	3
35	Kraka	D	Derivative of Kranich and Caribo	1982	5
36	Kronjuwel	D	Derivative of Caribo	1980	5
37	Maris Huntsman	GB	CI12633×Cappelle×Hybrid46×Marchall (CI12633= Timopheevi×Illinois×Chinese^2)	1975	3
38	Merlin	D	Derenburger Silber×HeineVII	1956	3
39	Monopol	D	Pantus×Admiral (Pantus= Triesd.St81/828×Carstacht)	1975	3
40	Obelisk	NL	Selection of synthetic of 36 cultivars	1990	1
41	Probus	CH	Trubilo×Plantahof	1946	2
42	Rektor	D	Derivative of Kormoran and Monopol	1980	5
43	Renan	F	F$_1$ (Mironov808×M. Huntsman)×F$_1$ ((VPM×Moisson) 1.5×Courtôt)	1991	8
44	Roazon	F	[(*Ae. ventricosa*×*T. persicum*)×Marne^3]×Moisson	1978	4
45	Sperber	D	(Robert×Merlin)×Kormoran	1982	6
46	Tamaro	CH	(Kormoran×NR72837)×Monopol	1982	1
47	US60xPrieur	F	US60(43)×Prieur {Prieur= [(Versailles^2×Etoile de Choisy)×Cappelle]}	1970	4
48	VPM	F	(*Ae. ventricosa*×*T. carthlicum*)×Marne^3		2
49	Vuka	D	(Toerring II× Merlin)×Carstacht	1975	8
50	Weique	D	*T. dicoccum* ×*Agr. intermedium*	1961	4
51	Zenith	CH	Can3842×HeineVII	1969	1
52	Zorba	D	{[(Tassilo×CarstensV)×?Thatcher]×[((Tassilo×CarstensV) ×(CarstensV×Marquillo))×Thatcher]}×Triticale		2

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[a] Power of 2, 3, and 4 refer to the first, second, and third backcross generation, respectively

[b] Year of release

[c] References: 1, H. Winzeler and M. Winzeler, personal communication; 2, Zeven and Zeven-Hissink 1976; 3, Hoeser et al. 1975; 4, G. Kleijer, personal communication; 5, Odenbach 1985; 6, H. Brunckhorst, F. v. Lochow Petkus GmbH, personal communication; 8, P. Franck, Pflanzenzucht Oberlimpurg Dr. Franck, personal communication

Table 1B Pedigree information of the nine spring wheat varieties and breeding lines used in the RFLP analysis

No.	Line	Origin	Pedigree	Year[a]	R[b]
53	Can3842	CAN	Unknown	1952	
54	Chinese Spring	PRC	Chinese land variety		2
55	FAP94779	CH	(Toropi×Gullo)×[Sappo×(B580×B664)]		1
56	FAP94883	CH	Bobwhite"S"×MS		1
57	FAP94885	CH	{(EXSR400×Solo)×[Sappo×(B580×B664)]}×(EXSR400×Selpek)	1	
58	FAP94892	CH	Kadett×Bobwhite"S"		1
59	Lona	CH	Red River68×Walter	1991	1
60	Thatcher	USA	(Marquis×Kanred)×Marquillo	1939	7
61	Walter	S	WW1369×WW4169	1979	1

[a] Year of release
[b] References: 1, H. Winzeler and M. Winzeler, personal communication; 2, Zeven and Zeven-Hissink 1976; 7, Lupton 1987

Table 1C Pedigree information of the 20 spelt varieties and breeding lines used in the RFLP analysis

No.	Line	Origin	Pedigree	Year[a]	R[b]
62	Altgold	CH	Oberkulmer×Sandmeier	1952	1
63	FAP65214	CH	(Ostro×Uniplanta80-23)×Ostro		1
64	FAP65237	CH	Male sterile wheat line×Ostro		1
65	FAP65246	CH	(Murirotkorn×Eiger)×Triticale50557		1
66	FAP65251	CH	Spelt-DPYT×(Ostro×Arina)		1
67	AP65273	CH	{[((GLH5062×Ostro)×H79R35)×MUT72202]×W'ST114-75} ×(Oberkulmer×Sardona)		1
68	FAP65289	CH	(Ostro×Arina)×Mutant72202 [72202= Hoeser52×(Cappelle×Atlas66)]		1
69	FAP65290	CH	Ardennen spelt×Mutant72202		1
70	FAP65291	CH	Oberkulmer×H79R35 [H79R35= (VPM×Moisson)×(V81.12×US60)]		1
71	Hercule	B	Rechbergs Früher×Ardenne (Ardenne= Virtue×Lignée24)		1
72	Hubel	CH	ALB62A×Uniplanta80-23	1992	1
73	Lueg	CH	Ostro×Uniplanta77-62	1990	1
74	LV1	D	Land variety from the Black Forest		1
75	LV2	D	Land variety from the Black Forest		1
76	LV3	D	Land variety from the Swabian Alb		1
77	LV4	D	Land variety from the Swabian Alb		1
78	Oberkulmer	CH	Selection from Swiss land variety	1948	1
79	Osmut	CH	Mutant of Ostro		1
80	Ostro	CH	Oberkulmer×Steiners Roter Tiroler	1978	1
81	Rouquin	B	(Lignée24×Ardenne)×Altgold		1

[a] Year of release
[b] References: 1, H. Winzeler and M. Winzeler, personal communication

entiate the winter from the spring wheat lines nor the wheat from the spelt lines. However, several probes (spread across the whole genome, Table 2) showed considerable differences in RFLP band frequency (≥0.5) between the wheat and the spelt lines. Probes Xpsr1196-3A, 3B, Xpsr598-3A, Xpsr906-5D, Xpsr546-6B, Xpsr303-7A, cMWG684-6B, and Xpsr596 (Fig. 1) detected one or more RFLP bands that occurred exclusively among the spelt lines but not among the wheat lines. Five probes (Xpsr931-3B, Xpsr911-5B, Xpsr637-5B, Xpsr546-6B and Xpsr392) revealed a difference in RFLP band frequency of at least 0.5 between the winter and the spring wheat lines.

Genetic distances among European wheat and spelt breeding material

Genetic distances (GD) based on RFLP data between all possible pairs of lines ranged from 0.01 between the two spelt lines FAP65214 (a backcross of Ostro) and Osmut (derived from Ostro by X-ray treatment) to 0.21 between winter wheat line FAP62420 and spelt line FAP65214 (Table 3). The mean GD was 0.11, i.e., two randomly chosen lines differed on average in 20 of 166 examined loci. The standard error of individual GD estimates ranged between 0.01 and 0.03. The genetic distance among the 52 winter wheat lines was smallest between the two Russian lines Bezostaja and its derivative Kavkas (0.01), greatest between Bezostaja and the German line Vuka (0.15), and was on average 0.083 (Table 3). The smallest GD value among the spring wheat lines was 0.07 found between the Swedish line Walter and its derivative Lona. The greatest GD value was found between Walter and the Canadian line Can3842 (0.15). On average, the genetic distance among the spring wheat lines was greater than among the winter wheat lines, reflecting the diverse geographic origin of this set of spring lines and the higher level of pedigree relatedness among the winter wheat lines. The spelt lines showed

Table 2 Polymorphism detected among the 81 wheat and spelt lines with the 58 probe-enzyme combinations used for RFLP analysis

Probe	Enzyme	Chr.[a]	Polymorph[b] for genome				Probe	Enzyme	Chr.	Polymorph for genome			
			A	B	D	X[c]				A	B	D	X
Xpsr601	*Dra*I	1	–	+	–		Xpsr332	*Xba*I	4				#
Xpsr544	*Hin*dIII	1	–	#	–		Xpsr144	*Hin*dIII	4	–	+	–	
Xpsr596	*Hin*dIII	1, 9	–			#	Xpsr163	*Eco*RV	4	–	–	–	
Xpsr908	*Eco*RV	1		+			Xpsr104	*Hin*dIII	4	+	–	+	
		2			#		Xpsr1051	*Eco*RI	4, 9	#	+	–	+
		6		#			Xpsr628	*Dra*I	5	–	–	–	
Xpsr1201	*Eco*RV	1	#				Xpsr1204	*Dra*I	5	–	–	–	
		5	–	+			Xpsr929	*Hin*dIII	5	–	–	–	
Xpsr666	*Eco*RV	2, 9	–	(+)	–	(+)	Xpsr906	*Eco*RI	5	–	–	(+)	
Xpsr131	*Hin*dIII	2	(#)	+	–		Xpsr574	*Dra*I	5	(+)	+	–	
Xpsr912	*Eco*RI	2, 9	–	(+)	–	+	Xpsr911	*Hin*dIII	5	#	+	–	
		5			–		Xpsr637	*Dra*I	5	–	+	+	
Xpsr107	*Eco*RI	2	(+)	(+)	–		Xpsr1194	*Xba*I	5				+
Xpsr112	*Hin*dIII	2, 9	–	–	–	(+)	Xpsr167	*Eco*RI	6, 9	–	#	(+)	+
Xpsr901	*Eco*RI	2	–	(+)	–		Xpsr662	*Eco*RV	6	(+)			
Xpsr1196	*Eco*RI	3	+	#	–				7	–	–	–	
Xpsr598	*Dra*I	3, 9	+	–	+	(+)	Xpsr312	*Hin*dIII	6	–	–	–	
Xpsr394	*Hin*dIII	3	–	–	(+)		Xpsr113	*Xba*I	6				–
Xpsr578	*Eco*RI	3, 9	–	+	–	+	Xpsr915	*Eco*RI	6, 9	(+)		+	#
Xpsr1077	*Dra*I	3	+	–	–		Xpsr142	*Hin*dIII	6, 9	–	–	–	–
Xpsr78	*Dra*I	3	+	–	–		Xpsr627	*Eco*RI	6	+	–	–	
Xpsr1205	*Eco*RV	3	–	+	–		Xpsr605	*Dra*I	6	–	+	+	
Xpsr931	*Eco*RI	3	–	+	–		Xpsr154	*Xba*I	6	–	–		
Xpsr926	*Xba*I	3				–	Xpsr546	*Eco*RV	6, 9		+	+	+
Xpsr689	*Dra*I	3, 9	–	+	–	#	Xpsr392	*Dra*I	7, 9	+		–	+
Xpsr910	*Xba*I	3	–	–	–		Xpsr690	*Eco*RV	7	–	–	–	
Xpsr1067	*Eco*RI	3			–		Xpsr129	*Xba*I	7	–	#	–	
Xpsr573	*Dra*I	4	+				Xpsr547	*Dra*I	7, 9	–	#	–	+
Xpsr580	*Eco*RI	4	–				Xpsr303	*Eco*RI	7	(#)	–	–	
		5		+	–		Xpsr149	*Xba*I	9				(+)
Xpsr115	*Eco*RV	4	#										
		5		–	–		cMWG684	*Eco*RV	6, 9		(+)		+
Xpsr1316	*Dra*I	4	–				cHS130	*Dra*I	9				–
		5		(+)									

[a] 9, homoeologous chromosome group unknown
[b] +, polymorphism was detected among the 52 winter wheat lines
(+), polymorphism was detected among the 81 lines but not among the 52 winter wheat lines
–, no polymorphism was detected among the 81 lines
#, difference in RFLP band frequency of at least 0.5 between the 52 winter wheat and 20 spelt lines
[c] X, genome unknown

Table 3 Mean, minimum, maximum, and standard deviation (SD) of genetic distance (GD) estimates within and between breeding groups of wheat and spelt lines

Breeding group	N[a]	Genetic distance			
		Mean	Minimum	Maximum	SD
Within winter wheat	1,326	0.083	0.01	0.15	0.02
Within spring wheat	36	0.108	0.07	0.15	0.03
Within spelt	190	0.096	0.01	0.15	0.03
Between winter and spring wheat	468	0.114	0.06	0.16	0.02
Between winter wheat and spelt	1,040	0.132	0.06	0.21	0.02
Between spring wheat and spelt	180	0.148	0.10	0.20	0.02

[a] N, number of pairwise line comparisons within each category

the same range of GD values as the winter wheat lines. GD estimates between lines of different breeding groups were on average greater than those within groups (Table 3). The increase in mean GD from within winter wheat compared to between winter and spring wheat was 0.031 and between winter wheat and spelt lines 0.049. The greatest mean GD estimate was found for line combinations between spring wheat and spelt.

Principal coordinate analysis of RFLP data

Associations among all 81 lines revealed by principal coordinate analysis (Fig. 2) reflect the general pattern observed with GD estimates. Wheat and spelt lines were separated by the first principal coordinate (PC1), which accounted for 19% of the total variation of the RFLP data. The older spelt cultivars, Oberkulmer, Ostro, and Altgold, and the land varieties were rather distant to the winter wheat lines, while spelt lines with pedigree relatedness to winter wheat (e.g., FAP65251, FAP65273, FAP65289, FAP65291, Hubel) were positioned in a direction towards the winter wheat lines. The second principal coordinate (PC2) explained 9% of the variation and mainly separates the spring from the winter wheat lines. The nine spring wheat lines formed a close cluster in the upper part of Fig. 2. The winter wheat lines were spread across PC2. The main progenitors of the European winter wheat breeding material (Bezostaja, Cappelle, Caribo, Carstacht, Derenburger Silber, HeineVII, Hoeser52, Probus, Zenith) represented the variation of winter wheat in both dimensions (PC1, PC2). Bezostaja and its derived lines Kavkas and FAP75337, as well as the French line US60xPrieur, were very distant from the other winter wheat lines and close to the spring lines.

Cluster analysis of RFLP data

While principal coordinate analysis was used to identify the most divergent groups of the European wheat and spelt breeding material, cluster analysis based on GD values was used to reveal pedigree relatedness among the 52 winter wheat lines (Fig. 3.). The lower hierarchy clusters shown in Fig. 3 were in good agreement with the origin of these lines and their pedigree information as far as available. One subcluster included the lines Iena, Cappelle, Champlein, Roazon, and VPM, all of French origin. The next cluster (Kanzler–Basalt) mainly consisted of German lines with pedigree relatedness to Carstacht (Kanzler–Vuka), Cappelle (Tamaro–Rektor) and Caribo (Bernina–Basalt). Lines derived from HeineVII and Derenburger Silber clustered together (Forno–Sperber) as well as lines related to

Fig. 1 Southern blot hybridization of the Xpsr596 probe with *Hin*dIII digested genomic DNA of nine winter wheat (*WW*), three spring wheat (*SW*), and seven spelt (*SP*) lines. *Arrows* indicate RFLP bands that occurred exclusively among spelt lines but were not found among wheat lines

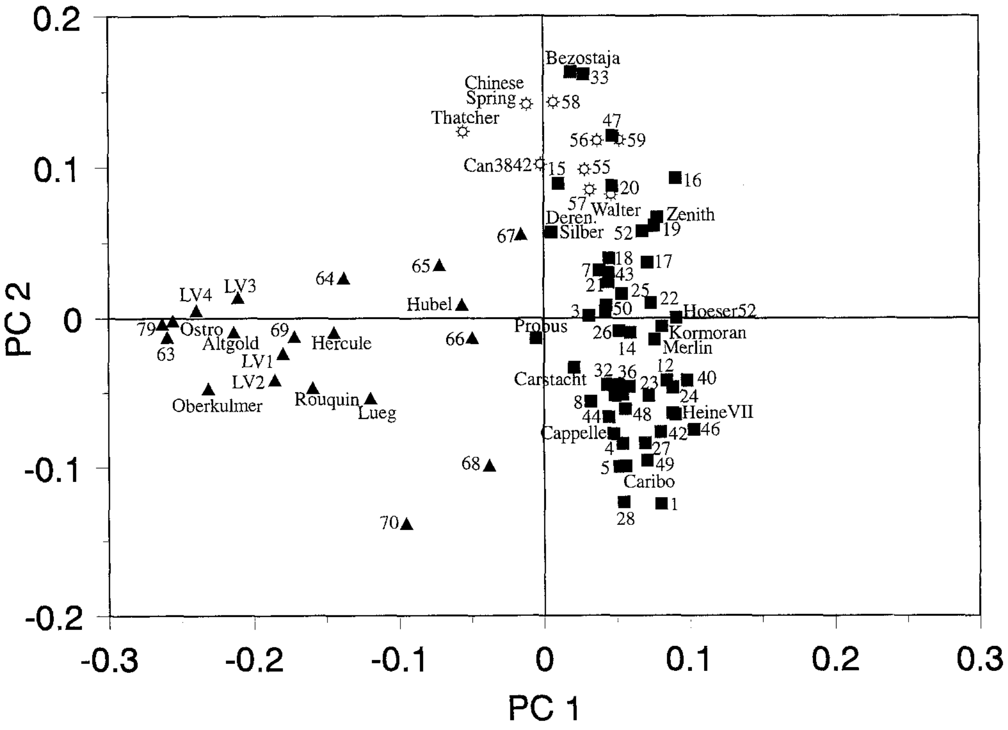
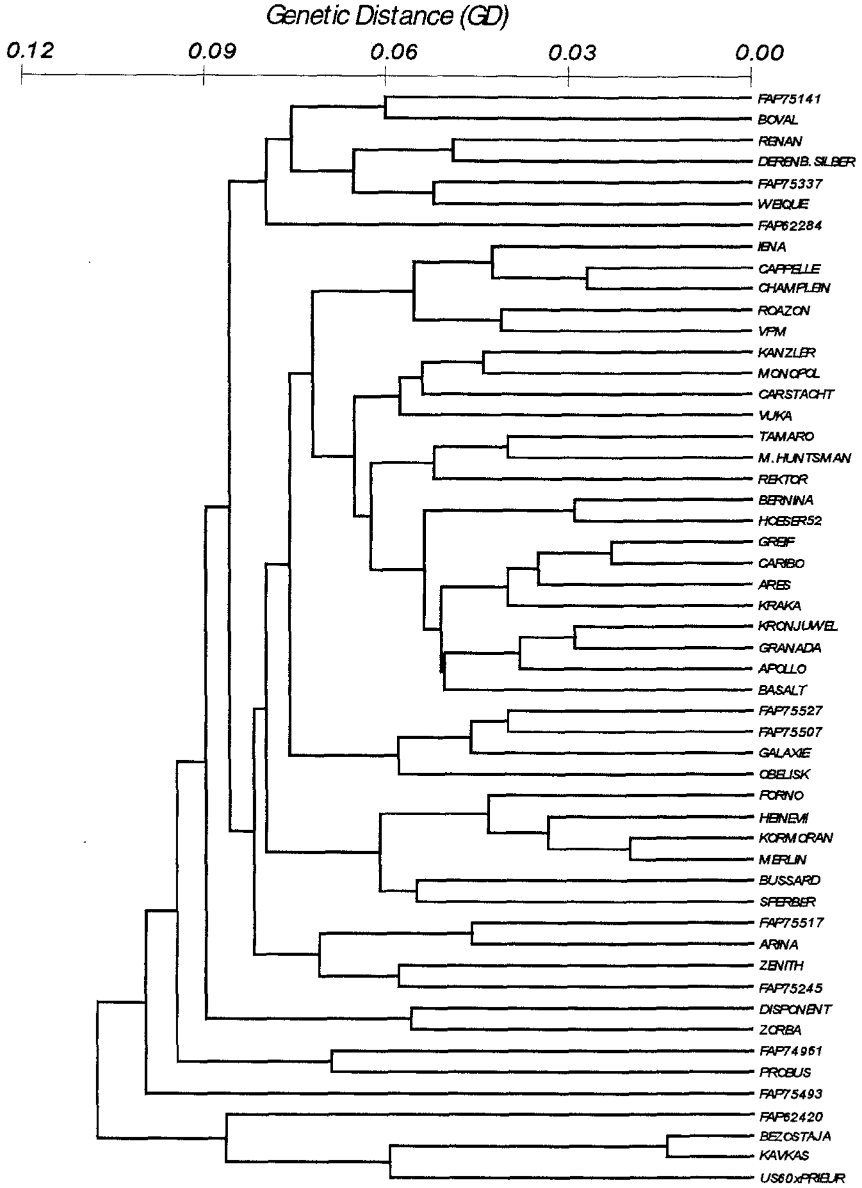


Fig. 2 Associations among the 81 wheat and spelt lines revealed by principal coordinate analysis based on RFLP data of 58 probe-enzyme combinations. Winter wheat, spring wheat, and spelt lines are indicated by *squares* ■, *circles* ☼, and *triangles* ▲, respectively. *Numbers* next to the symbols correspond to the number of the lines in Table 1

Fig. 3 Dendrogram of 52 winter wheat lines revealed by average linkage cluster analysis based on RFLP data of 58 probe-enzyme combinations



HeineVII and Arina (FAP75517–FAP75245). The smallest cluster was formed by Kavkas and its parent Bezostaja.

Genetic distance estimates between related lines

Examples of genetic distances between derived lines and their direct progenitors are given in Fig. 4. It is expected that the genetic distances of a derived line to each parent add up to the genetic distance between the parents and are of similar size in cases involving no selection pressure. This was observed for FAP75517 and Bernina. Merlin was closer to HeineVII than to its other parent, Derenburger Silber. The genetic distance between Caribo and its parent Carstacht was equal to the genetic distance between the parents indicating pedigree relationship to Cappelle but not to Carstacht. Zenith showed a similar relationship to both parents but not as close as expected. Both Caribo and Zenith showed RFLP bands that were not found in the parental lines.

Discussion

Frequency of RFLPs in wheat and spelt

A wide range of European wheat and spelt lines was evaluated for their degree of RFLPs. Forty-four percent of the assayed loci were polymorphic and yielded on average 2.4 different RFLP fragments within the 81 lines. However, only 11% of the loci were polymorphic in pairwise comparisons. This low level of polymorphism detected among cultivated wheat and spelt lines is consistent with other RFLP studies in wheat (Chao et al. 1989; Kam-Morgan and

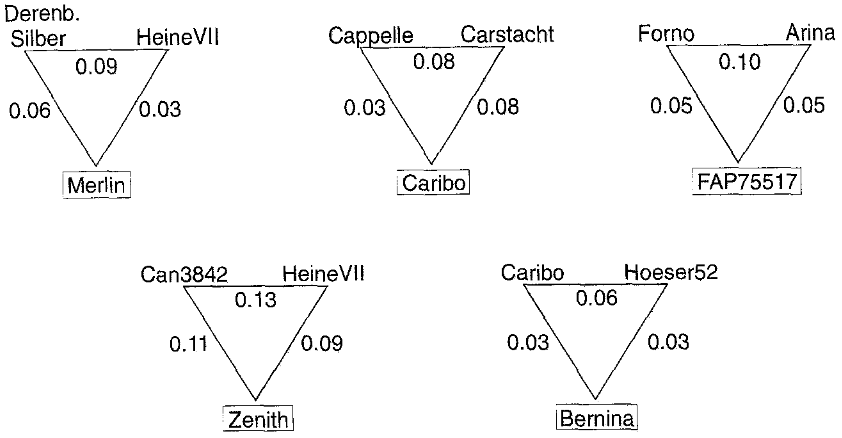


Fig. 4 Examples of genetic distance estimates of derived lines to their progenitors based on RFLP data of 58 probe-enzyme combinations. On the top of each triangle are the parental lines and their genetic distance; below is the derived line with its genetic distance to each progenitor given on the respective sides of the triangle

Gill 1989; Liu et al. 1990) and represents only a small proportion of the genetic variability present in the tribe Triticeae. Lubbers et al. (1991) found 80% polymorphic loci among 102 *Triticum tauschii* accessions and Monte et al. (1993) detected 2,647 DNA fragments among 16 Triticeae species with 63 probe-enzyme combinations; 97% of these bands were polymorphic.

The frequency of RFLPs in the B genome was 1.6 and 2.8 times greater than in the A and D genomes, respectively. This is in agreement with the results of Chao et al. (1989), who assayed the wheat homoeologous group 7 chromosomes with 18 RFLP probes and found chromosome 7B to be approximately three times more variable than chromosomes 7A and 7D. Liu and Tsunewaki (1991) detected twice the number of RFLPs in the A and B genomes compared to the D genome. A high degree of variation in the B genome compared to the D genome was also reported by Chen et al. (1988) using N-banding patterns of the wheat chromosomes. They speculated that the degree of genome differentiation may be related to the extent of heterochromatization or else may reflect the sequence of involvement of the three genomes during wheat evolution.

Each line could be distinguished from all other lines due to its unique RFLP profile. However, lines Osmut and FAP65214 differed in just one RFLP locus, implying that closer related lines may not be distinguishable by 58 RFLP probes. Therefore, RFLP assays with single-copy clones, as performed in our study, would be too elaborate for cultivar identification and protection in wheat and spelt. A higher level of polymorphism could be detected using specific clones in combination with frequently cutting restriction enzymes (Vaccino et al. 1993), repetitive DNA clones (Metzlaff et al. 1986; Liu et al. 1992), or RAPD markers (He et al. 1992; Vierling and Nguyen 1992).

The low degree of polymorphism for RFLPs and the large genome size of wheat represent a major obstacle for the genetic mapping of monogenic or polygenic inherited traits. Considering an estimated map size in wheat of 6,300 cM (Liu and Tsunewaki 1991), at least 210 polymorphic RFLP loci are needed to obtain a marker density of 30 cM. The most divergent winter wheat lines in the present study differed in 15% of the RFLP loci, i.e., in the best case, about 500 probes have to be screened for polymorphism before constructing a linkage map. Therefore, the level of polymorphism, as well as the traits of interest, have to be considered for the optimal choice of parents (Anderson et al. 1993).

Pedigree analysis based on RFLP data

Pedigree records can be verified by comparing the RFLP profile of a cultivar with its proposed parents. GD estimates of the derived lines FAP75517 and Bernina given in Fig. 4 fit very well with the expectation that a derived line obtains half of its genome from each parent. Merlin seems to have inherited a larger proportion of the genome from HeineVII than from Derenburger Silber possibly due to selection or genetic drift during line development. In contrast, the lines Caribo and Zenith had RFLP bands that were not present in the parents. This could be explained by (1) experimental errors of RFLP analysis, (2) erroneous pedigree records, or (3) remnant heterozygosity of the plants used for the respective crosses.

In most instances, lines with common ancestors revealed below-average GD estimates and grouped together (e.g., the Caribo-related lines and the HeineVII-related lines) in the cluster analysis (Fig. 3). Hence, it can be assumed that pairs of lines with unknown genetic background but small GD values (< 0.05) are related by pedigree. For example, line Apollo with an unknown pedigree shows a close relationship to Cappelle (GD=0.03) and to Caribo (GD=0.04). Line Kormoran with unclear pedigree records (Cappelle, Carstacht, Merlin) (Hoeser et al. 1975) showed a greater similarity to its progenitor Merlin (GD=0.02) than to the other two progenitors Cappelle (GD=0.07) and Carstacht (GD=0.08). Thus, Merlin seems to be a parent of Kormoran, while the other two lines are more likely grandparents.

Characterization of the European wheat and spelt breeding material

Monitoring of the level of genetic diversity in breeding material is essential to avoid genetic erosion and to ensure long-term selection progress. RFLP markers are a powerful tool for the estimation of genetic distances because differences among genotypes are detected directly at the DNA level. However, numerical values of GD estimates depend on the specific sample of probe-enzyme combinations used for RFLP analysis. The precision of GD estimates improves with the number of informative markers and their uniform distribution across the wheat genome. Due to the low level of variation for RFLPs in wheat and spelt, standard error estimates of individual GD values (0.01–0.03) were relatively large compared to the variation in GD (0.01–0.21). Therefore, 58 probes seem to be the minimum number for RFLP studies in wheat.

In this study emphasis was placed on agronomically important cultivars and breeding lines of the European winter wheat germplasm and their main progenitors. To evaluate the genetic diversity among winter wheat in relation to other breeding groups that could be used for introgression, spring wheat and spelt lines were also included in the analysis. Based on available pedigree information, 27, i.e., 60% of the winter wheat lines in this study were related to eight cultivars (Bezostaja, Cappelle, Carstacht, Derenburger Silber, HeineVII, Hoeser52, Probus, and Zenith) released between 1941 and 1969. These predominant ancestors are of diverse geographic origin (e.g., Bezostaja is a Russian line of unknown pedigree, Derenburger Silber derived from a complex cross of English squarehead, Swedish land variety, and US American spring wheat) and accounted for 93% of the observed RFLP bands in winter wheat. Ten RFLP bands occurred only in one of the 52 winter wheat lines: three of these bands were contributed by Probus (Trubilo×Plantahof), two by VPM (introgression of *Ae. ventricosa* genome) and two by Roazon (VPM×Moisson). Lines that originated from France and from Germany formed distinct groups in the cluster analysis, whereas lines from Switzerland were spread across the whole cluster (Fig. 3) indicating a greater genetic diversity within the Swiss breeding material. This was supported by groupings of the principal coordinate analysis performed on GD estimates of the 52 winter wheat lines (data not shown), where the French lines were separated from the German lines by the second principal coordinate while the Swiss lines were widely distributed. Bezostaja-related lines were divergent from all the other winter wheat lines. This was also true for the Swiss line FAP62420.

Despite the small sample of spring wheat lines, almost the same number of different RFLP fragments (235) were detected as among the winter wheat lines (238). Unique bands among the 81 lines were contributed by FAP94779 (a complex cross of lines of Brasilian, Argentinian, Swedish, and German origins), Chinese Spring, and Thatcher. Moreover, the mean of GD values within spring wheat was greater than within winter wheat. These are indications that the genetic diversity within the spring germplasm is greater than within the winter wheat germplasm perhaps, due, to the wide crosses employed for the introgression of resistance genes. Although the spring lines were genetically diverse to each other they grouped together in the principal coordinate analysis (Fig. 2) reflecting their distinctiveness to the winter wheat and spelt germplasm. However, screening of a larger set of spring wheat lines representative for the European germplasm is necessary to support or reject this hypothesis.

A greater amount of genetic diversity was found between the European spelt and winter wheat material. The spelt lines were clearly separated from the wheat lines by PC1 (Fig. 2) and large differences in RFLP band frequencies (≥0.5) between winter wheat and spelt lines were observed for 17 of the 73 polymorphic RFLP loci, especially for Xpsr596 and Xpsr303-7A. Spelt was also the most diverse cultivar among eight hexaploid *Triticum* accessions analyzed for RFLPs by Liu et al. (1990). They detected 17.3% polymorphism between *T.spelta* var. *duhamelianum* and Chinese Spring with 271 probe-enzyme combinations, which is very close to the average of 15.5% polymorphism found between Chinese Spring and the European spelt lines in the present study. These findings contradict the theory that the difference between spelt and wheat can be explained by just a few genes including the *Q* gene (Mac Key 1954) located on chromosome 5A (Liu and Tsunewaki 1991).

Over a few decades, winter wheat germplasm has been introgressed into spelt breeding material to develop improved spelt cultivars. This breeding strategy accounts for the smaller mean GD estimates found between winter wheat and spelt (0.132) compared to spring wheat and spelt (0.148). The mean GD of winter wheat lines to the traditional spelt lines was 0.144. The contribution of wheat germplasm in the novel spelt breeding lines (FAP65273, FAP65246, FAP65251, FAP65289, FAP65291, FAP65237) is reflected in their intermediate position between wheat and the older spelt lines (Fig. 2). This illustrates the potential power of RFLP-based principal coordinate analysis for the identification of genetically diverse breeding groups and the classification of new lines to these groups.

In conclusion, RFLP data are useful for the characterization and grouping of elite breeding material of wheat. Closely related cultivars as well as very divergent germplasm sources can be identified. Thus, RFLP data can assist in selecting appropriate parents for the development of improved cultivars and may help to maintain, or even broaden, the genetic diversity in breeding material.

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References

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME (1993) Optimizing parental selection for genetic linkage maps. Genome 36:181–186

Boppenmaier J, Melchinger AE, Brunklaus-Jung E, Geiger HH, Herrmann RG (1992) Genetic diversity for RFLPs in European maize inbreds. I. Relation to performance of flint×dent crosses for forage traits. Crop Sci 32:895–902

Botstein D, White RL, Skolnik M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymporphisms. Am J Hum Genet 32:314–331

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic map of wheat homoeologous group 7 chromosomes. Theor Appl Genet 78:495–504

Chen PD, Liu DJ, Pei GZ, Qi LL, Huang L (1988) The chromosome constitution of three endemic hexaploid wheats in western China. In: Miller TE, Koebner RMD (eds) 7th Int Wheat Symp. IPSR, Cambridge, England, pp 75–80

Devos KM, Gale MD (1992) The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84:567–572

Dweikat I, Mackenzie S, Levy M, Ohm H (1993) Pedigree assessment using RAPD-DGGE in cereal crop species. Theor Appl Genet 85:497–505

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Gale MD, Chao S, Sharp PJ (1990) RFLP mapping in wheat – progress and problems. In: Gustafson JP (ed) Gene manipulation in plant improvement II. Plenum Press, New York, pp 353–363

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34:362–374

Gower JC (1972) Measures of taxonomic distance and their analysis. In: Weiner JS, Huizinga J (eds) The assessment of population affinities in man. Clarendon Press, Oxford, pp 1–24

Graner A, Siedler H, Jahoor A, Herrmann RG, Wenzel G (1990) Assessment of the degree and the type of restriction fragment length polymorphism in barley (*Hordeum vulgare*). Theor Appl Genet 80:826–832

Hart GE, Gale MD, McIntosh RA (1993) Linkage maps of *Triticum aestivum* (hexaploid wheat, 2n=42, genomes A, B and D) and *T. tauschii* (2n=14, genome D). In: Hoisington D, McNab A (eds) Progress in genome mapping of wheat and related species. Proc 3rd Public Workshop Int Triticeae Mapping Initiative. El Batan, Mexico, DF: CIMMYT, pp 32–46

He S, Ohm H, Mackenzie S (1992) Detection of DNA sequence polymorphisms among wheat varieties. Theor Appl Genet 84:573–578

Hoeser K, Wenisch K, Oppitz K (1975) Die Entwicklung der wichtigsten deutschen Winterweizen-Marktsorten und ihr genetischer Hintergrund. Berichte über die Arbeitstagung der Arbeitsgemeinschaft der Saatzuchtleiter, Gumpenstein, pp 31–49

Kam-Morgan LNW, Gill BS (1989) DNA restriction fragment length polymorphisms: a strategy for genetic mapping of the D genome of wheat. Genome 32:724–732

Liu YG, Mori N, Tsunewaki K (1990) Restriction fragment length polymorphism (RFLP) analysis in wheat. I. Genomic DNA library construction and RFLP analysis in common wheat. Jpn J Genet 65:367–380

Liu YG, Tsunewaki K (1991) Restriction fragment length polymorphism (RFLP) analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. Jpn J Genet 66:617–633

Liu YG, Ikeda TM, Tsunewaki K (1992) Moderately repeated, dispersed, and highly variable (MRDHV) genomic sequences of common wheat usable for cultivar identification. Theor Appl Genet 84:535–543

Lubbers EL, Gill KS, Cox TS, Gill BS (1991) Variation of molecular markers among geographically diverse accessions of *Triticum tauschii*. Genome 34:354–361

Lupton FGH (1987) History of wheat breeding. In: Lupton FGH (ed) Wheat breeding. Chapman and Hall Ltd., London, New York, pp 51–70

Mac Key J (1954) Neutron and X-ray experiments in wheat and a revision of the speltoid problem. Hereditas 40:65–180

Metzlaff M, Troebner W, Baldauf F, Schlegel R, Cullum J (1986) Wheat specific repetitive DNA sequences – construction and characterization of four different genomic clones. Theor Appl Genet 72:207–210

Monte JV, McIntyre CL, Gustafson JP (1993) Analysis of phylogenetic relationships in the Triticeae tribe using RFLPs. Theor Appl Genet 86:649–655

Odenbach W (1985) 1.1.7. Abstammungslinien in der deutschen Winterweizenzüchtung. In: Hoffmann W, Mudra A, Plarre W (eds) Lehrbuch der Züchtung landwirtschaftlicher Kulturpflanzen. Band 2, 2nd edn., Paul Parey, Berlin Hamburg, pp 64–67

Rohlf FJ (1989) NTSYS-pc numerical taxonomy and multivariate analysis system. Version 1.5. Exeter Publishing Ltd., Setauket, New York

SAS Institute (1988) SAS procedure guide for personal computers. Release 6.03. SAS Institute, Cary, North Carolina

Schmidt T, Boblenz K, Metzlaff M, Kaemmer D, Weising K, Kahl G (1993) DNA fingerprinting in sugar beet (*Beta vulgaris*) – identification of double-haploid breeding lines. Theor Appl Genet 85:653–657

Sneath PHA, Sokal RR (1973) Numerical taxonomy. WH Freeman and Co., San Fransisco

Vaccino P, Accerbi M, Corbellini M (1993) Cultivar identification in *T. aestivum* using highly polymorphic RFLP probes. Theor Appl Genet 86:833–836

Vierling RA, Nguyen HT (1992) Use of RAPD markers to determine the genetic diversity of diploid wheat genotypes. Theor Appl Genet 84:835–838

Weining S, Langridge P (1991) Identification and mapping of polymorphisms in cereals based on the polymerase chain reaction. Theor Appl Genet 82:209–216

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Winzeler H, Rüegger A (1990) Dinkel: Renaissance einer alten Getreideart. Landwirtschaft Schweiz 3:503–511

Winzeler H, Schmid JE, Winzeler M, Rüegger A (1991) Neue Aspekte der Dinkelzüchtung (*Triticum spelta* L.) in der Schweiz. 2. Hohenheimer Dinkelkolloquium, Universität Hohenheim, pp 11–25

Zeven AC, Zeven-Hissink NC (1976) Genealogies of 14,000 wheat varieties. Issued by: The Netherlands Cereals Centre-NGC, Wageningen and The International Maize and Wheat Improvement Center-CIMMYT, Mexico