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Genetic diversity in Australian wheat varieties and breeding material based on RFLP data

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Abstract Restriction fragment length polymorphisms (RFLPs) have been used to characterise the genetic diversity of wheat (*Triticum aestivum*) germplasm. One hundred and twenty-four accessions comprising all major Australian wheat varieties and lines important for breeding purposes were assayed for RFLPs with clones of known genetic location and selected to give uniform genome coverage. The objectives of this study were to determine RFLP-based genetic similarity between accessions and to derive associations between agronomically significant traits and RFLP phenotypes. Ninety-eight probes screened against genomic DNA digested with five restriction endonucleases detected a total of 1968 polymorphic fragments. Genetic similarity (GS) calculated from the RFLP data ranged from 0.004 to 0.409 between accessions, with a mean of 0.18. Cluster analysis based on GS estimates produced four groupings that were generally consistent with available pedigree information. Comparisons of the RFLP phenotypes of accessions containing disease resistance genes present on introgressed alien segments enabled the identification of specific alleles characteristic of these regions. Associations were derived for a range of stem-rust, leaf-rust and yellow-rust resistance genes. These results suggest that RFLP analysis can be used for the characterisation and grouping of elite breeding material of wheat and RFLP profiling can identify chromosome segments associated with agronomic traits.

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Introduction

Genetic distance within crop species provides a measure of the average genetic divergence between cultivars. Relationships derived from agronomic traits have been shown to be useful for the analysis of variability (Smith 1984), the selection of parents for hybrid construction (Frei et al. 1986) and for the prediction of progeny performance (Grafius 1956). Genetic distance estimates derived from quantitative agronomic characters have also been used in the selection of superior parental combinations (Whitehouse 1969) and to predict the specific combining ability in F_1 progeny of spring wheat (Shamsuddin 1985). Genetic similarity between genotypes can be estimated indirectly by the coefficient of co-ancestry (Malécot 1948) based on pedigree information. Alternatively, genetic similarity can be obtained directly by measuring resemblance for biochemical or molecular markers (Smith et al. 1991).

Molecular markers offer an easily quantifiable measure of genetic variation within crop species. Of the molecular markers, Restriction Fragment Length Polymorphisms (RFLPs) are the most widely used due to their reliability and reproducibility, and they have been used both to derive genetic relationships and construct genetic linkage maps in a range of species. These studies have provided important information on genome organisation and evolution (Song et al. 1991; Slocum et al. 1990) and have enabled the location of loci controlling important agronomic traits (Kretschmer et al. 1997; Williams et al. 1996).

Wheat, however, displays a low level of polymorphism (Chao et al. 1989; Lui et al. 1990), thereby hampering the identification of RFLP markers linked to

agronomically important traits as well as the differentiation of varieties and the analysis of genetic variability. Polymerase chain reaction (PCR)-based marker systems (Saiki et al. 1988) have been proposed to overcome the problem of low levels of polymorphism. In particular, Randomly Amplified Polymorphic DNAs (RAPDs) (Williams et al. 1990) and microsatellites have been used with some success in cereals (Röder et al. 1995; Saghai Maroof et al. 1994). However, RAPDs were found to be ill-suited for use in wheat as a given primer can amplify a number of non-homologous sequences that may vary between wheat varieties (Devos and Gale 1992). Alternatively, microsatellites demonstrate a high level of polymorphism but have proved expensive to develop and have found only limited application to date (Plaschke et al. 1995).

While the low level of RFLP polymorphism may be problematic, it is possible that the level of genome conservation observed between varieties may offer an opportunity to identify markers associated with traits of interest. The rationale of the approach is based on 'linkage drag' (Hanson 1959; Brinkman and Frey 1977; Stam and Zeven 1981), a feature of chromosome behaviour whereby flanking DNA surrounding the target gene diminishes at a much slower rate than unlinked regions. Since varieties are so closely related, differences between related accessions may reflect differences in important agronomic traits. A comparison of the RFLP profile of accessions demonstrating a particular trait with those lacking the trait should facilitate the identification of linked markers. Loci linked to the trait of interest will show the same RFLP phenotype within each group, while unlinked loci will show a random distribution of RFLP alleles. This approach is analogous to bulk segregant analysis (Michelmore et al. 1991), but it is necessary to confirm the association between the gene of interest and the candidate RFLP markers using a segregating population.

Several studies have examined genetic variation within hexaploid wheat using morphological and molecular markers, however, no systematic survey of the genetic relationships among a large sampling of Australian cultivars using RFLPs has been reported. In the study presented here, we demonstrate the application of wheat RFLPs for the differentiation and estimation of genetic relationships among 124 wheat accessions that have been widely grown in Australia or have played a major role as progenitors of modern varieties. Further, we compare the data generated for usefulness in identifying markers associated with particular agronomic traits.

Materials and methods

Plant material

One hundred and twenty-four wheat cultivars and breeding lines were analysed (Table 1). Cultivars were selected on the basis of (1) being of historical significance to wheat breeding and production in Australia, (2) each accounting for over 2% of deliveries to silos in any Australian state since 1982, (3) recently released cultivars carrying traits of particular interest and (4) significant parents in current breeding programmes.

RFLPs

DNA clones were obtained through the Australian Triticeae Mapping Initiative (Table 2). DNA extraction, restriction digestion and Southern blotting and hybridisation were carried out as described by Guidet et al. (1991). Total genomic DNA was digested with *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV and *Hind*III. DNA membranes of the 124 wheat lines were screened with RFLP probes and the results analysed.

Data analysis

Genetic distances were calculated between pairs of accessions based on the method of Nei and Li (1979). Genetic distance is defined as the extent of genetic difference between cultivars, as measured by allele frequencies at a sample of loci (Nei 1987). Genetic similarity is defined as the converse of genetic distance; that is, the extent of similarity among cultivars. The distances used in this study were based on RFLP fragments scored in each of the cultivars. Because each of the probes used was believed to identify a distinct genetic locus, each RFLP fragment detected by Southern analysis was treated as a unique character. RFLP fragments detected by all probes for each accession were used to make comparisons with all of the other accessions. All fragments of the same size for a particular probe and enzyme combination were assumed to identify the same allele. The measure of distance or similarity among cultivars was the covariance of allele frequencies summed for all fragments scored. Parsimony analysis of RFLP data was carried out using PAUP (version 3.1.1), a Macintosh computer software package developed by Swofford (1993). The heuristic option of the PAUP programme using stepwise addition was used to generate phylogenetic trees.

Results

The 124 wheat accessions were evaluated using 119 probes with five enzyme combinations. These probes were selected on the basis of their known genetic location to give a uniform genome coverage. Of the probes 98 were found to be polymorphic, and a total of 1968 polymorphic fragments were scored, ranging from 0 to 14 polymorphisms per probe/enzyme combination. Most of the probes hybridised to 3 independent loci located on each of the three homeologous genomes (data not shown). Each accession was uniquely identified by the marker data, although no single probe discriminated among all accessions.

Genetic Distance (GD) based on all possible pairs of lines ranged from 0.004 to 0.409, with a standard error of 5×10^{-4}. The mean GD was 0.18, i.e. 2 randomly chosen lines differed on average in 354 of the 1968 examined loci. The least pairwise GD was between 2 accessions (AUS 2315 and AUS 885) of the nineteenth century European landrace Purple Straw (GD 4×10^{-3}) which differed at 8 loci, while the 2 cultivars

Table 1 Details of accessions tested, including source of seed and pedigree

Accession	Source[a]	Pedigree
3Ag # 14/5*Condor	PBI, U of S	3Ag # 14/5*Condor
3Ag # 3/4*Condor	PBI, U of S	3Ag # 3/4*Condor
Ag-Amigo/4*CSP44	PBI, U of S	Ag-Amigo/4*CSP44
Aroona	AUS 20992	WW15/Raven
Avocet	AUS 20601	WW119/WW15//Egret
Avocet R	PBI, U of S	seln from Avocet, YrA differential
Banks	AUS 20599	Thatcher/Ag.el//4*Heron(PWTH)/3/Cndr sib/4/2*Cndr
Barunga	AUS 25602	Halberd/Aroona//3*Schomburgk/3/2*Molineux
Bass	AUS 21960	Flinders sib/2*Cook
Batavia	QWRI	Brochis "S"/Banks
Bencubbin	AUS 1924	Gluyas Early/Nabawa
Bindawarra	AUS 20621	Mexico120/Koda//Raven
Bungulla	AUS 77	Bencubbin selection
Chinese Spring	Waite	
Cocamba	AUS 22605	AUS10894/4*Condor
Combination III	AUS 15358	Vernstein/CI12632
Condor	AUS 16036	WW80/2*WW15
Cook	AUS 20275	Timgalen/Condor sib//Condor
Corella	AUS 22655	Complex cross based on Egret
CS(Hope3B)	PBI, U of S	Intervarietal substitution line
CSP44	PBI, U of S	Condor selection plant 44
Cunningham	QWRI	3Ag # 3/4*Condor//Cook
Currawa	AUS 2229	Northern Champion/Cretan/Little Club
Dagger	AUS 22255	Sabre/Mec3//Insignia
Diaz	AUS 23326	CombIII/3*Oxley/3*Cook
Dirk	AUS 3662	Ford/Dundee
Dollarbird	AUS 23824	Wren/Gaboto//Kalyansona/Bluebird
Du Toits	AUS 2310	19th century introduction
Dundee	AUS 2300	Hard Federation/Cleveland/2/Sands
Early Purple Straw	AUS 183	19th century landrace from Tuscany
Early Purple Straw	AUS 2315	19th century landrace from Tuscany
Egret	AUS 16037	Heron/2*WW15
Egret FDN seln	PBI, U of S	seln from Egret homogeneous for YrA
Eradu	AUS 21110	Ciano/Gamenya
Excalibur	AUS 25292	RAC177/Uniculm 492//RAC311S
Falcon	AUS 206	Gular//Dundee/Gular/3/Bencubbin
Federation	AUS 218	Purple Straw/Yandilla
Festiguay	AUS 6113	Webster/Uruguay C10837
Flinders	AUS 99077	(PWTH)/Cndr sib//2*Cndr
Florence	AUS 10411	White Naples*2/Fife//Fife/Eden
Ford	AUS 3591	Fan/Comeback//Zealand/Tardent's Blue
Frame	AUS 25601	Molineux/3*Dagger
Free Gallipoli	AUS 2441	Club wheat/Yandilla King
Gabo	AUS 246	Bobin*2/Gaza (possibly)
Gamenya	AUS 256	Gabo/3/Gabo*5/Mentana//Gabo*2/Kenya117A
Gatcher	AUS 11621	Thatcher/SantaCatalina//Mayo48/3/Gabo*3/Charter
Ghurka	AUS 2494	Gallipoli/3/Currawa//Indian4E/Federation
Glenwari	AUS 279	Nabawa//Riverina/Hope
Gluyas Early	AUS 172	Wards Prolific selection
Grebe	AUS 23352	Skorospelka/3*Egret
Gutha	AUS 99084	Gamenya//Gabo*3/Khapstein/3/Falcon*3/Chile1B
Halberd	AUS 11612	Scimitar/KenyaC6042//Bobin/3/Insignia49
Harrier	AUS 99062	Norin10/Brevor(seln14)//Kite sib/3/Kite
Hartog	AUS 21533	Vicam71//Ciano'S'/SieteCer/3/Kalyansona/Bluebird
Heron	AUS 322	Doubbi/2*Ranee/2/Insignia/3/Insignia 49
Hudsons Early Pple Str	AUS 2565	19th century landrace from Tuscany
Insignia	AUS 2642	Ghurka/Ranee
Janz	AUS 24794	3Ag # 3/4*Condor//Cook
Kewell	AUS 99027	Peanut Oil Olympic Mutant132A/South African184
Kite	AUS 16035	N10/Br//4*Eureka2/3/T-A/3*Flcn/4/T-A/4*Flcn/5/T-A/5*Flcn
Koda	AUS 6116	Dundee/Kenya745//2*Bobin39/3/Gaza
Kulin	AUS 23163	Bodallin//Gamenya/Inia
Machete	AUS 23038	Mec-3/2*Gabo(RAC177)//Madden
Madden	AUS 16170	Gamenya//Gabo*3/Khapstein
Matong	AUS 21821	Kalyansona/Olympic
MeA4	Roseworthy	Pitic 'S' (Norin10/Brevor14//Yaktana54)

Table 1 Continued

Accession	Source[a]	Pedigree
Mec3	Roseworthy	Sonora 64 (Yaktana54//Norin10/Brevor/3/Yaqui54)
Meering	AUS 22606	Condor selection
Mentana	AUS 2997	Rieti/3/Rote Dikkop/Zeeuwse Witte//Squarehead/4/Akagomughi
Mexico120	AUS 546	Yaktana52//Norin 10/Brevor14
Millewa	AUS 20597	Sonora64/Yaqui50E//Gaboto/Mexico8156
Molineux	AUS 24457	Pitic62/Festiguay//2*Warigal
Nabawa	AUS 673	Gluyas Early/Bunyip
Norin 10	Roseworthy	Daruma/Fultz//Turkey Red
Norin10/Brevor	Roseworthy	Norin10/Brevor seln14
Olympic	AUS 3117	Baldmin/Quadrat
Osprey	AUS 22110	Cndr*2/WW33B = Thatcher/Ag.el/4*Hrn/2*(7165) WW80/WW15
Oxley	AUS 16461	Pen62/4*Gabo56//TPPN (WW80)/2*WW15
Pelsart	QWRI	Potam 70/4*Cook
Persia20	AUS 5206	Landrace
Phoenix	AUS 21907	WW15*2/WW80
Pinnacle	AUS 3168	Pindar selection (Pindar = Ghurka selection)
Pitic 62	AUS 804	Norin10/Brevor14//Yaktana54
Potam 70	AUS 22698	Lerma Rojo64/Sonora64//Napo63
Purple Straw	AUS 885	19th century landrace from Tuscany
Purple Straw	AUS 892	19th century landrace from Tuscany
Quadrat	AUS 3213	Major/Gallipoli/2/Currawa/3/Ghurka
RAC177	Roseworthy	Sonora64/TZPP//Yaqui54/3/2*Gabo
RAC416-1	Roseworthy	Condor/Petit Rojo
Ranee	AUS 1001	IndianF/Federation
Raven	AUS 1009	Dirk48/Orfed//Uruguay1064
Reeves	AUS 24851	Bodallin//Gamenya/Inia66
Rosella	AUS 23165	Farrolunga/Heron//2*Condor/3/Quarrion sib
Rowan	QWRI	Jarral66/Gamut//4*Hartog
Rye-Amigo/4*Condor	PBI, U of S	Rye-Amigo/4*Condor
Schomburgk	AUS 23325	W3589/Oxley//2*Warigal/3/2*Aroona
Shortim	AUS 20276	Sonora64/P41603//Timgalen sib*3
Siete Cerros	AUS 1214	Penjamo62 sib/Gabo55
Skorospelka	Roseworthy	Kanred/Fulcaster//Klein33
Songlen	AUS 17904	Lerma Rojo64/Sonora64A//Timgalen
Spear	AUS 22254	Sabre/Mec3//Insignia
Spica	AUS 1303	Three Seas/Kamburica//Pusa4/Flora
Spring Wheat	AUS 10894	Afghanistan landrace
Stiletto	AUS 25923	Veranapolis/3*RAC177//3*Spear/3/Dagger
Sunco	AUS 23455	SUN9E-27*4/3Ag#14//WW15/3/3*Cook
Suneca	AUS 21820	Ciano//Spica/Amber Mutant Sonora64
Sunkota	AUS 21113	Timson/IRN 67–451
Sunstar	AUS 22177	Condor/4/WW15/3/W199/WC356/2/LaPrevision
Tatiara	AUS 24317	Mexico120/Koda//Raven/3/Mengavi/4/Siete Cer
Tincurrin	AUS 20578	Gluclub/3/Chile1B//Insignia/Falcon
Trident	AUS 25924	VPM1/5*Cook//4*Spear
Vasco	AUS 23053	3Ag#14/4*Condor//5*Oxley
Veranapolis	AUS 1553	Trintecinco/B2017-37
Virest	AUS 11984	EST39-12/Virgilio
VPM/6*Cook	PBI, U of S	VPM1/6*Cook
Vulcan	AUS 23039	Condor/Pitic62//Condor sib
W3589	PBI, U of S	T. boeoticum/2*Spelmar//2*Steinwedel
Waratah	AUS 1613	Hudsons Early Purple Straw/Gluyas Early
Ward's Prolific	AUS 1630	DuToit selection
Warigal	AUS 20593	WW15/Raven
WW15	AUS 6919	Lerma Rojo//Norin 10/Brevor14/3/3*Andes
WW80	AUS 6921	Penjamo 64/4*Gabo56//TZPP/Nainari
Wyuna	AUS 22607	DX3-134/Olympic
Yarralinka	AUS 24350	Mengavi/SieteCer/3/Mengavi/SieteCer//Crim/4/CombIII/2*Wgl

[a] Source of seed tested in this experiment. AUS, Australian Winter Cereals Collection accession number; PBI, U of S, Plant Breeding Institute, University of Sydney; QWRI, Queensland Wheat Research Institute, Toowoomba; Roseworthy, Roseworthy Campus Wheat Breeding Programme, University of Adelaide; Waite, Waite Campus Wheat Breeding Programme, University of Adelaide

Table 2 Nomenclature, chromosomal location and source of RFLP probes

Probe	Chromosomal location	Source
ABC 154	1AS,1BS	Kleinhofs A
ABC 157	2L	
ABC 162	2	
ABC 309	7L	
ABG 373	5L	Kleinhofs A
ABG 453	3L	
AWBMA 1	5H	Langridge P
AWBMA 2	Unassigned	
AWBMA 3	5H	
AWBMA 6	Unassigned	
AWBMA 7	Unassigned	
AWBMA 14	2H	
AWBMA 19	6H	
AWBMA 21	2HL	
AWBMA 24	1H	
AWBMA 28	2H	
AWBMA 29	4H	
AWRM 1	1H	Langridge P
BCD 1	6L	Sorrels ME
BCD 111	2L	
BCD 127	3S	
BCD 129	7HS	
BCD 175	2HS	
BCD 207	1L	
BCD 240	2HL	
BCD 266	2HL	
BCD 269	6L/S	
BCD 276	6L	
BCD 292	2L	
BCD 304	1HL	
BCD 342	6S	
BCD 454	1L	
BCD 758	6L	
BCD 828	3L	
BCD 1130	5S	
BCD 1652	4L	
CDO 36	7HS	Sorrels ME
CDO 63	4HL	
CDO 64	2HS	
CDO 347	7HL	
CDO 366	2HL	
CDO 370	2HS/L	
CDO 373	2HL	
CDO 393	1HL	
CDO 395	3S	
CDO 464	1HS	
CDO 475	7S	
CDO 506	5HL	
CDO 537	2H	
CDO 541	4L	
CDO 580	1S	
CDO 595	7S	
CDO 673	1L/S	
CDO 680	2HL	
CDO 689	7HS	
CDO 795	4HL	
CDO 1174	3L	
XKSU B008	3S	Gill BS
XKSU E018	1DS	
XKSU G009	1DS	
XKSU G10	4D	
PSR 65	7AS, 7BS, 7DS	Gale MD
PSR 113	6AS, BS, 6DS	
PSR 119	4AL, 7AS, 7DS	
PSR 123	3S	
PSR 126	2AS, 2BS, 2DS	
PSR 128	5AL, 5BL, 5DL	
PSR 129	7AL, 7BL, 7DL	
PSR 131	2AS, 2BS, 2DS	
PSR 144	4AL, 4BS, 4DS	
PSR 149	6AL, 6BL, 6DL	
PSR 152	7AS, 7BS, 7DS	
PSR 154	6AL, 6BL, 6DL	
PSR 156	3AL, 3BL, 3DL	
PSR 158	1AL, 1BL, 1DL	
PSR 161	1AS, 1BS, 1DS	
PSR 162	1AL, 1BL, 1DL	
PSR 163	4AS, 4BL, 4DL	
PSR 167	6S, 6HS,	
PSR 311	7AL, 7BL, 7DL,	
PSR 326	5AS, 5BS, 5DS,	
PSR 381	1S	
PSR 544	1AL, 1BL, 1DL	
PSR 574	5AL, 5BL, 5DL	
PSR 578	3AL, 3BL, 3DL	
PSR 596	1AS, 1BS, 1DS	
PSR 598	3AS, 3BS, 3DS	
PSR 601	1AL, 1BL, 1DL	
PSR 609	2AL, 2BL, 2DL	
PSR 611	2L	
PSR 628	5AS, 5BS, 5DS	
PSR 666	2AS, 2BS, 2DS	
PSR 690	7AL, 7BL, 7DL	
PSR 754	3AL, 3BL, 3DL	
PSR 901	2AL, 2BL, 2DL	
PSR 902	3AS, 3BS, 3DS	
PSR 911	5AL, 5BL, 5DL	
PSR 915	6AL, 6BL, 6DL	
PSR 920	4AS, 4BL, 4DL	
PSR 928	2AS, 2DS, 7RL	
PSR 929	5AS, 5BS, 5DS	
PSR 931	3AL, 3BL, 3DL	
PSR 934	2AL, 2BL, 2DL	
PSR 949	1AS, 1BS, 1DS	
PSR 964	6BS, 6DS	
PSR 1051	4AS, 4BL, 4DL	
PSR 1196	3AS, 3BS, 3DS	
WG 110	3HL	Sorrels ME
WG 114	4BL, 4DL, 5AL	
WG 178	3HL	
WG 232	4HL	
WG 241	1HL	
WG 464	4HL	
WG 530	5HL	
WG 541	5HL	
WG 686	7HL	
WG 719	7HS	
WG 865		
WG 940	3HL	

'Insignia' and its back-cross derivative 'Heron' differed at only 14 loci (GD 7×10^{-3}). The greatest GD was between the cultivar 'Bass' and the breeding line W3589, which contains the stem rust resistance gene *Sr22*, derived from *T. boeoticum*. These lines differed at 757 polymorphic loci.

Cluster analysis of accessions based on genetic distance

Cluster analysis based on GD values provides the best estimates of relationships between close relatives and was used to reveal pedigree relatedness among accessions. The hierarchy clusters shown in Fig. 1 were in good agreement with the origin of these lines and their pedigree information as far as was known. The analysis placed the accessions into four main groups.

Group One consists almost exclusively of varieties and breeding lines with a high degree of pedigree relatedness. 'Gabo' and 'Gamenya', the 2 pivotal varieties in this group, were selected and released in New South Wales (NSW) in 1945 and 1960, respectively, and were widely grown throughout Australia with 'Gamenya' the dominant variety in Western Australia (WA) until 1985. The more recently released varieties in this group are products from the Roseworthy (South Australian) and WA breeding programmes and are cultivated almost exclusively in these two states.

All varieties in the second major group can be traced to either the landrace Purple Straw, believed to have originated from Tuscany and introduced to Australia in the 1860s (Wrigley and Rathjen 1981), or Du Toits, which was introduced from South Africa in 1881 (Macindoe and Walkden Brown 1968). The varieties 'Federation', 'Nabawa', 'Bencubbin', 'Insignia' and 'Halberd', in turn, dominated Australian wheat production from the beginning of the twentieth century until the release of semi-dwarf varieties in the 1970s and the introduction of stripe rust (*Puccinia striiformis*) in 1979. Recently released semi-dwarf varieties in this group are widely cultivated in South Australia and Western Australia but are not grown to any extent in other states. These semi-dwarf varieties carry the *Rht2* gene. Several distinct groupings occur within this major group, notably varieties released by the Roseworthy breeding program, including 'Halberd' and 'Spear', and a Victorian-derived cluster based on 'Currawa'. All varieties in both groups are tolerant to the high concentrations of boron which occur in the soils of large areas of these two states. The genetic source of this tolerance can be traced to 'Federation' (Paull 1990).

The third group actually comprised several minor groups and pairs of related lines that did not fall within the three other groups. In general, these minor groups were based on pedigree lines, for example the group based on 'Dundee' and 'Ford'. However, some of these varieties had a close pedigree relationship with varieties in Group One (e.g. 'Eradu'), Group Two (e.g. 'Matong' and 'Wyuna') and Group Four (e.g. 'Warigal', 'Molineux' and 'Barunga') but did not occur in the major groups due to major effect of the alternative parent. For example, 'Aroona' and 'Warigal' are sibs with the pedigree 'WW15/Raven', but 'Aroona' occurred within the WW15 group and 'Warigal' grouped with 'Raven'. A number of introductions carrying the *Rht* genes feature in the pedigrees of semi-dwarf varieties in these minor groups, and both *Rht1* and *Rht2* varieties are represented. The varieties in these groups originated from the five Australian states with wheat breeding programmes.

The fourth major group consists almost entirely of semi-dwarf varieties or breeding lines released since 1973 and derived from the CIMMYT line WW15. WW15 and most of the varieties in this group carry the *Rht1* gene (Gale and King 1988). The majority of varieties in this group were selected in New South Wales and Queensland (13 and 11 varieties each, respectively), with 2 each from South Australia and Victoria and none from Western Australia. As was the case for the other two major groups, this group could be subdivided, and varieties generally clustered according to pedigree; for example those derived from 'Cook'. Several breeding lines introduced from Mexico also occurred in this major group.

Association of disease resistance loci with marker alleles

Disease resistance genes from exotic germplasm and species related to *T. aestivum* have been back-crossed, often via bridging lines, into a number of Australian varieties. It was possible to identify RFLP markers associated with six segments of chromatin carrying disease-resistant genes by comparing the RFLP patterns of the breeding lines carrying the disease-resistant gene with the recurrent parent and the derived resistant variety/varieties (Table 3). This is illustrated for the *Sr38*, *Lr37*, *Yr17* gene complex derived from *Aegilops ventricosa*, located on chromosome 2AS of VPM1 (Bonhomme et al. 1995). The segment carrying these resistance genes was transferred to 'Cook' by five backcrosses. This line was then back-crossed to 'Spear' three times to produce the disease-resistant variety, 'Trident'. One RFLP identified with the probe BCD175, which maps to chromosomes of homoeologous group two (Heun et al. 1991), was unique to 'VPM/6*Cook' and 'Trident', indicating that the band in 'Trident' was derived from VPM and not 'Cook' or 'Spear' (Fig. 2). In view of the number of back-crosses involved in producing 'Trident', it is highly probable that the critical band is located in the chromatin carrying the three resistance genes, although linkage analysis is required to confirm this association.

Another important association was identified between an RFLP and a gene present in *T. aestivum* which confers resistance to Cereal Cyst Nematode (CCN) (*Heterodera avenae*). CCN is a destructive pest

Fig. 1 Dendrogram showing a cluster analysis of the 124 wheat accessions listed in Table I. A total of 1968 polymorphic fragments were used in the analysis

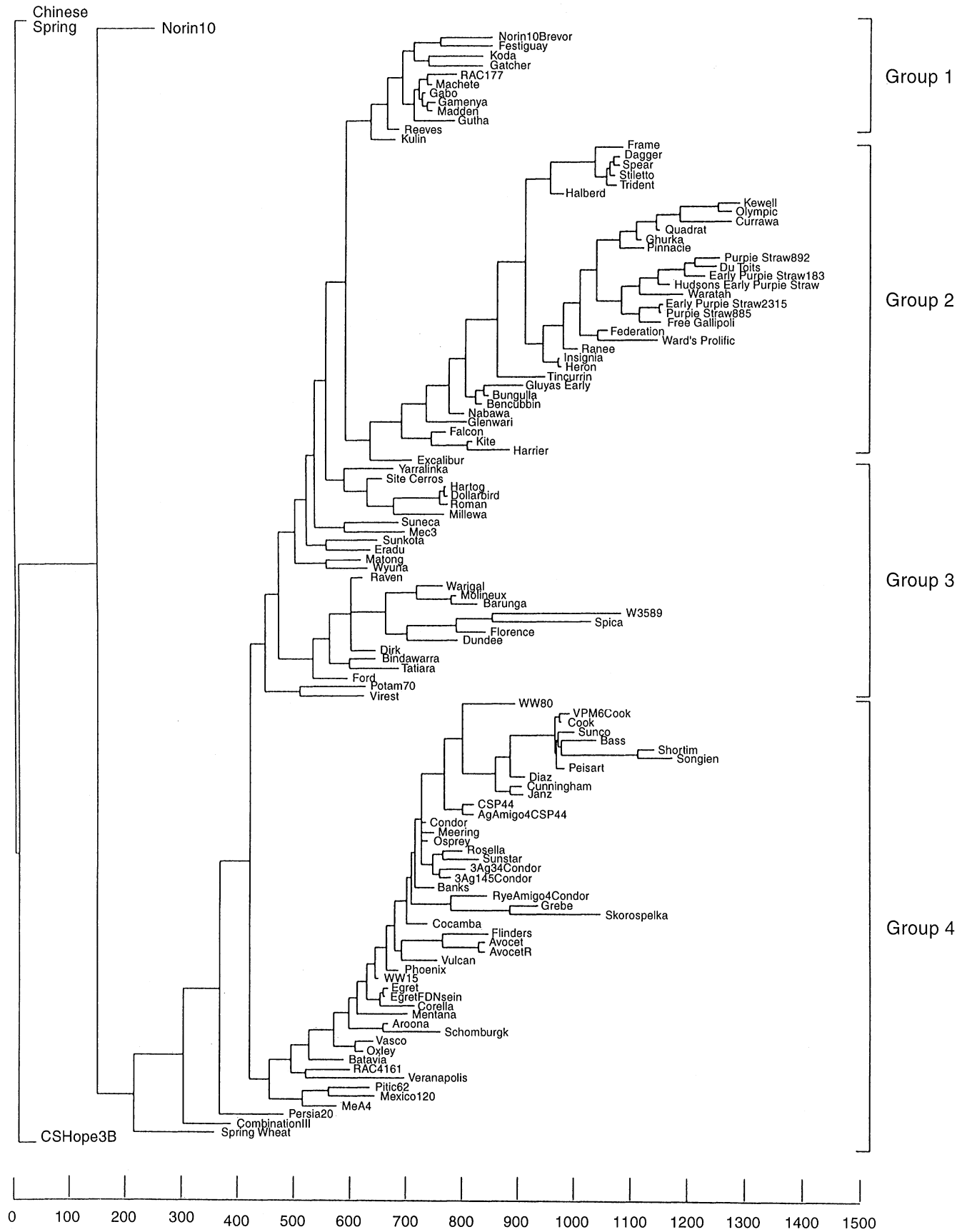
Chinese Spring
Norin10
Norin10Brevor
Festiguay
Koda
Gatcher
RAC177
Machete
Gabo
Gamenya
Madden
Gutha
Reeves
Kulin
Frame
Dagger
Spear
Stiletto
Trident
Halberd
Kewell
Olympic
Currawa
Quadrat
Ghurka
Pinnacle
Purpie Straw892
Du Toits
Early Purpie Straw183
Hudsons Early Purpie Straw
Waratah
Early Purpie Straw2315
Purpie Straw885
Free Gallipoli
Federation
Ward's Prolific
Ranee
Insignia
Heron
Tincurrin
Gluyas Early
Bungulla
Bencubbin
Nabawa
Glenwari
Falcon
Kite
Harrier
Excalibur
Yarralinka
Site Cerros
Hartog
Dollarbird
Roman
Millewa
Suneca
Mec3
Sunkota
Eradu
Matong
Wyuna
Raven
Warigal
Molineux
Barunga
W3589
Spica
Florence
Dundee
Dirk
Bindawarra
Tatiara
Ford
Potam70
Virest
WW80
VPM6Cook
Cook
Sunco
Bass
Shortim
Songlen
Peisart
Diaz
Cunningham
Janz
CSP44
AgAmigo4CSP44
Condor
Meering
Osprey
Rosella
Sunstar
3Ag34Condor
3Ag145Condor
Banks
RyeAmigo4Condor
Grebe
Skorospelka
Cocamba
Flinders
Avocet
AvocetR
Vulcan
Phoenix
WW15
Egret
EgretFDNsein
Corella
Mentana
Aroona
Schomburgk
Vasco
Oxley
Batavia
RAC4161
Veranapolis
Pitic62
Mexico120
MeA4
Persia20
CombinationIII
Spring Wheat
CSHope3B
Group 1
Group 2
Group 3
Group 4
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500

Table 3 Marker/trait associations based on pedigree

Resistant cultivar	Donor/ bridge-parent	Recurrent parent(s)	Locus	Chromosome	Probe
	T. boeoticum				
Schomburgk	W3589	Oxley Warigal Aroona	*Sr22*	7A	CDO464 CDO673 CDO689 PSR129 PSR152 PSR311 PSR65 PSR690 WG686 WG719
	A. elongatum				
Kite		Falcon	*Sr26*	6AL	ABC154
Harrier	Kite	Kite			BCD001
Bass	Flinders	Cook			BCD269
Flinders	PWTH	Condor			BCD276 BCD342 PSR149 PSR154 PSR915
	T. timopheevi				
Bass	Cook	Cook	*Sr36*	2BS	PSR131
Combination III	CI 12632				AWBMA14
Cook	Timgalen	Condor			
Diaz	Combination III, Cook	Cook			
Pelsart	Cook	Cook			
Shortim	Timgalen	Timgalen			
Songlen	Timgalen				
VPM/6*Cook	Cook	Cook			
Yarralinka	Combination III	Warigal			
	A. elongatum				
Cunningham	3Ag#3/4* Condor	Cook	*Sr24*, *Lr24*	6L	AWBMA19
Janz					PSR167
Sunco	3Ag#14/4* Condor	Cook			
Vasco		Oxley			
	Ae. ventricosa				
Trident	VPM/5* Cook	Spear	*Sr38*, *Lr37*, *Yr17*	2AS	PSR920 BCD175
	S. cereale				
Grebe	Skorospelka	Egret	*Sr31*, *Lr26*	1BL.1RS	PSR596 PSR949 CDO580 PSR161 KSUE18 CDO393 KSUG9
Molineux Barunga Frame	Festiguay	Warigal Aroona Schomburgk Dagger	*Cre3*	7HL	CDO347

of cereals in southern Australia. Resistance is available in wheat, rye, triticale, barley and oats, and the breeding of resistant cultivars is a priority in all breeding programmes. Two genetically independent sources of resistance have been used in wheat breeding in Australia, namely *Cre1* from AUS10894 and *Cre3* identified in the Australian cultivar 'Festiguay'. RFLP markers linked to *Cre1*, located on chromosome 2BS, were previously identified by Williams et al. (1996). However, the chromosomal location of *Cre3* is currently unknown. In this present study 'Festiguay' and 3 derived CCN-resistant cultivars ('Molineux', 'Barunga' and 'Frame') showed an allele that distinguished them from all other Australian cultivars when probed with

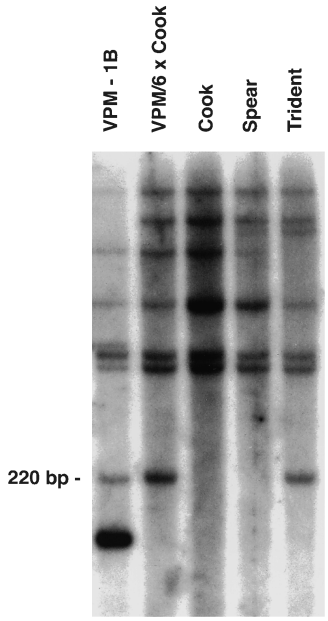

Fig. 2 RFLP profile of wheat accessions showing the association of a 220-bp fragment of BCD175 with a chromosomal segment derived from VPM

CDO347 (located to 7HL, Heun et al. 1991). The probability of this probe being linked to the *Cre3* gene is considered high because the derived resistant varieties were produced through backcrossing between 'Festiguay' and 3 different cultivars rather than all being sib-selections of back-crossing to a single cultivar. Again, as with the VPM locus, linkage analysis is required to confirm this association.

Parental contribution/pedigree tracing

The relative genetic contribution of parents to their progeny was calculated for varieties produced from either a single cross, or back-crossing, where both parents and the derived variety were included in the study (Table 4). The relative contributions were compared by chi-square analysis with those expected on the basis of polymorphisms shared between each parent and the progeny cultivar. For example, in the case of a single cross, it would be expected that if both parents contributed equally to the derived variety there would be the same number of RFLPs between each parent and the derived variety. This analysis was based on the assumptions that sufficient probes were tested to provide a near-complete coverage of the genome and that the RFLPs were randomly distributed over the genome. The greatest deviations from the expected values occurred for the varieties containing an alien segment, and in all cases there was less polymorphism between the

Table 4 Comparison of expected and observed contributions of parents to progeny varieties based on pedigree and RFLP information. The number of RFLPs observed refers to polymorphism between each parent and the progeny variety

Variety	Parents	Pedigree contribution	RFLPs		χ^2	*P*
			Observed	Expected		
Condor	WW80	1/4	188	218.75	16.9	***
	WW15	3/4	103	72.75		
Cocamba	AUS10894	1/16	360	423.75	153.5	***
	Condor	15/16	92	28.25		
Cunningham	3Ag#3/4* Condor	1/2	129	166	16.5	***
	Cook	1/2	203	166		
Janz	3Ag#3/4* Condor	1/2	145	170.5	7.6	**
	Cook	1/2	196	170.5		
Vasco	3Ag#14/4* Condor	1/32	159	213.1	439.9	***
	Oxley	31/32	61	6.9		
Bass	Flinders	1/4	310	306	0.2	ns
	Cook	3/4	98	102		
Pelsart	Potam 70	1/16	438	438.75	0.02	ns
	Cook	15/16	30	29.25		
Egret	Heron	1/4	313	283.5	12.3	***
	WW15	3/4	65	94.5		
Aroona	Raven	1/2	201	172.5	9.4	**
	WW15	1/2	144	172.5		
Warigal	Raven	1/2	141	180	16.9	***
	WW15	1/2	219	180		
Insignia	Ghurka	1/2	102	106	0.3	ns
	Ranee	1/2	110	106		
Matong	Olympic	1/2	190	190.5	0.00	ns
	Siete Cerros	1/2	191	190.5		
Trident	VPM1/5* Cook	1/16	536	544.7	2.2	ns
	Spear	15/16	45	36.3		

*,** and *** Significant at $P < 0.05$, 0.01 and 0.001, respectively; ns, not significant

parent carrying the alien segment and the derived variety than expected on the basis of pedigree contribution. This observation is probably the consequence of large segments of alien chromatin having been introgressed and remaining intact during back-crossing, possibly with a substantial amount of wheat chromatin associated through linkage drag, and there is a large degree of polymorphism between the alien chromatin and wheat.

Discussion

A wide range of Australian cultivars and breeding lines was evaluated for the degree of RFLPs. Of the assayed loci 98 were polymorphic and yielded on average 3.25 polymorphic fragments per probe/enzyme combination within the 124 accessions. However, only 18% of the loci were polymorphic in pairwise comparisons. Each accession was distinguishable from all others due to its unique RFLP profile. The most closely related accessions differed by at least 8 polymorphic fragments. The relatively low level of polymorphism detected between accessions of cultivated wheat is consistent with other RFLP studies. For example Kam-Morgan et al. (1989) found that polymorphisms were detected at approximately 33% of loci when a set of hexaploid wheats were assayed with four restriction enzymes, and Chao et al. (1989) found that 38% of 45 loci showed RFLP among six hexaploid wheats. However, the level of polymorphism found in hexaploid wheat represents only a small proportion of the genetical diversity present within the Triticeae. Monte et al. (1993) detected 97% polymorphism among 16 Triticeae species, and Lubbers et al. (1991) found 80% of loci were polymorphic among 102 *Triticum tauschii* accessions.

The direct measurement of genetic similarity based on isozymes in soybean and maize (Cox et al. 1985a; Smith and Smith 1989) and storage proteins and morphological characters in wheat (Cox et al. 1985b; Wrigley et al. 1982) has been used to identify genetic relationships. When these markers were used, it was possible to relate co-ancestry and genetic similarity, although only weak correlations were derived. Their usefulness in obtaining reliable estimates of genetic similarity is limited due to the lack of sufficient characters, the low level of polymorphism and poor genome coverage.

RFLPs overcome this limitation and provide an almost unlimited number of polymorphic markers with potentially complete genome coverage. Close associations between RFLP-based estimates of genetic similarity (GS) and co-ancestry have been reported in most of the studies with maize inbreds (Messmer et al. 1993). The ability of RFLPs to evaluate genetic diversity in maize with a high degree of accuracy is now accepted (Melchinger 1993).

Provided a good coverage of the genome is obtained, RFLP-based genetic similarity estimates would appear to reflect the true degree of genetic similarity more accurately than co-ancestry values as they take into account the effects of random genetic drift and selection during the breeding process. In this study, the average genetic distance based on RFLP data was in broad agreement with that predicted from the pedigrees. There were significant exceptions to this where lines that are genetically related based on pedigree did not group on the dendrogram.

Table 4 compares the observed and expected RFLP makeup of cultivars based on pedigree. In many cases, the RFLP patterns of the progeny differed significantly from those expected on the basis of contribution of the parents according to their pedigrees. While it is possible that the selection of RFLP loci screened may have introduced errors into the genetic similarity estimate, this is considered to be unlikely as the RFLP loci were selected to provide broad and even genome coverage. Because RFLPs sample variation at the genetic level, RFLP-based markers permit a representative, phenotype-independent sampling of the genome. Hence, RFLP-based GS estimates would appear more suited than co-ancestry to quantify the genetic relationships among wheat cultivars for many breeding applications. The information derived from the RFLP-based measure of GS therefore may have direct use in the breeding programme.

The identification of markers linked to a target genetic locus has routinely involved the construction of genetic linkage maps from appropriate segregating populations or the identification of differences between near-isogenic lines. However, the low degree of polymorphism for RFLPs and the large genome size of wheat represents a major obstacle for the genetic mapping of important traits. The high degree of genome conservation between accessions suggests that observed differences as revealed by RFLP may reflect differences in the phenotypic expression of traits between accessions. Monomorphic regions of the genome would be less likely to have differences in DNA sequence and consequently less likely to be segregating for agronomically important genes. In a study of genetic diversity among European barley cultivars, Melchinger et al. (1994) identified DNA clones with disjoint RFLP patterns for winter and spring cultivars. These clones map to the region of the *Sh3* locus, which is one of several genes controlling spring/winter habit in barley. This study would suggest that, in particular circumstances, RFLP-based genetic similarity assays can provide information about the linkage of those markers with genes controlling the expression of discrete traits with multigenic inheritance.

In view of the reportedly high level of polymorphism between hexaploid wheat and other members of the *Triticeae* species it should be relatively straight forward to identify markers linked to introgressed segments in

T. aestivum backgrounds. A number of Australian wheat cultivars contain a segment of chromatin derived from related *Triticeae* species that carry disease resistance genes. During the breeding process the alien segment was transferred by back-crossing to a bridging variety which was then used as a parent in further crosses. Paull et al. (1995) demonstrated that chromatin of chromosome 7A carrying *Sr22* could be traced by RFLP probes specific to homoeologous group 7 from the diploid *T. boeoticum* through tetraploid and hexaploid bridges to the cultivar 'Schomburgk'. In this present study, a number of other sets of bridging lines carrying an alien segment and the alternative parents and derived cultivars were included. By comparing the RFLP patterns of all parents and the resultant cultivar, we were able to identify markers for a number of alien segments carrying disease resistance genes (Table 3). For systematic application of this approach, the genotypes assayed have to be grouped into subsets with identical character expression. Provided the sample size of each subset is large enough, it is possible to identify markers associated with the trait of interest (Roders 1972). The markers showing an association close to 100% are expected to show close linkage to the trait. The locations of several genes identified by this method concur with previously published data. For example, *Sr26*, derived from *Agropyron elongatum*, is located on the distal portion of 6AL (Friebe et al. 1994). A number of probes, such as PSR149, PSR154 and PSR915, associated with this segment (Table 3) have also been mapped to the distal portion of chromosomes of group 6 (Gale et al. 1995).

In conclusion, we have demonstrated that RFLP analysis can be used for the characterisation and grouping of elite breeding material of wheat. Of particular usefulness is the fact that even closely related cultivars as well as diverse breeding lines can be distinguished. Furthermore, cluster analysis based on genetic distance in association with RFLP profiling can successfully identify chromosome segments associated with a particular trait. Thus, RFLP data can assist both in selecting appropriate parents for the development of new cultivars and in assessing the level of genetic diversity in breeding material.

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