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J. R. Russell · J. D. Fuller · M. Macaulay
B. G. Hatz · A. Jahoor · W. Powell · R. Waugh

Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs

Abstract RFLPs, AFLPs, RAPDs and SSRs were used to determine the genetic relationships among 18 cultivated barley accessions and the results compared to pedigree relationships where these were available. All of the approaches were able to uniquely fingerprint each of the accessions. The four assays differed in the amount of polymorphism detected. For example, all 13 SSR primers were polymorphic, with an average of 5.7 alleles per primer set, while nearly 54% of the fragments generated using AFLPs were monomorphic. The highest diversity index was observed for AFLPs (0.937) and the lowest for RFLP (0.322). Principal co-ordinate analysis (PCoA) clearly separated the spring types from the winter types using RFLP and AFLP data with the two-row winter types forming an intermediate group. Only a small group of spring types clustered together using SSR data with the two-row and six-row winter varieties more widely dispersed. Direct comparisons between genetic similarity (GS) estimates revealed by each of the assays were measured by a number of approaches. Spearman rank correlation ranked over 70% of the pairwise comparisons between AFLPs and RFLPs in the same order. SSRs had the lowest values when compared to the other three assays. These results are discussed in terms of the choice of appropriate technology for different aspects of germplasm evaluation.

Key words Barley · Genetic relationships · Molecular analysis · RFLP · AFLP · RAPD · SSR

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J. R. Russell · J. D. Fuller · M. Macaulay · W. Powell
R. Waugh (✉)
Department of Cell and Molecular Genetics, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK

B. G. Hatz · A. Jahoor
Technishe Universitat Munchen, Lehrstuhl fur Planzenbau und -zuchtung, 85350, Freising-Weihenstephan, Germany

Introduction

As one of the first crop plants to be domesticated, barley (*Hordeum vulgare* L.) remains one of the most important crops today. Ranking fourth in world acreage, barley is used for human consumption, as a fodder crop and as a raw material for brewing beer and whisky (Brown 1992). It belongs to the genus *Hordeum*, which comprises over 32 species, including diploid and polyploid, perennial and annual types, which are spread throughout the world. The genus can be divided into three groups of varying importance to cultivated barley improvement; the primary genepool (*H. vulgare* spp. *vulgare* and *H. vulgare* spp. *spontaneum*), the secondary genepool (*H. bulbosum*) and the tertiary genepool (all other *Hordeum* species). Presently more than 250,000 *Hordeum* accessions are held in genebanks throughout the world, and the number is increasing (IBPGR 1992). With the growth of the germplasm collection a need for procedures which will allow their more effective use is required. The 'Core Collection' concept, is one such method, which should provide users with a limited set of genetically distinct and representative accessions (Brown 1989). Recently this concept has been applied to barley, and the Barley Core Collection (BCC), consisting of a limited sample of accessions considered to represent the spectrum of genetic diversity available in the genus, was established (Hintum 1992).

In such collections, morphological data are the principle descriptors which have been used to detail the accessions held. With the development of molecular markers and their many perceived advantages, it is crucial that these techniques are applied to assess genetic diversity in germplasm collections in order to supplement and refine the morphological-based classification. However, in recent years, the number of molecular assays available for application in this area has increased dramatically, with each method differing in principle, in application, in the type and amount of polymorphism detected and in cost and time

requirements. The approaches include restriction fragment length polymorphism (RFLPs; Botstein et al. 1980), random amplified polymorphic DNA (RAPDs; Williams et al. 1990), simple sequence repeat polymorphisms or microsatellites (SSRs; Tautz 1989) and Amplified Fragment Length Polymorphism (AFLPs; Zabeau and Vos 1993).

Faced with this wealth of marker technology, it is appropriate to determine if the same patterns of variability are revealed by each and whether the observed molecular diversity reflects either co-ancestry or morphological classification. To address this we have evaluated and compared similarity measures obtained from the four above systems on a set of accessions which are representative of cultivated European barley germplasm. This has allowed us to compare the results obtained from molecular analysis with each other and with pedigree information. The results are discussed in relation to the overall genetic diversity observed and the features of the individual assays.

Materials and methods

Plant material and DNA isolation

Eighteen accessions (Table 1), representing the majority of ancestors European cultivated barley, were selected for this study. Total genomic DNA was isolated from fresh leaf material by a modification of the method described by Saghai-Maroof et al. (1984).

Marker analysis

RAPD

RAPD amplifications were performed as described by Barua et al. (1993). Fragments were separated on 1.5% agarose gels, stained with ethidium bromide, visualised with ultraviolet light and photographed. The presence or absence of polymorphic bands were scored. Twenty primers, which were polymorphic between the parents of a spring × spring cross ('Blenheim' and E224/3), were used in this study.

RFLP

RFLP profiles were detected according to the protocol described by Graner et al. (1991). DNA was digested with three restriction enzymes (*Bam*HI, *Eco*RI and *Hin*dIII), and restriction fragments were detected using 48 single-copy DNA clones selected from previous mapping experiments to give good genome coverage and levels of polymorphism (Graner et al. 1991). RFLP patterns were scored as presence or absence of bands.

SSR

Two sources of simple sequence repeats were used in this study: database-derived repeats and repeats derived from an enriched genomic library. The 6 database-derived SSRs are described in a recent publication by Becker and Heun (1995). The 7 library-derived SSRs are described by Macaulay et al. (in preparation). SSR assays were performed as described by Morgante et al. (1994). Allele lengths were determined by comparing the most intense band with an M13 DNA sequence marker.

Table 1 Pedigree information and country of origin of 18 barley accessions used in molecular analysis

Cultivar	Pedigree	Origin[a]
Spring type, two-rowed:		
Aramir	Volla × Emir	NL
Beka	Bethge XIII × Kniefel	F
Golden Promise	X-ray mutant from Maythorpe (Irish Goldthorpe × Maja)	GB
Grit	Langenstein-Nungesser (5547/67 × 46459/68) 480/68 or Hadml. 554-Emir-11191-Union-46495-Diamant 14008	D
Hora	Sultan × (Weihenstephaner 1206 Nacktgerste × Volla)	D
Krona	Complex cross including Triumph	D
Triumph	(Hadm.24566 × Diamant × 1402964/6) × ((Alsa × Abyssinian) × St. × Union)	D
Union	(Weihenstephaner Mehtauresistente II × Donaria) × Firlbecks III	D
Volga	Complex cross with eight varieties	F
Winter type, two-rowed:		
Igri	(Malta × Carlsberg 1427) × Ingrid	D
Marinka	(Alpha × SVP 674) × Malta	NL
Romanze	Weihenstephan 4622/73 × (Malta × Sonja)	D
Sonja	Tria × Malta	D
Winter type, six-rowed:		
Borwinia	Vogelsanger Gold × St. 7246	D
Express	Robur × Athene	D
Franka	(Vogelsanger Gold × Senta) × (Dura × Dea) × Vogelsanger Gold	D
Gaulois	Gerbel × Athene	F
Rondo	Tanaroo × Sisfor L. 90	I

[a] NL, The Netherlands; F, France; GB, Great Britain; D, Germany; I, Italy

AFLP

AFLP analysis was essentially as described by Vos et al. (1995). Briefly, 500 ng of genomic DNA was digested with *Eco*RI and *Mse*I and double-stranded adaptors ligated to the fragment ends. This was followed by a pre-amplification step using non-selective primers. Selective amplifications were performed on the pre-amplified fragment mixture using a total of six primer combinations. Only the *Eco*RI primer was radiolabelled with γ-[^{33}P] ATP (ICN), and all primers had three selective nucleotides. Amplification products were separated by denaturing 6% polyacrylamide gel electrophoresis (PAGE), visualised by autoradiography and manually scored for the presence or absence of bands.

All of the primer names and sequences used are available on request from the authors.

Data analysis

Diversity values were calculated for each locus as $(1 - \Sigma\, Pi^2$, where Pi is the phenotypic frequency for each assay unit (RFLPs-probe/enzyme combinations; RAPDs-primers; SSRs-primer pairs; AFLPs-primer combinations). Genetic similarities (GS) were calculated using the GENSTAT Version 531 software package according to Nei and Li's (1979) estimate of similarity. Similarities were expressed using the group average agglomerative clustering function of GENSTAT to generate principal co-ordinate plots (Kempton and McNicol 1990). Correlations between assays were calculated using [Procrustes rotational analysis (PR) on the principal co-ordinate data] Spearman rank correlation (SRC) and linear regression of the GS values.

Results

Fingerprinting

All of the molecular approaches used in this study were able to uniquely fingerprint each of the 18 cultivated barley accessions. The total number of assay units varied for each marker system from only 6 primer combinations for AFLPs to 144 probe/enzyme combinations for RFLPs (Table 2). Similarly, the number of bands scored ranged from 70 for SSRs to 299 for RFLPs. The percentage of polymorphic bands for each assay did not correlate to the total number of bands. For example, only 70 bands were scored for SSRs, which was the lowest number, but all 70 were polymorphic. In contrast, 297 AFLP bands were scored, and only 46.8% of those were polymorphic. RFLPs and RAPDs were intermediate with 83.2% and 66.3%, respectively, of all bands scored being polymorphic. There was wide variation in the average number of genotypes revealed by each marker system (Fig. 1). With RFLPs, for each probe/enzyme combination, an average of 2.37 genotypic classes could be distinguished. With AFLPs this figure increased to 17.2 as nearly all primer combinations were able to discriminate between the 18 accessions used. This is further reflected in the diversity index measures. Overall the highest diversity index was observed for AFLPs (0.937), and the lowest for RFLPs (0.322). RAPDs and SSRs were intermediate (0.521 and 0.566, respectively).

Genetic similarity

The cultivated barley genepool can be divided into spring and winter types. The winter barleys are mainly used for fodder and can be further divided into two- and six-rowed types. The spring barleys are mainly used for malting. The maximum, minimum and mean similarity estimates between the spring barleys and two-row and six-row winter barleys for each assay system are shown Table 3. The similarities ranged from 0.97 within spring types using AFLPs to 0.45 within six-row winter types using SSRs. Between assay systems the estimates of similarity followed the same pattern, i.e. higher estimates of similarity within the spring types (means: RFLPs = 0.843, AFLPs = 0.924, SSRs = 0.829) and lower estimates within the six-row winter types (means: RFLPs = 0.70, AFLPs = 0.877, SSRs = 0.657). Estimates with two-row winter types were intermediate. The situation with RAPDs was different, with spring and six-row winter types exhibiting equivalent mean similarities (0.879 and 0.897, respectively). Overall, SSRs revealed the lowest similarity values (0.93–0.45) and AFLPs the highest (0.97–0.81).

Some accessions can be traced to common ancestors. For example, Grit and Triumph have Union in their pedigrees and Krona has Triumph. Our expectation would therefore be that these 4 accessions should be closely related. Table 4 shows the genetic similarity

Table 2 Analysis of the RFLP-, RAPD-, SSRs- and AFLP-generated banding patterns

Marker	Number of assay units	Total no of bands	Number of polymorphic bands (%)	Number of bands per assay unit	Number of phenotypes per assay unit	Diversity index
RFLPs	114 (42 probes, 3 enzymes)	299	249 (83.2%)	2.62	2.37	0.322
RAPDs	22 (primers)	107	71 (66.3%)	4.86	3.41	0.521
SSRs	13 (primer pairs)	70	70 (100%)	5.38	5.38	0.566
AFLPs	6 (primer combinations)	297	139 (46.8%)	49.5	17.2	0.937

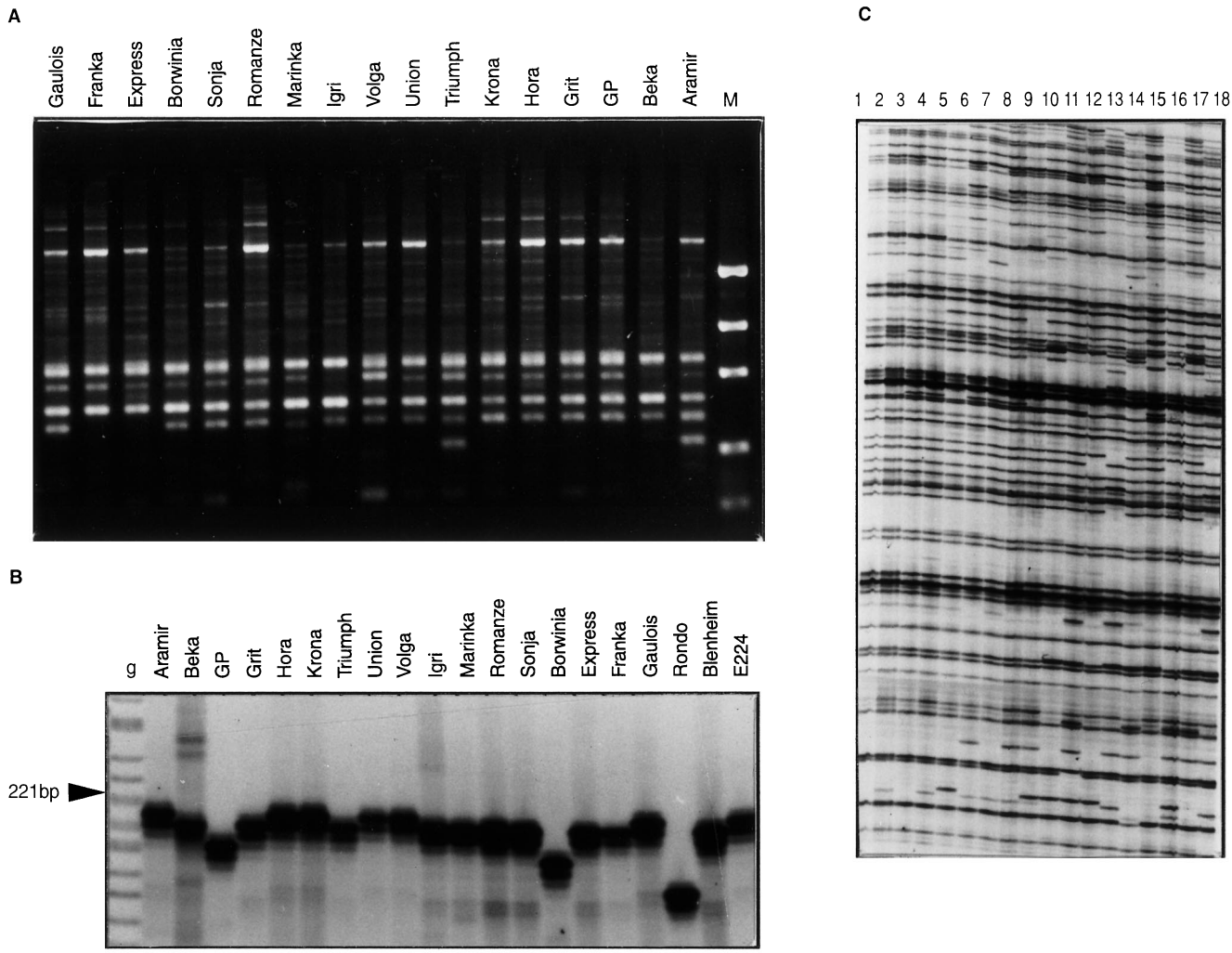

Fig. 1A–C An example of the different information content observed with RAPDS (**A**), SSRs (**B**) and AFLPs (**C**)

values for the comparisons of these 4 accessions with each of the molecular assays. With RFLPs, AFLPs and SSRs the genetic similarity values were higher than the mean values for all the spring types, and the highest similarity was between Triumph and Grit (RFLPs 0.93, AFLPs 0.97, SSRs 0.97). From the pedigree information in Table 1, Triumph and Grit share a number of parental lines including Union, Diamont and Hadm. With RAPDs, the genetic similarity values were less than the average, although the Triumph and Grit comparison was again the highest.

The genetic similarity values for the two-row winter varieties were intermediate between the spring and six-row winter types for RFLPs, AFLPs and SSRs. Sonja and Romanze were more similar than the other two-row winter types with values of 0.84 (SSRs), 0.93 (AFLPs), 0.89 (RFLPs) and 0.94 (RAPDs). This was not

Table 3 Maximum, minimum and mean genetic similarity estimates calculated from RFLP, RAPD, SSRs and AFLP data for winter and spring types

	RFLPs			RAPDs			AFLPs			SSRs			Parentage		
	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
Spring	93.0	76.0	84.3	95.0	84.0	87.9	97.0	88.0	92.4	93.0	66.0	82.9	0.330	0.020	0.133
Two-row winter	89.0	81.0	83.8	95.0	86.0	91.7	93.0	98.0	91.0	84.0	56.0	71.3	0.290	0.100	0.212
Six-row winter	85.0	60.0	70.0	95.0	88.0	89.7	91.0	81.0	87.7	90.0	45.0	65.7	0.350	0.000	0.111

Table 4 Genetic similarity values for the comparisons of 4 spring accessions with each of the molecular assays

	Grit	Krona	Triumph	Union	Maximum	Minimum	Mean
RAPDs:							
Grit	100.0						
Krona	88.0	100.0					
Triumph	90.0	85.0	100.0				
Union	88.0	88.0	84.0	100.0	95.0	84.0	87.9
RFLPs:							
Grit	100.0						
Krona	85.0	100.0					
Triumph	93.0	88.0	100.0				
Union	87.0	87.0	86.0	100.0	93.0	76.0	84.3
AFLPs:							
Grit	100.0						
Krona	91.0	100.0					
Triumph	97.0	92.0	100.0				
Union	93.0	94.0	94.0	100.0	97.0	88.0	92.4
SSRs:							
Grit	100.0						
Krona	84.0	100.0					
Triumph	97.0	87.0	100.0				
Union	69.0	70.0	94.0	100.0	97.0	66.0	82.9

unexpected as the co-efficient of parentage values were also the highest (0.290 for Sonja × Romanze compared to the mean for two-row winter type of 0.212). Both Sonja and Romanze are related through Malta, and Romanze has Sonja in its pedigree. The lowest genetic similarity values were observed for comparisons with Rondo.

Genetic relatedness

Associations among the 18 accessions were revealed by principal co-ordinate analysis (PCoA) (Fig. 2). The PCoA for the combined data (775 bands) clearly separated the winter from the spring accessions. Among the winter types, the two-rowed and six-rowed varieties formed two distinct groups, with the two-rowed types forming an intermediate group between the spring and six-rowed winter types. In the PCoAs generated by RFLP (299 bands) and AFLP (297 bands) data, a similar arrangement was observed. From the RAPD data, three distinct groups were again observed, although the spring types were more dispersed. Only a small group of spring types clustered together using SSR data, and two-row and six-row winter types were again more dispersed. On all of the PCoAs, Rondo appears in a remote position. In addition, 'Volga', a spring variety, was positioned between the rest of the spring and the two-rowed winter types.

Comparison between assays

To compare the results obtained with the four techniques, we tested correlations using Procrustes rotation (PR), linear regression of the pairwise GS values (LR) and Spearman rank correlation (SRC). The results for SRC (which compares how each system ranks pairwise similarities) are shown in Table 5. Comparisons using PR and LR showed the same general trends although the overall correlations were lower. Over 70% of the pairs of genotypes were ranked in the same order with RFLPs and AFLPs. This correlation is reduced to 10.9% when comparing RAPDs with AFLPs. SSRs were intermediate with over 50% of the genotypes ranking in the same order as that obtained with AFLPs and RFLPs.

Discussion

Given the proliferation of genetic markers, comparisons between techniques are inevitable. However, there is a need for such comparisons in order to decide on which technique is best suited to the issues being examined. In this study, three of the newer polymerase chain reaction (PCR)-based systems (RAPDs, SSRs and AFLPs) developed during the last 5 years have been compared with the well established RFLP system that was developed over 15 years ago. Each technique not only differs in principal, but also in the type and amount of polymorphism detected. The levels of polymorphism between the four techniques varied widely, ranging from a maximum of 100% (SSRs) to only 48.6% (AFLPs). Similar results were observed when Rus-Kortekaas et al. (1994) directly compared SSRs with RAPDs in tomato where the level of polymorphism was 40% with RAPDs compared to 100% with

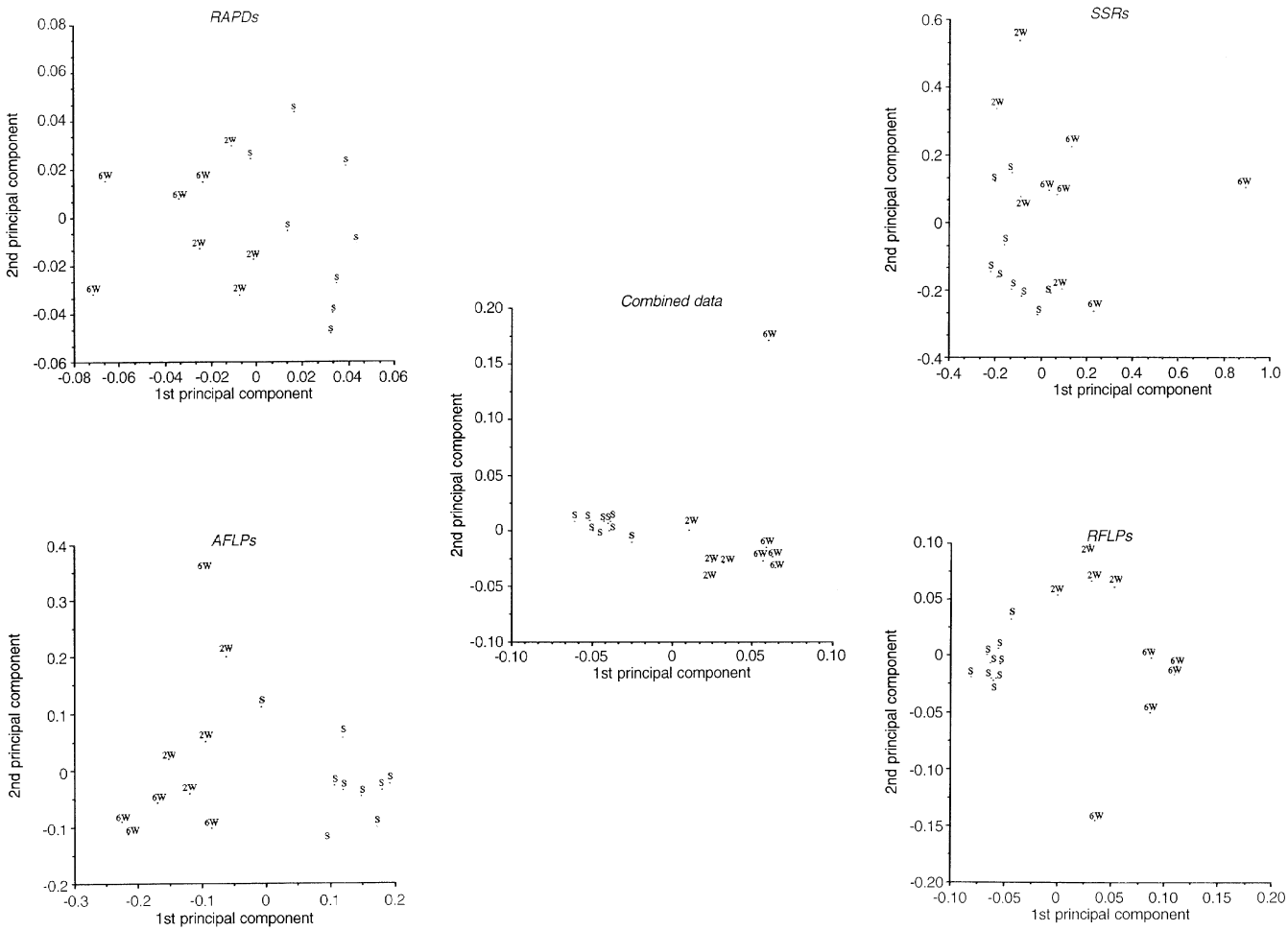

Fig. 2 Associations among the springs and winters cultivars revealed by principal co-ordinate analysis for each molecular assay

Table 5 Correlations obtained using RFLPs, RAPDs, AFLPs and SSRs based on Spearman's rank correlation and Procrustes rotation

SSR	1.000			
AFLP[a]	0.515	1.000		
RAPD[a]	0.235	0.109	1.000	
RFLP	0.505	0.708	0.201	1.000
	SSR	AFLP	RAPD	RFLP

[a] Rondo omitted from dataset for RAPD comparisons

SSRs. Indeed, whenever SSRs have been compared to other systems, they have always revealed the highest levels of polymorphism (Rus-Kortekaas et al. 1994; Salimath et al. 1995; Saghai Maroof et al. 1994; Powell et al. 1996; Maughan et al. 1995; Morgante et al. 1994; Wu and Tanksley 1993). The level of polymorphism detected using RFLPs in this study was higher (83.2%) than that observed in previous studies on barley using a similar selection of genotypes (46%) (Melchinger et al. 1994). This is probably due to pre-selection of polymorphic RFLP probes. The lowest level of polymorphism was associated with AFLPs. Becker et al. (1995) also observed that levels of polymorphism revealed by AFLPs were lower than by RFLPs. However, although AFLPs do not offer the highest level of polymorphism, they are the most efficient because they have the capacity to reveal many polymorphic bands in a single lane. The average number of bands per lane or per PCR for AFLPs was 49.5, compared to 1.0 band per lane or PCR for SSRs. Thus, when the overall diversity indices of the four techniques were compared, AFLP was the highest (0.937). Powell et al. (1996) introduced the concept of Marker Index as an overall measure of marker efficiency, and they demonstrated that, in *Glycine*, AFLPs had the highest Marker Index compared to other available marker systems. The high Marker Index or diversity index is a reflection of the efficiency of AFLPs to simultaneously analyse a large number of bands rather than the levels of polymorphism detected.

Barley germplasm can be divided into two genepools, winter and spring, based on morphology distinctions. Melchinger et al. (1994) using RFLPs observed a clear separation between the spring and winter

types. In this study, similar results were observed using RFLPs, AFLPs, RAPDs and SSRs. Furthermore, Melchinger et al. (1994) noted that sub-groups were also apparent for accessions with similar pedigrees, such as the compact grouping of two-row winter types intermediate between the spring and six-row winter types. With the exception of the SSR data, the two-row winter types form a sub-group between the six-row winter and the spring types for RFLP, AFLPs and RAPDs. With the SSR data there is a clear separation between the spring and winter types, but not within the winter types. This is not unexpected considering the low level of band sharing between accessions; even within groups the estimates of genetic similarity were much lower than any of the other assays.

Several previous studies have compared the use of RFLPs and RAPDs to examine genetic relatedness (Hallden et al. 1994; Thormann et al. 1994; Liu and Furnier 1993; dos Santos et al. 1994), and most of these show that RAPDs and RFLPs detect very similar relationships among the same group of accessions. Recently, other reports have compared RAPDs or RFLPs and SSRs on the same set of genotypes (Rus-Kortekaas et al. 1994; Wu and Tanksley 1993; Salimath et al. 1995; Maughan et al. 1995). Rus-Kortekaas et al. (1994) observed a lower percentage of band sharing in tomato accessions with SSRs compared to RAPDs and suggested that higher band sharing would make RAPDs more suitable for genetic relatedness studies. The results in this study would support the finding that SSRs may not be particularly well suited for pedigree relationship studies, although only a small number of SSRs were used.

Knowledge of genetic variation and the genetic relationship between genotypes is an important consideration for efficient rationalisation and utilisation of germplasm resources. Furthermore, it is important for the optimal design of plant breeding programmes, influencing the choice of genotypes to cross for the development of new populations. In barley, breeders have made crosses between highly selected genotypes with the result that the number of genotypes within the breeding genepool is very small. According to Graner et al. (1994) better knowledge and measures of genetic similarity of accessions could help to maintain genetic diversity. In the past, indirect estimates of similarity based on pedigree information have been widely used in many species including barley. Such estimates may not always reflect the true relationships between accessions (Graner et al. 1994). In this study we have used molecular markers to determine direct measures of genetic similarity between individuals. The estimates varied from 0.97 (AFLPs) to 0.45 (SSRs). Melchinger et al. (1994) reported GS values of 0.79 for unrelated barley pairs, based on RFLPs. The RFLP results reported in this paper were similar to these. Also, Tinker et al. (1993) observed GS values in a set of 27 North American barley cultivars using RAPDs which were similar to those found here (0.84–0.95). The values of GS based on SSRs in this present study are much lower than those based on RFLPs, AFLPs and RAPDs. Rus-Kortekaas et al. (1994) reported that the percentage of band sharing between tomato cultivars using SSRs was only 50.8% compared with 82.7% for RAPDs. Plaschke et al. (1995) observed even lower (0.31) estimates of genetic similarity when employing SSRs to examine wheat accessions and suggested that these low values are a reflection of the high information content provided by SSRs.

Although we have shown that molecular approaches can be used to group barley cultivars into morphologically distinct groups, and also further into sub-groups which have a similar genetic background, we have not addressed the issue of concordance of molecular-based estimates of GS and co-ancestry. Graner et al. (1994) compared RFLP-based estimates of GS with co-ancestry for a set of 48 cultivars. A very weak correlation was reported; $r_s = 0.21$ for winter and $r_s = 0.42$ for spring types. Similarly, using protein-based gliadin markers Cox et al. (1985) observed a correlation of $r_s = 0.27$. Both Graner et al. (1994) and Cox et al. (1985) agree that perhaps the reason for these poor correlations may be the high background similarity found for unrelated accessions using molecular markers. When related cultivars were used to investigate correlations between RAPD-based estimates of GS and co-ancestry a moderate correlation of $r_s = 0.61$ was observed between both measures (Tinker et al. 1993). Plaschke et al. (1995) observed similar results in wheat using SSR-based GS estimates and pedigree measures ($r_s = 0.55$). Although we have only a limited set of co-ancestry measures for the accessions studied here, several conclusions can be drawn from the correlations between molecular estimates of GS and the co-efficient of parentage. For example, the co-efficient of parentage for Rondo was 0 for all of the pairwise comparisons, and with all molecular measures Rondo had the lowest GS value. The low-to-moderate correlations between molecular measures of GS and pedigree estimates have led to the conclusion that pedigree information may not be as useful for certain applications for which they have been used in the past (Graner et al. 1994; Plaschke et al. 1995). In any case, molecular-based estimates of GS will provide more information than is available from pedigree information.

Having established that molecular-based estimates of GS will allow plant breeders to make informed decisions regarding the choice of genotypes to cross, we must ask the question as to which assay is most appropriate? Several studies have been described which address this question using isozymes, RFLPs and RAPDs (dos Santos et al. 1994; Thormann et al. 1994; Heun et al. 1994; Hallden et al. 1994). Heun et al. (1994) found that the correlation between RAPDs and isozymes among *Avena sterilis* accessions were moderately low

($r_s = 0.36$), although the overall representation of genetic relatedness was in considerable agreement. Beer et al. (1994) assessed genetic variation among *Avena sterilis* using morphological markers, isozymes and RFLPs and found a similarly low correlation ($r_s = 0.27$). A very different situation was observed among *Brassica* species. Thormann et al. (1994) reported correlations of $r_s = 0.969$ between RFLPs and RAPDs for a group of 18 accessions from different *Brassica* species. Dos Santos et al. (1994) also observed a significantly high correlation between RFLPs and RAPDs ($r_s = 0.745$) using genotypes within *Brassica oleracea*, although they did observe differences between the RFLP and RAPD dendrograms. When Spearman rank correlation was used, AFLPs and RFLPs ranked over 70% of the pairwise comparisons in the same order. This may well be because both techniques are based on restriction site changes, the major difference is that PCR is used in AFLPs rather than Southern analysis in RFLPs. In contrast SSRs and RAPDs have the lowest values when compared to the other assays. The low correlations observed with RAPDs could be a reflection of the choice of primers which we have previously used in the construction of a linkage map using a population derived from two related spring varieties. This may well have resulted in biased estimates of GS, which in turn has affected the ranking order of genotypes. For example, the lowest GS was between two spring types (Volga and Beka; GS = 0.84), whereas the lowest GS estimates for the other assays were between two winter types (even when Rondo was removed from all the data sets this still holds true).

The lack of correlation between SSRs and the other assays may not be fully unexpected, considering the high levels of polymorphism between pairwise comparisons. Powell et al. (1996) reported that SSRs were well-correlated with AFLPs and RFLPs at the interspecies level, however at the intraspecies level the correlation disappeared, emphasising the uniqueness of the SSR assay. Thus, while SSR analysis appeared to be the most polymorphic assay system, it did not seem to be particularly useful for assessing genetic relationships among cultivars. RFLPs were particularly valuable for assessing genetic relationships, but required several probe and enzyme combinations to discriminate between accessions. Both RFLPs and SSRs require an initial investment in terms of probe or sequence information, and according to Vos et al. (1995) the ideal fingerprinting assay should require no prior sequence knowledge. While only AFLPs and RAPDs meet these requirements, the lack of comparative information at each assayed locus (due to dominance) precludes an accurate assessment of true genetic relationships.

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