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The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin

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Abstract The potential of bulk analyses of RAPD and ISSR-PCR markers for fingerprinting purposes was evaluated using ten RAPD and ten ISSR primers. The phylogenetic relationships of 16 barley cultivars from different countries, and all having a known pedigree, were analysed using 353 PCR markers (125 RAPDs and 228 ISSRs). The band profiles generated were reproducible in spite of the different DNA extractions, PCR techniques, electrophoretic methods and gel scorings used. The RAPD primer S10 and four ISSR primers (811, 820, 835 and 881) were both able to distinguish all cultivars. A strong and quite linear relationship was observed between Resolving Power (Rp) of a primer and its ability to distinguish genotypes. The dendrograms obtained using these two molecular markers are in agreement with their known origin, showing clusters that separate very well the spring/winter and six-rows/two-rows cultivars. Thus, bulk analyses of RAPD and ISSR PCR markers provides a quick, reliable and highly informative system for DNA fingerprinting and also permit to establish genetic relationships which agree with, by other means, known origin of the cultivars.

Keywords RAPDs · ISSRs · Bulked analyses · Genetic diversity · Barley

Introduction

The cultivated barley, *Hordeum vulgare*, including its wild relative *H. vulgare* ssp. *spontaneum*, belongs to the primary gene pool of *Hordeum*. The total number of barley accessions in the genebanks, including redundant material, has been estimated about 280,000. Most of the genetic diversity that local or traditional varieties of cultivated crops possess is being lost. The new varieties are more genetically homogeneous and therefore more exposed to pathogens and adverse environmental conditions (Asins and Carbonell 1989).

Nowadays, there are several different molecular methods for the identification of cultivars. Restriction fragment length polymorphism (RFLP) and the polymerase chain reaction (PCR) are now widely used. The PCR methods using arbitrary primers has been widely utilised the last 10 years. Of these techniques, random amplified polymorphic DNAs (RAPDs) seems to be one of the most popular. RAPDs have been used for measuring genetic diversity in several plant species, i.e. broccoli and cauliflower (Hu and Quiros 1991), apple (Landry et al. 1994), wheat (Vierling and Nguyen 1992), commercial pea varieties (Bagheri et al. 1995), melon (García et al. 1998), *Triticum* (Cao et al. 1999), *Gossypium* (Khan et al. 2000), olive (Besnard et al. 2000) and *Oryza granulata* (Qian et al. 2001).

The inter-simple sequence repeat (ISSR) PCR using primers based on dinucleotide, tetranucleotide or pentanucleotide repeats has now become in fashion among the researchers (Zietkiewicz et al. 1994). ISSRs have been used for cultivar identification in maize (Kantety et al. 1995; Pejic et al. 1998), potatoes (Prevost and Wilkinson 1999), trifoliate orange (Fang et al. 1997), wheat (Nagaoka and Ogihara 1997), bean (Métais et al. 2000) and *Diplotaxis* (Martín and Sánchez-Yélamo 2000).

The assessment of genetic variability in barley germplasm has previously based on pedigree records, phenotypic traits, and also on biochemical and molecular markers. Studies about genetic diversity in barley have been performed using RFLP markers (Graner et al. 1994; Melchinger et al. 1994). However, the use of RFLPs in a great number of individuals has certain limitations, i.e. the amount of DNA required is relatively large (5–10 μg), and the technique is very expensive and time consuming. The PCR technology has offered new marker systems for diagnosis of genetic diversity in large scale studies (Saiki et al. 1988). The simple sequence re-

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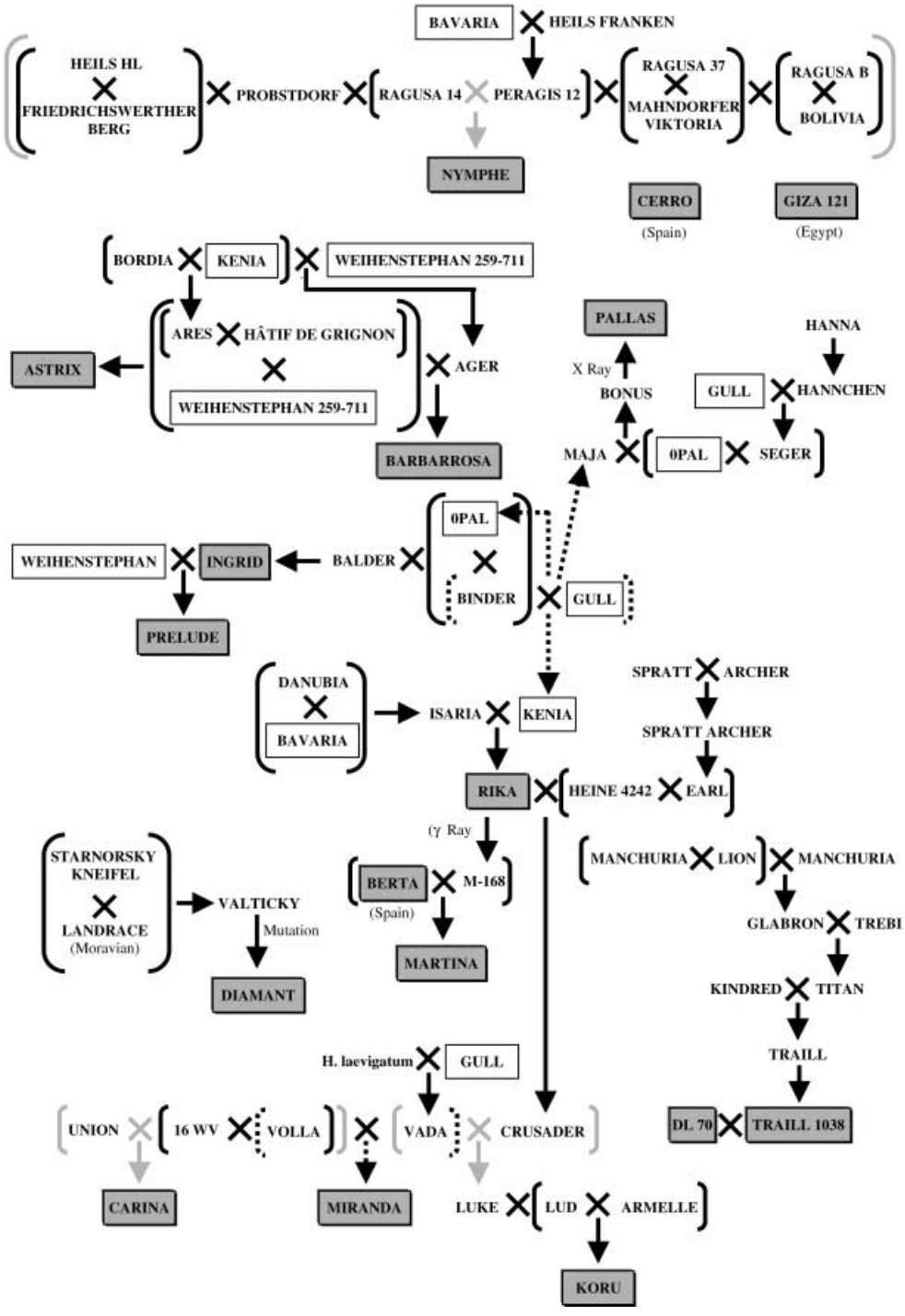

Fig. 1 Pedigree of the 16 barley cultivars used in this study. In the *grey boxes* are the cultivars analysed. The parental cultivars that appear more than one time in the pedigree are in *white boxes*

peat primers have been used to study genetic diversity in barley (Sánchez de la Hoz et al. 1996). RAPDs variation in wild populations of four species of the genus *Hordeum* (*Poacea*) have been studied using bulk analyses of 15 to 20 plants per populations (Tinker et al. 1993; Russell et al. 1997; De Bustos et al. 1998). Most lately, the microsatellite markers have been employed for the detection of genetic diversity in barley populations (Struss and Plieske 1998).

However, studies about the comparison between the known pedigree of commercial cultivars or varieties and the genetic relationships obtained using molecular markers like RAPDs and ISSRs are not frequent.

The objectives of the present study are: (1) to develop a set of RAPD and ISSR markers with relevance in bulk analyses, (2) to determine their variability, (3) to apply them for genome analysis, distinguishing between genotypes, and (4) to compare the genetic diversity obtained

using these markers in analyses of commercial barley cultivars of known pedigree.

Materials and methods

Plant materials

Sixteen barley (*H. vulgare* L.) cultivars from different countries and known pedigree were studied (Fig. 1). The sixteen barley cultivars studied were: 1 Astrix (A), 2 Barbarrosa (Ba), 3 Berta (Be), 4 Cerro (C), 5 Diamant (D), 6 Giza 121 (G), 7 Ingrid (I), 8 Carina (Ka), 9 Koru (Ko), 10 Martina (Ma), 11 Miranda (Mi), 12 Nymphe (N), 13 Pallas (P), 14 Prelude (Pr), 15 Rika (R) and 16 Traill 1038 × DL70 (T).

Genomic DNA extraction

Each cultivar was reduced to a pool of 20 plants, and 100 mg of young leaves from each plant were used to create the pool. These leaves were frozen in liquid nitrogen and stored at –80 °C. The extraction was carried out using a small-scale DNA isolation method (Dneasy Plant Mini Kit, from Qiagen).

Primers used in the PCRs

A total of ten 10-mer oligonucleotides from set S (Operon Technologies, Alameda, Calif.) were selected according to the number and consistency of amplified fragments (see Table 1).

For ISSRs, ten primers based on dinucleotide, tetranucleotide or pentanucleotide repeats were utilised following the previous criterion (see Table 1). These oligonucleotides were obtained from UBC primer set 100/9 (University of British Columbia).

Polymerase chain reactions (PCRs)

The protocol for RAPD analysis was adapted from Williams et al. (1990). The reactions were carried out in a 25-µl volume containing 0.4 units of Dynazyme polymerase (F501L), 8 ng of genomic DNA template, 15 pmol of primer, 2.5 mM of each dATP, dCTP, dGTP and dTTP, and 2.5 µl of 10 × Dynazyme reaction buffer. For ISSRs, we have adapted the protocol of Zietkiewicz et al. (1994). In this case, the reactions were carried out in a 25-µl volume containing 0.4 units of Dynazyme polymerase (F501L), 4 ng of genomic DNA template, 10 pmol of primer, 2.5 mM of each dATP, dCTP, dGTP and dTTP, and 2.5 µl 10 × Dynazyme reaction buffer.

DNA amplifications were performed in a PTC-100 thermocycler (MJ Research Inc) with a preliminary step of 5 min at 94 °C, 45 cycles of 60 s at 94 °C, 60 s at 36 °C and 2 min at 72 °C and a final step of 6 min at 72 °C for RAPDs. For ISSRs, with a initial step of 5 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 45 s at 52 °C and 2 min at 72 °C and a final 6-min extension at 72 °C.

Samples of 10-µl PCR products were analysed on 8% acrylamide gels in TBE buffer running at 100 V for 12.30 h. The gels were stained using silver nitrate.

Resolving Power (Rp)

According to Prevost and Wilkinson (1999) the Resolving Power (Rp) of a primer is: $Rp = \Sigma\ Ib$ where Ib (band informativeness) takes the value of: $1 - [2 \times (0.5 - p)]$, p being the proportion of the 16 genotypes (barley cultivars analysed) containing the band.

Band profile reproducibility

Three replicate DNA extractions (pool of 20 plants) from leaves of two different barley cultivars (Koru and Giza 121) were used to assess the consistency of the band profiles. RAPD and ISSR amplifications were repeated at least three times and only the repetitive PCR products were scored.

Analysis of amplification profiles

ISSR and RAPD bands behave as dominant markers and were scored for the presence (1) or absence (0) of homologous bands for all cultivars. The dendrograms were constructed by UPGMA cluster analysis using the simple matching coefficient (SM) (Sneath and Sokal 1973) and NTSYS-pc version 1.6 package (Rohlf 1992). SM = m/n, being m = shared present fragments (11) + shared absent fragments (00) and m = the total of obtained fragments.

One cultivar of rye, Ailés, was used as ‘out group’ element, providing a reference point against which genetic similarity of the barley cultivars could be measured for generating the trees.

Results

RAPD amplification (Fig. 2a and b)

DNAs from bulks from each of the 16 cultivars were amplified using the ten oligonucleotides indicated in Table 1. The total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (P%), number of different genotypes (NG), Resolving Power (Rp) and number of exclusive bands (NEB) obtained per each primer are shown in the same Table.

The total of amplified products was 125 (average of 12.5 bands per primer) ranging from 250 to 1,450 bp, 79 of them polymorphic (63%). Fourteen different barley cultivars (N, A, C, G, T, D, Mi, Ma and Ba) showed a total of 15 exclusive bands which can be converted into STS markers, of great value to detect mixes between cultivars and as DNA fingerprints.

The resolving power (Rp) of the ten RAPDs primers ranged from 1.376 for primer S9 to 8.880 for primer S10. Besides its high Rp value, RAPD primer S10 is able to distinguish all 16 barley cultivars.

Dendrogram obtained with RAPD markers

The dendrogram obtained using SM coefficient (matrix available on request) present two main clusters (A and B) with 12 and four cultivars each respectively (Fig. 3a). The cluster A has two subclusters (A1 and A2), having the first three cultivars with six rows and winter habit (Astrix, Barbarrosa and Nymphe) and also a fourth, Martina, with two rows and again with winter habit. The A2 subcluster contains the eight cultivars with two rows and spring habit. The cluster B has four cultivars, two of them with six rows and winter habit (Cerro and Berta) and the another two with six rows and spring habit (Giza 121 and Traill × DL).

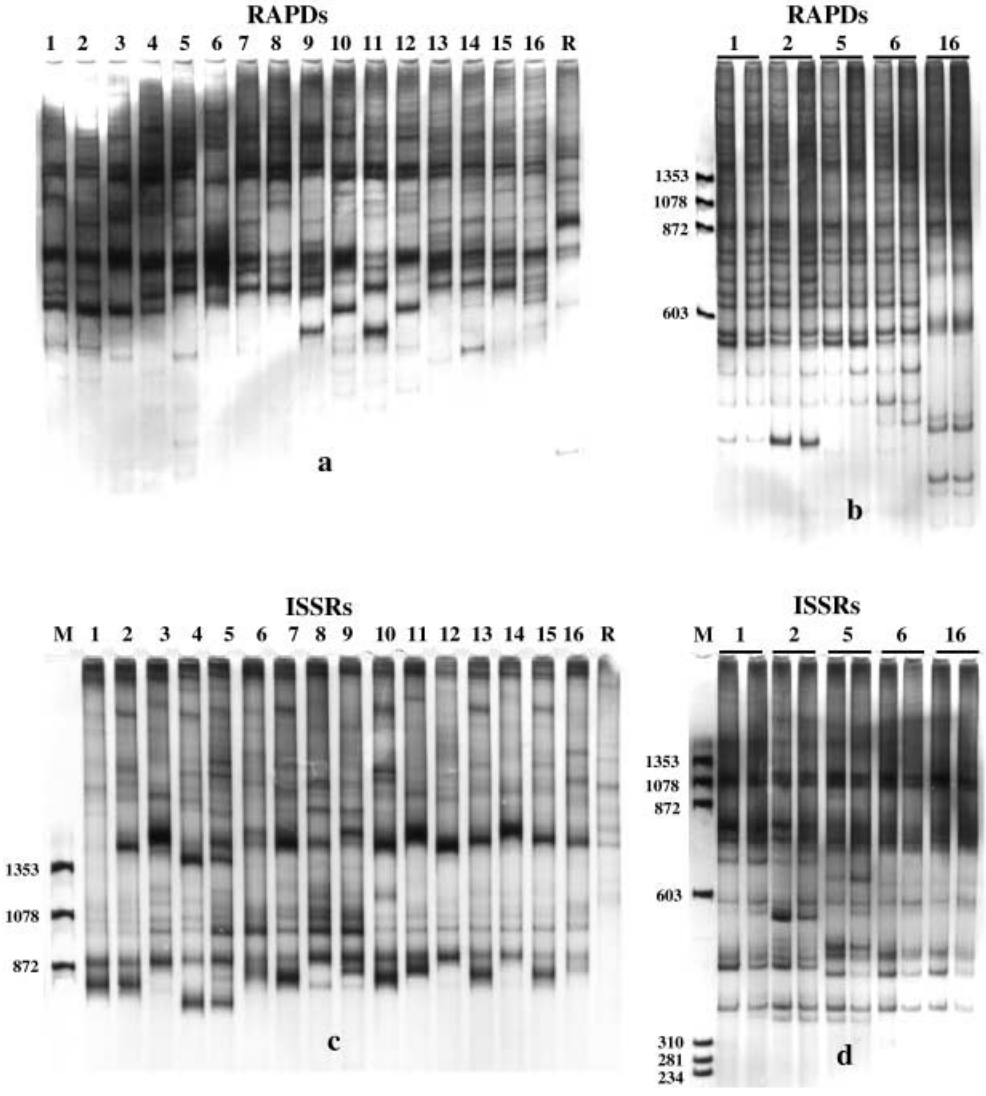

Fig. 2a–d RAPD and ISSR amplification products obtained from the sixteen cultivars studied: **a** S10 RAPD primer and **c** 820 ISSR primer. RAPD and ISSR markers generated from two different extractions of the same cultivar: **b** S19 RAPD primer and **d** 809 ISSR primer. *1* Astrix, *2* Barbarrosa, *3* Berta, *4* Cerro, *5* Diamant, *6* Giza 121, *7* Ingrid, *8* Carina, *9* Koru, *10* Martina, *11* Miranda, *12* Nymphe, *13* Pallas, *14* Prelude, *15* Rika, *16* Traill 1038 × DL70. *R* = "Ailés" rye cultivar (out group). *M* = the weight molecular marker was ØX174 DNA digested with *Hae*III

ISSR amplification (Fig. 2c and d)

The ten ISSR primers used here for the PCR amplifications of DNA bulks from 16 cultivars were selected among the only 31 primers from the set 100/9 UBC that give rise reproducible amplification products. The sequences of these 31 primers seem to indicate that microsatellites more frequent in barley contain the repeated dinucleotides (AG)*n*, (AC)*n*, (TC)*n*, (TG)*n* and the repeated pentanucleotides (GGAGA)*n* and (GGGTG)*n*.

The total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (P%), number of different genotypes (NG), Resolving Power (Rp) and number of exclusive bands (NEB) obtained per each primer are shown in Table 1. The total number of amplified products was 228 (an average of 22.8 bands per primer) ranging from 200 to 1,500 bp, being polymorphic 190 (83%) of them. Fourteen different barley cultivars (Mi, Pa, C, Ko, Ma, G, Pr, T, A, I, Be, N, Ca and Mi) showed a total of 34 exclusive bands also convertibles in STSs.

The resolving power (Rp) of the ten ISSR primers ranged from 3.754 for primer 841 to 20.632 for primer 811. Four ISSR primers (811, 881, 820 and 835) possess the highest Rp values (20.632, 17.880, 11.25 and 9.504, respectively) and are each able to distinguish all 16 barley cultivars.

Dendrogram obtained with ISSR markers

The dendrogram (Fig. 3b) obtained using SM coefficient (matrix available on request) has two main clusters (A and B); the first includes all the cultivars except Giza 121, coming from Egypt, which constitutes the second cluster (B). On the other hand, the first cluster is divided in two subclusters (A_1 and A_2). In one of them (A_1) are grouped the five cultivars with six rows and winter habit and Traill × DL (six rows and spring habit). The other subcluster (A_2) contains all the cultivars with two rows and spring habit and Martina (two rows and winter habit).

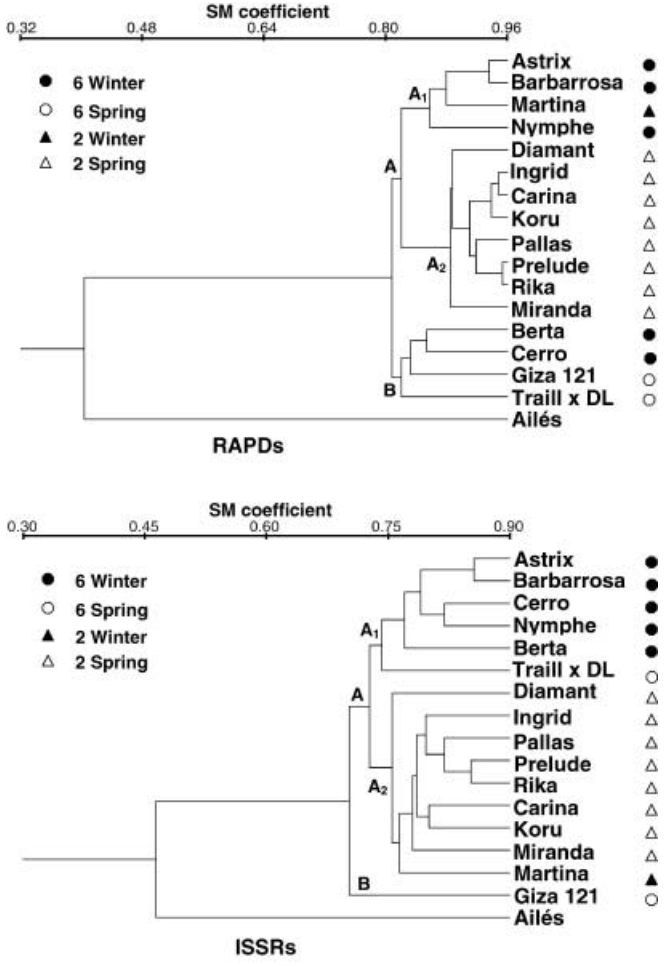

Fig. 3a, b UPGMA dendrograms showing the genetic relationships among the 16 barley cultivars based on the PCR markers (SM coefficient). "Ailés" rye cultivar (out group). **a** RAPD dendrogram and **b** ISSR dendrogram

Table 1 RAPD and ISSR primers selected from Set S (Operon) and UBC Set 100/9, respectively. Total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (P%), number of different genotypes or cultivars identified (NG), Resolving Power (Rp) and number of exclusive bands (NEB) obtained per RAPD primer and per ISSR primer

Primer	5′→3′	TNB	NPB	P%	NG	Rp	NEB
RAPDs							
S1	CTACTGCGCT	13	8	61	11	5,376	1
S2	CCTCTGACTG	13	6	46	12	3,748	0
S5	TTTGGGGCCT	13	9	69	8	3,002	2
S7	TCCGATGCTG	9	6	67	8	2,004	2
S8	TTCAGGGTGG	8	5	62	6	1,754	3
S9	TCCTGGTCCC	12	4	33	5	1,376	1
S10	ACCGTTCCAG	18	16	89	16	8,880	2
S11	AGTCGGGTGG	10	7	70	8	3,130	2
S13	GTCGTTCCTG	14	10	71	14	5,128	1
S19	GAGTCAGCAG	15	8	53	11	4,128	1
Total		125	79	63	99	38,526	15
ISSRs (Y = C,T)							
809	$(AG)_8G$	19	13	68	12	7,876	1
810	$(GA)_8T$	23	21	91	14	7,508	8
811	$(GA)_8C$	38	38	100	16	20,632	7
820	$(GT)_8C$	27	25	93	16	11,25	3
826	$(AC)_8C$	21	14	67	15	6,876	3
827	$(AC)_8G$	15	9	60	12	5,204	0
828	$(TG)_8A$	15	11	73	13	7,470	0
835	$(AG)_8YC$	18	17	94	16	9,504	2
841	$(GA)_8YC$	18	11	61	12	3,754	5
881	$GGG(TGGGG)_2TG$	34	31	91	16	17,880	5
Total		228	190	83	142	97,954	34

Discussion

RAPD and ISSR markers

Each cultivar was reduced to one tube that contained the same quantity of green tissue for each of 20 different plants. Therefore, all analyses were conducted using one bulk for each cultivar. Since bulk samples of DNA were used to produce RAPDs and ISSRs, a mixture of sequences with different degrees of homology with the primer could be amplified. The advantages and inconvenients of the bulk analysis have been discussed by Michelmore et al. (1991) and Loarce et al. (1996). Bulk analyses are economic and rapid but it is not possible to obtain information about the genetic variability inside the populations, it is only possible to get genetic variability between different populations.

In a parallel analysis of the same cultivars with 16 isozyme systems (data not shown) has been pointed out the existence of 33 loci, being 22 monomorphic and 11 polymorphic (33%). As expected in a autogamous crop, all the plants analysed were homozygous, although in nine of these cultivars the six plants analysed showed the same homozygous genotype, the remainder seven show mixes of different homozygous genotypes. Only one exclusive allele (*Est5*) was detected in the Diamant cultivar although at a low frequency (0.16).

The percentage of polymorphic bands is higher for ISSRs (83%) than for RAPDs (63%) and both molecular markers are more polymorphic than isozymes (33%). However, the individual isozyme analyses can detect intracultivar variability, whereas the bulk analyses only detect intercultivar variability. The mean number of amplification products obtained with RAPDs (12.5) is fewer than with ISSRs (22.8). Moreover, the total number of polymorphic bands detected with the ten ISSR primers (190) was surprisingly much higher than with the ten RAPD primers (79) (Table 1). Therefore, the ISSR markers are the most efficient marker system because of their capacity to reveal several informative bands in a single amplification (a mean of 19 informative bands per primer).

The average Resolving Power (Rp) of ISSR primers (9.79) was higher than RAPD primers (3.85). There is a seemingly linear relationship between the Rp of each primer and the number of genotypes or cultivars identified. This relationship was stronger for RAPDs (r^2 =

0.92) than for ISSRs ($r^2 = 0.73$). Prevost and Wilkinson (1999) have studied the nature of this relationship using a total of 371 hypothetical primers producing 8, 10 or 12 band positions ($r^2 = 0.98$). However, they have found a seemingly linear relationship between the Rp of real ISSR primers and the number of genotypes of potato cultivars identified ($r^2 = 0.65$). In our case, we have observed a greater correlation using RAPDs than ISSRs primers, probably due to the low number of cultivars analysed.

Dendrograms obtained using RAPDs and ISSRs

The dendrogram generated by the ISSR matrix agrees better with the genealogy of the barley cultivars studied than the dendrogram generated by the RAPD results (Figs. 1 and 3). So, for example, Astrix and Barbarrosa appear as the cultivars more narrowly related (0.854 of similarity) because they share several parentals like Weihenstephan 259-711, Ares and Hâtif de Grignon. However, although Berta and Cerro came from Spain and we have not found any relationship between them and Astrix, Barbarrosa and Nymphe, all are grouped in the same subcluster with six rows and winter habit (Fig. 3b).

The Ingrid, Pallas, Prelude and Rika cultivars came from the common cross Binder × Gull (Fig. 1) and Ingrid, Pallas and Prelude also share the Opal parental. In addition, Gull is involved two times in parental crosses of Pallas cultivar, appearing these four cultivars as very related in the ISSR dendrogram (Fig. 3b). The Koru, Carina Miranda and Martina are also related with the four previous cultivars, because Koru came from Rika and, Koru and Miranda share the Vada parental, and Gull is a parental of Vada. Moreover, the Volla cultivar is a parental of Carina and Miranda. The Martina cultivar came from (Berta × M-168), and M-168 is a γ-ray mutant from Rika.

All these cultivars are grouped in the subcluster A_2 that contains all the cultivars with two rows and spring habit (Ingrid, Pallas, Prelude, Rika, Carina, Koru and Miranda) and Martina (two rows and winter habit) (Fig. 3b).

The lowest similarities are found between Giza 121 (from Egypt) and the remaining cultivars and also between Traill (Traill 1038 × DL70) and the other 15 cultivars. We have not found relationships between Giza 121 and Traill with the remaining barley cultivars analysed in the germplasm database consulted. Therefore the results obtained in the ISSR dendrogram agree with the known pedigree.

However, Martina came from the cross (Berta × M-168), and Berta and Martina are in different subclusters. Martina is more related with Rika (the parental of M-168) than with Berta. In the comparison of the known pedigree with the ISSR dendrogram is necessary to remember that after every parental cross there are always several generations of selection and, during this selection process it is possible that two barley cultivars with a common origin accumulate many differences in a short period of time. On the other hand, a common original cross like Binder × Gull can originate three different barley cultivars (Kenia, Maja and Opal) using after the cross different selection programs. On the other hand, the relationships observed using molecular markers may provide information on the history and biology of cultivars, but it does not necessarily reflect what may be observed with respect to agronomic traits (Métais et al. 2000). The selection process leads to an accumulation of the best alleles for the traits under selection. RAPDs and ISSRs are dispersed throughout the genome and their association with agronomic traits is influenced by the breeder only in the region under selection pressure. The other loci are subjected to random genetic drift.

In general, the isozyme study reflected greater similarities between barley cultivars than those shown by molecular markers. This is explained by the conservative nature of these isozyme loci compared to RAPDs and ISSRs which might detect non-coding, and therefore, more polymorphic DNA. Also, the similarities detected with RAPDs are greater than the similarities observed with ISSRs. In summary, seems to exist a relation between the polymorphism of each kind of marker and the similarities detected between cultivars.

The differences found among the dendrograms generated by RAPDs and ISSRs could be partially explained by the different number of PCR products analysed (125 for RAPDs and 228 for ISSRs) reinforcing again the importance of the number of loci and their coverage of the overall genome, in obtaining reliable estimates of genetic relationships among barley cultivars. Similar results have been observed by Loarce et al. (1996), also in barley.

Another explanation could be the low reproducibility of RAPDs (Karp et al. 1997). The putatively similar bands originating for RAPDs in different individuals are not necessarily homologous although they may share the same size in base pairs. This situation may lead to wrong results when calculating genetic relationships.

With this study we can conclude that the bulk analyses of RAPD and ISSR markers were useful for study the genetic relationships between barley cultivars, providing the ISSR markers a powerful tool for the generation of potential fingerprinting diagnostic markers for cultivars. Also the phylogenetic analysis on the basis of ISSR-derived phenogram supports the known origin of the barley cultivars, in spite of the multiple generations of selection carried out after the ancestor crosses.

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References

Asins MJ, Carbonell EA (1989) Distribution of genetic variability in a durum wheat world collection. Theor Appl Genet 77: 287–294

Bagheri A, Paull JG, Landgridge P, Rathjen AJ (1995) Genetic distance detected with RAPD markers among selected Australian commercial varieties and boron-tolerant exotic germplasm of pea (*Pisum sativum*). Mol Breed 1:193–197

Besnard G, Baradat P, Bervillé A (2000) Genetic relationships in the olive (*Olea europea* L.) reflect multilocal selection of cultivars. Theor Appl Genet 102:251–258

Cao W, Scoles G, Huel P, Chibbar RN (1999) The use of RAPD analysis to classify *Triticum* accessions. Theor Appl Genet 98:602–607

De Bustos A, Casanova C, Soler C, Jouve N (1998) RAPD variation in wild populations of four species of the genus *Hordeum* (Poaceae). Theor Appl Genet 96:101–111

Fang DQ, Roose ML, Krueger RR, Federicé CT (1997) Fingerprinting trifoliate orange germplasm accessions with isozymes, RFLPs and inter-simple sequence repeat markers. Theor Appl Genet 95:211–219

García E, Jamilena M, Alvarez JI (1998) Genetic relationships among melon breeding lines revealed by RAPD markers and agronomic traits. Theor Appl Genet 96:878–885

Graner A, Bauer E, Kellermann A, Kirchner S, Muraya JK, Jahoor A, Wenzel G (1994) Progress of RFLP-map construction in winter barley. Barley Genet Newslett 23:53–59

Hu J, Quiros CF (1991) Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep 10:505–511

Kantety RV, Zeng XP, Bennetzen JL, Zehr BE (1995) Assessment of genetic diversity in Dent and Popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. Mol Breed 1:365–373

Karp A, Edwards K, Bruford M, Vosman B, Morgante M, Seberg O, Kremer A, Boursot P, Arctander P, Tautz D, Hewitt G (1977) Newer molecular technologies for biodiversity evaluation: opportunities and challenges. Nature Biotechnol 15:625–628

Khan SA, Hussain D, Askari E, Stewart JMcD (2000) Molecular phylogeny of *Gossypium* species by DNA fingerprinting. Theor Appl Genet 101:931–938

Landry BS, Li RQ, Cheung WY, Granger RL (1994) Phylogeny analysis of 25 apple rootstocks using RAPD markers and tactical gene tagging. Theor Appl Genet 89:847–852

Liu F, Bothmer R von, Salomon B (1999) Genetic diversity among East Asian accessions of the barley core collection as revealed by six isozyme loci. Theor Appl Genet 98:1226–1233

Loarce Y, Gallego R, Ferrer E (1996) A comparative analysis of genetic relationships between rye cultivars using RFLP and RAPD markers. Euphytica 88:107–115

Martín JP, Sánchez-Yélamo MD (2000) Genetic relationships among species of the genus *Diplotaxis* (*Brassicaceae*) using inter-simple sequence repeat markers. Theor Appl Genet 101:1234–1241

Melchinger AE, Graner A, Singh M, Messmer MM (1994) Relationships among european barley germplasm. I. Genetic diversity among winter and spring cultivars revealed by RFLPs. Crop Sci 34:1191–1199

Métais I, Aubry C, Hamon B, Jalouzot R (2000) Description and analysis of genetic diversity between commercial bean lines (*Phaseolus vulgaris* L.). Theor Appl Genet 101:1207–1214

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulk segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Nagaoka T, Ogihara Y (1997) Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor Appl Genet 94:597–602

Pejic I, Ajmone-Marsan P, Morgante M, Kozumplick V, Castiglioni P, Taramino G, Motto M (1998) Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. Theor Appl Genet 97:1248–1255

Prevost A, Wilkinson MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor Appl Genet 98:107–112

Qian W, Ge S, Hong DY (2001) Genetic variation within and among populations of a wild rice *Oryza granulata* from China detected by RAPD and ISSR markers. Theor Appl Genet 102: 440–449

Rohlf FJ (1992) NTSYS-pc, numerical taxonomy and multivariate analysis system. Version 1.6 Exeter Software, New York

Russell JR, Fuller JD, Macaulay M, Hatz BG, Jahoor A, Powell W, Waugh R (1997) Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor Appl Genet 95:714–722

Saiki RK, Gelfond DH, Stoffel S, Scharf SJ, Higuchi R, Horn BT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a termostable DNA polymerase. Science 239:487–491

Sánchez de la Hoz MP, Dávila JA, Loarce Y, Ferrer E (1996) Simple sequence repeat primers used in polymerase chain reaction amplifications to study genetic diversity in barley. Genome 39:112–117

Sneath PHA, Sokal RR (1973) Numerical Taxonomy. The principles and practice of numerical classification. W.H. Freeman and Co, San Francisco

Struss D, Plieske J (1998) The use of microsatellite markers for detection of genetic diversity in barley populations. Theor Appl Genet 97:308–315

Tinker NA, Fortin MG, Mather DE (1993) Random amplified polymorphic DNA and pedigree relationships in spring barley. Theor Appl Genet 85:976–984

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

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

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR) – Anchored Polymerase Chain Reaction Amplification. Genomics 20:176–183