Theor Appl Genet (1999) 98 : 721–727

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Development and characterization of *Hordeum chilense* chromosome-specific STS markers suitable for wheat introgression and marker-assisted selection

Received: 5 June 1998 / Accepted: 17 September 1998

Abstract RAPD markers were developed for octoploid × *Tritordeum* (amphiploid *Hordeum chilense* × *Triticum aestivum*) and its parents. Addition lines were used to identify specific RAPD markers for the *Hordeum chilense* chromosomes detectable in a wheat background. Twelve RAPD fragments have been cloned, sequenced and converted into STS markers. Eleven of these STSs have maintained both the chromosome specificity and the possibility of detection in a wheat background. The use of these markers in multiplexed PCRs facilitates both the efficient and reliable screening of new addition lines as well as the monitoring of introgression of *H. chilense* in bread and durum wheat.

Key words Addition lines · Multiplexed PCR · RAPD · Sequence tagged site · Tritordeum

Introduction

The wild barley *Hordeum chilense* belongs to a heterogeneous group of South American *Hordeum* species (Sec. *Anisolepsis* Nevski, Bothmer et al. 1995). It is very polymorphic and has been crossed with species from the genera *Aegilops*, *Agropyron*, *Dasypyrun*, *Hordeum*, *Secale* and *Triticum* (Fedak 1992).

The use of *H. chilense* in breeding programs has had two primary foci: (1) the development of hexaploid tritordeum (amphiploid between *H. chilense* and tetraploid wheat) to be used as a new cereal (Martín et al. 1996); (2) the introgression into wheat of genetic material from *H. chilense*, such as resistance to the root-knot nematode *Meloidogyne naasi* (Person-Dedryver et al. 1990). While chromosome addition lines of *H. chilense* in bread wheat have been developed and may serve as a tool for the transfer of wild barley genes to wheat (Miller et al. 1982), the development of addition lines is not the most efficient approach for chromosome transfer from a wild to a cultivated species. With the molecular tools currently available (mainly in situ hybridization and molecular markers), specific chromosomes from any *H. chilense* accession could be introduced into bread wheat, if the corresponding hexaploid tritordeum is available, through the hybrid wheat-tritordeum of genomic formula AABBDH and backcrossing to wheat (Martín et al. 1997).

In situ hybridization and morphological and isozyme markers are convenient tools for identifying addition lines or direct chromosome introgression. However, these tools have limited use in tracking small segments of *H. chilense* chromatin in a wheat background because they either lack resolution or mark only a small fraction of the *H. chilense* genome. Among the several marker systems currently available, random amplified polymorphic DNAs (RAPDs) (Welsh and McClelland 1990; Williams et al. 1990) represent a type of genetic marker that can be generated efficiently and without any previous knowledge of the genome to be studied. In the case of *H. chilense*, the use of RAPDs has not shown the limitations in the generation of polymorphism that have appeared with cultivated cereals like wheat (Devos and Gale 1992), and it has been possible to localize chromosome-specific RAPD markers detectable in a wheat background (Hernández et al. 1996).

Communicated by G. Wenzel

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Nevertheless, there are several problems associated with the slight but sometimes critical lack of reliability of the RAPD phenotypes (Weeden et al. 1992). These phenomena have been particularly evident in our breeding program, when we attempt to amplify specific markers across different genetic backgrounds. Thus, this source of error compromises the value of RAPDs for marker-assisted selection (MAS).

To overcome the problems associated with RAPDs and to improve their utility in MAS, investigators have developed longer, more specific primers from the sequences of RAPD fragments (Paran and Michelmore 1993). These longer primers generate a sequence tagged site (STS) in the terminology of Olsen et al. (1989), which can be particularly useful for following the inheritance of the marked region of the genome. Such markers have been developed for many plant species including lettuce (Paran and Michelmore 1993), common bean (Adam-Blondon et al. 1994), grape (Xu et al. 1995) and apple (Cheng et al. 1996). This conversion significantly improves the reproducibility and reliability of polymerase chain reaction (PCR) assays. In the study presented here we report the development of STS markers in the amphiploid × *Tritordeum* and their use for both the identification of six of the seven *H. chilense* chromosomes in a wheat background as well as the marker-assisted introgression of *H. chilense* in wheat.

Materials and methods

Plant material

We used a set of wheat (cv 'Chinese Spring') – *H. chilense* accession H1 addition lines (Miller et al. 1982) lacking chromosome 3H and the two parental lines (wheat and barley) for the chromosome location of *H. chilense*-specific RAPDs. Addition lines for chromosomes 4H, 5H, 6H and 7H were disomic. A ditelosomic addition line for the 1H short arm, an addition line of chromosome 1H lacking one of the long arms and a ditelosomic for 2H alpha arm were used.

To develop accession-specific markers we included the *H. chilense* accession H7. H1 and H7 belong to two very different morphological and ecophysiological groups of the species. The wheat background was tested using: (1) two accessions of bread wheat cv 'Chinese Spring' (referred to as T21) as well as an advanced wheat breeding line (referred to as T20); (2) the octoploid tritordeum lines HT20 (amphiploid H7 × T20) and HT21 (amphiploid H1 × T21). The F_2 from the cross H1 × H7 was developed to test the segregation of polymorphic RAPDs and corresponding STSs.

DNA extraction

DNA was extracted from young leaves using the method of Lassner et al. (1989) as modified by Torres et al. (1993). The concentration of each sample was estimated by comparing band intensity with lambda DNA controls of known concentrations after ethidium bromide staining of 0.8% agarose gels following electrophoresis.

RAPD analysis

Conditions for DNA amplification were standardized for all primers. PCR reactions (20 µl) contained 20–40 ng of genomic DNA, 1 U *AmpliTaq* DNA Polymerase Stoffel Fragment from Perkin-Elmer (Norwalk, Conn.), 100 µ*M* of each dNTP from Boehringer-Mannheim (Mannheim, Germany), 0.32 µ*M* primer, 4 m*M* $MgCl_2$, 50 m*M* KCl and 10 m*M* TRIS-HCl, pH 8.3. Ten-mer primers of arbitrary sequence were obtained from Operon Technologies (Alameda, Calif.). In order to increase polymorphism, we also tested mixtures of primers in pairwise combinations (0.32 µ*M* each). Amplification was performed in a System 9600 cycler from Perkin-Elmer. The cycling was performed as follows: 94°C for 3 min, followed by 40 cycles of amplification (94°C/20 s, 35°C/20 s, then increased to 72°C/1 min with a ramp of 1°C per second) and by a 7-min final extension at 72°C. Amplification products were maintained at 12°C until loaded onto a gel. The products were resolved by electrophoresis on gels consisting of 1% SeaKem agarose: 1% NuSieve agarose from FMC (Rockland, Me.) and TBE buffer. Gels were stained with 0.5 µg/ml ethidium bromide and photographed with a GDS 5000 system CCD camera from UVP (Cambridge, UK).

Cloning and sequencing RAPD products and sequence homology analyses

The RAPD bands were excised from agarose gels and soaked in 20 µl of sterile distilled milliQ water. One-microliter aliquots were re-amplified using the same PCR reaction mixture that was originally employed to generate the RAPD, but the number of cycles was reduced to 25. The products were checked on agarose gels, purified by selective precipitation and end-polished with *Pfu* DNA polymerase. The blunt-ended PCR products were inserted into the *pCR-Script* vector following the procedures of the *pCR-Script* Amp SK(+) Cloning Kit from Stratagene (La Jolla, Calif.). The RAPD fragment OPS20 was cloned using the 'TA Cloning Kit' (pUC18 plasmid vector) from Invitrogen (Carlsbad, Calif.) following the manufacturer instructions. Cloned amplification products were cycle-sequenced with the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with *AmpliTaq* DNA Polymerase, FS from Perkin-Elmer/Applied Biosystems (Foster City, Calif.) using the automated ABI 373 Stretch DNA sequencer from the same manufacturer and optimized electrophoresis conditions, as described by Lario et al. (1997). Double-stranded DNA sequencing was carried out using both the 21M13 and M13 forward/reverse primers.

Homology searches were performed using the BLAST algorithm at the URL ⟨http://www.ncbi.nlm.nih.gov⟩ with the program *blastn*.

Designing STS primers and subsequent specific-amplification of genomic regions

Based on the sequences of the cloned RAPD products, pairs of oligonucleotide primers ranging from 16 to 30 bases in length were designed using the programs OLIGO 5.0 for MacOS from National Biosciences (Plymouth, MN, USA) and PRIMERSELECT 3.03 for MacOS from DNAStar (Madison, Wis.) for specific amplification of the loci identified by RAPD markers. Care was taken to avoid possible primer dimer or secondary structure formation and false priming and also to match melting temperatures and to achieve appropiate internal stability. In order to make the development of multiplexed PCRs possible, the molecular weight of the amplification product was also considered. Primers were synthesized by Operon Technologies or by Perkin-Elmer.

Amplification reactions were carried out in 20-µl solutions containing 0.5 u *AmpliTaq* Gold DNA Polymerase (Perkin-Elmer), 100 µ*M* of each dNTP (Boehringer-Mannheim), 0.16 µ*M* of each

primer, 2.5 m*M* $MgCl_2$, 50 m*M* KCl and 10 mM TRIS-HCl, pH 8.3. Amplification was carried out under the following conditions: 94°C for 10 min; 35 amplification cycles (15-s denaturation at 94°C, 20-s annealing, 1 min of extension at 72°C); and a final extension of 6 min at 72°C. The annealing temperature was empirically optimized for each primer pair. Amplified products were resolved by electrophoresis on 2% agarose gels as previously described.

Multiplexed PCR

STS primer pairs with similar annealing temperatures and generating PCR products of different lengths were used for multiplexed PCR. Amplifications were carried out in 20-µl reaction mixtures containing the same components as those used for STS analysis, except that each of the primers was included at a concentration of 1 µ*M*.

Results

Identification of RAPD markers for the *Hordeum chilense* chromosomes detectable in wheat background

We have previously described 16 RAPD markers specific to the *H. chilense* chromosomes detectable in wheat backgrounds (Hernández et al. 1996). One hundred and twenty-five additional 10-mer RAPD primers and 345 pairwise combinations were screened with the aim of identifying more chromosome markers. Examples of the detection of chromosome-specific markers are shown in Fig. 1. For instance, a 701-bp band corresponding to chromosome 5H is shown on lanes T21A5H(H1), corresponding to the chromosome 5H addition line of *H. chilense* (line H1) in wheat T21, as well as on lanes H1, HT21 (amphiploid H1 × T21), H7 and HT20 (amphiploid H7 × T20). Chromosome-specific RAPD products chosen for cloning and sequencing and their associated STS markers are listed in Table 1.

Pairwise combinations of primers were used to generate more RAPD markers. This has been reported to increase the degree of polymorphism in other organisms (Welsh and McClelland 1991). In our study, the total number of runs yielding H1–H7 polymorphism increased from 71% to 81% when pairwise combinations were used instead of single primers. Thus, this remains our main mapping strategy for *H. chilense*. Surprisingly, in the case of chromosome-specific markers of *H. chilense* detectable in a wheat background, the number of useful runs decreased from 30% to 18%

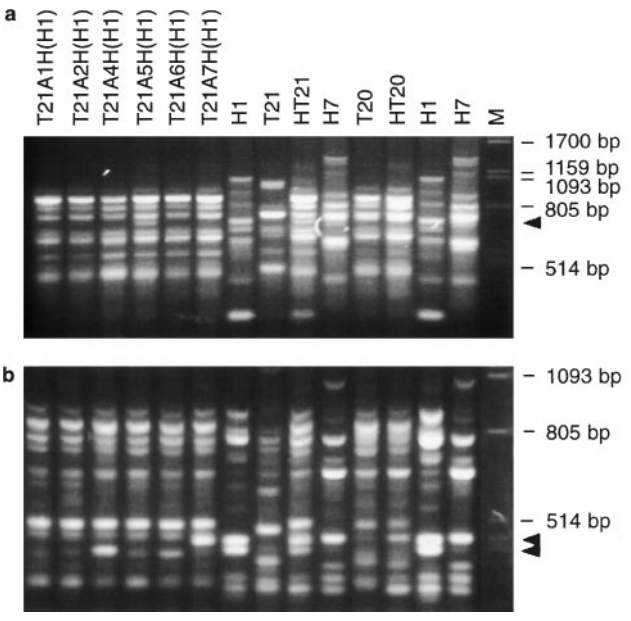

Fig. 1a, b RAPD amplification patterns. Primer OPS20 **a** generates a specific fragment for *H. chilense* chromosome 5H. Primer OPD16 **b** generates specific fragments for *H. chilense* chromosome 4H (accession H1) and 7H (accessions H1 and H7). *Lanes* from *left* to *right*: *T21A1H(H1) to T21A7H(H1)* *H. chilense* accession H1 in *T. aestivum* accession T21 addition lines (1H + telo, 2Hα, 4H, 5H, 6H, 7H), *H1 H. chilense* accession H1, *T21 T. aestivum* accession T21, *HT21 × Tritordeum* accession HT21, *H7 H. chilense* accession H7, *T20 T. aestivum* accession T20, *HT20 × Tritordeum* accession HT20, *M* molecular-weight marker. The position of the molecular markers in the addition line, *H. chilense* and × *Tritordeum*, are indicated by *arrows*

Table 1 RAPD products selected for sequencing and associated STSs

RAPD name	Chromosome	Accession		MW	Associated STS name	GenBank accession no.
		H1	H7			
R17-b	1	+	–	734	STHc1-3	AF028250
B6	2	+	+	335	STHc2-1	AF028252
E5-c	2	+	+	498	STHc2-2	AF028253
S13S14-a	4	+	+	363	STHc4-1	AF028254
K11K12-b	4	+	+	312	STHc4-2	AF028255
S20	5	+	+	701	STHc5-1	AF028258
S12-b	6	+	+	517	STHc6-1	AF028259
C13	6	+	–	320	STHc6-3	AF028261
D16-b	7	+	–	441	STHc7-1	AF028263
E5-a	7	+	+	848	STHc7-4	AF028266
S10S12-b	4, 7	+	+	277	STHc4&7-1	AF028268
D5D11-a	5	+	+	496	–	AF028272

upon the use of pairwise combinations. Nevertheless, it is still a cost-effective procedure to generate additional markers from available decamers.

Cloning and sequencing of PCR products, sequence homology searches, primer design and STS analysis

Twelve RAPD markers specific for the *H. chilense* chromosomes were chosen for cloning and sequencing (Table 1). Two different clones were forward and reversed sequenced for each RAPD product. Recombinant clones were obtained from most RAPD products using the *pCR-Script* SK (+) vector. Several attempts failed to produce recombinant clones from a fragment amplified by the primer OPR14, which is specific for chromosome 5H. Sequences were assembled using the SEQUENCHER 3.0 from Gene Codes (Ann Arbor, Mich.) software. The GenBank accession numbers of the consensus sequences appear in Table 1. As expected, the fragments carried one copy of the corresponding primer sequences at both ends. Sequence homology searches against nucleotide databases showed homologies with several plant protein sequences (Table 2).

Primer characteristics are shown in Table 3. Primers designed for the sequence AF028272 amplified the same fragment for both wheat and *H. chilense*, and therefore were not suitable for the detection of *H. chilense* chromosomes in wheat background. The rest of the primers successfully amplified the desired chromosome-specific fragment in a wheat background under the STS amplification conditions listed in Table 4.

STSs STHc4-1 (Fig. 2), STHc1-3, STHc1-4, STHc4-2, STHc5-1, STHc6-1, STHc6-3, STHc7-4, STHc7-5 and STHc4&7-1 (designated type 'a') produced a single band, whereas the STS STHc7-1 (type 'b', Fig. 2b), contained more than one specific band. The STS STHc2-1 (type 'c') showed one (durum) or two (bread) wheat amplification products in addition to the *H. chilense*-specific one.

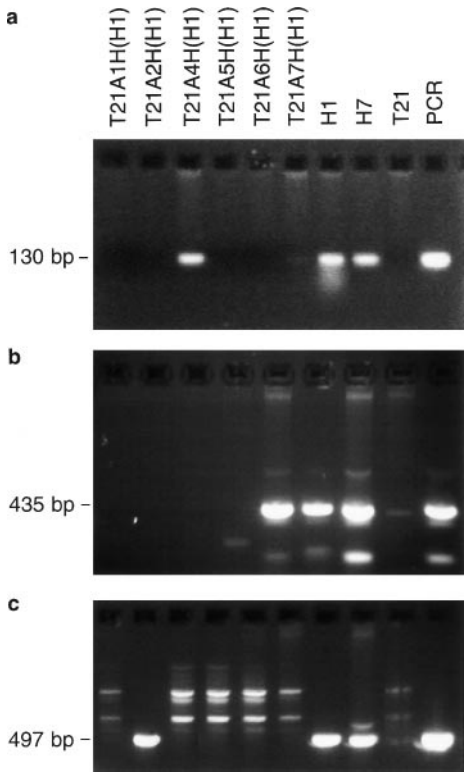

Fig. 2a–c STS amplification patterns. **a** shows an example of amplification type 'a', suitable for direct ethidium bromide detection (STHc4-1); **b** is the amplification of STHc7-1 (type 'b') and **c** is STHc2-2 (type 'c'). *Lanes* from *left* to *right*: *T21A1H(H1)* to *T21A7H(H1)* *H. chilense* accession H1 in *T. aestivum* accession T21 addition lines (1H + telo, 2Hα, 4H, 5H, 6H, 7H), *H1 H. chilense* accession H1, *H7 H. chilense* accession H7, *T21 T. aestivum* accession T21, *PCR* re-amplification of the excised original RAPD fragment

Up to three different *H. chilense* chromosomes were simultaneously detected in a wheat background by means of multiplexed PCR. An example is shown in Fig. 3.

Polymorphic STSs were tested in the F_2 from the cross H1 × H7; these cosegregated with their correspondent RAPD fragments and also displayed dominant expression. This is evidence, as expected, of the

Table 2 Homologies between the RAPD sequences and known plant proteins detected by the BLAST algorithm

RAPD sequence	Location (bases)	Homology (%)	Plant protein gene
AF028250	470-703	78	*Hordeum vulgare NK1558* gene for plastocyanin
	210-297	65	Barley *Amy* gene for α-amylase (clone gRAmy152)
AF028253	169-284	90	*Triticum aestivum* Cheynee α-gliadin gene
	276-377	60	
	40-79	80	
AF028259	235-272	81	*Brassica napus* (clone BnCh25) endochitinase gene
AF028272	36-459	50	*Antirrhinum majus TNP2* protein
	290-394	51	*Arabidopsis thaliana* extensin protein

Table 3 Primer sequences and positions

Primer name	Sequence (5′ → 3′)		Position[a] (5′ → 3′)	
	Forward primer	Reverse primer	Forward	Reverse
IAS-pHc1-3	ACGTAGAAGCGAAAGAAAAGATAAAGAGG	CGTACGTAGCCACAATGATATCCAA	5-33	710-734
IAS-pHc1-4	GGCATCCTGGACGTGAGTAATA	GTCTGAGCAGCCTCCTGTTC	86-107	252-271
IAS-pHc2-1	GCCCAAACCAACCTAACGAACCT	CCCTGGGGATGAATTAGGCACTT	7-29	286-308
IAS-pHc2-2	TCAGGGAGGTAATAATAGCATTTTTAA	CAGGGAGGTCGGAGGC	1-27	482-497
IAS-pHc4-1	TCCTGCCCTAAACCTCCTAGAC	TCAGCAAAATAAGCCAAGATGAG	6-27	113-135
IAS-pHc4-2	CTATGCCTAGCTAGGCGCATAA	GGATTTACTTGGCACAAACACTAA	54-75	194-217
IAS-pHc5-1	ACGGAGGATGTTCAAAATTGC	CGGAATATGAGGATTGATCTTGC	6-26	673-695
IAS-pHc6-1	GGTGAGTCTGGTTTACCTGCTG	GGTGAGTATTTTTTGGTGGTAGCTCT	4-25	490-514
IAS-pHc6-3	CTCGTCCACCACCTTCCA	CCTCGTCATAAAATAAATGATAACTTGC	5-22	290-317
IAS-pHc7-1	CGTAAGCCAGCCGTCCA	GGCGTAAGGTGCAAAAATGG	5-21	420-439
IAS-pHc7-4	GGAGGTTCAAGAAAAAACATACAATG	TCAGGGAGGTATGTTATATTATTGTTTAAC	5-30	819-848
IAS-pHc4&7-1	GGTGAGTATGTGAGAATTGAGATTGG	GTTCCAGCACCATCACAACC	4-29	256-275
IAS-pHcR3	GCTGTGTATGGGCACATGAAA	CTAGTCGAGTCATACATAATGAAACTCCT	5-25	469-497
IAS-pHcR4	GCCATTGTAACACTCAACTTGGA	GCGGACAAAGAAGAGAATTGG	4-26	473-493

[a] Position in the RAPD sequence

Fig. 3 Simultaneous detection of *H. chilense* chromosomes 1H, 6H and 7H in a wheat background by multiplexed PCR. Coamplification of the STSs STHc1-3 (specific for chromosome 1H), STHc6-3 (specific for chromosome 6H, line H1) and STHc7-1 (specific for chromosome 7H). *Lanes* from *left* to *right*: *T21A1H(H1)* to *T21A7H(H1)* *H. chilense* accession H1 in *T. aestivum* accession T21 addition lines (1H + telo, 2Hα, 4H, 5H, 6H, 7H), *H1 H. chilense* accession H1, *T21 T. aestivum* accession T21

collinearity between H1 and H7 STSs that allows the transfer of markers developed for these two accessions to the rest of the *H. chilense* species.

Discussion

Not very much is known about the nature of the DNA sequences amplified by the RAPD technique. In various plant species it has been reported that at least 45% of these sequences are highly repeated in the genome (Williams et al. 1990; Reiter et al. 1992). In this study, none of the sequenced RAPDs contained repeats. This is a logical outcome in view of the DNA fragment selection procedure employed to avoid shared sequences with both *H. chilense* and wheat genomes: the presence in only one of the addition lines plus the conditions of single *H. chilense* chromosome localization. These selection criteria have effectively eliminated the repetitive DNA sequences. In fact, genomic probing by Southern hybridization of total genomic DNA from barley-wheat amphiploid and parents with some biotinilated STSs amplification products have proved them to be single or low copy (data not shown).

Eleven RAPDs specific for different *H. chilense* chromosomes plus one specific for two chromosomes (4H and 7H) were selected for the development of STSs (Table 1). This approach has been quite efficient, permitting the development of chromosome-specific markers on a wheat background for each *H. chilense* chromosome except chromome 3H, whose addition line is not available. Of the 12 sequenced RAPDs 11 yielded the expected STSs. One of these (STHc2-1) was designed to amplify a chromosome 2H-specific marker for both H1 and H7 accessions. In addition to the target band, it amplified another one specific for accession H7. This additional DNA fragment is also linked to chromosome 2H. Considering that H1 and H7 are representative of the two very distinctive *H. chilense* morphological and ecophysiological groups (Martín

Table 4 Amplification conditions and other related information for each STS developed in this study

STS	Chromosome	Accession		T[a] (°C)	[Cl_2Mg] (m*M*)	MW of the PCR product	Primer position[b]	Amplification type[c]
		H1	H7					
STHc1-3	1	+	−	59	2.5	730	e	a
STHc1-4	1	−	+	68	2.5	186	i	a
STHc2-1	2	+	+	65	2.5	302	ei	c
	2	−	+					
STHc2-2	2	+	+	55	2.5	497	e	c
STHc4-1	4	+	+	67	2	130	ei	a
STHc4-2	4	+	+	68	2	164	i	a
STHc5-1	5	+	+	59	2.5	690	e	a
STHc6-1	6	+	+	59	2.5	511	e	a
STHc6-3	6	+	−	59	2.5	313	e	a
STHc7-1	7	+	+	59	2.5	435	e	b
STHc7-4	7	+	+	59	2.5	844	e	a
STHc4&7-1	4, 7	+	+	59	2.5	272	e	a

[a] Annealing temperature

[b] Primers were designed as an extension of the 10-mer RAPD primers (flanking the RAPD sequence); i, primers were internal to the RAPD sequence, ei, reverse primer was internal to the sequence, forward was flanking

[c] a, Amplification of a single band specific for an *H. chilense* chromosome; b, amplification of more than one *H. chilense*-specific product; c, amplification of one or two *H. chilense* chromosome-specific bands in addition to one or more wheat amplification products different from those of *H. chilense*

et al. 1997), the STS STHc2-1 has yielded both a general marker for the whole species and also an accession-specific one. As expected, this type of amplification has been observed in a sample of *H. chilense* acessions belonging to the H1 or H7 groups (data not shown). For instance, this and other STSs have been sucessfully used to identify H16 (type H1) addition lines on durum wheat. In the remaining 12 STSs, accession specificity has relied on the nature of the original RAPD polymorphism. Therefore, the STS approach has permitted the development of markers that can be used to assess the presence of *H. chilense* chromatin both in a wheat background as well as of a given accession.

Most of the chromosome 5H-specific RAPD markers appeared faint in the addition line profile. We sequenced 2 of these markers: OPS20 (AF028258) and D5D11-a (AF028272). The sequence information of the former led to the generation of a chromosome 5-specific STS (STHc5-1), and the latter amplified a DNA fragment both in wheat and barley. These results, the scarcity of specific markers and the subsequent amplification of wheat could suggest a greater homology of chromosome 5H with that of wheat. In addition, both sequences present some homology with maize retrotransposon sequences from the GenBank (data not shown).

STSs primer pairs, when derived from RAPD sequence information, are generally designed to include the 10-base sequence of the original RAPD primer. In this way, the amplification of the same original DNA fragment is obtained, requiring only sequence information for the ends of the amplicon. The significant sequence polymorphism presented between *H. chilense* and wheat genomes permitted the use of a number of sequences within the RAPD fragment for priming while maintaining the polymorphism observed after amplification. Thus, the design of compatible primers for multiplexed PCRs was facilitated.

To accomplish chromosome manipulation in the Triticeae, we must have cost-effective methods for the detection of chromosomes, chromatin or alien genes. Chromosome banding, in situ hybridization and RFLPs have become useful methods for obtaining addition and translocation lines. Nevertheless, they are laborious and technically demanding and, therefore, not the methods of choice when a large number of plants need to be assessed. In our experience, RAPDs have proven very useful markers when the genetic background is known and the amphiploid is available as a control (see Fig. 1). In spite of that, there are bands which are prominent in the *H. chilense* RAPD profile while very faint both in the amphiploid and in the corresponding addition line (data not shown). Furthermore, there are RAPD amplification products that cannot be scored when the wheat genetic background is changed. Sometimes there is a wheat product of the same molecular weight which interferes with the detection of the *H. chilense* products. On other occasions, the dissapearance of a *H. chilense* product on a wheat background is the result of a competence or a "context" effect, due to the competition for primers between many PCR products (McClelland and Welsh 1995). In this study, there is an additional complexity due to the genetic background and ploidy level of the genotype where a given RAPD product is to be detected. *H. chilense* RAPD profiles are relatively simple in comparison to those of wheat for the same oligonucleotide. In many cases, a prominent *H. chilense* product is

difficult to detect in a wheat background. The development of STSs offers the possibility to solve these problems easily, economically and efficiently. Currently, we are successfully using the STSs presented in this paper for the development of introgressions and sets of addition lines of *H. chilense* in both bread and durum wheat.

Out of the 11 STSs, 8 produced the amplification of a single band for *H. chilense*. STSs STHc2-1 and STHc2-2 generated one or more wheat amplification products. When the wheat amplification products are robust (e.g. STHc2-1, showing one amplification product in durum wheat and two in bread wheat) they serve as positive controls of amplification both for the STS alone and in multiplexed PCRs, as they are always present when there is wheat DNA in the reaction. Amplifications produced by the STS STHc2-2 (Fig. 2c) also contain an internal amplification control, even when the wheat product does not appear when the target band is present (i.e. in the addition line).

Three of the sequenced RAPDs show homologies at different degrees with known plant protein sequences (Table 2). We consider that only 1 of these could have a biological meaning: AF028250. It is located on *H. chilense* chromosome 1. It presents two zones of homology with *H. vulgare* genes also located on chromosome group 1: the first one (bases 470–703) with a 'high score' of 399 with the plastocyanin gene (the highest found in this study) and the second (bases 480–645) with a 'high score' of 170 with an α-amylase gene (Table 2). The first homology has a reading initiation codon at reading phase 6 (position 480).

In summary, the development of STS markers from RAPDs has proven to be a robust, quick and efficient methodology for evaluating the introgression of *H. chilense* in wheat. This result is particularly relevant given the scarcity of sequence information in this species, thus paving the way for future marker–assisted selection wheat breeding programs.

Acknowledgements The first author acknowledges the "Consejo Superior de Investigaciones Científicas" for the award of a fellowship. Financial support from the CICYT, Madrid, Spain (Project numbers AGF95-0964-C02-01 and AGF98-0945-C02-01) is also acknowledged. The experiments described in this work comply with the current laws of Spain and the USA.

References

Adam-Blondon AF, Sévignac M, Bannerot H, Dron M (1994) SCAR, RAPD and RFLP markers linked to a dominant gene (*Are*) conferring resistance to anthracnose in common bean. Theor Appl Genet 88:865–870

Bothmer RV, Jacobsen N, Baden C, Jorgensen RB, Linde-Laursen I (1995) An ecogeographical study of the genus *Hordeum* vol 7. IPGRI, Rome

Cheng FS, Weeden NF, Brown SK (1996) Identification of codominant RAPD markers linked to fruit skin color in apple. Theor Appl Genet 93:222–227

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

Fedak G (1992). Intergeneric Hybrids with *Hordeum*. In: Shewry PR (ed) Barley: genetics, biochemistry, molecular biology and biotechnology, vol 5. C.A.B. Int, Wallingford, UK, pp 45–68

Hernández P, Rubio MJ, Martin A (1996) Development of RAPD markers in tritordeum and addition lines of *Hordeum chilense* in *Triticum aestivum*. Plant Breeding 115:52–56

Lario A, González A, Dorado G (1997) Automated laser-induced fluorescence DNA sequencing: equalizing signal-to-noise ratios significantly enhances overall performance. Anal Biochem 247:30–33

Lassner MW, Peterson P, Yoder JI (1989) Simultaneous amplification of multiple DNA fragments by polymerase chain reaction in the analysis of transgenic plants and their progeny. Plant Mol Biol Rep 7:116–128

Martín A, Martínez-Araque C, Rubiales D, Ballesteros J (1996) Tritordeum: triticales's new brother cereal. In: Guedes-Pinto H, Darvey N, Carnide VP (eds) Triticale: today and tomorrow. Kluwer Academic Publ, Dordecht, pp 57–72

Martín A, Martín LM, Cabrera A, Ramírez MC, Giménez MJ, Rubiales D, Hernández P, Ballesteros J (1997) The potential of *Hordeum chilense* in breeding Triticeae species. In: Jaradat AA (ed) Int Triticeae Symp. Science Publishers, Inc. Enfield, NH, USA, pp 377–386

McClelland M, Welsh J (1995) DNA fingerprinting using arbitrarily primed PCR. In: Dieffenbach CW, Dveksler GS (eds) PCR primer. A laboratory manual. Cold Spring Harbor Laboratory Press, New York, pp 203–211

Miller TE, Reader SM, Chapman V (1982) The addition of *Hordeum chilense* chromosomes to wheat. In: Broertjes C (ed) Proc Int Symp Eucarpia Induced Variability Plant Breed. Pudoc, Wageningen, pp 79–81

Olsen M, Hood L, Cantor C, Botstein D (1989) A common language for physical mapping of the human genome. Science 245:1434–1435

Paran I, Michelmore R (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Person-Dedryver F, Jahier J, Miller TE (1990) Assessing the resistance to cereal root-knot nematode, *Meloidogyne naasi*, in a wheat line with the added chromosome arm 1HchS of *Hordeum chilense*. J Genet Breed 44:291–296

Reiter RS, Williams JGK, Feldmann KA, Rafalski JA, Tingey SV, Scolnick K (1992) Global and local genome mapping of *Arabidopsis thaliana* by using recombinant inbreds and random amplified polymorphic DNAs. Proc Natl Acad Sci USA 89:1477–1481

Torres AM, Weeden NF, Martín A (1993) Linkage among isozyme, RFLP and RAPD markers in *Vicia faba*. Theor Appl Genet 85:937–945

Weeden NF, Timmerman GM, Hemmat M, Kneen BE, Lodhi MA (1992) Inheritance and reliability of RAPD markers. In: Applications of RAPD technology to plant breeding. Crop Science Society of America, Minneapolis, Minn., pp 12–17

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Welsh J, McClelland M (1991) Genomic fingerprinting with AP-PCR using pairwise combinations of primers: application to genetic mapping of the mouse. Nucleic Acids Res 19:5275–5279

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

Xu H, Wilson DJ, Arulsekar S, Bakalinsky AT (1995) Sequence-specific polymerase chain-reaction markers derived from randomly amplified polymorphic DNA markers for fingerprinting grape (*Vitis*) rootstocks. J Am Soc Hortic Sci 120:714–720