Theor Appl Genet (2002) 104:735–739 © Springer-Verlag 2002

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Utility of barley and wheat simple sequence repeat (SSR) markers for genetic analysis of *Hordeum chilense* and tritordeum

Received: 20 November 2000 / Accepted: 12 April 2001

Abstract A selection of 36 wheat and 35 barley simple sequence repeat markers (SSRs) were studied for their utility in *Hordeum chilense*. Nineteen wheat and nineteen barley primer pairs amplified consistent *H. chilense* products. Nine wheat and two barley SSRs were polymorphic in a *H. chilense* mapping population, producing codominant markers that mapped to the expected homoeologous linkage groups in all but one case. Thirteen wheat and 10 barley primer pairs were suitable for studying the introgression of *H. chilense* into wheat because they amplified *H. chilense* products of distinct size. Analysis of wheat/*H. chilense* addition lines showed that the *H. chilense* products derived from the expected homoeologous linkage groups. The results showed that wheat and barley SSRs provide a valuable resource for the genetic characterization of *H. chilense*, tritordeums and derived introgression lines.

Keywords *Hordeum chilense* · Tritordeum · Introgression · Marker-assisted selection · Mapping · Microsatellite · SSR

Introduction

Wild species have great variability and are potential sources of novel genetic variation for crop improvement. The characterization of genetic variability in wild species and the development of tools to introduce it into cultivated crops are important plant-breeding goals. *Hordeum chilense* Roem et Schult. (2n=2*x*=14) belongs to a heterogeneous group of South American *Hordeum* species (Sec. *Anisolepsis* Nevski, Bothmer et al. 1995). It exhibits great morphological variability that has been sampled by collection expeditions (Tobes et al. 1995; Giménez et al. 1997). Its crossability with bread and durum wheat (*Triticum aestivum* and *Triticum durum*, respectively) makes it a valuable source of new traits for wheat breeding. Important traits already identified include resistance to the root-knot nematode *Meloidogyne naasi* on chromosome $1H^{ch}S$; tolerance to salt on chromosomes $1H^{ch}$, $4H^{ch}$ and $5H^{ch}$; resistance to *Septoria tritici* on chromosome $4H^{ch}$; and high carotenoid pigment content and resistance to common bunt, both located on chromosome $7H^{ch}$ (Martín et al. 1998). In addition, the potential of octoploid and hexaploid tritordeums (amphiploids between *H. chilense* and hexaploid or tetraploid wheats, respectively) to be used as new cereals (Martín 1988) is an additional source of interest in the species.

The introgression of novel traits requires the identification of recombinants between *H. chilense* and wheat chromosomes. In situ hybridization readily distinguishes *H. chilense* chromosomes or chromosome segments from those of wheat (Cabrera et al. 1995), but trait transfer would be greatly facilitated if introgressions could be characterized by molecular markers. RAPD markers (random amplified polymorphic DNA: Welsh and McClelland 1990; Williams et al. 1990) are easy to produce, and *H. chilense* chromosome-specific RAPD markers detectable in a wheat background have been developed (Hernández et al. 1996). However, their use in wheat introgression is limited because their amplification is very much affected by the wheat background (Hernández et al. 1996). This problem can be overcome by converting selected RAPD bands to SCAR markers (sequence-characterized amplified regions: Paran and Michelmore 1993; Hernández et al. 1999).

Simple sequence repeat (SSR or microsatellite) markers are potentially superior to RAPDs because of their co-dominant nature and suitability for automation. SSRs can be identified using DNA database searches

Communicated by H.C. Becker

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(Akkaya et al. 1992; Devos et al. 1995) but this is not feasible for *H. chilense* where relatively few sequences are currently available. High development costs also make it impractical to develop them directly from wild species like *H. chilense*. However, if SSR markers developed in related crop species can be used, genetic analysis of *H. chilense* could advance rapidly.

In the present work, SSR markers from barley (*Hordeum vulgare*) and wheat (*T. aestivum*) were tested to determine their usefulness for mapping, introgression and variability studies in *H. chilense*. Wheat SSR markers were predominantly from the D-genome because previous work suggested that *H. chilense* is more closely related to the D- than to the A- or B-genomes. This was shown by means of in situ hybridization with probe pAs1 (Cabrera et al. 1995) as well as the ability of *H. chilense* chromosomes to compensate for D wheat chromosomes in substitution lines (S. Reader, personal communication).

Material and methods

Plant material

H. chilense lines H1 and H7, *H. vulgare* cv 'Betzes' and hexaploid wheat cv 'Chinese Spring' were used for the initial SSR marker-transferability study. For the chromosome location of the transferred SSR markers, a set of wheat (cv 'Chinese Spring')/*H. chilense* accession H1 addition lines (Miller et al. 1982) was used together with the two parental lines. Addition lines for chromosomes $4H^{ch}$, 5 H^{ch}, 6 H^{ch} and 7 H^{ch} were disomic. A ditelosomic addition line for 1 $H^{ch}S$, an addition line of chromosome 1 H^{ch} lacking one of the long arms, and a ditelosomic for the 2 H^{ch} alpha arm were also used. Linkage analysis was performed using an F_2 population of 114 plants from an H1×H7 cross.

DNA extraction

DNA was extracted from young frozen leaf tissue using the CTAB method of Murray and Thompson (1980) as modified by Hernández et al. 2001. The concentration of each sample was estimated by comparing their band intensities with lambda DNA controls of known concentrations after ethidium bromide staining of 0.8% agarose gels subjected to electrophoresis.

PCR amplification and SSR analysis

PCR primer sequences from bread wheat (Röder et al. 1998) and barley (Liu et al. 1996) were tested. Amplification reactions were carried out in 10-µl volumes containing 25 ng of DNA, 0.5 units of *Taq* DNA Polymerase (Roche, Basel, Switzerland), 200 µM of each dNTP (Roche), 0.2 µM of each primer, 2.5 mM $MgCl_2$, 50 mM KCl and 10 mM Tris-HCl, pH 8.3. Cycling conditions were as follows: an initial denaturation step of 4 min at 94°C was followed by 30 amplification cycles (30-sec denaturation at 95°C, 1-min annealing, 1-min extension at 72°C) and a final extension of 5 min at 72°C. Annealing temperatures and other details are given in Tables 1 and 2. Aliquots of each PCR reaction (2.5 µl) were run on sequencing gels (6% polyacrylamide, 8 M urea) under standard conditions, and the products were visualized by silver staining using the method described by Bassam et al. (1991) with minor modifications.

Results

Analysis of wheat SSR markers

H. chilense DNA from accessions H1 and H7 was tested with 36 wheat SSR primer pairs developed by Röder et al. (1998) and the results are summarized in Table 1. All the markers, with the exception of gwm6, were from the D-genome of wheat but four (gwm165, gwm205, gwm249 and gwm639) amplified from more than one wheat genome.

Nineteen primer pairs (53%) gave reproducible amplification products from *H. chilense* DNA and would be useful for mapping and germplasm assessment. Six of the nineteen (gwm6, gwm165, gwm249, gwm469, gwm565 and gwm639) gave PCR products of a similar size range in *H. chilense* and wheat, while in the remainder the *H. chilense* products were smaller. Eight (22%) showed polymorphism between H1 and H7 (Fig. 1) and

Table 1 Characterization of wheat SSR markers in *H. chilense*

SSR	T_{ann}	Product size range in wheat[a]	Product size range in *Hordeum chilense*	Chromosome location[b]	Mapped[c]
gwm6	55	196–207	182–205	4B, $4H^{ch}$	Yes ($4H^{ch}$)
gwm37	60	189	172–178	7D	No
gwm165	60	188–261	180–205	4 A, 4B, 4D, $4H^{ch}$	Yes ($4H^{ch}$)
gwm192	60	191–232	125–148	4D, $4H^{ch}$	Yes ($4H^{ch}$)
gwm205	60	143–158	124	5 A, 5D, $5H^{ch}$	No
gwm249	55	150–180	175–190	2 A, 2D, $2H^{ch}$	Yes ($2H^{ch}$)
gwm271	60	179	134–154	5D, $5H^{ch}$	No
gwm314	55	171–182	115–123	3D, $6H^{ch}$	Yes ($6H^{ch}$)
gwm469	60	118–172	114–210	6 A, 6D	No
gwm565	60	142–150	135–150	5D, $5H^{ch}$	Yes ($5H^{ch}$)
gwm608	60	144–181	108–111	2D, 4D, $4H^{ch}$	No
gwm639	55	130–170	135–145	5 A, 5B, 5D, $5H^{ch}$	Yes ($5H^{ch}$)
gwm642	60	179–187	155–179	1D, $1H^{ch}$	Yes ($1H^{ch}$)

[a] From Röder et al. (1998)
[b] From analysis of wheat 'Chinese Spring'/*H. chilense* H1 addition lines
[c] Mapped in the *H. chilense* H1×H7 F_2 population

Table 2 Characterization of barley SSR markers in *H. chilense*

SSR	T_{ann}	Product size in barley[a]	Product size range in *H. chilense*	Chromosome location[b]	Mapped in *H. chilense*[c]
HVM4	55	198	150–155	7H, $7H^{ch}$	No
HVM5	55	202	145–205	7H	No
HVM20	55[c]	151	95–105	1H, $1H^{ch}$	No
HVM22	55[d]	167	160–170	6H, $6H^{ch}$	No
HVM23	60[e]	246	226	2H, $2H^{ch}$	No
HVM31	55[c]	163	166–167	6H, $6H^{ch}$	No
HVM54	55[c]	159	135–165	2H, $2H^{ch}$	Yes ($2H^{ch}$)
HVM64	55[c]	253	210–215	1H, $1H^{ch}$	No
HVM65	55[c]	129	120–123	6H, $6H^{ch}$	Yes ($6H^{ch}$)
HVLEU	55	166	166	5H, $5H^{ch}$	No

[a] From Liu et al. (1996)
[b] From analysis of wheat 'Chinese Spring'/*H. chilense* H1 addition lines
[c] Mapped in the *H. chilense* H1×H7 F_2 population
[d] A 18-cycle touchdown of 1°C decrease per cycle starting at 64°C was incorporated
[e] A 18-cycle touchdown of 1°C decrease per cycle starting at 69°C was incorporated

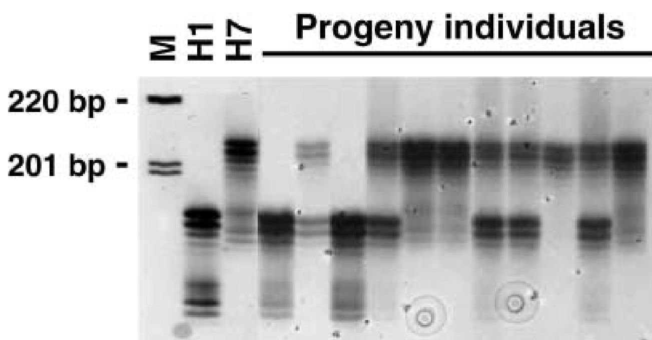

Fig. 1 Polymorphism for SSR gwm165 between *H. chilense* H1 and H7, and segregation in 11 F_2 progeny

were mapped in the F_2 population. Their chromosome locations were consistent with the results from wheat, suggesting that they amplified homoeologous sequences (Table 1).

Thirteen primer pairs (36%) were useful for introgression studies because the *H. chilense* amplification products in tritordeums or addition lines were clearly distinguishable in size from those of the wheat parent (Fig. 2). The remaining six primer pairs (17%) gave no *H. chilense* band from tritordeum or the addition lines, even though the *H. chilense* products had smaller molecular weights than wheat and would therefore be expected to amplify efficiently. The reason for the lack of amplification of the *H. chilense* sequence was probably competition from wheat sequences with higher homology to the primers. Even in cases where the *H. chilense* product was successfully amplified from tritordeum or addition lines, it was usually less intense than in *H. chilense* itself. For example, the *H. chilense* PCR fragment was amplified more efficiently in lanes H1 and H7 than in the addition line T21A5H(H1) (Fig. 2). The same effect is observed in the amphiploid (lane HT21). The amphiploid DNA amplification, representing an equimolecular 'mix' of the *H. chilense* genome and the three wheat genomes, can be considered as a control, and the

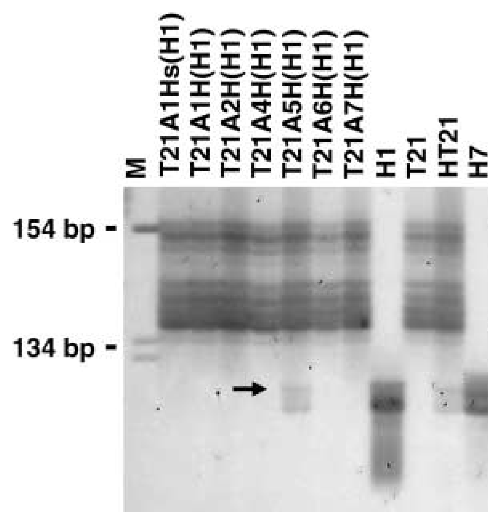

Fig. 2 Location of SSR gwm205 to chromosome $5H^{ch}$ by amplification of wheat/*H. chilense* addition lines. The main *H. chilense* amplification product is indicated by an *arrow*. *Lanes* (left to right) are as follows: *1* molecular-weight marker, *2 to 8 T. aestivum* (accession T21)/*H. chilense* (accession H1) addition lines $1H^{ch}s$, $1H^{ch}$+telo, $2H^{ch}\alpha$, $4H^{ch}$, $5H^{ch}$, $6H^{ch}$ and $7H^{ch}$; *9 H. chilense* accession H1; *10 T. aestivum* accession T21; *11* HT21 tritordeum amphiploid (H1×T21); *12 H. chilense* accession H7

less-efficient amplification of the *H. chilense* fragment is also attributable to an effect of competition in the PCR reaction.

Analysis of barley SSR markers

Thirty five barley SSR markers (Liu et al. 1996) were tested using the same approach and gave comparable results (Table 2). Nineteen (54%) primer pairs gave reproducible amplification products from *H. chilense* DNA. However, only two (6%) showed polymorphism between H1 and H7 and could be mapped in the progeny of the H1×H7 cross. Their chromosome location was consistent with the corresponding barley marker.

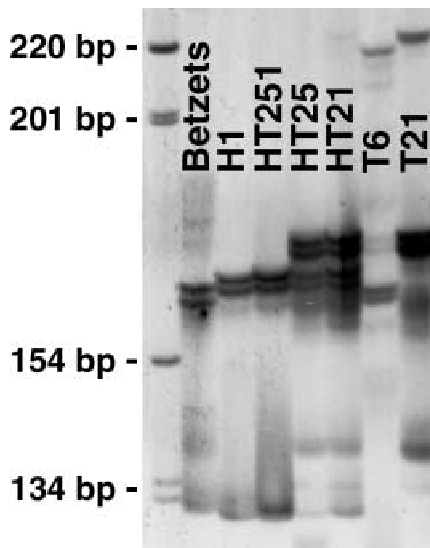

Fig. 3 Amplification of SSR HVM31 in *H. chilense*, wheat and tritordeum, showing its usefulness in assessing introgression. *Lanes* (left to right) are as follows: *H. vulgare* 'Betzes'; *H. chilense* accession H1; tetraploid tritordeum HT251 (H108×T6), hexaploid tritordeum HT25 (H1×T22); octoploid tritordeum HT21 (H1×T21); *Aegilops squarrosa* accession T6; *T. aestivum* accession T21

Ten barley primer pairs (29%) were useful for studying introgression. This is illustrated in Fig. 3 which shows the amplification of HVM31 in a series of tritordeums and parental lines. Analysis of addition lines showed that the *H. chilense* products were amplified from the expected homoeologous chromosomes (Table 2). Eight of the ten gave amplification products of similar sizes in *H. chilense* and barley, while HVM4 and HVM20 gave smaller amplification products in *H. chilense*. As with the wheat SSR markers, amplification of the *H. chilense* product was usually weaker when wheat sequences were also present, probably because of competition in the PCR.

The remaining nine markers (17%) failed to show the *H. chilense* product in tritordeums or the addition lines. In common with the wheat SSR markers that behaved in the same way, the *H. chilense* amplification products were smaller than those from barley. Competition from wheat sequences was a likely reason for failure to amplify the *H. chilense* product.

Overall levels of transferability

The present study yielded 23 SSR markers suitable for studying the introgression of *H. chilense* into wheat, which was 32% of the primer pairs tested. In addition, 11 codominant markers (15%) were found that could be mapped in the H1×H7 cross. These levels of transferability show that wheat and barley SSRs are a valuable source of markers for studying tritordeums and derived introgression lines, and are suitable for genetic mapping and germplasm characterisation within *H. chilense* itself.

Discussion

Previous work has shown that SSR primer pairs developed for one species can be used in close relatives (see Peakall et al. 1998 for a review), but transferability in the Gramineae has generally been poor where this has been tested (e.g. Brown et al. 1996). This is illustrated by studies in hexaploid wheat where most SSR markers are genome specific and few amplify from related species such as barley (Bryan et al. 1997; Röder et al. 1998; Stephenson et al. 1998). However, this is not always the case as a recent study has found a high level of transferability of SSR markers between maize and *Miscanthus* (Hernández et al. 2001).

There is little information available to predict the transferability of SSR markers, but their ease of assay enables them to be tested empirically. As large numbers of SSR markers are being developed in wheat and barley, even a relatively low level of transferability provides a valuable marker resource. In the present case more than 50% of wheat and barley primer pairs amplified from *H. chilense* and would be applicable for mapping and germplasm surveys. Some of the *H. chilense* products failed to amplify in tritordeum or the addition lines, probably because of preferential amplification of the wheat sequences, but 36% of wheat and 29% of barley markers could be used for analysis of tritordeums and derived introgression lines. These figures clearly show that wheat and barley SSRs constitute efficient and cost-effective sources of molecular markers for *H. chilense*.

The results are in accordance with the general trend in mammalian SSRs, which have been reported to amplify shorter, less-polymorphic fragments in related species (Ellegren et al. 1997). This can be clarified by analysis of a larger sample of *H. chilense* accessions.

Transferable SSR markers help to link the genetic maps of *H. chilense* to those of barley and wheat, which in turn provide a link to other grasses. Thus, the *Xgwm165* locus from wheat (Röder et al. 1998) has proved useful for aligning the group-4 maps of wheat and barley (Ivandic et al. 1999) as well as *H. chilense*. There is, however, an exception: *Xgwm314* has a different chromosome location in wheat (3D) than in *H. chilense* ($6H^{ch}$). This could be a case of amplification of different loci, as has been reported for wheat where some SSR markers amplify non-homoeologous loci in different genomes (Röder et al. 1998).

In conclusion, wheat and barley SSR markers are a valuable source of polymorphic markers for analysing the relatively unknown *H. chilense* gene pool, for studying *H. chilense* introgression into wheat, for genetic mapping and for alignment of genetic maps with other Gramineae species. Twenty three *H. chilense* chromosome-specific markers have become available in this study and many more should become available from current efforts to generate larger collections of wheat and barley SSR markers.

Acknowledgements Financial support from the 'Ministerio de Ciencia y Tecnologia', Spain, from the Spanish-British concerted action HB1998–0199 and from the CICYT, Spain (project number AGF98–0945-C02–01), is gratefully acknowledged. The authors are grateful to Dr. Marion Röder, IPK, Germany, for providing SSR primers. The experiments carried out in this work complied with the laws of the UK.

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