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A core genetic map of *Hordeum chilense* and comparisons with maps of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*)

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Abstract The first genetic map of the wild South American barley species *Hordeum chilense* is presented. The map, based on an F_2 population of 114 plants, contains 123 markers, including 82 RAPDs, 13 SSRs, 16 RFLPs, four SCARs, two seed storage proteins and two STS markers. The map spans 694 cM with an average distance of 5.7 cM between markers. Six additional SSRs and seven additional SCARs which were not polymorphic were assigned to chromosomes using wheat/*H. chilense* addition lines. Polymorphisms were revealed by 50% of the RAPD amplifications, 13% of wheat and barley SSR primers, and 78% of the Gramineae RFLP anchor probes. The utility of SSR and RFLP probes from other Gramineae species shows the usefulness of a comparative approach as a source of markers and for aligning the genetic map of *H. chilense* with other species. This also indicates that the overall structure of the *H. chilense* linkage groups is probably similar to that of the B and D genomes of wheat and the H genome of barley. Applications of the map for tritordeum and wheat breeding are discussed.

Keywords Anchor probes · Comparative mapping · Marker-assisted breeding · Microsatellites · RAPD · RFLP · SCAR

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Introduction

Hordeum chilense Roem et Schult. (2n = 2*x* = 14) is an extremely variable species belonging to a heterogeneous group of South American *Hordeum* species (Sec. *Anisolepsis* Nevski, Bothmer et al. 1995). It can be crossed with species from the genera *Aegilops*, *Agropyron*, *Dasypyrun*, *Hordeum*, *Secale*, and *Triticum* (Fedak 1992), and disomic addition lines of *H. chilense* in bread wheat (*Triticum aestivum*) have been developed (Miller et al. 1982). The use of *H. chilense* in breeding programmes has focused on two main areas. The first is the development of octoploid and hexaploid tritordeums (amphiploids between *H. chilense* and hexaploid or tetraploid wheats, respectively) to be used as new cereals (Martín 1988). The second is the introgression into wheat of novel traits including: 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* on chromosome $4H^{ch}$; and high carotenoid pigment content and resistance to common bunt, both located on chromosome $7H^{ch}$ (Martín et al. 2000).

The addition lines are a useful starting point for the transfer of novel traits to wheat, but the introgression of specific characters requires the identification of recombinants that remove unwanted regions of the *H. chilense* chromosome. The physical size of introgressed segments can be assessed by in situ hybridization, which readily distinguishes *H. chilense* chromosomes from those of wheat (Cabrera et al. 1995), but introgression would be greatly facilitated by the availability of a genetic map of *H. chilense* which would provide molecular markers linked to the genes of interest. A genetic map would also establish the extent of colinearity between *H. chilense* chromosomes and those of wheat. If translocations or inversions are present, introgression may produce chromosomally unbalanced progeny that would be expected to have reduced fitness. The implications of translocations for introgression have been discussed with reference to rye (*Secale cereale*) by Devos et al. (1993).

Genetic maps are fundamental to understanding the genetic control of plant characters and have numerous applications in research and plant breeding. Many important crop plants have well-developed genetic maps, but their wild relatives are generally poorly understood despite their potential value as sources of novel traits for crop improvement. Genetic mapping of wild species is facilitated by a comparative genetic approach which utilizes markers developed in crop species. cDNA clones used as RFLP (restriction fragment length polymorphism) probes have proved particularly effective for aligning the genetic maps of different cereals (Ahn et al. 1993; Kurata et al. 1994; Moore et al. 1995; Van Deynze et al. 1995). RFLPs are reproducible, co-dominant and locus-specific. However, they present some drawbacks that limit their later use in marker-assisted selection, including the use of radioisotopes, the laboriousness of the technique, and the large amount of DNA needed.

PCR-based methods, in contrast, are simple to use and require very small amounts of DNA. RAPD markers (random amplified polymorphic DNA, Welsh and McClelland 1990; Williams et al. 1990) are easy to produce, but their usefulness in mapping is limited by their dominant nature and their non-random distribution in genetic maps (Nilsson et al. 1997, Saliba-Colombani et al. 2000). Moreover, they have limited use in wheat introgression because they are very affected by the wheat background (Hernández et al. 1996). These problems can be overcome by converting selected RAPD bands to SCAR markers (sequence-characterized amplified regions: Paran and Michelmore 1993; Hernández et al. 1999b). Simple sequence repeat (SSR or microsatellite) markers are superior to RAPDs and SCARs because of their co-dominant nature and their potential for automation, but their high development costs makes it impractical to derive them from wild species like *H. chilense*.

The approach taken in this work was therefore to use RAPDs to develop a series of markers quickly, and to use RFLP and SSR markers from other cereals to consolidate the linkage groups and to align them with existing maps. *H. chilense* addition lines were used to assign markers to specific chromosomes (Hernández et al. 1995, 1999a). This paper describes the production and characteristics of a preliminary *H. chilense* map and briefly discusses its applications for breeding tritordeum and wheat.

Materials and methods

Plant material

The population used for linkage analysis was an F_2 of 114 plants from the cross H1 × H7. H1 and H7 belong to very different morphological and ecophysiological groups of *H. chilense* (Martín et al. 1998). Five of the 114 initial plants (4%) died a t different developmental stages and did not reach maturity. For the chromosome location of *H. chilense* specific 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. The 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, a ditelosomic for 2 H^{ch} alpha arm, and ditelosomic lines for 6H^{ch} S and 6H^{ch}L were also used.

DNA extraction

DNA was extracted from young frozen leaf tissue using the CTAB method of Murray and Thompson (1980) with some modifications. Tissue was frozen, ground in liquid nitrogen and, before the tissue thawed, 3 ml of CTAB buffer containing 0.5% sodium bisulfite and 25 mM of dithiothreitol (DTT) were added. The solution was incubated for 40 min at 65°C with occasional mixing. After the tissue was extracted once with an equal volume of 24:1 chloroform/octanol, DNA was precipitated with isopropanol, spooled out using a glass hook, washed in 70% ethanol and air-dried. The DNA was dissolved in 500 µl of TE buffer. RNase was added (0.03 mg) and the DNA was dissolved overnight at 4°C. The DNA stock solution s were diluted 1:20 in water for PCR-amplification. For RFLP analysis, the DNA solution was extracted once with phenol:chloroform:isoamylalcohol (24:24:1) and alcohol-precipitated.

RFLP analysis

Genomic DNA from the parents and the mapping population wa s digested with *Eco*RI, *Eco*RV, *Hind*III, *Bam*HI or *Dra*I from Roche (Basel, Switzerland). Electrophoresis, Southern blotting and hybridization after labelling of probes with [^{32}P] were carried out following standard procedures and using 12µg of DNA per lane. The mapped RFLP probes include 16 from the ''anchor'' sets used for comparative mapping of grass genera (Van Deynze et al. 1998), the barley genomic clone PSB44, and the probes XRHch2–1 and XRHch6–1 derived from cloned RAPD markers (unpublished results).

RAPD analysis

Approximately 20–40 ng of genomic DNA were used in amplification reactions, following the protocol described by Hernández et al. (1996). Amplifications were initiated either by single primers or by pairwise combinations of the oligonucleotides. Random 10-mer primers were purchased from Operon Technologies (Alameda, Calif., USA) and the *AmpliTaq* DNA Polymerase Stoffel Fragment from PE Biosystems (Foster City, Calif., USA). Amplification was performed in a System 9600 thermocycler from the same manufacturer. The amplified products were resolved by electrophoresis on gels consisting of 1% (w/v) SeaKem agarose: 1% (w/v) NuSieve agarose from FMC (Rockland, Me., USA), and TBE buffer. Gels were stained with 0.5 µg of ethidium bromide/ml, and photographed with a GDS 5000 system CCD camera from UVP (Cambridge, UK). RAPD marker names correspond to the primer/primer combination ID (OP_) followed by the band order from higher to lower molecular weight.

SCAR and STS analysis

Amplification reactions were carried out in 20-µl solutions containing 0.5 units of *AmpliTaq* Gold DNA Polymerase from PE Biosystems, 100 µM of each dNTP from Roche, 0.16 µM of each primer, 2.5 mM $MgCl_2$, 50 mM 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 primer sequences and annealing temperatures used appear in Hernández et al. (1999a, b) or are available from the first author. Amplified products were resolved by electrophoresis in 2% agarose gels as described above.

Microsatellite analysis

PCR primers were obtained from PE Biosystems. Amplification reactions were carried out in 20 µl as described in the previous section. SSR primer sequences and amplification conditions appear in Liu *et al*. (1996) or Röder et al. (1998). Amplified products were resolved by electrophoresis in 3.5% metaphor agarose gels (FMC). When the polymorphisms were not resolved in agarose gels, forward primers were labelled with either blue (6-FAM), green (TET) or yellow (HEX) fluorescent tags (PE Biosystems), and resolved on an ABI 310 capillary DNA sequencer (P-E Biosystems). Amplification reactions were then carried out in 8-µl volumes.

Seed storage proteins analysis

Endosperm protein extraction was performed as reported in Alvarez et al. (1993). Electrophoresis was carried out in vertical SDS-PAGE gels at a 10% (w/v, C=1.28%) polyacrylamide concentration, and 4 M urea in a Tris-HCl/glycine buffer system (Laemmli 1970). Gels were stained overnight with 12% (w/v) trichloroacetic acid solution containing 5% (v/v) ethanol and 0.05% (w/v) Coomassie Brilliant Blue R-250.

Linkage analysis

Estimation of linkage used JoinMap version 2.0 software (Stam and Van Ooijen 1995). All the RAPD markers that did not fit the expected 3:1 ratio were excluded for the assignment of markers to linkage groups. Grouping of markers into linkage groups was based on a LOD score ≥4. A LOD of 0.01 and a recombination threshold of 0.49 was used for mapping. In a third step, RAPDs showing skewed segregation ratios were assigned to the linkage groups. Map distances were based on Kosambi's mapping function.

Results and discussion

Map construction

Polymorphisms were revealed by 50% of 470 RAPD amplifications. A total of 261 RAPD bands were scored of which 196 fitted a 3:1 segregation ratio and were selected for mapping. This initial data set produced 12 linkage groups. In general, markers in coupling were not merged with those in repulsion phase. In order to complete the seven linkage-group map, additional marker types were studied. This showed that 13% of 101 wheat and barley SSR primers, 78% of 37 Gramineae RFLP anchor probes, and 2% of 50 the wheat and barley STS markers tested were polymorphic in the *H. chilense* cross. The addition of ten SSRs, 15 RFLPs, three SCARs, two seed storage protein polymorphisms and one STS marker consolidated the maps into seven linkage groups, each of which could be assigned to a specific *H. chilense* chromosome by reference to markers detected in the addition lines.

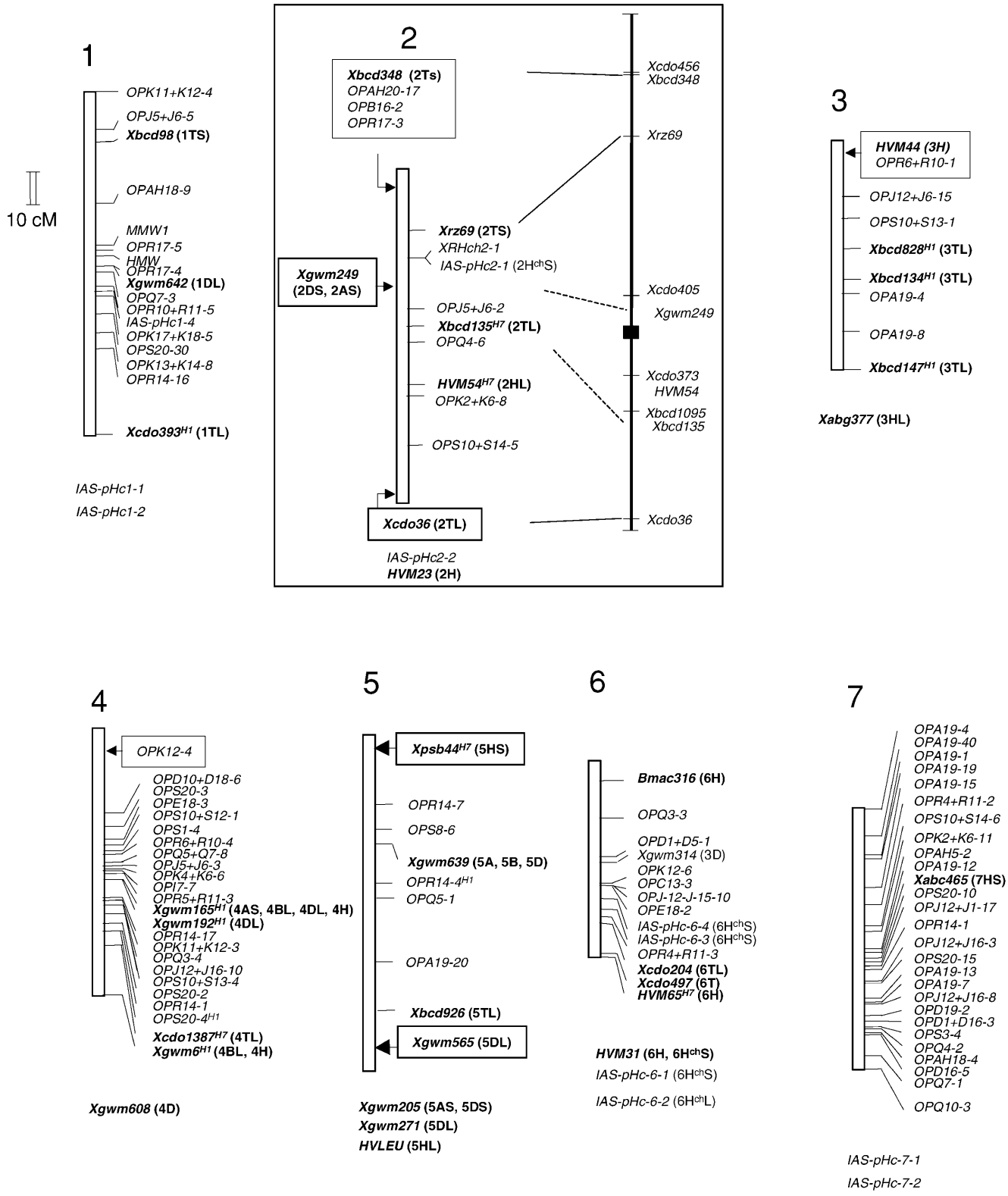
Although seven linkage groups were produced, their genetic lengths were unexpectedly high and RAPD markers in coupli ng and repulsion phases were sub-clustered. A similar clustering pattern was observed when data were analyzed with Mapmaker (Lander et al. 1987). Difficulties in mapping using RAPD datasets have been reported previously due to their dominant nature (Säll and Nilsson 1994) or the effects of competition (Hallden et al. 1996). Similar clustering effects were studied in detail in a high-density map of sugar beet (Nilsson et al. 1997). In that work, more than a third of the segregating RAPD markers were discarded during the mapping procedure compared to only 4% of the RFLPs.

In addition to problems arising directly from the dominant nature of RAPD markers, we suspected that the resolution power of agarose gels was inadequate. To test this we ran individual RAPD reactions in agarose and in 6% non-denaturing polyacrylamide gels. About 40% of the bright-strong bands in agarose gels were resolved as two to five bands in polyacrylamide gels, showing that this may be an additional source of error when RAPDs are used for mapping. Consequently, the RAPD data set was reanalyzed to discard 'noisy' markers. This was performed by using the JoinMap jmdia module. Markers not fitting the hyperbolic curve (LOD scores vs recombination values) were discarded. In this way, the RAPD data set was reduced to 42% (83) of the initial 3:1 markers.

After re-examining the RAPD data a total of 113 markers comprising 79 RAPDs, ten SSRs, 15 RFLPs, three SCARs, two seed storage proteins and one STS could be placed unambiguously on the genetic map (Fig. 1). This core map spanned 694 cM with an average distance of 5.7 cM between markers. Additionally, three RAPDs, one RFLP, one SCAR, two SSRs and one STS were placed only in their most-likely intervals, because they increased map size or because JoinMap did not include them at a LOD>4. This gave a map of 119 markers. Six additional SSRs and seven additional SCARs were assigned to chromosomes using the available addition line s. Thus, Fig. 1 shows 132 markers in total.

Genetic distortion

As described in the Materials and methods section, 4% of the progeny of the H1×H7 cross died at different developmental stages before flowering. This fact, together with the high segregation distortion found in some chromosomal regions (Fig. 1), may suggest genetic differentiation between groups within *H. chilense*. There are additional data supporting this suggestion including cytoplasm differentiation and reaction against diseases. H1 cytoplasm gives rise to male sterility when, after backcrossing, alloplasmic bread wheat lines are obtained, while H7 cytoplasm is phenotypically indistinguishable from wheat. With respect to disease resistance, *H. chilense* accessions belonging to the H7 type show an avoidance mechanism against leaf rust of similar effect to the non-host reaction, while H1 type accessions are hosts to leaf rust (Martín et al. 1998). Additionally, the different viability of addition lines in bread wheat for accessions H1 and H7 also suggests differentiation between the populations. As an example, the addition line of chromosome 3 for H1 has not been obtained, while it has been obtained for population H7 (unpublished results).


1
10 cM
OPK11+K12-4
OPJ5+J6-5
Xbcd98 (1TS)
OPAH18-9
MMW1
OPR17-5
HMW
OPR17-4
Xgwm642 (1DL)
OPQ7-3
OPR10+R11-5
IAS-pHc1-4
OPK17+K18-5
OPS20-30
OPK13+K14-8
OPR14-16
Xcdo393H1 (1TL)
IAS-pHc1-1
IAS-pHc1-2
2
Xbcd348 (2Ts)
OPAH20-17
OPB16-2
OPR17-3
Xgwm249 (2DS, 2AS)
Xrz69 (2TS)
XRHch2-1
IAS-pHc2-1 (2HchS)
OPJ5+J6-2
Xbcd135H7 (2TL)
OPQ4-6
HVM54H7 (2HL)
OPK2+K6-8
OPS10+S14-5
Xcdo36 (2TL)
IAS-pHc2-2
HVM23 (2H)
Xcdo456
Xbcd348
Xrz69
Xcdo405
Xgwm249
Xcdo373
HVM54
Xbcd1095
Xbcd135
Xcdo36
3
HVM44 (3H)
OPR6+R10-1
OPJ12+J6-15
OPS10+S13-1
Xbcd828H1 (3TL)
Xbcd134H1 (3TL)
OPA19-4
OPA19-8
Xbcd147H1 (3TL)
Xabg377 (3HL)
4
OPK12-4
OPD10+D18-6
OPS20-3
OPE18-3
OPS10+S12-1
OPS1-4
OPR6+R10-4
OPQ5+Q7-8
OPJ5+J6-3
OPK4+K6-6
OPI7-7
OPR5+R11-3
Xgwm165H1 (4AS, 4BL, 4DL, 4H)
Xgwm192H1 (4DL)
OPR14-17
OPK11+K12-3
OPQ3-4
OPJ12+J16-10
OPS10+S13-4
OPS20-2
OPR14-1
OPS20-4H1
Xcdo1387H7 (4TL)
Xgwm6H1 (4BL, 4H)
Xgwm608 (4D)
5
Xpsb44H7 (5HS)
OPR14-7
OPS8-6
Xgwm639 (5A, 5B, 5D)
OPR14-4H1
OPQ5-1
OPA19-20
Xbcd926 (5TL)
Xgwm565 (5DL)
Xgwm205 (5AS, 5DS)
Xgwm271 (5DL)
HVLEU (5HL)
6
Bmac316 (6H)
OPQ3-3
OPD1+D5-1
Xgwm314 (3D)
OPK12-6
OPC13-3
OPJ-12-J-15-10
OPE18-2
IAS-pHc-6-4 (6HchS)
IAS-pHc-6-3 (6HchS)
OPR4+R11-3
Xcdo204 (6TL)
Xcdo497 (6T)
HVM65H7 (6H)
HVM31 (6H, 6HchS)
IAS-pHc-6-1 (6HchS)
IAS-pHc-6-2 (6HchL)
7
OPA19-4
OPA19-40
OPA19-1
OPA19-19
OPA19-15
OPR4+R11-2
OPS10+S14-6
OPK2+K6-11
OPAH5-2
OPA19-12
Xabc465 (7HS)
OPS20-10
OPJ12+J1-17
OPR14-1
OPJ12+J16-3
OPS20-15
OPA19-13
OPA19-7
OPJ12+J16-8
OPD19-2
OPD1+D16-3
OPS3-4
OPQ4-2
OPAH18-4
OPD16-5
OPQ7-1
OPQ10-3
IAS-pHc-7-1
IAS-pHc-7-2

RFLP, RAPD and SSR markers: which is more convenient?

The highest level of polymorphism was detected with RFLPs, whereas the RAPD technique was very simple and should allow analysis of a large part of the genome and the identification of bands suitable for conversion to SCARs, which would be more suitable for marker-assisted selection. However, a large proportion of RAPDs had to be discarded. The advantages of SSRs compared to RAPD and RFLP markers are well documented (Gupta et al. 1999) but the high cost of development is not justified for a wild species like *H. chilense*, with limited direct agricultural value. However, given the large number of SSR markers being developed in wheat and barley, the levels of transferability observed should be sufficient for many *H. chilense* applications.

Alignment of linkage groups with other species

The data from marker order and the assignment of markers to addition lines suggested an overall similarity between *H. chilense* chromosomes and those of the B and D genomes of wheat and the barley H genome (Fig. 1). For example, there was no evidence of the known chromosome 4 A pericentric inversion of wheat (Nelson et al. 1995a), marked by microsatellite *Xgwm165* (Röder et al. 1998). Comparison of the $2H^{ch}$ map with a consensus map of the group-2 chromosomes of wheat was possible and this suggests that colinearity is maintained (Fig. 1). Overall similarity is consistent with the ability of *H. chilense* chromosomes to compensate for wheat chromosomes in substitution lines (S. Reader, personal communication). However, the number of RFLP and SSR markers studied is limited and several chromosome arms contain no such ''anchors''. Therefore, it will be necessary to analyse additional markers to determine the full relationship of *H. chilense* chromosomes to those of other Triticeae species.

These conclusions are consistent with previous work which suggested that *H. chilense* is more closely related to the D genome of wheat than to the A and B genomes. This was shown by means of in situ hybridization with the probe pAs1 (Cabrera et al. 1995) as well as the preferential transferability of D-genome microsatellites (P. Hernandez, unpublished results), the efficacy of chromosomal substitutions (S. Reader, personal communication), and the high fertility of amphiploids between *H. chilense* and wheats at the three ploidy levels (Martín et al. 1998).

H. chilense possesses traits that could be of interest for wheat (durum and bread) breeding programs, including male sterility, quality traits (pigments and proteins) and resistance to biotic and abiotic stresses. The present map provides a useful starting point for the practical use of *H. chilense* germplasm in marker-assisted wheat and tritordeum breeding projects.

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◀ **Fig. 1** Linkage map of *H. chilense* obtained from an F_2 population derived from a cross between lines H1 and H7. *Boxes* on the left of the chromosome represent the most-likely location of markers excluded from the map because they increased map size, or because they were placed at a LOD <3. Markers with a *suffix* H1 or H7 showed significant segregation distortion in favour of the H1 or H7 parent, respectively. Marker nomenclature: *Xbcd*, *Xcdo*, *Xrz* and *Xpsb* are loci detected using Gramineae RFLP probes; *RHch* are RFLP probes derived from *H. chilense* RAPD markers, *OP_* RAPD markers (operon primer followed by band order from higher to lower molecular weight), *Xgwm_* wheat microsatellite markers (Röder et al. 1998); *HVM_* barley microsatellite markers (Liu et al. 1996), *Bmac316* barley microsatellite marker from the Scottish Crop Research Institute, *IAS-pHc_ H. chilense* SCARs, *Xabc465*, *Xabg377* barley STS s (http://greengenes.cit.cornell.edu). ''Anchor'' markers (http://greengenes.cit.cornell.edu:80/anchors/) were previously mapped in wheat (A, B or D), barley (H) or both (T) and so allow alignment of the maps. Comparison between *H. chilense* chromosome $2H^{ch}$ and wheat is also shown (*boxed*). *Xbcd*, *Xcdo* and *Xrz* markers on the consensus map are from the Graingenes database (http://wheat/pw.usda.gov/ggpages/linemaps/Wheat/Trit2.html). Placement of *Xgwm249* was by alignment with the wheat 2 A and 2D maps of Röder et al. (1998) with the consensus map. Placement of *Xbcd135* was by alignment of the map of Nelson et al. (1995b) with the consensus map. The barley SSR marker *HVM54* could not be aligned to the wheat consensus map directly

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