

Development of wild barley (*Hordeum chilense*)-derived DArT markers and their use into genetic and physical mapping

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Received: 5 July 2011 / Accepted: 18 October 2011 / Published online: 3 November 2011
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Abstract Diversity arrays technology (DArT) genomic libraries were developed from *H. chilense* accessions to support robust genotyping of this species and a novel crop comprising *H. chilense* genome (e.g., tritordeums). Over 11,000 DArT clones were obtained using two complexity reduction methods. A subset of 2,209 DArT markers was identified on the arrays containing these clones as polymorphic between parents and segregating in a population of 92 recombinant inbred lines (RIL) developed from the cross between *H. chilense* accessions H1 and H7. Using the segregation data a high-density map of 1,503 cM was constructed with average inter-bin density of 2.33 cM. A subset of DArT markers was also mapped physically using a set of wheat–*H. chilense* chromosome addition lines. It allowed the unambiguous assignment of linkage groups to chromosomes. Four segregation distortion regions (SDRs) were found on the chromosomes 2H^{ch}, 3H^{ch} and 5H^{ch} in

agreement with previous findings in barley. The new map improves the genome coverage of previous *H. chilense* maps. *H. chilense*-derived DArT markers will enable further genetic studies in ongoing projects on hybrid wheat, seed carotenoid content improvement or tritordeum breeding program. Besides, the genetic map reported here will be very useful as the basis to develop comparative genomics studies with barley and model species.

Introduction

Cereals account for over half of all calories consumed by humans. The progressive narrowing of the genetic basis in crops (Tanksley and McCouch 1997; Warburton et al. 2006) is a serious threat and constitutes an important limitation in breeding novel traits. Wild species serve as a resource for breeding since they usually exhibit large genetic variability which can be introduced into breeding programs. *Hordeum vulgare* spp. *spontaneum* is being used in barley breeding (Alsop et al. 2011; Inostroza et al. 2009; Matus et al. 2003) and *Aegilops tauschii*, donor of the D genome of common wheat, is being used to develop new synthetic wheat (van Ginkel and Ogbonnaya 2007). Other distant relatives such as *Triticum urartu*, donor of the A genome, have been also proposed for durum wheat breeding (Alvarez et al. 2009; Rodríguez-Suárez et al. 2011c).

Hordeum chilense Roem. et Schult. is a diploid wild barley included in the section *Anisolepis* Nevski, native to Chile and Argentina. *H. chilense* shows potentially useful traits for wheat breeding including resistance to several diseases and pests and abiotic stress tolerance (Martin et al. 1996). Besides, it could widen the genetic basis for grain quality traits of wheat due to its variability in prolamins (Alvarez et al. 1999a; Atienza et al. 2000, 2005) or high

Communicated by M. Xu.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-011-1741-2) contains supplementary material, which is available to authorized users.

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seed carotenoid content (Alvarez et al. 1999b; Atienza et al. 2007b; Ballesteros et al. 2005; Rodríguez-Suárez et al. 2011a). *H. chilense* also offers interesting variability at cytoplasm level. Indeed, *H. chilense*–wheat alloplasmic lines, i.e., lines with the same nucleus but cytoplasm from different species, may result in either self-fertile (Atienza et al. 2007d) or male-sterile lines (Martin et al. 2008) depending on the *H. chilense* accession used as cytoplasm donor. As a consequence, a new source of cytoplasmic male sterility (CMS) for hybrid wheat production is being studied at present for its exploitation, as fertility restorer lines have been obtained introgressing *H. chilense* chromatin onto wheat (Martin et al. 2008, 2009, 2010).

A major interest in this species relates to its high crossability with other members of the Triticeae tribe: *Aegilops*, *Agropyrum*, *Dasypirum*, *Secale*, *Triticum*, and \times *Triticosecale* (Von Bothmer et al. 1986; Martin et al. 1998). Crosses between *H. chilense* and wheat lead to fertile amphiploids named tritordeums. Hexaploid tritordeum (\times *Tritordeum* Ascherson et Graebner $2n = 6x = 42$; AABBH^{ch}H^{ch}) is the amphiploid derived from the cross between *H. chilense* and durum wheat. At present, a breeding program is being conducted at IAS-CSIC aimed to develop tritordeum as a new crop (Atienza et al. 2007c). In addition to its new crop potential, tritordeum is also useful as a genetic bridge for introducing genetic variability from *H. chilense* into wheat (Martin et al. 1996).

The efficient exploitation of the full potential of *H. chilense*, either for tritordeum or for wheat breeding, requires application of modern breeding tools. Molecular markers are useful tools for genetic studies, and have enhanced the precision of genetic analysis in a number of crops. Molecular markers, including RAPDs (Hernández et al. 2001) and AFLPs (Vaz Patto et al. 2001) have been developed de novo for *H. chilense*. The advance in model crop species have allowed the development of candidate gene approaches to tag specific traits of interest such as threshing ability (Gil-Humanes et al. 2009), endosperm storage proteins (Piston et al. 2004, 2005, 2006) or carotenoid content (Atienza et al. 2007a). Besides, comparative genomics have allowed the transfer of wheat and barley molecular markers to *H. chilense* (Castillo et al. 2008, 2010a; Hagrais et al. 2005a, b; Nasuda et al. 2005). These markers proved useful for physical mapping (Atienza et al. 2007c; Cherif-Mouaki et al. 2011; Said and Cabrera 2009) and diversity studies (Castillo et al. 2008, 2010a) but their density is insufficient for detailed genetic studies.

Linkage mapping is essential in the identification of loci affecting quantitative traits such as grain quality. Preliminary *H. chilense* maps with RAPDs and RFLPs (Hernández et al. 2001) and AFLPs (Vaz Patto et al. 2003) were developed using a F₂ population derived from the cross between *H. chilense* accessions H1 and H7. These maps

allowed some progress in QTL identification for leaf rust resistance (Vaz Patto et al. 2003) or endosperm yellow pigment content (Atienza et al. 2004). However, they do not provide sufficient genome coverage to enable precise genetic dissection of complex characters. Until now, the high costs of technology development have made establishment of molecular markers directly from wild species like *H. chilense* impractical (Castillo et al. 2008). However, the recent trend in cereal molecular breeding is to use high-throughput and cost-efficient hybridization-based markers such as single nucleotide polymorphism (SNP) and diversity array technology (DArT). While barley SNP markers (OPA1) (Close et al. 2009) are not directly transferable to *H. chilense* (own unpublished results), DArT markers offer an interesting alternative since they do not require previous knowledge of the sequence. Indeed, this technology has been successfully applied to a large number of species with limited information at the sequence level such as rye (Supriya et al. 2011), oat (Oliver et al. 2011), pearl millet (Badea et al. 2011) or the *Festuca/Lolium* complex (Bartoš et al. 2011).

We report the development of *H. chilense*-derived DArT markers; the physical mapping of a set of these markers to be used as anchor points for genetic mapping; the development of a genetic map of the RIL population H1 \times H7. We also report the existence of segregation distortion zones in chromosomes 2, 3 and 5 of *H. chilense*.

Materials and methods

Plant materials

The study was based on *H. chilense* (lines H1 and H7), common wheat cv. Chinese Spring (CS), wheat (CS)-*H. chilense* addition lines for complete chromosomes 1H^{ch}, 4H^{ch}, 5H^{ch}, 6H^{ch} and 7H^{ch} (named CS MA 1H^{ch}-1 H^{ch}S, CS DA 4H^{ch}, CS DA5H^{ch}, CS DA6H^{ch} and CS DA7H^{ch}, respectively, where MA refers to monosomic addition and DA means disomic addition), the wheat- (CS)-*H. chilense* ditelosomic addition lines CS DA1H^{ch}S, CS DA2H^{ch}S, CS DA5H^{ch}L, CS DA6H^{ch}S, CS DA7H^{ch} α , CS DA7H^{ch} β , and the recombinant inbred line population (RIL population, RI₇) derived from the cross H1 \times H7 and having 92 progeny.

DArT analyses

Complexity reduction

Genomic representations for microarray preparation and for genotyping were prepared using two complexity reduction methods as previously described (Jaccoud et al. 2001; Wenzl et al. 2004; Akbari et al. 2006).

DNA preparation and genomic representations

DNA was isolated from 1 g of bulked leaf tissue of seedlings. Each *H. chilense* accession (both parental and RILs) was represented by five plants. A sample of H1 and H7 was used for making the representation in order to develop the DArT arrays. These accessions belong to two different germplasm groups as reported in diversity studies (Castillo et al. 2010a, b). Importantly, these two accessions are the parents of our mapping population.

Hordeum chilense-derived markers were produced by Diversity Arrays Technology Pty. Ltd. with standard DArT protocols for *PstI/TaqI* ('bPt' markers) and *PstI/BstMI* ('bPb' markers) digestion-based array development (Jaccoud et al. 2001; Wenzl et al. 2004; Akbari et al. 2006).

The mapping progeny and the H1 and H7 parents were genotyped using arrays containing 15,360 clones from various cultivated and wild *H. vulgare* barley libraries including 5,376 DArT markers from *H. vulgare* and 9,984 *H. chilense*-derived markers. A total of 94 samples were assayed (H1, H7 and 92 progenies). The same arrays were applied in a parallel work on tritordeum diversity including the wheat–*H. chilense* addition lines described in the plant materials. Chromosome addition lines were analyzed in duplicate. Markers with a *P* value (*P* is the variance of the relative hybridization intensity between allelic states as a percentage of the total variance of the relative hybridization intensity, Wenzl et al. 2006) and a call rate both greater than 80% and discordance lower than 0.005 were selected. Markers with a quality parameter *P* between 77 and 80 were incorporated on a case-by-case basis.

Other markers used in this study

A set of 70 conserved orthologous set (COS) markers (Quraishi et al. 2009) was used for amplification in *H. chilense*. PCR reactions were performed in a total of 100 µl reaction mixture consisting of 50 ng of genomic DNA, 2.4 U of Taq (Biotools B&M labs, Madrid), 1 × PCR buffer, 1.5 mM MgCl₂, 0.32 mM dNTPs (Promega, Madison, WI, USA) and 0.24 µM of each primer. PCR amplifications were carried out as follows: an initial denaturation step at 94°C for 5 min, 35 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, with a final extension step at 72°C for 7 min. Digestion with restriction enzymes *AfaI*, *ApaI*, *BamHI*, *BglI*, *BglIII*, *BspT104I*, *Clal*, *Dra I*, *EcoRI*, *EcoRV*, *EcoT14I*, *HaeIII*, *HapII*, *HindIII*, *HinfI*, *KpnI*, *MvaI*, *PstI*, *PvuII*, *SacI*, *Sall*, *SmaI*, *XbaI* and *XhoI* (Takara Bio Inc., Japan) were carried out according to manufacturer's instructions using 10 µl of PCR in a final volume of 20 µl.

PCR reactions for EST amplification were performed in a total 20 µl reaction mixture consisting of 10 ng of

genomic DNA, 0.6 U of Taq (Biotools B&M Labs, Madrid), 1 × PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs (Promega, Madison, WI, USA) and 0.225 µM of each primer. PCR amplifications were carried out as follows: 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 50.5°C and 1 min at 72°C, followed by 7 min at 72°C. Barley SSR markers were amplified as described by (Varshney et al. 2007). *Psy1_Hch* was genotyped as described by (Atienza et al. 2007a).

Segregation and linkage analysis

JoinMap 4.0 mapping software (Van Ooijen 2006) was used for segregation and linkage analysis. Redundant markers (with the same scoring patterns) were automatically excluded from the linkage analysis by JoinMap. Mendelian segregation was tested by Chi-square goodness-of-fit to a 1:1 ratio. Markers significantly deviating from mendelian segregation were excluded in the first mapping round. LOD thresholds (from LOD 6.5 to LOD 12.5) were tested to group the markers, until a LOD threshold was obtained that resulted in the optimum number of markers in linkage group in which physical mapped markers were correctly separated. Linkage groups were assigned to chromosomes using markers physically mapped using wheat–*H. chilense* addition lines.

Maps were constructed using the ELM algorithm implemented in JoinMap 4.0. The map was calculated using the Monte Carlo Maximum Likelihood Mapping algorithm (ML–Mapping) module of JoinMap and the Kosambi mapping function. For each chromosome, several rounds of mapping were performed by excluding markers producing ambiguities (definitely excluded) or co-segregating until a stable order was obtained. Redundant markers were included in the final map in the same position as the mapped marker with identical segregation pattern. Heavily distorted markers were used in linkage analyses after the initial map was finished. Several linkage groups were recalculated considering these markers and the process described above (see “Results” section). The program RECORD 2.0 (Van Os et al. 2005) was used to compare the order of molecular markers within linkage groups. All map figures included in the manuscript were produced utilizing MapChart software (Voorrips 2002).

Results

DArT development and mapping dataset

The DArT marker discovery phase produced over 11,000 clones using two complexity reduction methods where *PstI* was used for fragment selection as rare cutter (adapters

were ligated only to *PstI* ends) and “frequent cutters” *BstNI* and *TaqI* were used to further reduce the complexity of *PstI* representations. Around 40% of markers in each method are in common, with the remainder being method specific. A set of 2,209 DArT markers selected for the quality criteria explained in the “Materials and methods” section were used for mapping. Out of these, 968 were ‘bPb’ markers (derived from *PstI/BstNI* method) and 1,241 were ‘bPt’ markers (derived from *PstI/TaqI* method). This set of markers was mainly composed of *H. chilense*-derived markers (95%) but it also included *H. vulgare* markers (5%).

A limited number of other (non-DArT) markers were added to a final set of 2,270 markers. A set of 70 COS markers (Quraishi et al. 2009) were tested. These markers were uniformly distributed across wheat chromosomes according to physical mapping. Ten markers per chromosome were selected (Supplementary File 1). Fifty-seven COS amplified in *H. chilense* which corresponds to a potential transferability rate of 81.4% for this marker type. However, only 9 markers were polymorphic between H1 and H7 which supposes a 12.85% of utility for mapping in our progeny. The remaining 13 either did not amplify or yielded non-specific amplification and were excluded from further analysis. Five markers produced direct polymorphism while the rest were tested for cleaved amplified polymorphism (CAP) using a set of 24 restriction enzymes. In spite of this significant effort invested in this SNP search polymorphisms were only uncovered for seven additional markers. The mapping dataset was complemented with three barley SSRs (GBM1411-1H; GBM1432-7H and GBM1464-7H, one gene from the carotenoid pathway and 1 SCAR (Hernández et al. 2001). Forty-three loci derived from barley ESTs were also included (Supplementary File 1). These markers were derived from primers yielding more than one amplification product, and thus these markers are not useful for direct comparative mapping without sequencing.

Physical mapping of a subset of markers

A subset of 450 DArT markers was used in a parallel work on application of DArT markers to tritordeum breeding (to be published elsewhere) where the wheat *H. chilense* chromosome addition lines were included. This allowed the physical mapping of this subset of markers. Each line was genotyped in duplicate with the exceptions of CS MA-1H^{ch}-(single analysis) and CS DA-1H^{ch}S (analyzed in triplicate). A total of 41 markers gave signal in CS and thus, they could not be physically mapped. Additionally, 122 markers were derived from H7, the other parent in our mapping population, and thus they were not useful for physical mapping since the chromosome addition lines

were developed using H1 as donor. The remaining 287 markers were physically mapped to a specific *H. chilense* chromosome (Fig. 1; Supplementary File 2). Since chromosomes 3H^{ch} and 2H^{ch}L are not available in the set of chromosome addition lines, markers found in H1 and absent in the complete set of chromosome addition lines were preliminary assigned to chromosomes 2-3H^{ch}. Nevertheless, differential methylation between *H. chilense* (H1 line) and wheat-*H. chilense* chromosome addition lines would also explain for markers present in H1 and absent in addition lines. Six markers gave signal in 5H^{ch} but they also gave signal in one replicate of CS DA-7H^{ch} and CS DA-7H^{ch}β. Thus they were coded as suspected (5H^{ch}?) in Supplementary File 1 and excluded from further analysis. The candidate gene *Psyl_H^{ch}* was also physically mapped to chromosomes 7H^{ch}α (Atienza et al. 2007a). A chromosome code was added to physically mapped DArT to allow a quick inspection of their location after the linkage analysis (Supplementary File 2). Most markers tentatively assigned to chromosomes 2-3H^{ch} were mapped into chromosomes 2H^{ch}L and 3H^{ch}. However, three markers were mapped into chromosomes 1H^{ch} (2) and 4H^{ch} which might indicate differential methylation as indicated above.

Map construction and features

A set of 755 redundant markers were automatically excluded from the linkage analysis by JoinMap before linkage analyses. Additionally, 195 markers significantly departing from mendelian inheritance were also excluded in a first step. Stringent LOD thresholds were considered (from LOD 6.5 to LOD 12.5) to group the markers. Linkage group analysis resulted in nine linkage groups which were assigned to chromosomes using markers physically mapped. Chromosomes 1H^{ch} and 7H^{ch} were split in two different groups. The group 1H^{ch}b corresponded to 1H^{ch}L while 7H^{ch}b belongs to chromosome 7H^{ch}α as determined from physically mapped markers.

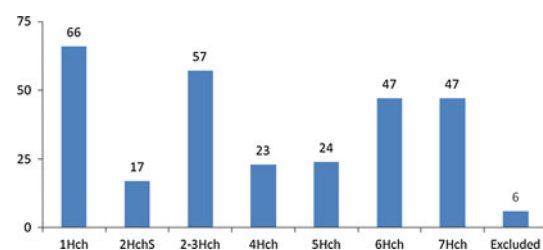


Fig. 1 Physical mapping of a subset of 287 DArT markers using wheat Chinese Spring (CS)-*H. chilense* (H1) chromosome addition lines. Markers present in H1 and absent in all the chromosome addition lines and CS were preliminary assigned to chromosomes 2-3H^{ch}, since there is no chromosome addition line for chromosomes 2H^{ch}L and 3H^{ch}. Six markers were excluded since they gave signal in two chromosomes

The best order of loci within each linkage group was established after several mapping sessions by excluding co-segregating markers and discarding problematic markers in a case-by-case basis. The final order was inspected using RECORD 2.0. Minimal changes between both programs were found (data not shown).

The set of 195 markers initially excluded due to distortion were used for a second linkage analyses. These markers were not evenly distributed among the different chromosomes but mapped preferentially to chromosomes 2H^{ch}, 3H^{ch} and 5H^{ch}. Thus, linkage groups corresponding to these chromosomes were recalculated by including the distorted markers.

The segregation data were used to inspect the segregation distortion along chromosome positions for these chromosomes. The heavily distorted markers mapped to four segregation distortion regions (SDRs), two of them on chromosome 2H^{ch} (Fig. 2). The maternal allele (H1) was favored in three of these regions while the allele of the pollen donor was only favored in the distal SDR of chromosome 2H^{ch}L.

The final map was constructed by adding the co-segregating markers excluded either by JoinMap or during the establishment of the best order within linkage groups (Supplementary Fig. 1, Supplementary File 3). To do this, each marker was assigned the same position than the marker it was identical to. The final map spans 1,503.5 cM (Table 1; Supplementary Fig. 1). The length of the resulting chromosomes varied between 152.1 cM for chromosome 4H^{ch} and 259.2 for chromosome 2H^{ch} which is in correspondence to chromosome size. A total of 2,032 markers were mapped to 646 unique positions (bins) which results in an average distance of 2.33 cM/marker (Table 1; Supplementary File 3).

Marker densities were visualized by constructing histograms along map distance (Fig. 3). Centromeres position was estimated by considering high marker density regions; low inter-bin distance and physical mapping of markers in the telosomic addition lines (Fig. 3). The centromere of chromosomes 6H^{ch} and 7H^{ch} was precisely established at 84.03 and 56.56 cM, respectively (Fig. 3), thanks to telosomic addition lines. Markers physically mapped to different chromosome arms (Supplementary File 2) co-segregated at these positions. A certain degree of marker accumulation was also observed in the distal part of chromosomes 1H^{ch}S, 2H^{ch}S and 6H^{ch}S.

Discussion

DArT markers: a new resource for *H. chilense* genomics

A set of *H. chilense*-derived DArT markers has been developed and used for genetic and physical mapping in

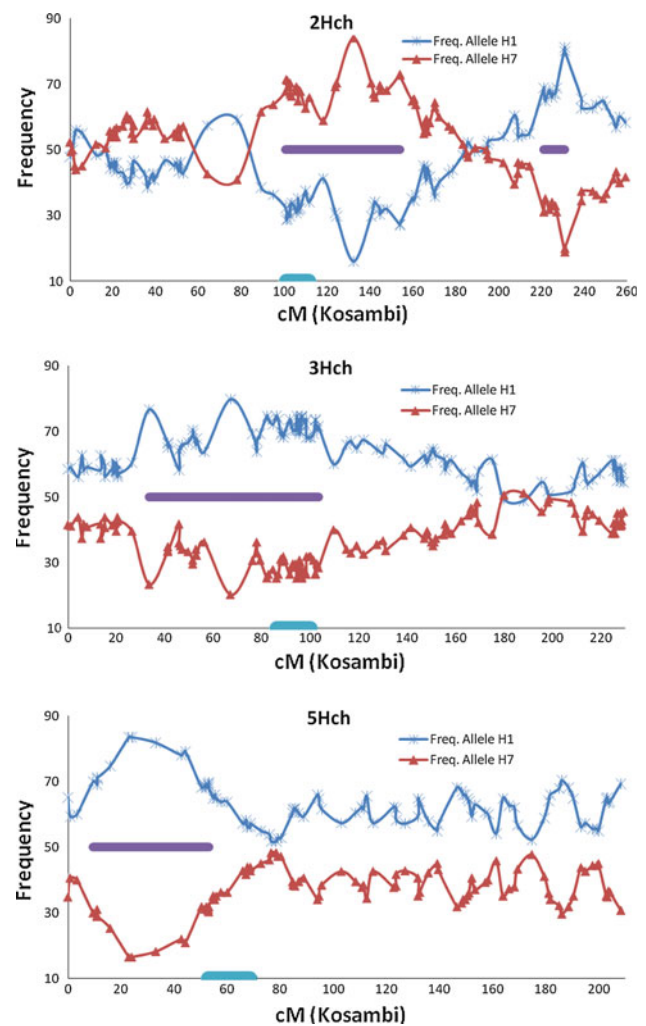


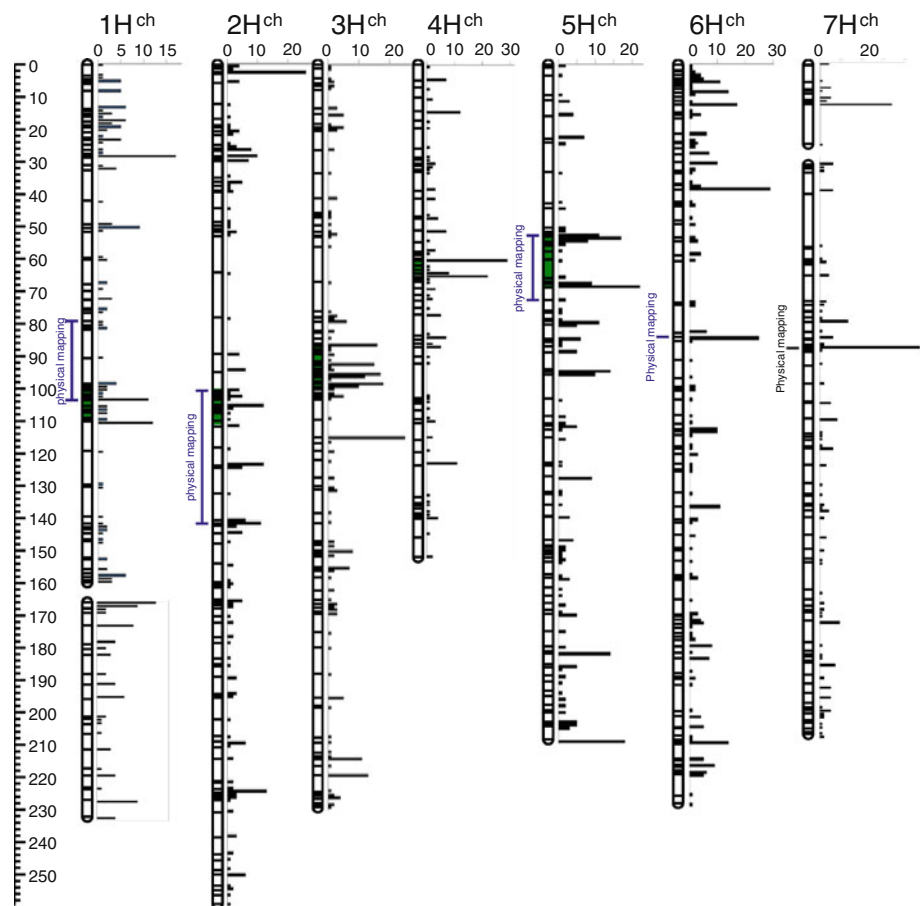
Fig. 2 Identification of segregation distortion regions (SDRs) in chromosomes 2, 3 and 5 of *H. chilense*. Allelic frequency for the maternal parent (H1) is denoted with an *asterisk* while allelic frequency for the pollen donor (H7) is shown with a *triangle*. For each chromosome, the line in the middle of the vertical axis represents the position of the markers distorted. The line at the bottom of the graph depicts the estimated position of the centromere. Each chromosome is oriented such that the short arm is at the left

H. chilense. Although around 3,000 markers were identified in this study, more markers are available for further work since more than 11,000 clones from *H. chilense* were obtained. These markers have proved to be very useful for genetic and physical mapping in *H. chilense*. The map spanned a total length of 1,503.5 cM being much larger than the previous maps developed using the F₂ population derived from the cross H1 × H7. The first map of the species was mainly based in RAPD markers and spanned 649 cM with an average distance between markers of 5.7 cM (Hernández et al. 2001). Similarly, the AFLP-derived map spanned 619.6 cM. It consisted of 213 unique bins with an inter-bin distance of 2.91 cM (Vaz Patto et al. 2003). Thus, the new map presented in this work improves

Table 1 Map features of the H1 × H7 linkage map

Linkage group	Chromosome	Mapped markers	Unique positions	Distance (cM)	Average density	Larger gap
1Hch	1H ^{ch}	193	79	159.865	2.024	10.442
1Hchb	1H ^{ch}	83	22	66.280	3.013	5.938
2Hch	2H ^{ch}	294	100	259.219	2.592	13.828
3Hch	3H ^{ch}	322	107	229.391	2.144	10.754
4Hch	4H ^{ch}	200	63	152.069	2.414	10.594
5Hch	5H ^{ch}	301	90	208.402	2.316	9.664
6Hch	6H ^{ch}	360	103	228.082	2.214	12.878
7Hch	7H ^{ch}	221	72	175.769	2.441	16.346
7Hchb	7H ^{ch}	58	10	24.462	2.446	12.356
Total		2,032	646	1,503.539	2.327	

Fig. 3 Visualization of marker-dense regions in *H. chilense* chromosomes. The 2,032 loci were collapsed into 646 bins. Each bin is represented by a horizontal line across a chromosome shown in schematic view. Horizontal bars lengths (on the right of each chromosome) depict the number of co-segregating markers within each bin. The interval marked *physical mapping* to the left of the chromosomes (except for 3H^{ch} and 4H^{ch}) indicate the position of the last marker physically mapped to the short arm (*upper limit*) and the first marker mapped to the long arm (*lower limit*). The estimated centromere position is shown as a colored segment within each chromosome. In chromosomes 6H^{ch} and 7H^{ch} centromeres were located in a single position since markers belonging to different chromosome arms mapped at the same position (color figure online)



the genome coverage of previous *H. chilense* maps. Besides, the map had only 9 gaps between 10 and 17 cM and an average inter-bin distance of 2.33. This is quite good for a wild species where limited information is available at the sequence level. As expected, the marker density is lower than in cultivated barley (Marcel et al. 2007; Stein et al. 2007; Varshney et al. 2007; Wenzl et al. 2004, 2006) but comparable to recent works in rye (Bolibok-Bragoszewska et al. 2009), barley × *H. vulgare*

subsp. *spontaneum* (Alsop et al. 2011), sorghum (Mace et al. 2008) or wheat (Mantovani et al. 2008).

Hordeum chilense-derived DArT markers have been very useful for physical mapping. It has allowed the unambiguous assignment of linkage groups to chromosomes which is not always possible as recently reported in oat (Tinker et al. 2009). Besides, the physically mapped markers have been very useful for map development. First, they have allowed establishing a stringent LOD threshold

for group definition. Second, the assignment of markers to specific chromosome arms has allowed inspecting the order within linkage groups. In addition, the use of physically mapped markers helped to estimate the position of the centromeres.

Redundancy

Since DArT markers are obtained by cloning random fragments of genomic representations (Wenzl et al. 2004), some degree of marker redundancy is expected. The 2,032 mapped markers could be collapsed into 646 bins, which suggest a maximum redundancy over 65%. However, as the size of the mapping population genotyped on the new array was small (below 100 individuals) and high level of linkage disequilibrium is expected in this material, this number is obviously an overestimation. DArT redundancy between 30 and 40% has been reported in barley (Wenzl et al. 2006) and wheat (Francki et al. 2009) but values of true redundancy as low as 10% have been reported in wheat (Akbari et al. 2006). Thus, it is expected that a similar level may be found in *H. chilense*, especially since the size of the marker libraries developed for this species is still several times smaller than the one for *H. vulgare* or wheat. The high number of co-segregating markers found in our work is not surprising; however, marker redundancy will always be higher than sequence redundancy due to closely linked but physically different markers (Alsop et al. 2011). In our case, the relatively small size of the mapping population hampers the discrimination between closely linked loci. Indeed, we observed co-segregating markers in the opposite allelic phase which clearly demonstrates that they are not redundant clones. Furthermore, markers physically mapped to different chromosome arms mapped in the same genetic position in chromosomes 6H^{ch} and 7H^{ch}. This demonstrates that these markers are not redundant and suggests that the centromere is located at this position. This hypothesis is reinforced by the clustering of markers detected in both chromosomes at these positions as expected for centromeric regions. In barley, it has been previously shown that DArT markers tend to cluster around centromeres as deduced from the larger number of loci per bin and the shorter inter-bin distances in the vicinity of centromeres (Wenzl et al. 2006). It has been proposed that this clustering reflects centromeric suppression of recombination (Tanksley et al. 1992; Wenzl et al. 2006). This clustering tendency along physical mapping allowed us to estimate the position of the centromeres in the chromosomes 1H^{ch} to 5H^{ch} in a window between 5 cM for chromosome 4H^{ch} and 40 cM for chromosome 2H^{ch}. The relative position of centromeres within each chromosome was similar to the position in barley (Stein et al. 2007; Wenzl et al. 2006), including the relatively small size of

5H^{ch}S compared to 5H^{ch}L. The density of DArT markers also appeared to be higher in distal regions in chromosomes 1H^{ch}S and 6H^{ch}S as described in barley in chromosomes 1H, 2H, 6H and 7H (Wenzl et al. 2006). Whether this is caused by a moderate bias of *Pst*I-derived markers towards hypomethylated areas as proposed in barley cannot be concluded without further studies.

Segregation distortion regions (SDRs)

Markers showing segregation distortion also tend to cluster (Graner et al. 1991; Li et al. 2010; Marcel et al. 2007) into segregation distortion regions (SDRs). Segregation distortion is defined as a deviation of the observed allelic frequencies from their expected values. It can be caused by a variety of mechanisms including pollen abortion; pollen tube competition; competitive fertilization or zygotic selection (reviewed by Xian-Liang et al. 2006). SDRs have been identified in different species including wheat (Kumar et al. 2007) and rice (Harushima et al. 2001).

Segregation distortion has also been reported in barley (Graner et al. 1991; Li et al. 2010; Marcel et al. 2007; Stein et al. 2007). In our case, markers showing segregation distortion mapped to 4 SDRs in chromosomes 2H^{ch}, 3H^{ch} and 5H^{ch}. Interestingly, the first RFLP map in barley also detected SDR in chromosomes 2H, 3H and 5H in the same relative positions within the chromosomes (Graner et al. 1991) and these SDRs were further validated by Stein et al. (2007). The allele from the pollen donor (Franka, 6-rowed) was favored in chromosome 2H while the allele from the cytoplasm donor (IGRI, 2-rowed) was favored in SDRs located in chromosomes 3H and 5H as happens with *H. chilense*. Recently, the construction of a high-density composite map in barley based on DArT markers has allowed the identification of a total of 14 SDRs in barley (Li et al. 2010). The SDRs detected in *H. chilense* would correspond to barley SDR2.1, SDR2.3, SDR3.1 and SDR5.1 described by Li et al. (2010). Most SDRs detected in barley have been identified using double-haploid populations and they usually tend to favour the allele of the cultivar with a better response to in vitro culture. In our case, the segregation distortion cannot be attributed to this factor. However, segregation distortion is usually observed in populations derived from wide crosses including interspecific crosses in rice (*indica-japonica*) (Harushima et al. 2001). The accessions H1 and H7 are very different even at the cytoplasmic level. Indeed, the development of *H. chilense*-wheat alloplasmic lines gives rise to either fertile or male-sterile lines when H7 or H1 is used as cytoplasm donor, respectively (Atienza et al. 2007; Martin et al. 2008). This indicates a crucial difference at the cytoplasmic level between both lines. Thus, a certain degree of nucleus × cytoplasm incompatibility between

H1 and H7 cannot be excluded as responsible for the SDRs detected. Indeed, during the development of the RIL populations some lines were lost due to sterility (data not presented).

Final remarks

In summary, the purpose of this study was to enhance *H. chilense* genetic studies by developing novel markers in a cost-effective manner, and to demonstrate their potential for both physical and genetic mapping in this species. DArT markers will enable further genetic studies in ongoing projects on hybrid wheat (Martín et al. 2010), seed carotenoid content improvement (Rodríguez-Suárez et al. 2010), development of *H. chilense*–durum wheat chromosome substitution lines (Rodríguez-Suárez et al. 2011b) or in tritordeum breeding program. Besides, the genetic map reported here will be very useful to develop comparative genomics studies by using COS markers (Quraishi et al. 2009), barley ESTs (Sato et al. 2009) or identifying candidate genes from the barley genome. DArT markers will be utilized as a high-throughput and fast platform for *H. chilense* and tritordeum-related projects.

Acknowledgments This research was supported by grants (to S.G. Atienza) AGL2008-03720, 200840I137 and P09-AGR-4817 from the Spanish Ministry of Science and Innovation (MSI), CSIC, Junta de Andalucía and FEDER. We are grateful to E. León for her technical assistance. C. Rodríguez-Suárez acknowledges financial support from CSIC (JAE-Doc program). The authors thank Prof. Tsujimoto (Tottori University, Japan), for providing barley EST primers.

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