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

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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. Beside, 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 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 ×*Triticosecale* (Bothmer et al. 1986; Martin et al. 1998). Crosses between *H. chilense* and wheat lead to fertile amphiploids named tritordeums. Hexaploid tritordeum (×*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; Hagras et al. 2005a; 2005b; 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 F_2 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 × H7. We also report the existence of segregation distortion zones in chromosomes 2, 3 and 5 of *H. chilense*.

Material 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 DA$5H^{ch}$, CS DA$6H^{ch}$ and CS DA$7H^{ch}$ respectively, where MA refers to monosomic addition and DA means disomic addition), the wheat- (CS)-*H. chilense* ditelosomic addition lines CS DA$1H^{ch}S$, CS DA$2H^{ch}S$, CS DA$5H^{ch}L$, CS DA$6H^{ch}S$, CS DA$7H^{ch}\alpha$, CS DA7Hchβ, and the Recombinant Inbred Line Population (RIL population, RI_7) derived from the cross H1×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 5 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; 2010b). Importantly, these two accessions are the parents of our mapping population.

H. chilense-derived markers were produced by Diversity Arrays Technology Pty. Ltd. with standard DArT protocols for *Pst*I/*Taq*I ('bPt' markers) and *Pst*I/*Bst*NI ('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 COS (Conserved Orthologous Set) 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 $MgCl_2$, 0.32 mM dNTPs (Promega, Madison, WI, USA) and 0.24 µM of each primer. PCR amplifications were carried our as follows: an initial denaturation step at 94ºC for 5 min, 35 cycles at 94ºC for 1 min, 55ºC for 1 min, 72ºC for 1 min, with a final extension step at 72ºC for 7 min. Digestion with restriction enzymes *AfaI*, *ApaI*, *BamHI*, *BglI*, *BglII*, *BspT104I*, *ClaI*, *Dra I*, *EcoRI*, *EcoRV*, *EcoT14I*, *HaeIII*, *HapII*, *HindIII*, *HinfI*, *KpnI*, *MvaI*, *PstI*, *PvuII*, *SacI*, *SalI*, *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 $MgCl_2$, 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 94ºC, 35 cycles of 1 min at 94ºC, 1 min at 50.5ºC and 1 min at 72ºC, followed by 7 min at 72ºC. 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 *Pst*I ends) and "frequent cutters" *BstNI* and *TaqI* were used to further reduce the complexity of *Pst*I 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 Material and Methods section were used for mapping. Out of these, 968 were 'bPb' markers (derived from *Pst*I/*BstNI* method) and 1,241 were 'bPt' markers (derived from *Pst*I/*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 (Additional 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 thirteen 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 7 additional markers. The mapping data set was complemented with 3 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 (Additional 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 (Figure 1,

Additional 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-$7H^{ch}\beta$. Thus they were coded as suspected ($5H^{ch}$?) in additional file 1 and excluded from further analysis. The candidate gene *Psy1_H^{ch}* was also physically mapped to chromosomes $7H^{ch}\alpha$ (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 (additional file 2). Most markers tentatively assigned to chromosomes 2-3Hch 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, one hundred and ninety five 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 9 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 $7H^{ch}\alpha$ as determined from physically mapped markers.

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 $2H^{ch}$ (Figure 2). The maternal allele (H1) was favoured in three of these regions while the allele of the pollen donor was only favoured 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 (Suplementary figure 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 figure 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 (Figure 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 (Figure 3). The centromere of chromosomes $6H^{ch}$ and $7H^{ch}$ was precisely established at 84.03 and 56.56 cM respectively (Figure 3) thanks to telosomic addition lines. Markers physically mapped to different chromosome arms (Suplementary file 1) 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 *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 F_2 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 the genome coverage of previous *H. chilense* maps. Besides, the map had only nine gaps between 10 and 17cM 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).

H. 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 30-40 % 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, since 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 *PstI*-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 favoured in chromosome 2H while the allele from the cytoplasm donor (IGRI, 2-rowed) was favoured 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 inter-subspecific 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. 2007d; 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.

Table 1. Map features of the H1xH7 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	

Caption Figures.

Figure 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.

Figure 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 purple line in the middle represents the position of the markers distorted. The blue 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.

Figure 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 coloured segment within each chromosome. In chromosomes 6Hch and $7H^{ch}$ centromeres were located in a single position since markers belonging to different chromosome arms mapped at the same position.

Caption supplementary files

Supplementary Figure 1. Genetic linkage map of the cross H1 × H7 containing 2,032 loci. Loci with physical mapping are indicated with a chromosome-code. The estimated centromere position is indicated as a green segment. For chromosomes $6H^{ch}$ and $7H^{ch}$, where the centromere was positioned in a single map position, markers mapping to the centromere are shown in red colour.

Supplementary file 1. Set of COS and EST markers used in this work.

Supplementary file 2. Physical mapping of 287 DArT markers using *H. chilense* (H1)-wheat (Chinese Spring) chromosome and telosomic addition lines.

Supplementary file 3. Features of loci of the linkage map. Excel spreadsheet containing a list of all mapped loci and their features. Data include locus position (in both Kosambi and Haldane), physical mapping when available and segregation in the RIL population.

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