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Cytological and molecular characterization of wheat-*Hordeum chilense* chromosome 7H^{ch} introgression lines

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Abstract Chromosome 7H^{ch} of *Hordeum chilense* carries the *Phytoene synthase 1* (*Psy1*) gene encoding the first step in the carotenoid biosynthetic pathway. As such it can be used in the improvement of seed carotenoid content in wheat. Four introgressions of chromosome 7H^{ch} into wheat have been characterized by in situ hybridization of labeled DNA probes and by several sets of DNA markers. Chromosome-specific SSR were used for the identification of wheat chromosomes. Besides 113 conserved orthologous set (COS) markers were tested for homoeologous group 7, of which 97 amplified in *H. chilense* and 32 were polymorphic between *H. chilense* and wheat, and 28 expressed sequence tag (EST) barley markers previously allocated to chromosome 7. A total of 60 markers (32 COS and 28 EST) were allocated to chromosome 7H^{ch} with 28 assigned to 7H^{ch}S and 22 to 7H^{ch}L. A combination of in situ probing and marker genotyping have shown that among the four introgressions there was a substitution line 7H^{ch} (7D), a ditelosomic addition line for the long arm of 7H^{ch} and two homozygous centric translocations 7H^{ch}S·2DS and 7H^{ch}S·5AL. The *Psy1* gene was localized on the short arm of 7H^{ch}. The positions of markers from the international barley consortium map (IBSC2012) were determined and the comparative arm location between *H. chilense* and *H. vulgare* is discussed. The genetic stocks characterized here include new wheat-*H. chilense* recombinations useful for genetic studies and with a potential for breeding.

Keywords Wheat · *Hordeum chilense* · Introgression lines · Cytogenetics · FISH · Barley

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Introduction

The reduction of genetic variability in crops is a serious threat to agriculture. The use of wild relatives in breeding programs of cultivated species provides plant breeders with a pool of useful genes to develop new varieties with an improved agronomic performance or quality characteristics. These resources are especially useful in the Triticeae. For instance, the development of synthetic wheat from the crosses

between durum wheat and *Aegilops tauschii* Coss. represents a considerable effort for the use of the diversity available in wild relatives (van Ginkel and Ogbonnaya 2007) in wheat improvement. Similarly, alien resources have been widely used both in durum wheat (reviewed by Ceoloni et al. 2014) and bread wheat (reviewed by Khlestkina 2014) for the development of new varieties or for genetic studies.

Hordeum chilense Roem. et Schult is a diploid wild barley native to Chile and Argentina (Bothmer et al. 1995). It has a potential for breeding Triticeae species due to its high crossability with other members of this tribe, including both durum and common wheat (Martín et al. 1998). The development of introgression lines of *H. chilense* into wheat has potential for wheat breeding. Chromosome addition and substitution lines of *H. chilense* in common wheat were developed by Miller et al. (1982). These lines have been successfully used to determine the chromosome location of genes coding for important traits including resistance to greenbug (*Schizaphis graminum*) (Castro et al. 1996) and endosperm prolamins (Payne et al. 1987) located on chromosome $1H^{ch}$; resistance to *Septoria tritici* on chromosome $4H^{ch}$ (Rubiales et al. 2000); tolerance to salt on chromosomes $1H^{ch}$, $4H^{ch}$ and $5H^{ch}$ (Forster et al. 1990), or restoration of fertility on chromosome $6H^{ch}$ (Martín et al. 2008).

Wheat-*H. chilense* addition lines have also been used to transfer alien chromosome segments carrying genes of interest such as the resistance to cereal root-knot nematode (*Meloidogyne naasi*) from *H. chilense* to wheat (Person-Dedryver et al. 1990). Recently, Calderón et al. (2012) have reported the development of $4H^{ch}$ introgression lines in durum wheat that may serve as a source of resistance to *Septoria tritici* blotch (STB) in wheat, although the characterization of these lines for STB resistance is still pending.

The yellow pigment content (YPC) is caused by carotenoids, mainly lutein (Atienza et al. 2007b; Mellado-Ortega and Hornero-Mendez 2012). The chromosome $7H^{ch}$ has a potential for improving the seed carotenoid content in wheat since it carries the *Phytoene synthase 1* (*Psy1*) which plays a major role in this trait (Ficco et al. 2014; Rodríguez-Suárez et al. 2014). The availability of molecular markers capable of distinguishing between *H. chilense* chromatin in wheat background constitutes an important tool for selection. Barley expressed sequence tag (EST) have been successfully transferred to *H. chilense* (Hagras et al. 2005a, b) and they have been used for physical mapping of chromosomes $4H^{ch}$ (Said and Cabrera 2009), $1H^{ch}$ (Cherif-Mouaki et al. 2011) and $3H^{ch}$ (Said et al. 2012). In addition, the development of the conserved orthologous set (COS) (Quraishi et al. 2009) signifies an additional source of markers potentially useful for *H. chilense*. EST and COS markers allow comparative studies with barley and thus their transference to *H. chilense* is an important goal.

In the present work, four wheat-*H. chilense* chromosome $7H^{ch}$ introgression lines were cytologically characterized using FISH/GISH and chromosome-specific SSR markers. COS markers were assessed for transferability to *H. chilense*. The arm location within $7H^{ch}$ was determined for COS and EST markers and compared to their location in barley 7H.

Materials and methods

Plant material

Hordeum chilense lines H1 and H7, common wheat 'Chinese Spring' (CS) and *H. vulgare* 'Betzes' were used as controls. Four wheat (CS)-*H. chilense* introgression lines involving chromosome $7H^{ch}$ developed at the University of Córdoba were characterized. These lines were obtained by pollinating tritordeum line HT31 (amphiploid *H. chilense* and durum wheat, $AABBH^{ch}H^{ch}$, $2n = 6x = 42$) with a wheat disomic addition line for gametocidal chromosome 2C from *Aegilops cylindrica* Host and F_1 plants ($AABBDH^{ch} + 2C$) monosomic for the gametocidal 2C were backcrossed with CS followed by five generations of selfing (Cifuentes et al. 2005). The breeding procedure used to obtain these cytogenetic stocks was that described in (Said et al. 2012).

Fluorescence in situ hybridization (FISH)

Root tips were prepared as described by Said et al. (2012). Preparation of somatic metaphase chromosomes and FISH protocol were carried out as described by Cabrera et al. (2002). For chromosome identification, the pAs1 sequence (1 kb) isolated from *A. tauschii* Coss. by Rayburn and Gill (1986), the GAA-satellite sequence isolated from barley by Pedersen and Langridge (1997) and *H. chilense*

genomic DNA were used as probes. The pAs1 probe hybridizes strongly to D-genome chromosomes of wheat (Mukai et al. 1993) and H^{ch}-genome chromosomes from *H. chilense* (Cabrera et al. 1995). The GAA-satellite sequences probe allows identification of both A- and B-genome chromosomes (Pedersen and Langridge 1997). Using nick translation, the pAs1 and GAA-satellite sequences were labeled with biotin-16-dUTP (Roche Corporate) and total *H. chilense* DNA with digoxigenin-11-dUTP (Roche Corporate). Chromosome preparations were hybridized simultaneously with both pAs1 and *H. chilense* genomic DNA or GAA-satellite sequences and *H. chilense* genomic DNA as probes.

Biotin- and digoxigenin-labeled probes were detected with streptavidin-Cy3 conjugates (Sigma, St. Louis, MO, USA) and anti-digoxigenin-FITC (Roche Corporate) antibodies, respectively. Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and mounted in Vectashield (vector Laboratories, Inc). Signals were visualized using a Leica DMR epifluorescence microscope and images were captured with a SPOT CCD camera supported by the SPOT 2.1 software (Diagnostic Instruments, Inc., Sterling Heights, Michigan, USA). PhotoShop 7.0 software (Adobe Systems Inc., San José, California, USA) was used for processing the images.

Molecular markers analysis

A set of chromosome-specific single sequence repeats (SSR) for A- and D-genomes from wheat was used for the verification of the introgression lines (Supplementary file 1) (Sourdille et al. 2004; Röder et al. 1998). Amplifications were performed as described at GrainGenes, http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker).

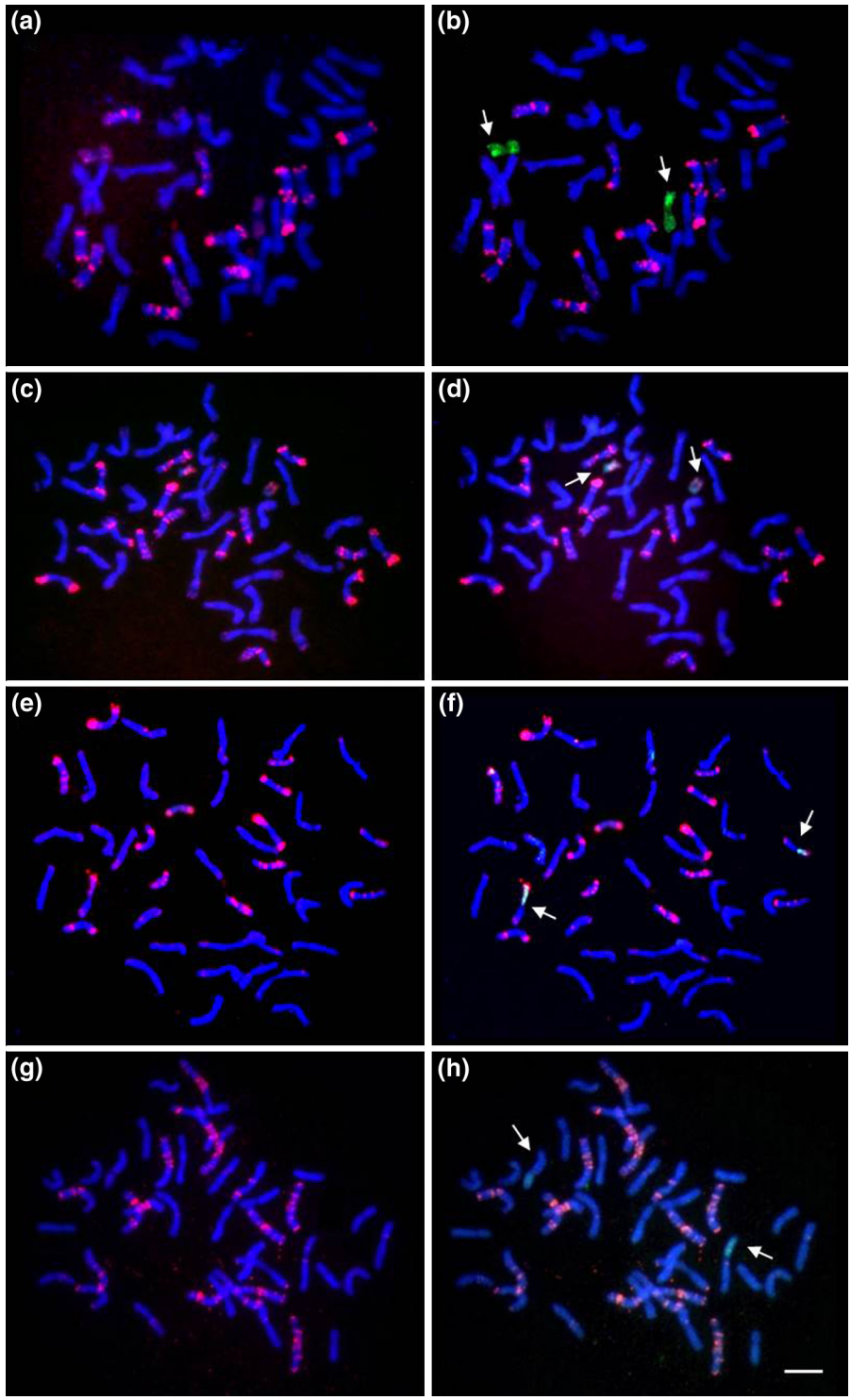
The presence of *Psy1* gene from *H. chilense* was evaluated using the diagnostic cleaved amplified polymorphism (CAP) marker developed by Atienza et al. (2007a). A set of 113 COS markers (Quraishi et al. 2009) from wheat homoeologous group 7 was assessed for their utility in *H. chilense* (Supplementary file 2). In addition, twenty-eight barley EST markers previously transferred to *H. chilense* (Hagras et al. 2005a) were also screened (Supplementary file 3).

Total genomic DNA was isolated from young frozen leaf tissue using the CTAB method (Murray and Thompson 1980). Samples were stored at −20 °C until PCR amplification was carried out. The concentration of each sample was estimated using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA). Amplifications were made using a TGradient thermocycler (Biometra, Göttingen, Germany) and performed with 40 ng of template DNA, in a 25 µl volume reaction including 2.5 µl of 10× PCR Buffer, 0.5 µM of each primer, 1.5–2.0 mM $MgCl_2$, 2.4 mM dNTPs and 0.25 U of *Taq* DNA Polymerase (BIOTOOLS B&M Laboratories, Madrid, Spain). PCR conditions of COS markers were as follows: 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at optimal annealing temperature (Supplementary file 2), and 1 min at 72 °C. EST markers were amplified using the touchdown protocol previously reported by (Nasuda et al. 2005; Hagras et al. 2005a).

Amplification products were resolved in 2 % agarose gels (ESTs and SSRs) or vertical PAGE gels at 8 % (w/v, C: 2.67 %) (COS and CAPs), stained with ethidium bromide or SafeView Nucleic Acid Stain (NBS Biologicals, Huntingdon, UK) incorporated in the gel. A 100 bp DNA Ladder (Solis BioDyne, Tartu, Estonia) was used as a standard molecular weight marker. Amplicon lengths were determined using Kodak Digital Science 1D software (version 2.0).

Comparative mapping

For COS markers (Quraishi et al. 2009), the rice locus (RAP) used to design each COS was considered. The RAP locus identifier was retrieved using the ID Converter tool (http://rapdb.dna.affrc.go.jp/tools/converter) (Supplementary file 2). COS markers were located in the Barley Genome Zipper (Mayer et al. 2011) using the RAP locus (Supplementary file 4). The barley full length cDNA corresponding to each rice locus was used for the determination of the barley Unigene corresponding to each COS marker. The Unigene sequences were aligned in Barleymap (Cantalapiedra et al. 2014, in press) to obtain their positions in the International Barley Sequencing Consortium map (IBSC2012) (Mayer et al. 2012) or the POPSEQ map (Mascher et al. 2013) (Supplementary file 5). For some COS, the RAP locus has no barley sequences in the Genome Zipper. In these cases, the positions were estimated considering the barley flanking sequences in the barley Genome Zipper.


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)

◀**Fig. 1** FISH with the pAs1 (*red*) probe to mitotic metaphase chromosomes: **a** disomic substitution $7H^{ch}$ (7D) in CS; **c** ditelosomic addition of $7H^{ch}L$ chromosome arm in CS and; **e** homozygous translocation $7H^{ch}L{\cdot}2DS$ in CS; In (**b**, **d**, **f**) double FISH signals with the pAs1 (*red*) and *H. chilense* genomic DNA (*green*) as probes hybridized to the same metaphase as in (**a**, **c, e**), respectively; **g** metaphase chromosomes of homozygous $7H^{ch}S{\cdot}5AL$ translocation line probed with GAA-satellite sequence (*red*); **h** double FISH signals with GAA-satellite sequence (*red*) and *H. chilense* genomic DNA (*green*) as probes hybridized to the same metaphase as in **g**. *H. chilense* chromatin is visualised by *green* FITC fluorescence whereas wheat chromosomes are counterstained with *blue* DAPI. *Scale bar* is 10 μm. (Color figure online)

The clone name used to design each EST marker (kindly provided by Prof. Tsujimoto, personal communication) was used to identify the corresponding barley Unigene at http://www.ncbi.nlm.nih.gov/unigene/. The Unigene sequence was used for alignment at Barleymap (http://floresta.eead.csic.es/barleymap) (Cantalapiedra et al. 2014, in press) as described for COS markers. Detailed information for BAWU markers is provided in Supplementary file 3.

Results

Cytogenetic and molecular characterization of wheat-*H. chilense* introgression lines involving chromosome $7H^{ch}$

FISH analysis with both pAs1 and *H. chilense* genomic DNA as probes allowed the identification of a pair of chromosomes $7H^{ch}$ and the absence of wheat chromosomes 7D pair in one line with 42 chromosomes indicating that it was disomic for the substitution $7H^{ch}$(7D) (Fig. 1a, b). The absence of 7D was verified using Xcfd66-7DS (Fig. 2a) and Xbarc111-7DL (Fig. 2b) molecular markers. A pair of telocentric chromosomes for the long arm of chromosome $7H^{ch}$ was identified in one line with 42 + 2t chromosomes, showing that this line was ditelosomic for the $7H^{ch}L$ (Fig. 1c, d). Two centromeric translocations involving chromosome $7H^{ch}S$ arm and wheat chromosomes were identified in the remaining two lines. One of these lines was homozygous for the $7H^{ch}S{\cdot}2DS$ translocation and it was also ditelosomic for 2DL chromosome arm and nullisomic for chromosome 7D (Fig. 1e, f). Chromosome-specific markers showed the absence of 7D chromosome (Fig. 2a, b) and the presence of 2DS and 2DL (Fig. 2d, e). The other line was homozygous for the $7H^{ch}S{\cdot}5AL$ translocation as demonstrated by double FISH with GAA-satellite sequence and genomic DNA from *H. chilense* as probes (Fig. 1g, h). Molecular markers specific for chromosome 5A confirmed the absence of 5AS and the presence of 5AL chromosome arms, respectively (Fig. 2c). All lines were fertile and vigorous. Table 1 shows the chromosome constitutions of wheat-*H. chilense* introgression lines involving chromosome $7H^{ch}$ characterized in this study.

A set of 113 COS markers corresponding to homoeologous group 7 of wheat (Quraishi et al. 2009) was used for characterizing the introgression lines (Supplementary file 2). Ninety-seven (85.8 %) COS markers were successfully amplified in *H. chilense*. Out of the remaining 16, six only amplified in wheat and ten failed to amplify in both wheat and *H. chilense*. Thirty-two COS (32.9 %) were polymorphic between *H. chilense* and wheat, which permitted the characterization of the cytogenetic stocks described above (Table 2). All the polymorphic COS were located on chromosome $7H^{ch}$ as demonstrated by their presence in $7H^{ch}$ (7D) substitution line. The translocation lines $T7H^{ch}S{\cdot}2DS$ and $T7H^{ch}S{\cdot}5AL$ and the ditelosomic line Dt $7H^{ch}L$ were used for the assignation of markers to a specific arm of chromosome $7H^{ch}$. Twenty-one COS were assigned to $7H^{ch}S$ and nine to $7H^{ch}L$. The remaining two COS were simultaneously amplified in both $7H^{ch}S$ and $7H^{ch}L$ arms. A set of 28 barley ESTs markers (coded BAWU) located in $7H^{ch}$ (Hagras et al. 2005a) was also used (Supplementary file 3). All these markers were successfully amplified in the substitution line $7H^{ch}$ (7D). Seven markers were located in $7H^{ch}S$, thirteen in $7H^{ch}L$ arm, and eight markers were found in both arms (Table 2). Examples of amplification of EST and COS markers are given in Fig. 3. A total of sixty markers allowed the distinction of chromosome $7H^{ch}$ in wheat background and they are therefore useful for the selection of introgressions of this chromosome. The four wheat-*H. chilense* introgressions were genotyped for the presence of *Psy1* gene (Fig. 4). The presence of *Psy1* gene on the wheat substitution $7H^{ch}$ (7D) line and both $T7H^{ch}S{\cdot}2DS$ and $T7H^{ch}S{\cdot}5AL$ translocation lines as well as their absence in the ditelosomic line for the $7H^{ch}L$ arm confirmed the location of this gene on the short arm of chromosome $7H^{ch}$ (Atienza et al. 2007a).

The Align tool implemented in BarleyMap (http://floresta.eead.csic.es/barleymap) (Cantalapiedra et al.

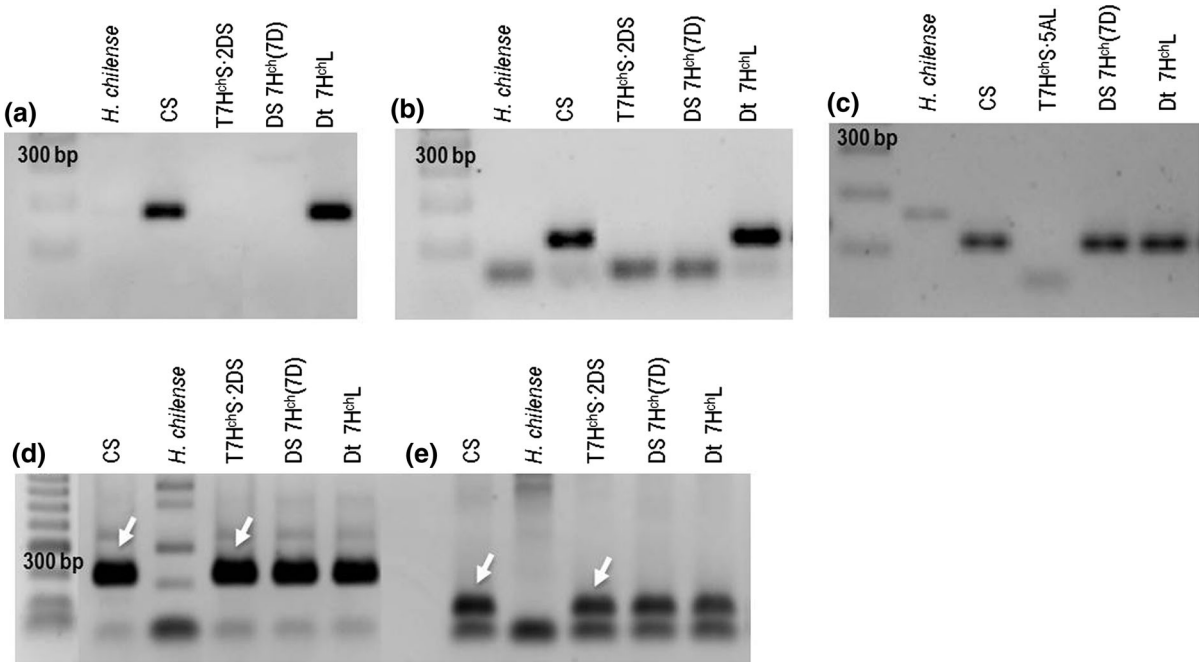


Fig. 2 Molecular characterization with chromosome-specific SSR markers. **a** Xcfd66-7DS; **b** Xbarc111-7DL; **c** Xbarc56-5AS; **d** Xgwm261-2DS and **e** Xgwm157-2DL

Table 1 Chromosome constitution of wheat-*H. chilense* introgression lines involving chromosome $7H^{ch}$

Lines	Number of chromosomes from each genome			2n
	AB	H^{ch}	D	
DS $7H^{ch}$ (7D)[a]	28	2	12	42
Dt $7H^{ch}L$[b]	28	2 telos	14	42 + 2t
$T7H^{ch}S{\cdot}5AL$[c]	26 + 2T	2T	14	42
$T7H^{ch}S{\cdot}2DS$[d]	28	2T	10 + 2T + 2 telos	40 + 2t

[a] Disomic substitution $7H^{ch}$ (7D) in CS wheat

[b] Ditelosomic addition of $7H^{ch}L$ in CS wheat and ditelosomic for 2DL

[c] Homozygous centromeric translocation $7H^{ch}S{\cdot}5AL$ in CS wheat

[d] Homozygous centromeric translocation $7H^{ch}S{\cdot}2DS$ in CS wheat, ditelosomic for 2DL and nullisomic for 7D

2014, in press) was used to determine the positions of COS and EST in barley maps (Table 2, Supplementary files 2 and 4). The majority of the markers unambiguously assigned to $7H^{ch}S$ and $7H^{ch}L$ were located in the same barley 7H arm (Table 2). The most notable exceptions were *Psy1*, which is located in the distal part of 7HL, and BAWU21 and BAWU763, which are located in the distal part of 7HS in barley. Besides, two BAWU markers (BAWU302 and BAWU857) were not found in Barleymap and three (BAWU381, BAWU559 and BAWU752) were located on different barley chromosomes, as previously reported (Hagras et al. 2005b). Four of the COS markers (COS557, COS561, COS562 and COS571) were not found in the Barley Genome Zipper (Mayer et al. 2011) so that their position in barley could not be investigated.

Discussion

The cytogenetic stocks analyzed in this work were obtained using the chromosome $2C^c$ from *Ae.*

Table 2 PCR analysis showing the presence (+) or absence (−) of *Psy1* gene and DNA markers on chromosome $7H^{ch}$ of *H. chilense* and comparison with their position (cM) in *H. vulgare*

Marker[a]	DS $7H^{ch}$ (7D)	T7H^{ch}S·5AL	T7H^{ch}S·2DS	Dt $7H^{ch}$L	Polymorphism[b]	Physical location in $7H^{ch}$	Location in $7H^{vc}$ (cM)
COS557	+	+	+	−	b	7H^{ch}S	n.g.z
COS562	+	+	+	−	b	7H^{ch}S	n.g.z
COS571	+	+	+	−	b	7H^{ch}S	n.g.z
COS519	+	+	+	−	b	7H^{ch}S	49.72
COS518	+	+	+	−	b	7H^{ch}S	54.82
COS536	+	+	+	−	b	7H^{ch}S	54.82
COS526	+	+	+	–	b	7H^{ch}S	59.61
BAWU829	+	+	+	–	a	7H^{ch}S	61.47
COS368	+	+	+	–	b	7H^{ch}S	61.72
COS539	+	+	+	−	b	7H^{ch}S	62.39
COS528	+	+	+	−	b	7H^{ch}S	65.12
BAWU680	+	+	+	−	a	7H^{ch}S	67.42
BAWU127	+	+	+	−	a	7H^{ch}S	67.68
COS535	+	+	+	−	b	7H^{ch}S	67.78
COS530	+	+	+	−	b	7H^{ch}S	68.06
COS534	+	+	+	−	b	7H^{ch}S	68.06
BAWU406	+	+	+	−	b	7H^{ch}S	68.09
COS312	+	+	+	−	b	7H^{ch}S	68.38
COS543	+	+	+	−	b	7H^{ch}S	69.26
COS579	+	+	+	−	b	7H^{ch}S	69.97
COS551	+	+	+	−	b	7H^{ch}S	70.18
BAWU104	+	+	+	−	a	7H^{ch}S	70.96
BAWU550	+	+	+	−	a	7H^{ch}S	70.96
COS313	+	+	+	−	b	7H^{ch}S	70.96
COS577	+	+	+	−	b	7H^{ch}S	70.96
COS572	+	+	+	−	b	7H^{ch}S	71.25
BAWU522	+	+	+	−	a	7H^{ch}S	74.29
COS316	+	+	+	−	b	7H^{ch}S	74.29
Psy1	+	+	+	−	c	7H^{ch}S	140.44
BAWU763	+	−	−	+	a	7H^{ch}L	12.75
BAWU21	+	−	−	+	a	7H^{ch}L	15.37
BAWU709	+	−	−	+	a	7H^{ch}L	69.05
COS567	+	−	−	+	b	7H^{ch}L	70.18
BAWU681	+	−	−	+	a	7H^{ch}L	70.25
BAWU79	+	−	−	+	a	7H^{ch}L	70.68
BAWU379	+	−	−	+	a	7H^{ch}L	70.68
COS563	+	−	−	+	b	7H^{ch}L	70.68
COS592	+	−	−	+	b	7H^{ch}L	70.68
BAWU514	+	−	−	+	a	7H^{ch}L	70.96
BAWU165	+	−	−	+	a	7H^{ch}L	76.56
BAWU872	+	−	−	+	a	7H^{ch}L	76.56
COS594	+	−	−	+	b	7H^{ch}L	76.56
COS581	+	−	−	+	b	7H^{ch}L	76.70

Table 2 continued

Marker[a]	DS $7H^{ch}$ (7D)	$T7H^{ch}S{\cdot}5AL$	$T7H^{ch}S{\cdot}2DS$	Dt $7H^{ch}L$	Polymorphism[b]	Physical location in $7H^{ch}$	Location in $7H^{vc}$ (cM)
BAWU166	+	−	−	+	a	$7H^{ch}L$	76.91
COS373	+	−	−	+	b	$7H^{ch}L$	89.63
BAWU576	+	−	−	+	a	$7H^{ch}L$	97.31
BAWU566	+	−	−	+	a	$7H^{ch}L$	98.55
BAWU631	+	−	−	+	a	$7H^{ch}L$	100.00
COS374	+	−	−	+	b	$7H^{ch}L$	114.52
COS318	+	−	−	+	b	$7H^{ch}L$	140.7
COS561	+	−	−	+	b	$7H^{ch}L$	n.g.z
BAWU302	+	+	+	+	a	$7H^{ch}S + 7H^{ch}L$	n.f.
BAWU381	+	+	+	+	a	$7H^{ch}S + 7H^{ch}L$	d.c.
BAWU559	+	+	+	+	a	$7H^{ch}S + 7H^{ch}L$	d.c.
BAWU752	+	+	+	+	a	$7H^{ch}S + 7H^{ch}L$	d.c.
BAWU857	+	+	+	+	a	$7H^{ch}S + 7H^{ch}L$	n.f.
COS369	+	+	+	+	b	$7H^{ch}S + 7H^{ch}L$	39.68
COS537	+	+	+	+	b	$7H^{ch}S + 7H^{ch}L$	61.47
BAWU517	+	+	+	+	a	$7H^{ch}S + 7H^{ch}L$	69.26
BAWU764	+	+	+	+	a	$7H^{ch}S + 7H^{ch}L$	77.97
BAWU123	+	+	+	+	a	$7H^{ch}S + 7H^{ch}L$	131.59

[a] EST (BAWU, Hagras et al. 2005a); conserved orthologous set (COS, Quraishi et al. 2009); Gene (*Psy1*, Atienza et al. 2007a)

[b] *a* Amplification of a single band in *H. chilense* chromosome $7H^{ch}$, *b* different band size polymorphism between *H. chilense* and common wheat, *c* Cleaved amplified polymorphism

[c] Position in barley determined using Barleymap (http://floresta.eead.csic.es). *n.f.* not found in barley map, *d.c.* different chromosome, *n.g.z.* not in Genome Zipper

cylindrica which induces chromosome breaks in wheat (Endo 1988). This approach was subsequently applied to barley (Shi and Endo 1999) and rye (Friebe et al. 2000). The chromosome $2C^c$ induces frequent breakage in the centromeric region of barley chromosomes, with high rate of telocentric chromosomes and whole-arm translocations (Endo 2009). Accordingly, this strategy has allowed the development of structural changes in *H. chilense* for chromosomes $4H^{ch}$ (Said and Cabrera 2009), $1H^{ch}$ (Cherif-Mouaki et al. 2011) and $3H^{ch}$ (Said et al. 2012) of *H. chilense*.

Alien introgression is an important strategy in both common (Khlestkina 2014) and durum wheats (Ceoloni et al. 2014). In particular, alien introgressions for homoeologous group 7 have received significant attention for the use of *Lr19* and *Sr25* from *Thynopirum ponticum* (Popd.) Barkworth & D.R., Dewey (2n = 10x = 70) or a fusarium head blight resistance locus from *Thynopirum elongatum* (Ceoloni et al. 2014). Similarly, the development of wheat-*Thynopirum* recombinants for chromosome 7 has also resulted in improved seed carotenoid content in durum (Ceoloni et al. 2014) and common wheat (Marais and Marais 1990; Ahmad et al. 2013) presumably due to the presence of alien phytoene synthase genes. To our knowledge, no previous translocation lines involving the chromosome $7H^{ch}$ into wheat have been described and, thus, these cytogenetic stocks constitute an important resource for studying the effect of this chromosome on wheat grain color. Besides, these cytogenetic stocks have allowed the localization of a set of DNA-based markers to specific arms in *H. chilense*. These markers would facilitate the selection of new genotypes carrying shorter regions of chromosome $7H^{ch}$. Some COS and EST markers were found in both arms which suggest that they are located in the pericentromeric region as was concluded in barley when analyzing chromosome arms derived from flow-sorting (Muñoz-Amatriaín et al. 2011).

The high transference rate of COS markers to *H. chilense* (85.8 %) was expected since these markers were intended for comparative studies among grasses

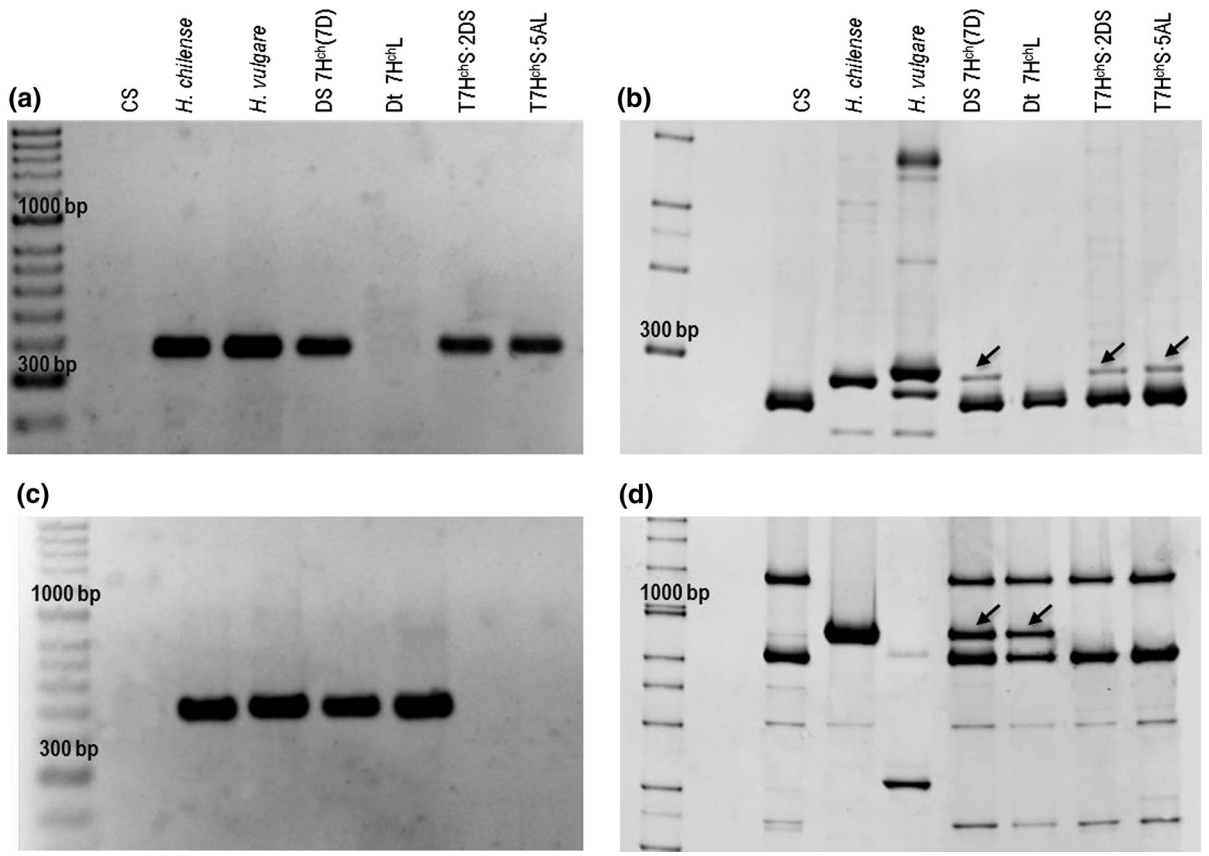


Fig. 3 Examples of PCR amplification profiles used for physical location of DNA molecular makers on chromosome $7H^{ch}$: **a** BAWU680 and **b** COS536 mapped on the short arm of chromosome $7H^{ch}$; **c** BAWU166 and **d** COS374 mapped on the long arm of chromosome $7H^{ch}$

(Quraishi et al. 2009). All the EST markers used in this work were previously assigned to chromosome $7H^{ch}$ (Hagras et al. 2005a). Barley ESTs show a lower transference rate to *H. chilense* than COS. Indeed, from a set of 1,165 barley markers, a total of 510 (43.8 %) successfully amplified in *H. chilense* (Hagras et al. 2005a). Nevertheless, these markers are very useful for the identification of *H. chilense* chromatin into wheat background (Atienza et al. 2007c; Castillo et al. 2014; Said and Cabrera 2009; Cherif-Mouaki et al. 2011; Said et al. 2012). Previous studies have yielded a limited set of markers useful for *H. chilense* including RAPDs, SCARs, SSRs and STS (reviewed by Hernandez 2005) with barley and wheat SSRs providing a transferability rate of 53.5 % (Hernandez et al. 2002). The transferability of COS markers reported in this work clearly outperforms both ESTs and SSRs. Besides, these markers show a good collinearity among different grasses and they are therefore expected to be highly informative for comparative mapping between *H. chilense* and barley in future studies.

Until now, comparative mapping of carotenoid-related genes (Rodríguez-Suárez and Atienza 2012) and polyphenol oxidase genes (Rodríguez-Suárez and Atienza 2014) has shown good collinearity between *H. chilense* and Triticeae species. Nevertheless, breaks in the collinearity between *H. chilense* and barley have been previously reported (Hagras et al. 2005b; Said and Cabrera 2009). In this work, the arm location of COS and EST markers in *H. chilense* showed a good correspondence with barley. The most notorious change refers to the distal parts of barley 7H since the markers located in these regions are found in the opposite arms in *H. chilense.* This is especially important in the case of *Psy1* since this gene influences grain carotenoid content

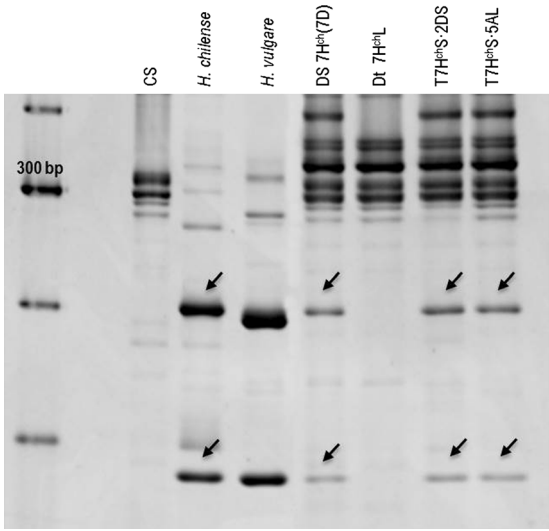


Fig. 4 PCR amplification of *Psy1* gene after digestion with enzyme *HinfI*. *Arrows* show bands from *H. chilense* genome

in wheat and tritordeum (Ficcó et al. Ficco et al. 2014; Rodríguez-Suárez et al. 2014). This gene was located in 7H^{ch}S using *H. chilense*-wheat chromosome addition lines (Atienza et al. 2007a; Rodríguez-Suárez et al. 2011), whereas it is found in the long arm of the homoeologous group 7 of wheat and barley (Ficco et al. 2014; Rodríguez-Suárez et al. 2011). On the contrary, the COS318 marker is mapped in the distal part of 7H^{ch}L (Rodríguez-Suárez et al. 2012; Rodríguez-Suárez and Atienza 2012) in agreement with the position in barley (Mascher et al. 2013). COS318 is located close to *Psy1* in barley whereas *Psy1* and COS318 are located in different arms in *H. chilense*. Furthermore, the EST markers at the distal positions of 7HS are located in 7H^{ch}L. These results might indicate an inversion between the distal parts of 7H^{ch}S and 7H^{ch}L. Nevertheless, further studies would be required to confirm this hypothesis. In any case, inter-specific hybrids between *H. vulgare* and *H. chilense* are possible, but are always sterile, and chromosome doubling to produce amphiploids has been unsuccessful (Thomas and Pickering 1985). Inversion affecting homoeologous chromosomes could be responsible for failures in chromosome pairing leading to the lack of fertility in interspecific hybrids between *H. vulgare* and *H. chilense*.

In conclusion, the genetic stocks characterized here include new wheat-*H. chilense* recombinations useful for genetic studies and with a potential for breeding. Furthermore, these lines allowed the identification of new molecular markers useful for selecting new introgressions involving 7H^{ch}. Finally, the distal parts of 7H and 7H^{ch} do not maintain the collinearity, which includes the position of *Psy1*.

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