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RESEARCH ARTICLE

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Sequence variations of *PDHA1* gene in Triticeae species allow for identifying wheat-alien introgression lines

Abstract In order to develop a molecular marker for the detection of alien chromatin, an allele-specific primer targeting pyruvate dehydrogenase (*PDHA1*) gene was used to analyze 12 taxa representing different basic genomes in Triticeae. Amplification products with different sizes were generated among species. The sequence alignments indicated that the *PDHA1* genes contained some deletions/insertions of Miniature Inverted-repeat Terminal Elements (MITE) and simple sequence repeats (SSRs), thus suggesting that the Triticeae genomes have been rapidly evolving during speciation. The genome-specific amplicons and chromosomal location of *PDHA1* gene on Triticeae genomes can be used to trace the corresponding alien chromatins from *Aegilops*, *Secale* and *Dasypyrum* species in wheat background.

Keywords *PDHA1* gene, Triticeae species, marker assisted selection, phylogeny

1 Introduction

The tribe Triticeae includes the important cereal crops such as wheat, barley, and rye as well as other species. Compared with barley and rye, both only regionally important, wheat can claim to be one of the most important species in agriculture globally. The tribe Triticeae provides a vast gene pool for most agronomically important traits including some that do not exist in wheat (Dewey, 1984). Wheat breeders have made use of wild relatives in several of the genera within the tribe.

The strategy of marker assisted selection (MAS) has been employed in breeding cereals in the recent two decades which not only makes it possible for identification of quantitative trait loci, but also provides a new way of breeding. There are different marker systems used in MAS, such as restriction-fragment length polymorphisms (RFLPs), sequence-tagged site (STS), amplified-fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs) (Merker, 1992). Due to the availability of large expressed sequence tag (EST) datasets, it has become possible to systematically search for SSRs in EST sequences with the help of bioinformatics tools (Rajeev et al., 2004). Recently, new marker systems named 'amplified consensus genetic marker' (ACGM) (Brunel et al., 1999; Fourmann et al., 2002) and 'PCR based landmark unique gene' (PLUG) (Ishikawa et al., 2007, 2009), both based on conservative regions in coding sequences and variations in introns, were developed. Since these markers are derived from ESTs, corresponding to the transcribed component of a gene unit, they have been shown to possess a high potential for interspecific transferability (Rajeev et al., 2004).

PDHA1 is one component of the pyruvate dehydrogenase (PDH) complex, a nuclear-encoded, mitochondrial matrix multienzyme system that provides the primary link between glycolysis and the tricarboxylic acid (TCA) cycle by catalyzing the irreversible conversion of pyruvate into acetyl-CoA (Randall et al., 1977; Børglum et al., 1997). Analysis of genomic DNA provided evidence of a single copy of an active *PDHA1* gene in all diploid plant and animal genomes (Patel and Korotchkina, 2006).

Since the coding regions of *PDHA1*genes are conserved but the lengths and the position of the introns are variable among sequenced grass (*Poaceae*) genomes, it allows production of molecular markers to target the *PDHA1* gene in the Triticeae species. In the present study, we analyzed the sequence variations of the second intron of *PDHA1* gene among the Triticeae species, and localized the *PDHA1* gene in different chromosomes from *Aegilops*, *Thinopyrum* and *Dasypyrum* genomes. In addition, we also present the feasibility of the molecular marker of *PDHA1* gene to trace the specific alien chromatin introduced into the wheat genome by chromosome manipulation.

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2 Materials and methods

2.1 Plant materials

A total of seventeen accessions or cultivars including diploid, tetraploid and hexaploid Triticeae species for PDHA1 gene sequencing are listed in Table 1. The wheat cv. Chinese Spring (CS) Nullisomic-tetrasomic (NT) lines and CS-*Dasypyrum villosum* addition lines (CS1V-CS7V) were kindly provided by the Wheat Genetics Resource Center, Kansas State University. CS-*Lophopyrum elongatum* addition lines (CS1E-CS7E) were obtained from Dr. Dvorak at UC Davis. The *Yr17*-resistant near isogenic lines (NIL) of cv. Avocet (*Yr17*/6*Avocet) and *Lr37*-resistant NIL of cv. Thatcher (*Lr37*/6*Thatcher), which carry a translocation between an *Aegilops ventricosa* 2NS segment and the chromosome arm 2AS of wheat were provided by Dr. Peigao Luo of Sichuan Agricultural University and Dr. Hongfei Yan of Hebei Agricultural University, respectively. An F_2 population of 120 plants was issued from the cross between L2 (resistant parent) and SY95–71 (susceptible parent).

2.2 DNA extraction, amplification, cloning, and sequencing

Total genomic DNA was prepared from young leaves according to Yang et al. (2005). The DNA concentration was determined by using a Sizhumen DNA-protein photometer. Based on the sequences of alignment of *PDHA1* genes from *Oryza sativa* (Os04g0119400), *Zea mays* (Gene ID: 100272834), *Brachypodium distachyon* (Bradi5g01420.1) and *Sorghum bicolor*(Gene ID: 8065978) as well as the EST homologies of *Hordeum vulgare* (BE420726) and wheat (CK167138), the primers PDHA1-F (5′-GGAGAGGTGAAGGACCAACTC-3′) and PDHA1-R (5′-CCTTGCAGCGTAGTGAGATTT-3′) for amplifying the second intron of *PDHA1* gene were designed and synthesized by Invitrogen, Shanghai, China. Total reaction volume was 25 μL containing 10 mmol Tris–HCl (pH 8.3), 2.5 mmol $MgCl_2$, 0.2 mmol·L^{-1} dNTP, 100 ng template DNA, 0.3 U *Taq* polymerase (Takara, Japan), and 400 nmol primer. The PCR cycle consisted of an initial 5 min denaturation at 95°C, followed by 32 cycles of 95°C for 30 s, 53°C–63°C (optimum 58°C) for 30 s, and 72°C for 2 min, followed by a final extension at 72°C for 7 min. The amplification products were separated on 1.0% agarose gels in 1×Tris-acetic-EDTA (TAE) buffer and detected by staining with 10 μg·mL^{-1} ethidium bromide. The target PCR products were purified on the agarose gels using a gel extraction kit (Qiagen, Valencia, Calif.). The purified fragment was ligated into the pMD18-T vector using T4 ligase, and then used to transform the competent cells of *Escherichia coli* JM109. The positive clones were sequenced on an automatic DNA sequencer (Invitrogen Biotech). The BLAST program in the GenBank database was used to search for sequence similarities with the DNA.

Table 1 Accessions or cultivars of Triticeae species and the *PDHA1* gene sequences

species	genome	accession	origin	GenBank accession	length/bp
Pseudoroegneria spicata	St	PI232131	NPGS, USA	GU211260	1161
Lophopyrum elongatum	E	PI153179	NPGS, Turkey	GU211258	2179
Aegilops biuncialis	U^bM^b	–	China	GU211254	1190
Aegilops ventricosa	D^vN^v	PI542385	NPGS, Turkey	GU211255	1121
Dasypyrum breviaristatum (2×)	V^b	—	WGRC, USA	GU211263	1185
Dasypyrum breviaristatum (4×)	V^bV^b	PI546317	NPGS, Morrocoi	GU211262	1124
Dasypyrum villosum	V	PI546317	NPGS, Morrocoi	GU211257	1191
Secale africanum	R	CN41211	MBG, South Africa	GU211256	1536
Secale cereale subsp. *cereale*	R	PI436168	NPGS, Chile	GU332270	1360
Secale cereale cv. CISE45	R	PI535171	NPGS, Germany	GU332271	1335
Secale strictum	R	PI317593	NPGS, Turkey	GU211265	1338
Secale sylvestre	R	PI401400	NPGS, Iran	GU211264	1536
Secale vavilovii	R	PI618682	NPGS, Poland	GU332272	1360
Secale cereale cv. Kustro	R	–	Germany	GU332273	1364
Aegilops tauschii	D	KD20042	China	GU211253	1350
Hordeum vulgare	H	PI401365	NPGS, USA	GU211259	1118
Triticum aestivum cv. Chinese Spring	ABD	–	China	GU191338	1223
				GU211251	1533
				GU211252	1355

2.3 Phylogenetic analysis

The sequences were aligned using the ClustalX 2.0 program (Larkin et al., 2007). Phylogenetic trees were constructed using maximum parsimony methods, in which the sequence of *Oryza sativa* ssp. *japonica* (GenBank accession number AJ301799) was used as outgroup. Maximum parsimony tree analyses were conducted with MEGA4.0 (Tamura et al., 2007), using the heuristic search procedure. The robustness of the trees was assessed by the bootstrap method (Felsenstein, 1985) with 10000 replications.

3 Results

3.1 PCR and sequences length variation

The PCR primers PDHA1-F and PDHA1-R were used to amplify the DNA of the Triticeae species (Table 1). The different sizes of PCR products were clearly observed among the tested species (Fig. 1). The longest sequence with 2179 bp was from *Lo. elongatum*, and the shortest one with 1118 bp was from *H. vulgare*. Most accessions had a length of about 1200 bp (Table 1, Fig. 2).

The alignment of sequences showed that the $(CT)_3$ was conserved in the exon of gene *PHDA1* in all except *O. sativa.* However, extensive variations on SSRs were observed in the intron region. Based on the program 'SSR Search', we found that rice contained only one SSR locus $(CA)_3$ at the 3′ part of the intron. However, *Lo. elongatum* had 7 different SSRs in the middle of the intron region, which led to a longer intron sequence compared with those in other species. The sequences within the *Triticum*, *Secale* and *Dasypyrum* displayed apparent variations of SSR in number and position (Fig. 2).

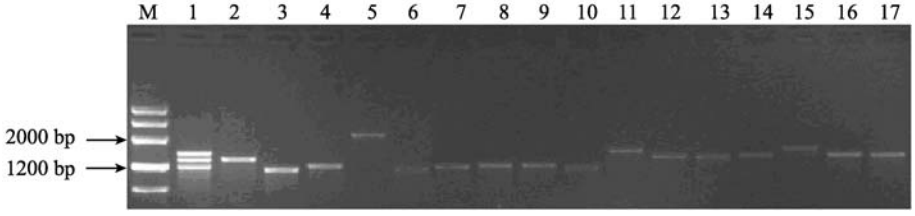

Fig. 1 Amplified products of *PDHA1* gene sequences from Triticeae species by PDHA1 primers
Note: M is 100 bp DNA ladder. 1–17 represent Chinese Spring (CS), *Ae. tauschii, Ae. ventricosa*, *Ae. biuncialis*, *Lo. elongatum*, *H. vulgare, Ps. spicata*, *D. villosum, D. breviaristatum* (2×), *D. breviaristatum* (4×), *S. africanum*, *S. cereale* ssp. *cereale*, *S. cereale* cv. CISE45, *S. cereal* cv. kustro, *S. sylvestre*, *S. vavilovii*, and *S. strictum*, respectively.

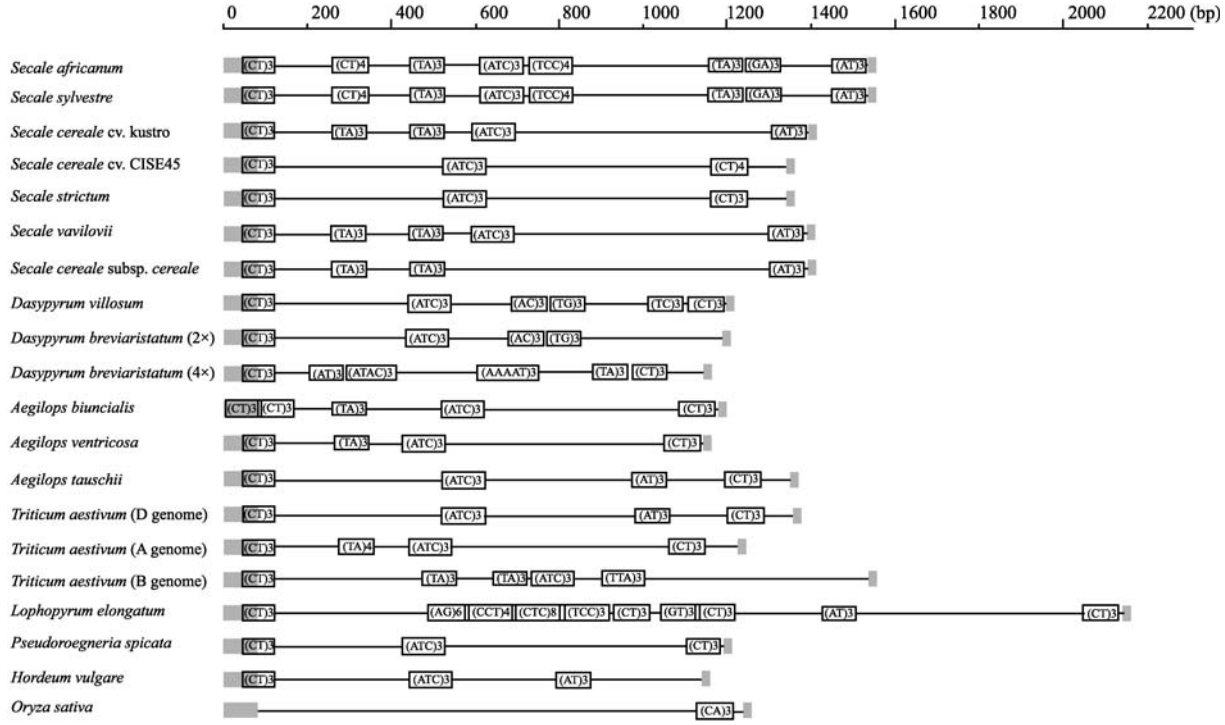

Fig. 2 Structure of the *PDHA1* sequence intron showing the location of SSR
Note: The scale indicates length of gene (bp), the gray parts indicate exons and the boxes indicate SSR.

Sequence comparison among *Secale* species revealed a 159 bp Miniature Inverted-repeat Terminal Elements (MITE) *stowaway* transposon insertion at position 1097 bp of *S. africanum* and *S. sylvestre* (Fig. 3(a)). The 5′ flanking sequences of the repeated sequence were duplicated TACT. The secondary structure of this element showed that four inverted repeat regions formed a more-or-less hairpin-like structure (Fig. 3(b)).

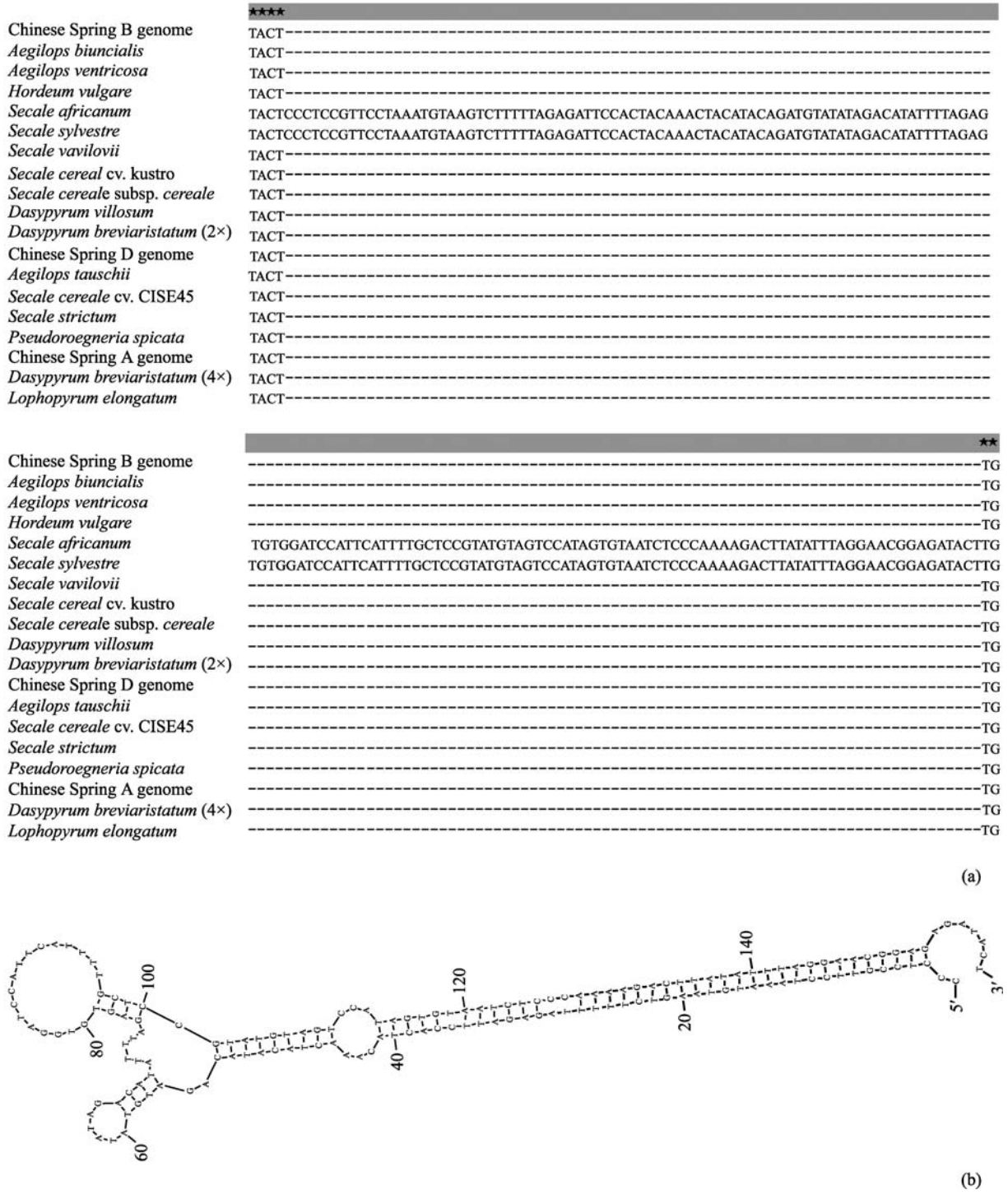

Fig. 3 The alignment and secondary structure of 159 bp MITE *stowaway* transposon insertion in the *PDHA1* gene of *S. africanum* and *S. sylvestre*

Note: (a) and (b) represent alignment structure and secondary structure, respectively.

3.2 Phylogenetic analysis

The *PDHA1* sequences were used to construct the phylogeny of *Triticum-Aegilops* (Fig. 4(a)) and the *Secale* and *Dasypyrum* species (Fig. 4(b)) using rice as the outgroup. Parsimony analysis revealed *Ae. tauschii* and common wheat cv. CS D genome formed one group, with strong supporting evidence that the D genome of common wheat was derived from *Ae. tauschii*. CS B genome was positioned remotely from other species and clustered into a separate group from A and D genomes. This difference was also indicated from the SSR distribution patterns in Fig. 2. As for the phylogenetic tree in *Secale* and *Dasypyrum* species, it clearly indicates that *S. strictum* and *S. cereale* cv. CISE45 evolved into *S. sylvestre* and *S. africanum* through MITE (Fig. 3) and the insertion of SSRs (Fig. 2). *S. vavilovii*, *S. cereale* subsp. *cereale*, and *S. cereale* cv. kustro form one group with the 100% bootstrap, supporting that *S. vavilovii* and *S. cereale* may be divergent from the ancient *Secale* at a similar evolutionary time. The phylogenetic tree also showed that the tetraploid *D. breviaristatum* was separated from the clade of the diploid *D. breviaristatum* species, suggesting great variation among the *Dasypyrum* species.

3.3 Chromosomal location of *PDHA1* gene

As shown in Fig. 5(a), the three bands from 2B, 2D, and 2A found in common wheat CS are clearly indicated. With the help of nullisomic-tetrasomic (NT) stocks of CS, we have assigned the wheat *PDHA1* gene to the homoeologous group 2 chromosomes. To further determine the chromosome location of *PDHA1* gene in other species, we used a set of CS-*D. villosum* and CS-*Lo. elongatum*

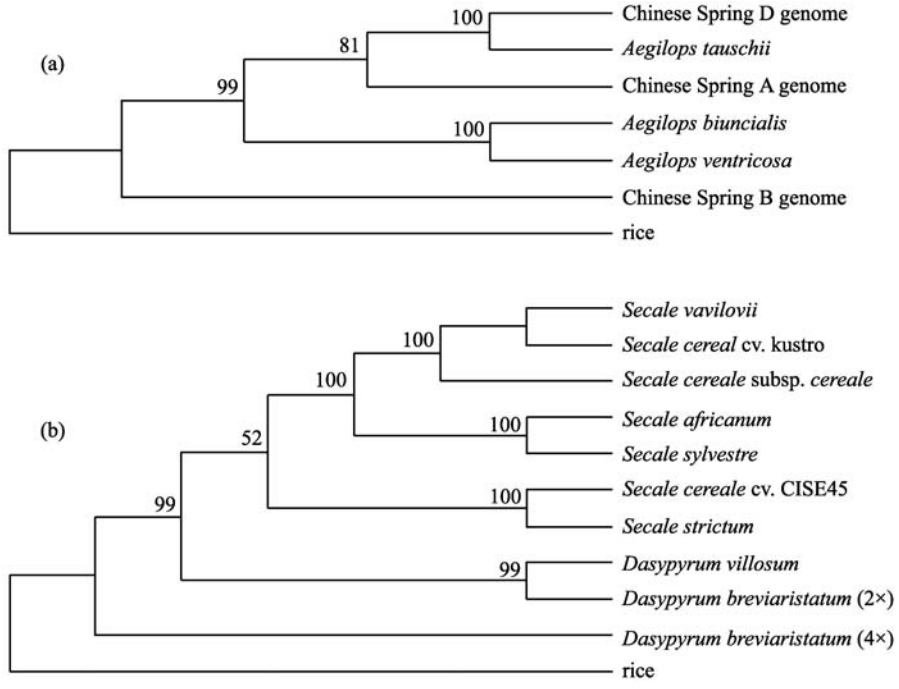

Fig. 4 Maximum parsimony trees generated from the second intron sequences of *PDHA1* gene
Note: Numbers on branches are bootstrap value > 50%.

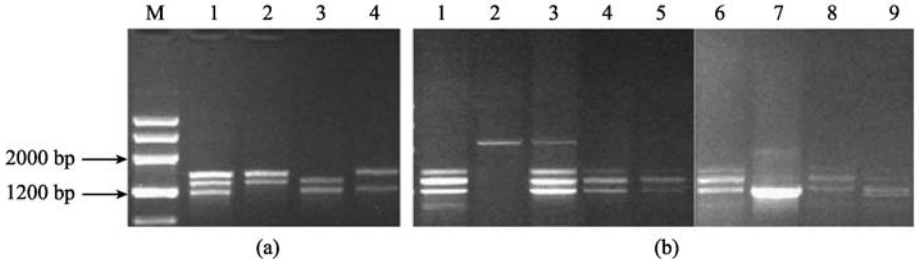

Fig. 5 Chromosomal location of *PDHA1* gene in CS (a), *D. villosum* and *Lo. elongatum* (b)
Note: M is 100 bp DNA ladder. 1–4 in Fig. 5(a) are CS, N2A-T2D, N2B-T2A and N2D-T2B and in Fig. 5(b) 1 is CS, 2 is *Lo. elongatum*, 3–5 are CS1E-CS3E, and 6–9 represent CS, *D. villosum*, CS1V and CS2V, respectively.

addition lines as materials. PCR amplification results showed that the *PDHA1* gene was located in chromosome 2V of *D. villosum* and chromosome 1E of *Lo. elongatum,* respectively (Fig. 5(b)).

Since the marker PDHA1 was located on group 2 of the wheat chromosomes, we tested the marker yellow rust resistance gene *Yr17*, which was transferred from *Ae. ventricosa* chromosome 2N to wheat chromosome 2A. We detected the DNA of *Yr17* NILs and its derived F_2 population by PCR, and found that marker PDHA1 was linked to *Yr17*. Furthermore, we cloned the target bands from *Yr17*/6*Avocet, a resistant pool of the F2 population and *Ae. ventricosa* PI542385 (Fig. 6). Alignment results showed that the PDHA1 sequences linked to the resistance gene were identical to that from *Ae. ventricosa*. This result suggested that the marker PDHA1 can be used to target the *Yr17* gene from the 2NS chromosome from *Ae. ventricosa* in marker assisted selection.

4 Discussion

Comparative studies on DNA sequence variation provide a means for analysing phylogenetic relationships over a wide range of taxonomic levels (Käss and Wink, 1997). The coding sequences, such as 5sRNA (Kellogg and Appels, 1995), *rpoA* (Petersen and Seberg, 1997), *DMC1*, *EF-G* (Petersen et al., 2006), *ACC* (Faris et al., 2001; Huang et al., 2002; Fan et al., 2009), and *RPB2* gene (Sun et al., 2007, 2008) were used for reconstruction of evolutionary trees in tribe Triticeae. However, the coding sequences were less informative with respect to determining relationships among closely related species within a genus (Gielly et al., 1996). The non-coding regions, i.e., rDNA ITS sequences were successfully used in phylogenetic analysis in the genus *Secale* (Bustos and Jouve, 2002), *Elymus* (Liu et al., 2006) and tetraploid wheat (Zhang et al., 2002). In the present work, we used the sequence variation of the *PDHA1* intron to analyze the phylogenetic relationship of some Triticeae species from *Triticum-Aegilops* complex, *Secale*, and *Dasypyrum* genera. The extensive length variations of *PDHA1* intron, due to the accumulation of SSRs and insertion of MITE, indicated that Triticeae genomes among species evolved fast. The phylogenetic analysis of seven *Secale* species (R genome) suggested that the evolutionary speciation process had two steps. The result agrees with the previous results of Bustos and Jouve (2002) based on rDNA ITS sequences. The unique MITE insertion of *PDHA1* gene made distinguishing the wheat-*S. africanum* introgression from wheat-*S. cereale* derivatives, as an alien gene transfer, remarkably easy. Moreover, the *PDHA1* sequence alignments revealed that *D. breviaristatum* (2×) and *D. villosum* formed one group, while *D. breviaristatum* (4×) was clustered into the other group. These results indicate that there was similar genomic structure and evolutionary time between *D. breviaristatum* (2×) and *D. villosum*, and that *D. breviaristatum* (4×) was not an autoploid of *D. breviaristatum* (2×). The results are also consistent with the morphological (von Bothmer and Claesson, 1990), cytological (Linde-Laursen and Frederiksen, 1991; Blanco et al., 1996) and molecular (Li et al., 2009) observations.

Effective MAS will be of importance to identify the alien chromatin transfer from various Triticeae species to cultivated wheat. The detection of the alien chromatin by molecular markers at an early generation can use the genomic specific PCR markers. After the addition or substitution lines are obtained, the distribution of the homoleogous group of alien chromatin will be essentially established. Recently, the EST-PCR primers were assigned in different homoleogous groups of wheat (Qi et al., 2004). The previous comparative genome analysis revealed that there was a high collinearity level of the coding regions existing between genomes from rice, *Brachpodium* and wheat (Ramakrishna et al., 2002; Yan et al., 2003; Chantret et al., 2004). The extensive accumulation of barley and wheat ESTs can be used to effectively generate useful functional markers in tribe Triticeae. Wang et al. (2009) found that two markers, which were designed based on EST sequences, distributed on homology group 1 chromosomes of *T. aestivum*, and can be used to screen specific markers for chromosome 1RS of *S. cereale*. Song et al. (2008) found one EST-SSR marker for discriminating chromosome arms 6AS, 6BS, 6DS, 6VS, and 6SS. In the present work, we identified the *PDHA1* gene on chromosomes 2A, 2B and 2D of hexaploid wheat-CS. Using a set of CS-*D. villosum* addition lines, we identified the *PDHA1* gene on chromosome 2V of *D. villosum*. The *Yr17* and *Lr37* were located on the translocation chromosome 2NS-2AS·2AL, and the *PDHA1* linked to the *Yr17* gene was also assigned to the 2NS chromosome while the *PDHA1* gene was identified on chromosome 1 of *Lo. elongatum*

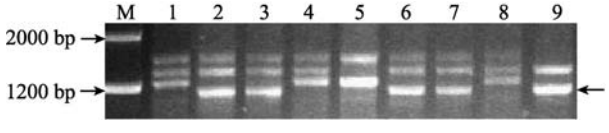

Fig. 6 DNA fragments amplified by primer PDHA1

Note: M is 100 bp DNA ladder and 1–9 represent CS, P1 (L2), Br (resistant pool), Bs (susceptible pool), P2 (SY95–71), *Yr17*/6*Avocet (*Yr17*-resistant NIL of cv. Avocet), *Lr37*/6*Thatcher (*Lr37*-resistant NIL of cv. Thatcher), Thatcher and *Ae. ventricosa* PI542385, respectively. The right arrow indicates the band is linked to *Yr17* derived from 2NS.

using a set of CS-*Lo. elongatum* addition lines. It is therefore prudent to note that the polymorphism of a single copy gene in the conserved chromosomal regions can be used as a candidate to produce molecular markers for identifying the progenies from wide crosses.

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