Genes & Genomics (2012) 34: 67-75
DOI 10.1007/s13258-011-0153-7

RESEARCH ARTICLE

Genomic rearrangement between wheat and *Thinopyrum elongatum* revealed by mapped functional molecular markers

Li-Jun Hu · Cheng Liu · Zi-Xian Zeng· Guang-Rong Li · Xiao-Jin Song · Zu-Jun Yang

Received: 27 July 2011 / Accepted: 19 September 2011 / Published online: 13 February 2012
© The Genetics Society of Korea and Springer 2012

Abstract

Thinopyrum elongatum serves as an excellent gene pool for wheat improvement. Genes for resistance to many biotic and abiotic stresses have been transferred from *Th. elongatum* to wheat through chromosome manipulation. For breeding programs, molecular markers enable screening of a large number of genotypes for alien chromosome introgressions. The main objective of the present study was to develop and characterize EST (expressed sequence tags) and PLUG (PCR-based Landmark Unique Gene) markers that can distinguish *Th. elongatum* chromatin from the wheat genomes. A total of 258 mapped EST primer pairs and 46 PLUG primer pairs were tested on DNA from wheat Chinese Spring (CS) and CS-*Th. elongatum* addition lines. The results showed that 43 primer pairs could be effectively mapped to specific *Th. elongatum* chromosomes. Twenty-two of the 43 markers displayed similar homoeologous chromosome locations to hexaploid wheat. Nine markers mapped to different linkage groups between wheat and *Th. elongatum*, while 12 makers mapped on two or three different *Th. elongatum* chromosomes. A comparison of molecular marker locations indicated that *Th. elongatum* genome was closely related to the D genome of wheat, and chromosome rearrangements and duplication had occurred in *Th. elongatum* and the wheat genomes. The markers will be useful in comparative gene mapping, chromosome evolutionary analysis, and gene introgression for wheat improvement using *Th. elongatum* accessions as gene donors.

Keywords Expressed sequence tags; GISH; *Thinopyrum elongatum*; Molecular marker

L.-J. Hu · C. Liu · Z.-X. Zeng · G.-R. Li · X.-J. Song · Z.-J. Yang(✉)
School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, 610054 Sichuan, China
email: yangzujun@uestc.edu.cn

Introduction

Wild relatives of common wheat (*Triticum aestivum* L.) have been used extensively as sources of useful genes for agriculture (Friebe et al., 1996; Trethowan and Mujeeb-Kazi, 2008). These alien-derived genes have been transferred into wheat via wide hybridization and make it possible to increase resistance and tolerance to biotic and abiotic stresses, respectively (Fedak, 1999; Feuillet et al., 2007). *Thinopyrum elongatum* (Host) A. Löve (2n = 14, genome E^eE^e) was determined to have many agronomical useful traits for wheat improvement, such as tolerance to salt (Dvorak et al., 1988; Zhong and Dvorak, 1995) and drought (Roundy, 1985), resistance to leaf rust (Friebe et al., 1996), stripe rust (Ma et al., 1999; Yang and Ren, 2001), *Fusarium* head blight (Shen et al., 2004; Jauhar et al., 2009) as well as high protein (Garg et al., 2009). With the aim to introduce useful genes from *Th. elongatum* to wheat, *T. aestivum-Th. elongatum* amphiploids and several sets of alien chromosome addition and substitution lines were developed as germplasm stocks (Dvorak and Knott, 1974; Dvorak and Chen, 1984; Tuleen and Hart, 1988; Mujeeb-Kazi et al., 2008).

From the last century, wheat breeders have crossed E-genome-containing species with wheat in the attempt to improve wheat's disease resistance or quality. Numerous resistant *Trititrigia* lines have been produced (Sharma and Knott, 1966; Sun, 1981; Li et al., 1985; Sharma et al.,1989; Friebe et al., 1994; Zhang et al., 2005). In order to identify the resistant gene(s) quickly and efficiently, the development of molecular marker assisted-chromosome manipulation has played a vital role (Gale et al., 1989; Hohmann et al., 1996; Rogowsky et al., 1991; Schwarzacher et al., 1992). Randomly amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR), microsatellite markers or simple sequence repeat (SSR) were widely applied to develop genome- or chromosome-specific markers for *Th. elongatum* species (Liu et al., 1998; You et al., 2003; Chen et al., 2007, 2010). Since the interpretation of RAPD data is sometimes limited by poor re-

peatability and the SCAR markers and genomic SSR markers lack transferability among related species, attention was focused on the PCR markers based on EST data (Mullan et al., 2005). To date, more than one million wheat expressed sequence tags (ESTs) have been registered in public databases (Wheeler et al., 2006). The abundant EST sequences and in particular the representing unigenes allowed for designing PCR primers to target the chromosomal regions of wheat (Qi et al., 2003; Ishikawa et al., 2009). EST-SSR is recognized as an efficient and stable method to develop chromosomal molecular markers depended on its association with conserved expression sequences. The transferability of EST-based PCR markers among the related species allowed it to trace the introduced alien chromatin easily (Varshney et al., 2005; Sim et al., 2009).

In this paper, we report the development of 43 molecular makers specific to *Th. elongatum* chromosomes, which reveals extensive genomic rearrangements in *Th. elongatum*. These markers proved to be powerful for detecting *Th. elongatum* chromatin in wheat background, can be widely used in wheat breeding programs.

Material and Methods

Plant materials

T. aestivum L. cv. Chinese Spring (CS, genome AABBDD, $2n$=42), *Th. elongatum* (Host) D. Dewey, CS-*Th. elongatum* amphiploid (Ag-CS-1, genome AABBDDEE, $2n$=56), and 7 disomic additions of individual *Th. elongatum* chromosome in CS (DA1E-DA7E) were kindly provided by Dr. J. Dvorak, University of California, Davis, CA, USA. *Pseudoroegneria spicata* (Pursh) A. Love (St genome, $2n = 2x = 14$) accession PI 232131were obtained from the USA National Small Grains Collection at Aberdeen, Idaho. Wheat-*Th. ponticum* partial amphiploid 7430 developed by Li et al. (1985), and were obtained by Dr. Z. J. Chang of the Shanxi Academy of Agricultural Science, China. Wheat-*Th. intermedium* ssp. *trichophorum* partial amphiploid 1520 was developed by our lab (Yang et al., 2006) and identified in this study.

DNA extraction and PCR analysis

Total genomic DNA was prepared from young seedling leaves after following the procedure of Yang et al. (2006). A total of 258 pairs of EST-SSR primers (listed in Table 1) were synthesized according to Xue et al. (2008). EST-SSR analysis was performed in total reaction volume of 25 μℓ, containing 10 mmol Tris-HCl (pH 8.3), 2.5 mmol $MgCl_2$, 200 μmol of each dNTP, 400 nmol primer, 50 ng template DNA and 0.5 U Taq polymerase (Takara, Japan). EST-SSR amplification was carried out in a MyCycleTM Thermal Cycler (Bio-RAD, USA). The cycling parameters were, 94 °C for 3 min to pre-denature; followed by 40 cycles of 94 °C for 45sec, 55 °C for 45sec, and 72 °C for 1 min; and then a final extension at 72 °C for 10 min. The PCR products were fractionated on 39:1 polyacrylamide gel by silver-staining.

PCR-based landmark unique gene (PLUG) primer sequences were developed by Ishikawa et al. (2007), the primers were listed in Table 1. PLUG primer analysis was performed in total reaction volume of 25 μℓ, containing 10 mmol Tris-HCl (pH 8.3), 2.5 mmol $MgCl_2$, 200 μmol of each dNTP, 400 nmol primer, 50 ng template DNA and 0.5 U Taq polymerase (Takara, Japan). EST-SSR amplification was carried out in a MyCycleTM Thermal Cycler (Bio-RAD, USA). The cycling parameters were 94 °C for 3 min to pre-denature; followed by 40 cycles of 94 °C for 45sec, 55 °C for 45sec, and 72 °C for 2min; and then a final extension at 72 °C for 7 min. An 8 μℓ aliquot of the product was digested for two hours with 1.0 U of *Taq*I (60 °C), *Hpa*Ⅱ(37 °C), *Hae*III (37 °C) in incubators, respectively. Digested fragments were fractionated by electrophoresis on a 3% agarose gel in TAE buffer.

GISH analysis

Pseudoroegneria spicata (St genome) genomic DNA was labeled with digoxigenin-11-dUTP by nick translation according to the manufacturer's instruction (Roche Diagnostics, USA), and Chinese Spring (CS) genomic DNA for blocking. The probe was diluted to a final concentration of 1 μg/mℓ in the hybridization solution and the hybridization mixture was prepared as described by Mukai et al. (1993). The digoxigenin labeled genomic DNA signal was detected with fluorescein-conjugated antidigoxigenin antibody (Roche Diagnostics). The slide was finally mounted in Vectashield antifade solution (Vector Laboratories, USA) with (0.25 μg/mℓ) propidium iodide for only FITC detection. Photomicrographs of GISH chromosomes were taken with an Olympus BX-51 microscope.

Results

Development of *Th. elongatum* chromosome specific markers

An initial set of 258 EST-SSR primer pairs mapped on wheat individual chromosomes by Xue et al. (2008) were applied to reveal amplified polymorphisms between Ag-CS-1 and CS. Only 100 primer pairs out of a total of 258 primer pairs amplified polymorphic bands between Ag-CS-1 and CS. These 100 primer pairs were used to develop specific markers for detecting individual *Th. elongatum* chromosomes in wheat background using *Th. elongatum*, a set of disomic addition lines (DA1E to DA7E) and CS as materials. Of the original 100 primer pairs, only 37 primer pairs could amplify polymorphic bands between wheat and *Th. elongatum*, and the remaining 63 primer pairs could not be mapped to the disomic addition lines (DA1E to DA7E). Thirteen primer pairs were from wheat A genome, 21 from B genome, and 3 from D genome. Less

Table 1. The detail information of 43 primer pairs which can be used to generated *Thinopyrum elongatum* chromosomal specific marker.

Primer pair name	Original Acession	Wheat or rice Chromosomal location	E-genome location	Amplification size	Amplification in amphiploids	Sources
Xmag3489	BM140589	1A	1E	330bp	N/A	[1]
Xmag834	TC79494	1A	1E	500bp	N/A	[1]
Xmag2137	CD876028	1D	1E	350bp	N/A	[1]
Xmag3253	CN010914	2A	2E	330bp 600bp	7430	[1]
Xmag2090	CV774559	2B	2E	350bp	1520	[1]
Xmag3616	CN008451	2D	2E&3E	250bp	N/A	[1]
Xmag905	TC95858	3A	3E	250bp	N/A	[1]
Xmag620	TC97429	3A	5E	300bp	N/A	[1]
Xmag2134	Authors' lab	3A	7E	250bp	1520, 7430	[1]
Xmag4137	BM138418	3B	2E&3E	500bp	N/A	[1]
Xmag3113	BM135674	3B	6E	450bp	N/A	[1]
Xmag574	BQ743434	3D	7E	170bp	7430	[1]
TNAC1248	CK209182	3S	3E	700bp	N/A	[2]
Xmag3733	BQ903542	4A	4E&6E	300bp	N/A	[1]
Xmag1682	CJ527931	4B	4E	850bp	1520, 7430	[1]
Xmag2055	CD373975	4B	4E	500bp	N/A	[1]
Xmag1293	TC255212	4B	5E	850bp	1520	[1]
Xmag2071	-	4B	1E&4E&7E	450bp	N/A	[1]
Xmag1426	TC263658	5B	5E	650bp	1520, 7430	[1]
Xmag4013	BQ902006	5B	5E	370bp 650bp	7430 1520	[1]
Xmag532	TC86119	5B	5E	250bp	N/A	[1]
Xmag3137	BQ161813	5B	7E	120bp 300bp	N/A	[1]
Xmag1625	-	5B	1E&2E	300bp	N/A	[1]
TNAC1503	CK209224	5S	5E	700bp	1520,7430	[2]
TNAC1559	AY847708	5S	5E	1180bp	1520,7430	[2]
A19	BF474700	5S	5E	725bp	N/A	This study
Xmag1470	TC252107	6A	1E&6E	200bp	N/A	[1]
Xmag3534	CN010475	6A	1E&6E	600bp 700bp	1520	[1]
Xmag1579	CV771791	6B	5E	500bp	N/A	[1]
Xmag3221	BM138303	6B	5E	1000bp	N/A	[1]
Xmag2276	BE498763	6B	6E	250bp	N/A	[1]
Xmag3017	BM135029	6B	6E	270bp	1520, 7430	[1]
Xmag521	TC88776	6B	1E&6E	700bp	1520,	[1]
Xmag590	TC101185	6B	1E&6E	350bp	1520	[1]
STS-CDO29	CDO29	6B	1E&6E	400bp	N/A	[1]
Xmag749	TC67842	6B	1E&6E	250bp	N/A	[1]
TNAC1674	CK211350	6S	6E	700bp	1520	[2]
TNAC1702	CK157364	6S	1E&6E	500bp	N/A	[2]
Xmag1715	BE406627	7A	5E	150bp	N/A	[1]
Xmag1809	CD896941	7A	7E	500bp	7430,	[1]
Xmag278	CF133375	7A	7E	400bp	1520, 7430	[1]
Xmag3284	BI750867	7A	7E	400bp 500bp	1520, 7430,	[1]
Xmag1932	CD880294	7B	7E	300bp 700bp	N/A	[1]

Note: [1]=Xue et al (2008); [2]=Ishikawa et al. 2009; N/A=No amplification

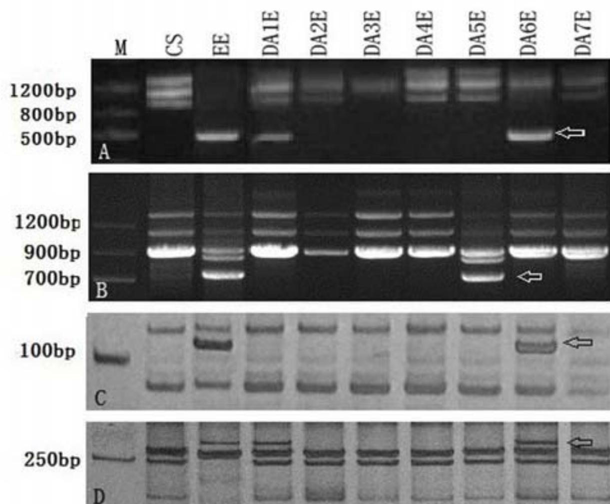

Figure 1. PCR amplification patterns of primer pairs TNAC1702 (A), A19 (B), Xmag3137 (C) and Xmag1470 (D) on wheat, *Th.. elongatum* and wheat-*Th.. elongatum* addition lines.

Note: Fig.1 A and B were from agarose gel electrophoresis, Fig.1 C and D were from PAGE stain.

than 10% (3/37) of the primer pairs could distinguish the wheat D genome from *Th. elongatum* E genome implied that *Th. elongatum* E genome and wheat D genome had a very close relationship. In comparison, over 50% (21/37) primer pairs from wheat B genome could amplify polymorphic bands, indicating that *Th. elongatum* E genome was relatively distant to wheat B genome. As shown in Figure 1C, the amplification pattern of EST-PCR primer Xmag3137 showed that one strong additional band in *Th. elongatum* and DA6E was generated compared to other material tested, suggesting that it might be a marker for 6E chromosome. However, the amplification patterns of Xmag1470 (Fig. 1D) showed that polymorphism DNA bands could be found in both DA1E and DA6E simultaneously, indicating that the primer pairs were able to detect both 1E and 6E in wheat background.

The PLUG primers are designed based on rice syntenic region, and presumably amplify fragments corresponding to the similar linkage group(s) of wheat relatives (Ishikawa et al., 2007). If verified, they will be useful markers to identify the conserved regions and produce alien chromosome-specific markers. Of 46 PLUG primers, 5 markers (11%) gave rise to the specific amplification of *Th. elongatum* DNA. As shown in Figure 1A, primer pair TNAC1702 clearly generated 6E-specific bands. Table 1 shows the specific homoeologous relationships between wheat and *Th. elongatum.*

Among the initial set of 258 EST-SSR primer pairs, 37 were found to amplify additional DNA bands from *Th. elongatum* compared to the control CS. Among these 37 primer pairs, 17 (45.9%) were mapped to the same homoeologous chromosome groups of *Th. elongatum* as with hexaploid wheat, indicating that *Th. elongatum* and wheat possessed well-conserved synteny in those chromosome regions. Nine primers (24.3%) were mapped to different linkage groups of wheat and *Th. elongatum*, suggesting that chromosome rearrangement had occurred between *Th. elongatum* and wheat genome during the evolution. Eleven primers (29.7%) gave rise to additional amplification bands in other chromosome groups of the E genome, besides those that corresponding with groups of wheat. This result indicated either a degree of gene/chromosome segmental duplication occurred in *Th. elongatum,* or segmental deletion in the wheat genome (Table 1).

Application of markers to identify wheat-*Thinopyrum* derivatives

One wheat-*Th. ponticum* partial amphiploid ‘7430’, containing 16 J or J^s (E^e and/or E^b) chromosomes (Fig. 2A) in wheat background, was reported to resistance to strip rust and powdery mildew (Chen, 2005). However, the homoeologous group

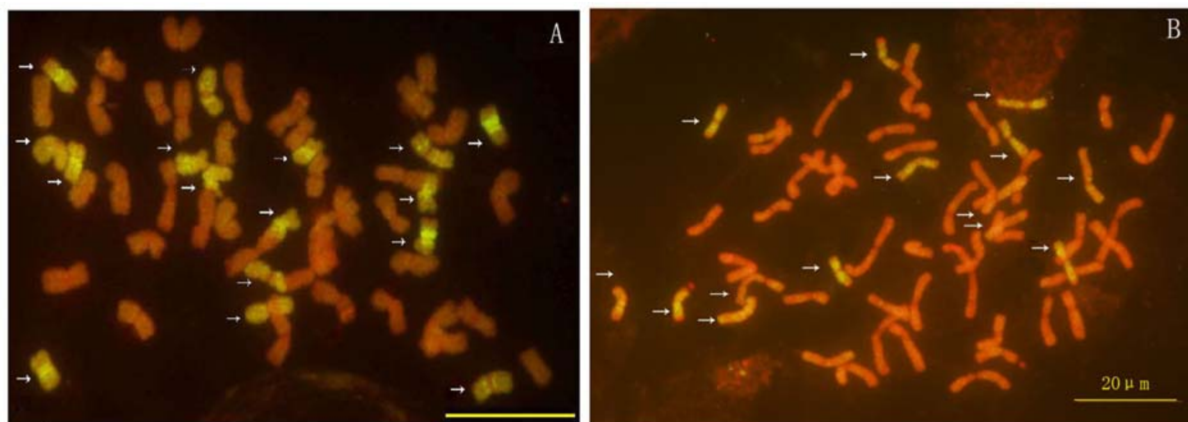

Figure 2. GISH patterns of wheat-*Th. ponticum* partial amphiploid 7430 (A) and wheat-*Th. intermedium* ssp. *trichophorum* partial amphiploid 1520 (B) using St genomic DNA as probe. Arrowed indicated the *Thinopyrum* chromosomes. Bars indicated 20μm.

of alien chromosomes in '7430' remains unknown. In order to determine the detailed genome constitution, the *Th. elongatum* (E genome) specific markers were used to characterize line '7430'. Nine markers (listed in Table 1) clearly amplified DNA from 7430 with corresponding E genome specific bands (figure not shown), which suggested possibly that 7430 contained chromosomes $2E^e$, $4E^e$, $5E^{e,}$ $6E^e$ and $7E^e$. A wheat and *Th. intermedium* ssp. *trichophorum* partial amphiploid 1520 carrying stripe rust and powdery mildew resistances was studied by GISH. In our study, the result indicated that Line 1520 possessed 14 alien chromosomes including St, J or J^s (E^e and/or E^b) (Fig. 2B). The amplification of the markers also showed that Line 1520 may have chromosomes $1E^e$, $5E^e$, $6E^e$ and $7E^e$. It is thus feasible to apply the E genome specific markers to determine the genomic designations of *Th. intermedium* and *Th. ponticum* chromatin in wheat background.

Discussion

The availability of wheat chromosome bin mapped sequences, including EST-SSRs and PLUGs, has promoted the development of molecular markers for detection of alien chromatin in a wheat background (Ishikawa et al., 2007; Mohan et al., 2007; Zhuang et al., 2008; Xue et al., 2008). EST-SSR and PLUG primers reveal the polymorphism among wheat's A, B and D genomes. The STS-SSR and PLUG markers are useful for facilitating chromosome engineering of alien chromatin in wheat background (Jia et al., 2011; Hu et al., 2011). Depending on the alien species and chromosomes involved, the ratio of informative markers for alien chromosomes developed from wheat EST-SSRs or PLUGs varies; 9.1% (4/44) for *Hordeum chilense* $4H^{ch}$ chromosome (Said and Cabrera, 2009), 12.5% (7/56) for *D. breviaristatum* (Liu et al., 2010) and 45% (29/64) for *Aegilops* species which have N, M, U and C genomes (Bandopadhyay et al., 2004). In the present study, we identified 37 polymorphic EST-SSR markers specific for *Th. elongatum* chromosome from 258 selected EST-SSRs (14.3%), and 5 PLUG markers from 48 PLUGs (10.4%), which revealed a similar ratio as the reports of *H. chilense* and *D. breviaristatum*, but much lower than that of *Aegilops* species. It is suggested that *Aegilops* genomes are closely related to wheat genomes, and *Th. elongatum* genome is more distantly related to wheat genomes.

Until now, wheat-*Th. elongatum* amphiploids, chromosome addition, substitution and translocation lines have been developed. *Th. elongatum*-specific chromosome markers will be of importance for further chromosome manipulation in wheat breeding programs. Recently, several molecular markers were established to identify the *Th. elongatum* chromosomes (Table 2), based on the published markers we concluded that there were over 200 specific primers for *Th. elongatum* chromosomes. Nevertheless, stable and accurate PCR-based molecular markers still needed to be developed and verified. In this study, we developed 43 stable molecular markers specific to individual chromosomes of 1E through to 7E. In addition, we identified some of these markers which can be applied to other wheat-*Thinopyrum* partial amphiploids. Interesting, wheat-*Thinopyrum* partial amphiploids often contain translocations between alien genomes, as shown in Figure 2A. The J^s chromosomes in Line 7430 were translocation chromosomes apparently derived from interchanges between St and J (E^e and/or E^b) chromosomes, and the result complies with the early study (Chen, 2005), other studies showed that translocations appeared frequently in *Th. intermedium* partial amphiploids (Fedak et al., 2000; 2005) which suggested that there are close relationships between St chromosomes and J (E^e and/or E^b) chromosomes (Chen, 2005). According to results in Table1, the markers we established not only detect the E chromosome of *Th. elongatum* but also trace the E chromosome into wheat-*Thinopyrum* partial amphiploids Line 7430 and Line 1520 we created. In addition, the markers will be used in characterized the E chromosome in progeny of wheat-*Thinopyrum*.

Wide hybridization between common wheat and its relatives is recognized as a classical approach to improve wheat quality through non-GM methods. The genus *Thinopyrum (Agropyron)* is an excellent genetic resource for wheat improvement. Several rust resistance genes, such as *Lr19*, Lr24, *Lr29*, *Sr24*, *Sr25* and *Sr26* had already been transferred from *Thinopyrum spp.* to wheat via translocation or recombinant lines (Jiang et

Table 2. Other specific markers for *Th. elongatum* chromosomes.

Marker type	Chromosomes location	Number of marker	Reference
repetitive DNA	E genome	-	McIntyre et al. 1988; Zhang et al. 1990
RAPD	1E and 3E	4	Liu et al. 1998;
RAPD	E genome	-	Zhang et al. 1998;You et al. 2002
Biochemical markers	E genome	5	Liu et al. 1999
SSR	E genome	25	You et al. 2003
RGAP	1E-7E chromosomes	30	Chen et al. 2007
RGA	E genome	20	Chen et al. 2010
EST-SSR	E genome	150	Mullan et al. 2005; Wang et al. 2010
AFLP and STS	E genome	28	Zhang et al. 2007
CAPS markers	E genome	-	Li et al. 2007

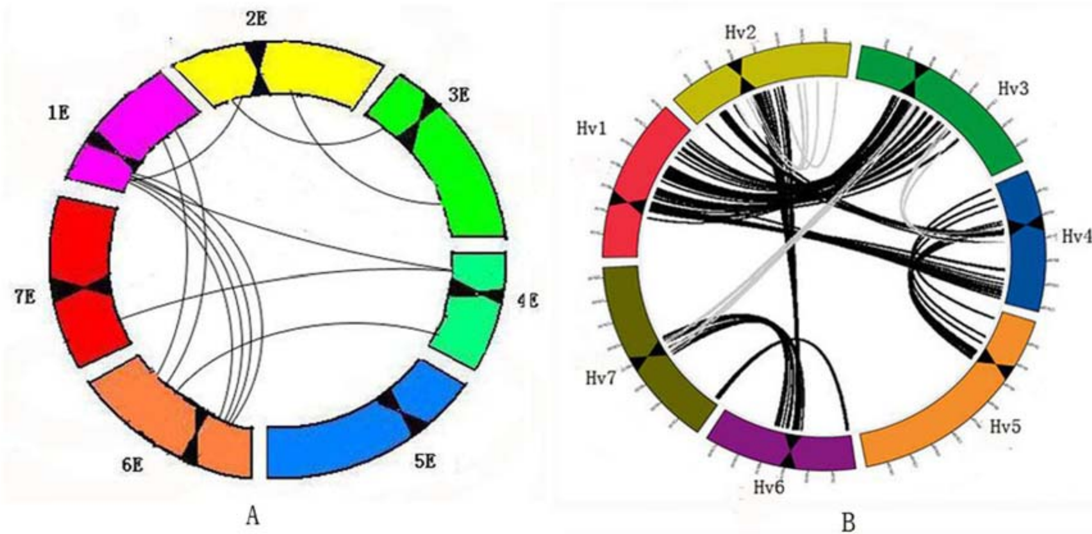

Figure 3. The putative *Th. elongatum* chromosome segmental duplication (A) compared with barley (B, Mayer et al, 2011) genomes

al., 1994, Friebe et al., 1996). The majority of these translocation lines involve wheat D chromosomes (Friebe et al., 1996). Using genomic Southern and in situ hybridization, Liu et al. (2007) found that E genomes DNA readily hybridized to wheat D genome than A and B genomes, indicating the close affinity of the E genome to the D genome. Because molecular markers based on the synteny of different genomes are useful for determining the homoeologous relationships of chromosomes from different species by comparative mapping (van Deynze et al., 1998; Heslop-Harrison, 2000), in the present research, the EST-SSR and PLUG primers were choose to reveal the polymorphism between wheat and *Th. elongatum* genomes. The marker data support the view that E genome is more close to wheat D genome compared to A and B genome, and this may indicate why most of the spontaneous wheat-*Th. elongatum* translocations and substitutions usually happen in the D genome, but rarely involving the A and B genomes.

Based on the evolutionary analysis, the grass family Poaceae displays a high degree of variation in overall genomic size, ploidy level, and chromosome number (Devos, 2005). A series of whole-genome and segmental duplications, chromosome fusions, and translocations drives the rapid evolution of grass genomes. Salse et al. (2008) provided evidence that 12 ancestral chromosomes of grass species arose from duplications and rearrangements of a basic number of five chromosomes. Anderson et al. (1992) estimated that 25 to 30% gene duplications existed for the wheat genome by RFLP markers. Mohan et al. (2007) also found that 19% of detected wheat EST-SSR markers localized on multiple loci on non-homoeologous chromosomes of *Secale cereale* L., which might represent duplicated DNA sequences in rye. Recently, Mayer et al. (2011) identified nine major duplications (212 paralogous pairs) that cover 48% of the barley genome, and six of these corresponded to previously described ancestral segmental duplications shared between grass genomes. In the present study, we found that 11 primer pair markers that could amplify fragments from different homologous group chromosomes of *Th. elongatum* (Table 1), indicating chromosome duplication also occurred in *Th. elongatum.* Based on the molecular marker data, a putative *Th. elongatum* chromosome segmental duplication draft was constructed (Fig. 3A). We constructed a scaffold based on conserved synteny for all wheat chromosomes, (Fig. 3A) showing several chromosome segmental duplications between *Th. elongatum* chromosomes 1E and 6E, and mild duplication involving chromosomes 2E and 3E, 4E and 6E, 4E and 7E etc. We have not found any duplications involving chromosome 5E. The *Th. elongatum* chromosome 2E -3E duplication was also revealed by Udall et al. (2005), which was consistent with our findings. Compared with the duplication in individual chromosomes of barley genome (Fig. 3), the present studies showed that *Th. elongatum* contained unique chromosome duplication patterns. The *Th. elongatum* genome appears to have presumable translocations (such as 2E and 3E etc.) and frequent rearrangements (such as 1E and 6E) involved in the rapid genomic evolution. Extensive duplication apparently occurred in cereal chromosome linkage groups 1 and 6 based on the evolutionary analysis of seeds protein gene families (Xu and Messing, 2009). Our previous studies also indicated that the *Th. elongatum* genome displays rapid genomic divergence by analysis of the gliadin gene family in *Th. elongatum* compared to wheat gliadin genes (Li et al., 2010). Also, there were 100 pairs of primers could amplify the polymorphic bands between Ag-CS-1 and CS, but only 37 pairs of primers located to the disomic addition lines (DA1E to DA7E), This result possibly suggests

that chromosomal structural changes had occurred during the process of producing the alien chromosome addition lines from the amphiploid. During this experimental procedure, EST sequences may have been deleted during the process of *Th. elongatum* chromosomes being added to the wheat background, which is possibly similar to the studies on sequences loss occurred when *Secale cereale* chromosomes introgressed to wheat (Ma et al., 2004; Tang et al., 2009). Detailed genomic analysis of the diploids of genera *Secale*, *Dasypyrum* and *Thinopyrum*, provide valuable insights into the long history of chromosome rearrangements and segmental duplications that may have occurred during Triticeae genomic evolutionary history.

Acknowledgement We particularly thank Dr. I. Dundas (The University of Adelaide, Australia) and other two anonymous editors for their reviewing of the manuscript. We thank the National Natural Science Foundation of China (No. 30170502, 31101143, 31171542), Open Foundation of state Key lab of CAS (2010-PCCE-KF-03), and Young Scholars Foundation from the Science and Technology Committee of Sichuan (2008-31-371) for financial support.

References

Anderson JA, Ogihara Y, Sorrells ME, and Tanksley SD (1992) Development of a chromosomal arm map for wheat based on RFLP markers. Theor. Appl. Genet. 83: 1035-1043.

Bandopadhyay R (2004) DNA polymorphism among 18 species of *Triticum-Aegilops* complex using wheat EST-SSRs. Plant. Sci. 166: 349-356.

Chen GY, Dong P, Wei YM, He K, Li W, and Zheng YL (2007) Development of E^e -chromosome- specific RGAP markers for *Lophopyrum elongatum* (Host) A. Love in wheat background by using resistance gene analog polymorphism. Acta. Agron. Sin. 33: 1782-1787.

Chen GY, Wei YM, Liu YX, Wei L, Wang JR, and Zheng YL (2010) Identification of resistance gene analogues (RGA) and development of E chromosome-specific RGA markers in wheat-*Lophopyrum elongatum* addition lines. Crop & Pasture. Sci. 61: 929-937.

Chen Q (2005) Detection of alien chromatin introgression from *Thinopyrum* into wheat using S genomic DNA as a probe - A landmark approach for *Thinopyrum* genome research. Cytogenet. Genome. Res. 109: 350-359.

Devos K (2005) Updating the ‘crop circle’. Curr. Opin. Plant. Biol. 8: 155-162.

Dvorak J and Knott D (1974) Disomic and ditelosomic additions of diploid *Agropyron elongatum* chromosomes to *Triticum aestivum*. Can. J. Genet. Cytol. 16: 399-417.

Dvorak J and Chen KC (1984) Distribution of nonstructural variation between wheat cultivars along chromosome arm *6Bp*: evidence from the linkage map and physical map of the arm. Genetics 106: 325-333.

Dvorak J, Edge M, and Ross K (1988) On the evolution of the adaptation of *Lophopyrum elongatum* to growth in saline environments. Proc. Acad. Sci. USA 85: 3805-3809.

Fedak G (1999) Molecular aids for integration of alien chromatin through wide crosses. Genome 42: 584-591.

Fedak G, Chen Q, Conner RL, Laroche A, Petroski R, and Armstrong KW (2000) Characterisation of wheat-*Thinopyrum* partial amphiploids by meiotic analysis and genomic in situ hybridization. Genome 43: 712-719.

Fedak G and Han FP (2005) Characterization of Derivatives from Wheat-*Thinopyrum* Wide Crosses. Cytogenet. Genome. Res. 109: 360-367.

Feuillet C, Langridge P, and Waugh R (2007) Cereal breeding takes a walk on the wild side. Trends in Genetics 24: 24-32.

Friebe B, Jiang J, Knott DR, and Gill BS (1994) Compensation indices of radiation-induced wheat-*Agropyron elongatum* translocations conferring resistance to leaf rust and stem rust. Crop. Sci. 34: 400-404.

Friebe B, Jiang JM, Raupp WJ, McIntosh RA, and Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91: 59-87.

Friebe B, Tuleen NA, Badaeva ED, and Gill BS (1996) Cytogenetic identification of *Triticum peregrinum* chromosomes added to common wheat. Genome 39: 272-276.

Gale MD, Sharp PJ, Chao S, and Law CN (1989) Applications of genetic markers in cytogenetic manipulation in wheat. Genome 31: 137-142.

Garg M, Tanaka H, Ishikawa N, Takata K, Yanaka M, and Tsujimoto H (2009) *Agropyron elongatum* HMW-glutenins have the potential to improve wheat end product quality through targeted chromosome introgression. J. Cereal. Sci. 50: 358-363.

Heslop-Harrison JS (2000) Comparative genome organization in plants: from sequence and markers to chromatin and chromosomes. Plant Cell 12: 617-635.

Hohmann U, Badaeva K, Busch W, Friebe B, and Gill BS (1996) Molecular cytogenetic analysis of *Agropyron* chromatin specifying resistance to barley yellow dwarf virus in wheat. Genome 39: 336-347.

Hu LJ, Li GR, Zeng ZX, Chang ZJ, Liu C, and Yang ZJ (2011) Molecular characterization of a wheat-*Thinopyrum ponticum* partial amphiploid and its derived substitution line for resistance to stripe rust. J. Appl. Genetics. 52: 279-285.

Ishikawa G, Yonemaru J, Saito M, and Nakamura T (2007) PCR-based landmark unique gene (PLUG) markers electively assign homoeologous wheat genes to A, B and D genomes. BMC Genomics 8: 135.

Ishikawa G, Nakamura T, Ashida T, Saito M, Nasuda S, Endo TR, Wu J, and Matsumoto T (2009) Localization of anchor loci representing five hundred annotated rice genes to wheat chromosomes using PLUG markers. Theor. Appl. Genet. 118: 499-514.

Jauhar PP, Peterson TS, and Xu SS (2009) Cytogenetic and molecular characterization of a durum alien disomic addition line with enhanced tolerance to Fusarium head blight. Genome 52: 467-483.

Jia JQ, Li GR, Liu C, Lei MP, and Yang ZJ (2011) Characterization of wheat yellow rust resistance gene Yr17 using EST-SSR and rice syntenic region. Cereal. Res. Commun. 39: 88-99.

Jiang JM, Friebe B, and Gill BS (1994) Recent advances in alien gene transfer in wheat. Euphytica 73: 199-212.

Li GR, Liu C, Zhang T, Zhou JP, and Yang ZJ (2010) The genomic evolution of the *Thinopyrum* and *Dasypyrum*: Evidence from α-gliadin sequences. Annual Wheat Newsletter 56: 41-44.

Li XM, Lee BS, Mammadov AC, Koo BC, Mott IW, and Wang RRC (2007) CAPS markers specific to E^b, E^e, and R genomes in the tribe Tritieae. Genome 50: 400-411.
Li ZS, Rong S, Zhong GC, Chen SY, and Mu SM (1985) Wheat wide-cross. Beijing: Science Press 52-83.
Liu C, Li GR , Sehgal KS , Jia JQ , Yang ZJ, Friebe B, and Gill BS (2010) Genome relationships in the genus *Dasypyrum*: evidence from molecular phylogenetic analysis and in situ hybridization. Plant. Syst. Evol. 288: 149-156.
Liu SB, Jia JZ, Wang HG, Kong LR, and Zhou RH (1998) Special chromosome markers for E genome and DNA polymorphism between *Agropyron elongatum* (2n=14) and common wheat detected by RAPD marker. Acta. Agro. Sin. 24: 687-690.
Liu SB, Jia JZ, Wang HG, Kong LR, and Zhou RH (1999) Identification of homology between the *Elytrigia elongatum* (2n = 14, EE) and wheat chromosomes using biochemical and molecular markers. Acta. Genetica. Sinica. 1: 37-42.
Liu Z, Li D, and Zhang X (2007) Genetic Relationships Among Five Basic Genomes St, E, A, B and D in Triticeae Revealed by Genomic Southern and in situ Hybridization. J. Integr. Plant. Biol. 49: 1080-1086.
Ma JX, Zhou RH, Dong YS, and Jia JZ (1999) Gene location for resistance to wheat strip rust derived from *Lophopyrum elongatum* (Host) A. Love. Chin. Science. Bull. 44: 65-69.
Ma XF, Fang P, and Gustafson JP (2004) Polyploidization-induced genome variation in triticale. Genome 47: 839-848.
Mayer KFX, Martis M, Hedley PE, Simkova H, Liu H, Morris JA, Steuernage B, Taudien S, Roessner S, Gundlach H et al. (2011) Unlocking the barley genome by chromosomal and comparative genomics. Plant Cell 23: 1249-1263.
McIntyre CL, Clarke BC, and Appels R (1988) Amplification and dispersion of repeated DNA sequences in the Triticeae. Plant. Syst. Evol. 160: 39-59.
Mohan A, Goyal A, Singh R, Balyan HS, and Gupta PK (2007) Physical mapping of wheat and rye expressed sequence tag-simple sequence repeats on wheat chromosomes. The Plant Genome. 1: 3-13.
Mujeeb Kazi A, Cortes A, Gul A, Farooq M, Majeeb F, Ahmad I, Bux H, William M, Rosas V, and Delgado R (2008) Production and cytogenetics of a new *Thinopyrum elongatum*/*Triticum aestivum* hybrid, its amphiploid and backcross derivatives. Pak. J. Bot. 40: 565-579.
Mukai Y, Friebe B, Hatchett JH, Yamamoto M, and Gill BS (1993) Molecular cytogenetic analysis of radiation-induced wheat-rye terminal and intercalary chromosomal translocations and the detection of rye chromatin specifying resistance to Hessian fly. Chromosoma 102: 88-95.
Mullan DJ, Platteter A, Teakle NL, Appels R, Colmer TD, Anderson JM, and Francki MG (2005) EST-derived SSR markers from defined regions of the wheat genome to identify *Lophopyrum elongatum* specific loci. Genome 48: 811-822.
Qi LL, Echalier B, Friebe B, and Gill BS (2003) Molecular characterization of a set of wheat deletion stocks for use in chromosome bin mapping of ESTs. Funct. Integr. Genom. 3: 39-55.
Rogowsky PM, Guidet FLY, Langridge P, Shepherd KW, and Koebner RMD. (1991) Isolation and characterization of wheat-rye recombinants involving chromosome arm 1DS of wheat. Theor. Appl. Genet. 82: 537-544.
Roundy BA (1985) Emergence and establishment of Basin wild rye *Elymus*-Cinereus and tall wheatgrass *Agropyron-Elongatum* in relation to moisture and salinity. J. Range Manage. 38: 126-31.
Salse J, Bolot S, Throude M, Jouffe V, Piegu B, Quraishi UM, Calcagno T, Cooke R, Delseny M, and Feuillet C (2008) Identification and characterization of shared duplications between rice and wheat provide new insight into grass genome evolution. Plant Cell 20: 11-24.
Said M and Cabrera A (2009) A physical map of chromosome $4H^{ch}$ from *H. chilense* containing SSR, STS and EST-SSR molecular markers. Euphytica 167: 253-259.
Schwarzacher T, Heslop-Harrison JS, Anamthawat Jónsson K, Finch RA, and Bennett MD (1992) Parental genome separation in reconstructions of somatic and premeiotic metaphases of *Hordeum vulgare* × *H. bulbosum*. J. Cell. Sci. 101: 13-24.
Sharma D and Knott DR (1966) The transfer of leaf rust resistance from *Agropyron* to *Triticum* by irradiation. Can. J. Genet. Cytol. 8: 137-143.
Sharma HC, Ohm H, Liste, R, Foster JE, and Shukle RH (1989) Response of wheatgrasses and wheat × wheatgrass hybrids to barley yellow dwarf virus. Theor. Appl. Genet. 77: 369-374.
Shen X, Kong L, and Ohm H (2004) Fusarium head blight resistance in hexaploid wheat (*Triticum aestivum*)-*Lophopyrum* genetic lines and tagging of the alien chromatin by PCR markers. Theor. Appl. Genet. 108: 808-813.
Sim SC, Yu JK, Jo YK, Sorrels ME, and Jung G (2009) Transferability of cereal EST-SSR markers to ryegrass. Genome 52: 431-437.
Sun SC (1981) The approach and methods of breeding new varieties and new species from *Agrotriticum* hybrids. Acta. Agro. Sin. 7: 51-58.
Tang Z, Fu S, Ren Z, and Zou Y (2009) Rapid evolution of simple sequence repeat induced by allopolyploidization. J. Mol. Evol. 69: 217-228.
Trethowan RM and Mujeeb-Kazi A (2008) Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat. Crop. Sci. 48:1255-1265.
Tuleen NA and Hart GE (1988) Isolation and characterization of wheat *Elytrigia* elongate chromosome 3E and 5E addition and substitution line. Genome 30: 289-292.
Udall JA, Quijada PA, and Osborn TC (2005) Detection of chromosomal rearrangements derived from homoeologous recombination in four mapping populations of *Brassica napus* L. Genetics 169: 967-979.
Van Deynze AE, Sorrells ME, Park WD, Ayres NM, Fu H, Cartinhour SW, Paul E, and McCouch SR (1998) Anchor probes for comparative mapping of grass genera. Theor. Appl. Genet. 97: 356-369.
Varshney RK, Sigmund R, Borner A, Korzun V, Stein N, Sorrells ME, Langridge P, and Graner A (2005) Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. Plant. Sci. 168: 195-202.
Wang RRC, Larson SR, and Jensen KB (2010) Analyses of *Thinopyrum bessarabicum*, *T. elongatum*, and *T. junceum* chromosomes using EST-SSR markers. Genome 53: 1083-1089.
Wheeler DL, Barrett T, Benson DA, Bryant SH, Canese K, ChetverninV, Church DM, DiCuccio M, Edgar R, Federhen S et al. (2006) Database resources of the National Centerfor Biotechnology Information. Nucleic. Acids. Res. 34: 173-180.
Xu JH and Messing J (2009) Amplification of prolamin storage protein genes in different subfamilies of the Poaceae. Theor. Appl. Genet. 119: 1397-1412.

Xue S, Zhang Z, Lin F, Kong Z, Cao Y, Li C, Yi H, Mei M, Zhu H, Wu J et al (2008) A high-density intervarietal map of the wheat genome enriched with markers derived from expressed sequence tags. Theor. Appl. Genet. 117: 181-189.

Yang ZJ, Li GR, Chang ZJ, Zhou JP, and Ren ZL (2006) Characterization of a partial amphiploid between *Triticum aestivum* cv. Chinese Spring and *Thinopyrum intermedium* ssp. *trichophorum*. Euphytica 149: 11-17.

Yang ZJ and Ren ZL (2001) Chromosomal distribution and genetic expression of *Lophopyrum elongatum* (Host) A. Löve genes for adult plant resistance to stripe rust in wheat background. Genet. Resour. Crop. Ev. 48: 183-187.

You MS, Li BY, Tang ZH, Liu SB, Song JM, Mao SF, and Liu GT (2002) Establishment of E-genome-specific RAPD and SCAR markers for *Thinopyrum ssp*. J. Chin. Agricu. Univ. 7: 1-6.

You MS, Li BY, Tian ZH, Tang ZH, Liu SB, and Liu GT (2003) Development of specific SSR markers for E^e-genome of *Thinopyrum* ssp. by using wheat microsatellites. J. Agric. Biotech. 11: 577-581.

Zhang HB and Dvorak J (1990) Isolation of repeated DNA sequences from *Lophopyrum elongatum* for detection of *Lophopyrum* chromatin in wheat genomes. Genome 33: 283-293.

Zhang XY, Dong YC, Li P, and Wang RRC (1998) Distribution of E- and St- specific RAPD fragments in few genomes of Triticeae. Acta. Genet. Sin. 25: 131-141.

Zhang WJ, Lukaszewski AJ, Kolmer J, Soria MA, Goyal S, and Dubcovsky J (2005) Molecular characterization of durum and common wheat recombinant lines carrying leaf rust resistance (*Lr19*) and yellow pigment (Y) genes from *Lophopyrum ponticum*. Theor. Appl. Genet. 111: 573-582.

Zhong GY and Dvorak J (1995) Chromosomal control of the tolerance of gradually and suddenly imposed salt stress in the *Lophopyrum elongatum* and wheat, *Triticum aestivum* L., genomes. Theor. Appl. Genet. 90: 229-236.

Zhang L, Yan HZ, Zheng YL, Liu DC, Dai SF, Zhang LQ, and Wei YM (2008) Development of E^e-chromosome specific AFLP and STS molecular marker for *Lophopyrum elongatum* in Chinese Spring wheat background. J. Agric. Biotech. 16: 465-473.

Zhuang LF, Song LX, and Qian BL (2008) Development and chromosome mapping of new wheat EST-SSR markers and application for characterizing rye chromosomes added in wheat. Acta. Agron. Sin. 34: 926-933.