Theor Appl Genet (2005) 111: 1617–1622
DOI 10.1007/s00122-005-0093-1

ORIGINAL PAPER

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BAC libraries of *Triticum urartu*, *Aegilops speltoides* and *Ae. tauschii*, the diploid ancestors of polyploid wheat

Received: 14 June 2005 / Accepted: 22 August 2005 / Published online: 23 September 2005

Abstract *Triticum urartu, Aegilops speltoides* and *Ae. tauschii* are respectively the immediate diploid sources, or their closest relatives, of the A, B and D genomes of polyploid wheats. Here we report the construction and characterization of arrayed large-insert libraries in a bacterial artificial chromosome (BAC) vector, one for each of these diploid species. The libraries are equivalent to 3.7, 5.4 and 4.1 of the *T. urartu*, *Ae. speltoides, Ae. tauschii* genomes, respectively. The predicted levels of genome coverage were confirmed by library hybridization with single-copy genes. The libraries were used to estimate the proportion of known repeated nucleotide sequences and gene content in each genome by BAC-end sequencing. Repeated sequence families previously detected in Triticeae accounted for 57, 61 and 57% of the *T. urartu*, *Ae. speltoides* and *Ae. tauschii* genomes, and coding regions accounted for 5.8, 4.5 and 4.8%, respectively.

Introduction

The availability of a large-insert genomic library is considered an absolute prerequisite for positional gene cloning. Large-insert libraries are also indispensable for studies of genome structure. Bacterial artificial chromosomes (BACs) have proven to be the most versatile vectors for the construction of such libraries (Shizuya et al. 1992). They are easy to maintain and reproduce, have low levels of chimerism, and are easy to screen by DNA hybridization.

Here we report the construction and characterization of BAC libraries for each of the three immediate diploid relatives of common wheat. Wheat species form a classical polyploid series consisting of a hexaploid, tetraploid and diploid levels. The hexaploid *Triticum aestivum* (genomes AABBDD) is globally one of the most important crops. It originated via hybridization of tetraploid wheat, *T. turgidum* (genomes AABB), with the diploid goatgrass *Aegilops tauschii* (genomes DD) (Kihara 1944; McFadden and Sears 1946). Tetraploid wheat originated via hybridization of diploid wild einkorn wheat, *T. urartu* (genomes AA), with a species closely related to the extant *Aegilops speltoides* (genomes SS), which contributed the wheat B genome (Sarkar and Stebbins 1956; Dvorak et al. 1993). While *T. urartu* has not been exploited for wheat breeding, the other two ancestors of polyploid wheats, *Ae. tauschii* and *Ae. speltoides*, were shown to be rich sources of resistance to wheat diseases (Dvorak 1977; Kerber and Dyck 1979; Gill et al. 1986). Wheat diploid ancestors are also an invaluable resource in studies of wheat genome structure and evolution.

Polyploid wheat and its diploid relatives have large genomes that range in size from 4.2–4.9 Gb for *Ae. tauschii* (Bennett and Smith 1976; Arumuganathan and Earle 1991) to 16 Gb for hexaploid wheat (Bennett and Smith 1976). Because of the large sizes of these genomes, the construction of arrayed large-insert libraries for these species is laborious. One arrayed library was reported for durum wheat, *T. turgidum* ssp. *durum*, and two were reported for bread wheat, *T. aestivum* ssp. *aestivum* (Allouis et al. 2003; Cenci et al. 2003; Nilmalgoda et al. 2003). An arrayed large-insert library was also reported for diploid wheat, *T. monococcum* (Lijavetzky et al. 1999). Because the A genome of all polyploid wheats was contributed by *T. urartu*, not by *T. monococcum* (genomes A^m A^m) (Dvorak et al. 1993), the lack of a *T. urartu* BAC library represents a serious limitation for wheat genomic studies. DNA sequence comparisons indicate that *T. urartu* and *T. monococcum* diverged between 0.5 and 1.0 million years ago (Huang et al. 2002), which is reflected by greatly reduced recombination between the wheat A-genome chromo-

Communicated by P. Langridge

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somes and *T. monococcum* chromosomes (Dubcovsky et al. 1995; Luo et al. 2000). Divergence of the A and A^m genomes is also apparent at the DNA sequence level (Wicker et al. 2003). *Aegilops tauschii*, the ancestor of the wheat D genome, consists of two genepools, S and T, loosely affiliated with ssp *strangulata* and ssp. *tauschii* (Dvorak et al. 1998). Accessions Aus18913 (Moullet et al. 1999) and AL 8/78 (Xu et al. 2002) were used for the construction of arrayed large-insert libraries of *Ae. tauschii*. Both accessions belong to the S genepool; no library exists for the T genepool. Finally, no large-insert library is available for *Ae. speltoides*.

Materials and methods

Plant materials

Accession G1812 was selected for the development of the *T. urartu* library. This accession was shown by RFLP to have the closest relationship of 315 *T. urartu* accessions to the A genome of hexaploid wheat (Ming-Cheng Luo and Jan Dvorak, unpublished). Accession AS75 from China of *Ae. tauschii* ssp. *tauschii* was chosen for the construction of the *Ae. tauschii* ssp. *tauschii* library. This accession showed the greatest genetic distance to accession AL8/78 and the D-genome of Chinese Spring wheat among 172 accessions characterized by RFLP at 55 loci (Dvorak et al. 1998). AS75 is a parent of a high-resolution mapping population currently used for anchoring AL8/78 BAC contigs on the *Ae. tauschii* genetic map (Karin Deal, Ming-Cheng Luo and Jan Dvorak, unpublished). While *T. urartu* and *Ae. tauschii* are self-pollinating species, and their libraries could be constructed for essentially homozygous lines, *Ae. speltoides* is a cross-pollinating species. F_4 family no. 134 from the cross *Ae. speltoides* 2-12-4-8-1-1-1 × *Ae. speltoides* PI36909-12-II was used for the construction of the library. The family was homozygous for two *Ph1* suppressors that have been discovered in this species (Chen and Dvorak 1984) and for spikes with terminal-awns.

BAC library construction and characterization

Nuclei were isolated from leaves of one-month-old plants according to Fisher and Goldberg (1982), with modifications described by Dvorak et al. (1988). They were embedded in agarose plugs, lysed and DNA was extracted according to Zhang et al. (1995).

Partial digestion of DNA with *Mbo*I restriction endonuclease, ligation to *Bam*HI linearized dephosphorylated pECBAC1 vector, and transformation of *E. coli* DH10B competent cells were performed at Amplicon Express Inc. (Pullman, Washington, USA) following previously described procedures (Tao and Zhang 1998).

Transformed DH10B cells were plated on LB agar medium in Q-Trays (Genetix) containing 90 μg/ml IPTG, 90 μg/ml X-gal, and 12.5 g/ml of chloramphenicol. The amount of plated cells was adjusted to be no more than 2,000 colonies per Q-Tray, according to manufacturer's recommendations. Plates were incubated at 37°C overnight or until the blue and white colonies became easily distinguishable. White colonies were collected into 384-well plates (Genetix) using a Q-Bot robot (Genetix). White colonies that were missed by the Q-Bot were picked manually. The 384-well plates contained 80 μl of medium containing 10 g/l of bactotryptone, 5 g/l of yeast extract, 0.17 M sodium chloride, 36 mM K_2HPO_4, 13.2 mM KH_2PO_4, 1.7 mM sodium citrate, 0.4 mM $MgSO_4$, 6.8 mM $(NH_4)_2SO_4$, 4.4% glycerol, and 12.5 μg/ml of chloramphenicol. Plates were incubated overnight at 37°C and stored at −80°C. This copy of a library, referred to as an original copy, was used to prepare two more replicas of the library: a pristine copy and a working copy. The original and pristine copies of the libraries were stored in different freezers at −80°C.

The sizes of inserted DNA fragments were estimated for about 100 clones from each library. Plasmid DNA was digested with *Not*I restriction endonuclease and fragments were sized with pulse-field gel electrophoresis in 1% agarose gel in 1x Tris-borate buffer.

The working copy of each library was used for the preparation of high-density BAC membranes. Colonies were double printed on 22 cm × 22 cm Hybond N^+ nylon membranes (Pall-Gelman) using Q-Bot following the manufacturer recommendations. Membranes with arrayed colonies were placed on the surface of LB agar medium with 12.5 μg/ml of chloramphenicol and grown for at least 12 h at 37°C. To lyse the cells, the filters were placed on Whatman paper saturated with 0.5 M NaOH and 1.5 M NaCl for 7 min and then transferred for 7 min on a Whatman paper saturated with 0.5 M Tris-HCl (pH 8.0) and 1.5 M NaCl. Membranes were dried for 1 h at room temperature and placed for 7 min on Whatman paper saturated with 0.4 M NaOH and then transferred on a paper saturated with 4X SSPE for 7 min. Membranes were then dried overnight at room temperature.

Eight clones were hybridized with BAC high-density membranes (Table 1). Inserted DNA fragments were prepared free of plasmid sequences either by excising the insert with a restriction enzyme or by PCR using primers that amplified only the insert DNA. Contamination of libraries with chloroplast DNA was determined by hybridization of two membranes from each library with fragments of the *rbc*L and *pet*D chloroplast genes as described by Cenci et al. (2003). DNA was labeled with ^{32}P (Feinberg and Vogelstein 1983) and hybridization was performed in a buffer consisting of 0.36 M Na_2HPO_4, 0.14 M NaH_2PO_4, 7% sodium dodecyl sulfate, 1% BSA, 3 mM EDTA, and 0.1% of sheared salmon sperm DNA.

Table 1 Clones used for DNA hybridization with BAC high-density screening membranes

Probe	Forward/Reverse primers	Length, bp	Number of positive clones in BAC libraries[a]		
			T. urartu	*Ae. speltoides*	*Ae. tauschii*
PSR920	Excised with *Pst*I	669	2	–	–
BE471272	agcccaaaactccaaagaag/cagagtgacctttgccgcatga	198	4	–	2
BG274853	ccatattagcaggccgacgt/ggtcacctcagaatctccgaat	359	3	–	4
PINA	ccctgtagagacaaagctaa/tcaccagtaatagccaatagtg	330	–	–	2
BE494877	gcgcaggcaagagaggagac/gcaactccgcgcaaacatatat	668	–	7	–
BE500570	cccgtccggccgtcagattt/ggccgaaccaagaacaaccagag	794	–	3	1
BE591682	tgtttcactcatgcttggctatc/ccggtcacgccctaaggta	629	–	4	1
BE498189	ggcgcaccatcagcaacatc/ataaggaacagacaatgccaaatca	359	–	4	–

[a]In *T. urartu* library, 9 membranes were hybridized with PSR920 and 8 with BE471272 and BG274853; in *Ae. tauschii* library, three membranes were hybridized; in *Ae. speltoides* BAC library, 10 membranes were hybridized

BAC-end sequencing and sequence analysis

The percent of empty clones and sequence composition of genomes were estimated by BAC-end sequencing of 96 clones from each library. Bacterial cells were grown in 2X YT medium in 96-well plates at 37°C overnight and plasmid DNA was isolated using R.E.A.L. Prep 96 Plasmid Kit (QIAGEN). Plasmid DNA was dissolved in 20 µl of deionized water, and 5 µl was used in sequencing reaction with BigDye v 3.1(Applied Biosystems). Five microliters of plasmid DNA was mixed with 1 µl of 50% DMSO, 1 µl of T7 universal sequencing primer (3.2 pmole/µl), 1.5 µl of 5x sequencing buffer (Applied Biosystems), 1 µl of BigDye v 3.1, and 0.5 µl of deionized water. Sequencing reaction and purification of sequencing products were performed according to manufacturer's specifications. Trace files generated by an ABI 3730xl Genetic Analyzer were processed using Sequencher 4.2 software (Gene Codes).

The proportion of each genome represented by known repetitive elements was estimated by homology search in databases of repetitive elements for matches with BAC-end sequences (BESs). The database of Triticeae Repeat Sequences (TREP) was downloaded from GrainGenes (wheat.pw.usda.gov/GG2/index.shtml) and searched using the BLASTn program with the *E*-value threshold *E*-10. Matches were also searched in the collection of repetitive elements at the CENSOR web server of Genetic Information Research Institute (http://www.girinst.org) (Jurka et al. 1996). Homology-based search was used to estimate the coding potential of BES. The BESs with masked repetitive elements were used in homology search in the NCBI collection of plant ESTs using BLASTn program with *E*-value threshold *E*-10.

Results and discussion

Triticum urartu, *Ae. speltoides* and *Ae. tauschii* BAC libraries

The BAC library of *T. urartu* consisted of 163,200 clones, 140,000 picked with the Q-Bot and 23,200 picked manually, arrayed into 425 plates. The clones were arrayed onto 9 high-density membranes, each containing 18,432 double-printed clones; one membrane was incomplete. The *Ae. speltoides* BAC library contained 237,312 clones, 200,000 picked with the Q-bot and 37,312 picked manually, in 618 plates. The clones were printed in duplicates on 13 high-density membranes. The *Ae. tauschii* library contained 181,248 clones, 151,000 picked with the Q-Bot and the rest picked manually, in 472 plates. Clones from this library were printed in duplicates on 10 high-density membranes.

Characterization of BAC libraries

The estimated average sizes of inserts in *T. urartu*, *Ae. speltoides* and *Ae. tauschii* libraries were 110 kb, 115 kb and 115 kb, respectively. These insert sizes were similar to insert sizes in other BAC and BiBAC libraries constructed for species in the tribe Triticeae (Lijavetzky et al. 1999; Yu et al. 2000; Allouis et al. 2003; Cenci et al. 2003; Nilmalgoda et al. 2003) but were lower than those reported for the *Ae. tauschii* AL8/78 libraries (Xu et al. 2002).

The percent of empty clones was determined by BAC-end sequencing of 96 clones from each library. Empty clones comprised 2.2, 3.2 and 5.3% of the clones in the *T. urartu, Ae. speltoides, Ae. tauschii* libraries, respectively (Table 2). Clones containing chloroplast DNA represented 0.89, 0.79 and 0.88% of the *T. urartu*, *Ae. speltoides* and *Ae. tauschii* libraries, respectively. Taking into account the average sizes of inserts in the libraries, the number of empty clones and the number of clones with chloroplast DNA, the *T. urartu* library represented 3.7 genome equivalents, the *Ae. speltoides* library represented 5.4 genome equivalents, and the *Ae. tauschii* library represented 4.1 genome equivalents. These library sizes yielded 97.3, 99.4 and 98.0% probability, respectively, to recover any gene in the genome.

To assess empirically the predicted level of genome coverage, the number of BAC clones hybridizing with clones of loci present only once per genome was determined. From three to nine high-density BAC filters from

Table 2 Characterization of *T. urartu*, *Ae. speltoides* and *Ae. tauschii* BAC libraries

Species	Genome size[a] (Gb)	Clones (no.)	Average insert size (kb)	Empty clones (%)	cpDNA (%)	Genome coverage
T. urartu	4.9	163,200	110	2.2	0.89	3.7
Ae. speltoides	5.1	237,312	115	3.2	0.79	5.4
Ae. tauschii	4.9	181,248	115	5.3	0.88	4.1

[a]According to Bennett and Smith (1976)

each library were hybridized with three to five single-copy genes (Table 1). The average number of BAC clones hybridizing with single-copy genes in the *T. urartu* BAC library was close to the predicted 3.7X coverage. The predicted genome coverage of the *Ae. speltoides* and *Ae. tauschii* BAC libraries was slightly lower than suggested by empirical data (Table 2), which could be a sampling variation.

BAC-end sequencing

The total lengths of sequenced BAC ends were 38,051 bp for *T. urartu*, 50,736 bp for *Ae. speltoides* and 49,883 bp for *Ae. tauschii* (Table 3). GC base pair represented 45.6, 46.2 and 46.2% of the *T. urartu*, *Ae. speltoides*, *Ae. tauschii* genomes, respectively. These estimates were very similar to those obtained previously for the *T. monococcum* and rice genomes (SanMiguel et al. 2002; Yu et al. 2002).

According to previous reports, about 60–80% of the grass genome sequences are repetitive elements (Flavell et al. 1974; SanMiguel and Bennetzen 1998; SanMiguel et al. 2002). All previous BAC-based estimates of the number of repeat elements in wheat genomes were derived from a nonrandom set of BAC clones selected for one or another reason for sequencing. In contrast, BAC-end sequences are essentially a random sample of genomic sequences. A search performed against the TREP database detected fewer repeated sequences in BESs than a search against the GIRI database (Table 3). Moreover, using the TREP database, the number of repeated elements was 10% lower in the *Ae. tauschii* genome than in the *T. urartu* and *Ae. speltoides* genomes. The repeated sequence content determined by searching the GIRI database yielded higher content of repeated sequences in each genome than by searching the TREP database. The GIRI search indicated that the *Ae. speltoides* genome had 4% more repeated sequences than the *T. urartu* and *Ae. tauschii* genomes (Table 3). The latter observation was consistent with the observation that *Ae. speltoides* has a greater number of different repetitive elements in the genome than *T. urartu* and *Ae. tauschii* (Dvorak and Zhang 1992). The difference between the two databases probably reflected a bias generated by preferentially focusing on the A genomes in previous wheat BAC sequencing projects.

The repetitive DNA content in the *T. urartu*, *Ae. speltoides* and *Ae. tauschii* genomes (Table 3) was similar to that in maize. Maize BES suggested that 58% of the genome is represented by repetitive elements (Messing et al. 2004). If we assume that the difference in genome sizes between maize and wheat is mostly due to the difference in the content of repeated sequences, then about 80% of wheat genomes should be represented by repetitive elements. The fact that our estimated repeat content was lower than expected may have reflected an under-representation of certain classes of wheat repetitive elements in the databases. Since the analysis of complete BAC sequences of wheat yielded a repeat content that was close to a predicted value (SanMiguel et al. 2002; Gu et al. 2004), the discrepancy found here suggests that different representation of fractions of a genome in BESs is due to uneven distribution of *Mbo*I restriction sites used for BAC library construction. Messing et al. (2004) reported that maize BAC libraries prepared using *Hind*III restriction enzyme recovered retrotransposons better than BAC libraries prepared using *Eco*RI and *Mbo*I restriction enzymes.

An important fact revealed by BES analyses is that a large proportion of the *T. urartu, Ae. tauschii* and *Ae. speltoides* genomes do not show any homology to known repetitive elements or coding sequences in NCBI database. This proportion was 34.2% for *Ae. speltoides*, 37.4% for *T. urartu* and 38.4% for *Ae. tauschii* in our libraries. These numbers are comparable to a similar estimate (34.5%) for the maize genome (Messing et al. 2004). It is currently unknown as to what these sequences represent.

Relatively short lengths of BESs precluded application of gene-predicting programs to detect their coding potential. We therefore utilized homology-

Table 3 Repeat and gene content of BAC-end sequences

BAC library	Total no. of base pairs	GC (%)	Repetitive elements having match in TREP	Repetitive elements having match in GIRI	Coding regions
T. urartu	38,051	46.2	20,502 bp (53.9%)	21,608 bp (56.8%)	2,206 bp (5.8%)
Ae. speltoides	50,736	45.6	26,423 bp (52.1%)	31,132 bp (61.4%)	2,256 bp (4.5%)
Ae. tauschii	49,883	46.2	21,795 bp (43.7%)	28,328 bp (56.8%)	2,406 bp (4.8%)

based approach for gene prediction. According to the number of hits in the EST database, the coding regions represented 5.8% of the *T. urartu* genome, 4.5% of the *Ae. speltoides* genome and 4.8% of the *Ae. tauschii* genome (Table 3). In maize, with a genome size equivalent to one-half of the genomic studies here, the BES indicated that 7.5% of the genome had a coding property.

Genomic resource

The successful recovery of all single-copy genes used for hybridization indicated that the three BAC libraries will be valuable tools for genomic studies and gene isolation. Two of the libraries (*T. urartu* and *Ae. speltoides*) fill gaps in the availability of libraries of wheat immediate ancestors, and the *Ae. tauschii* library is the only library available for the T (''tauschii'') genepool of this species. Clones and BAC filters of each library are available upon request from the authors.

Acknowledgements We thank USDA/CSREES/NRICGP for financial support by grant 2001-35301-10594.

References

Allouis S, Moore G, Bellec A, Sharp R, Faivre Rampant P, Mortimer K, Pateyron S, Foote TN, Griffiths S, Caboche M, Chalhoub B (2003) Construction and characterisation of a hexaploid wheat (*Triticum aestivum* L.) BAC library from the reference germplasm 'Chinese Spring'. Cereal Res Comm 31:331–338

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Bennett MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Philos Trans R Soc Lond B 274:227–274

Cenci A, Chantret N, Kong X, Gu Y, Anderwson OD, Fahima T, Distelfeld A, Dubcovsky J (2003) Construction and characterization of a half million clone BAC library of durum wheat (*Triticum turgidum* ssp. *durum*). Theor Appl Genet 107:931–939

Chen KC, Dvorak J (1984) The inheritance of genetic variation in *Triticum speltoides* affecting heterogenetic chromosome pairing in hybrids with *Triticum aestivum*. Can J Genet Cytol 26:279–287

Dubcovsky J, Luo M-C, Dvorak J (1995) Differentiation between homoeologous chromosomes 1A of wheat and $1A^m$ of *Triticum monococcum* and its recognition by the wheat *Ph1* locus. Proc Natl Acad Sci USA 92:6645–6649

Dvorak J (1977) Transfer of leaf rust resistance from *Aegilops speltoides* to *Triticum aestivum*. Can J Genet Cytol 19:133–141

Dvorak J, Zhang HB (1992) Reconstruction of the phylogeny of the genus *Triticum* from variation in repeated nucleotide sequences. Theor Appl Genet 84:419–429

Dvorak J, McGuire PE, Cassidy B (1988) Apparent sources of the A genomes of wheats inferred from polymorphism in abundance and restriction fragment length of repeated nucleotide sequences. Genome 30:680–689

Dvorak J, di Terlizzi P, Zhang HB, Resta P (1993) The evolution of polyploid wheats: identification of the A genome donor species. Genome 36:21–31

Dvorak J, Luo M-C, Yang Z-L, Zhang H-B (1998) The structure of *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theor Appl Genet 97:657–670

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Fisher RL, Goldnerg RB (1982) Structure and flanking regions of soybean seed protein genes. Cell 29:651–660

Flavell RB, Bennett MD, Smith JB, Smith DB (1974) Genome size and the proportion of repeated nucleotide sequence DNA in plants. Biochem Gen 12:257–269

Gill BS, Raupp WJ, Sharma HC, Browder LE, Hatchett JH, Harvey TL, Moseman JG, Waines JG (1986) Resistance in *Aegilops squarrosa* to wheat leaf rust, wheat powdery mildew, greenbug, and Hessian fly. Plant Dis 70:553–556

Gu YQ, Coleman-Derr D, Kong X, Anderson OD (2004) Rapid genome evolution revealed by comparative sequence analysis of orthologous regions from four Triticeae genomes. Plant Physiol 135:459–470

Huang S, Sirikhachornkit A, Su X, Faris J, Gill BS, Haselkorn R, Gornicki P (2002) Genes encoding plastid acetyl-CoA carboxylase and 3-phopshoglycerate kinase of the *Triticum*/*Aegilops* complex and the evolutionary history of polyploid wheat. Proc Natl Acad Sci USA 99:8133–8138

Jurka J, Klonowski P, Dagman V, Pelton P (1996) CENSOR - a program for identification and elimination of repetitive elements from DNA sequences. Comput Chem 20:119–122

Kerber ER, Dyck PL (1979) Resistance to stem rust and leaf rust of wheat in *Aegilops squarrosa* and transfer of a gene for stem rust resistance to hexaploid wheat. In: Ramanujam S (ed) Proceedings of the 5th international wheat genetics symposium, Indian Soc Genet Plant Breed. Indian Agric Res Inst, New Delhi, India, pp 358–364

Kihara H (1944) Discovery of the DD-analyser, one of the ancestors of *Triticum vulgare* (Japanese). Agric Hortic (Tokyo) 19:13–14

Lijavetzky D, Muzzi G, Wicker T, Keller B, Wing R, Dubcovsky J (1999) Construction and characterization of a bacterial artificial chromosome (BAC) library for the A genome of wheat. Genome 42:1176–1182

Luo M-C, Yang Z-L, Kota R-S, Dvorak J (2000) Recombination of chromosomes $3A^m$ and $5A^m$ of *Triticum monococcum* with homeologous chromosomes *3A* and *5A* of wheat: The distribution of recombination across chromosomes. Genetics 154:1301–1308

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37:81–89, 107–116

Messing J, Bharti AK, Karlowski WM, Gundlach H, Kim HR, Yu Y, Wei FS, Fuks G, Soderlund CA, Mayer KFX, Wing RA (2004) Sequence composition and genome organization of maize. Proc Natl Acad Sci USA 101:14349–14354

Moullet O, Zhang HB, Lagudah ES (1999) Construction and characterisation of a large DNA insert library from the D genome of wheat. Theor Appl Genet 99:305–313

Nilmalgoda SD, Cloutier S, Walichnowski AZ (2003) Construction and characterization of a bacterial artificial chromosome (BAC) library of hexaploid wheat (Triticum aestivum L.) and validation of genome coverage using locus-specific primers. Genome 46:870–878

SanMiguel P, Bennetzen JL (1998) Evidence that a recent increase in maize genome size was caused by the massive amplification of intergene retrotransposons. Ann Bot 82:37–44

SanMiguel PJ, Ramakrishna W, Bennetzen JL, Busso CS, Dubcovsky J (2002) Transposable elements, genes and recombination in a 215-kb contig from wheat chromosome $5A^m$. Funct Integr Genom 2:70–80

Sarkar P, Stebbins GL (1956) Morphological evidence concerning the origin of the B genome in wheat. Amer J Bot 43:297–304

Shizuya H, Birren B, Kim U-J, Mancino V, Slepak T, Tachiiri Y, Simon M (1992) Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. Proc Natl Acad Sci USA 89:8794–8797

Tao Q, Zhang HB (1998) Cloning and stable maintenance of DNA fragments over 300 kb in *Escherichia coli* with conventional plamid-based vectors. Nucl Acid Res 26:4901–4909

Wicker T, Yahiaoui N, Guyot R, Schlagenhauf E, Liu ZD, Dubcovsky J, Keller B (2003) Rapid genome divergence at orthologous low molecular weight glutenin loci of the A and A^m genomes of wheat. Plant Cell 15:1186–1197

Xu Z, Deal KR, Li W, Covaleda L, Chang Y-L, Dvorak J, Luo M-C, Gill BS, Anderson OD, Zhang HB (2002) Construction and characterization of five large-insert BAC and BIBAC libraries of *Aegilops tauschii*, the diploid donor of the wheat D genome. In: 10th international plant and animal genome conference, San Diego, USA, p 101

Yu Y, Tomkins JP, Waugh R, Frisch DA, Kudrna D, Kleinhofs A, Brueggeman RS, Muehlbauer GJ, Wise RP, Wing RA (2000) A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes. Theor Appl Genet 101:1093–1099

Yu J, Hu SN, Wang J, Wong GKS, Li SG, Liu B, Deng YJ, Dai L, Zhou Y, Zhang XQ, Cao ML, Liu J et al. (2002) A draft sequence of the rice genome (Oryza sativa L. ssp indica). Science 296:79–92

Zhang HB, Zhao X, Ding X, Paterson AH, Wing RA (1995) Preparation of megabase size DNA from plant nuclei. Plant J 7:175–184