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Chromosome markers in the tetraploid wheat *Aegilops ventricosa* analysed by in situ hybridization

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Abstract Three lines of the tetraploid wheat *Aegilops ventricosa* Tausch (2n = 4*x* = 28), which contains good resistance to eyespot, were analysed using fluorescent in situ hybridization. Probes used included rDNA, cloned repeated sequences from wheat and rye, simple-sequence repeats (SSRs) and total genomic DNA. The banding patterns produced could be used to distinguish most chromosome arms and will aid in the identification of *Ae. ventricosa* chromosomes or chromosome segments in breeding programmes. All lines had a single major 18S-25S rDNA site, the nucleolar organizing region (NOR) in chromosome 5N and several minor sites of 18S-25S rDNA and 5S rDNA. A 1NL.3DL, 1NS.3DS translocation was identified, and other minor differences were found between the lines.

Key words Karyotype evolution · Genome evolution · In situ hybridization · Plant breeding · Alien genes · *Triticum ventricosum*

Introduction

The wild Triticeae grass, *Aegilops ventricosa* Tausch (synonyms *Ae. fragilis* Parl., *Triticum ventricosum* Ces.; 2n = 4*x* = 28) is important in wheat breeding as a source of resistance to a number of diseases. The species is a tetraploid with a genetic composition of DDNN (Kimber and Tsunewaki 1988). The N genome (Yen and Kimber 1992) is derived from *Ae. uniaristata* Vis (Kimber et al. 1983; using the alternative earlier designation of M, sometimes extended to M^v; Badaeva et al. 1996a,b). The D genome is derived from *Ae. tauschii* Cosson (*Ae. squarrosa* L.; 2n = 2*x* = 14; Lilienfeld 1951). Valuable agronomic genes have been found in both the D- and N-genome chromosomes in *Ae. ventricosa* and transferred into hexaploid bread wheat (*Triticum aestivum* L., ABD genome constitution). In particular, the French line VPM1 carries resistance to eyespot, stripe rust, leaf rust and stem rust from *Ae. ventricosa* (Maia 1967; Bonhomme et al. 1995; Kimber 1967).

The value of the genes carried by chromosome segments derived from *Ae. ventricosa* means that markers are required to follow whole chromosomes or chromosome segments through breeding programmes. C-banding is unable to identify most individual chromosomes (Cunado et al. 1986). In situ hybridization using cloned repetitive DNA sequences (Mukai et al. 1990; Castilho et al. 1996), synthetic simple-sequence repeats (di-, tri- and tetranucleotide repeats; Pedersen et al. 1996; Cuadrado and Schwarzacher 1998) and total genomic DNA (Schwarzacher et al. 1992) as probes has enabled the identification of chromosomes and aliens in triticeae species and hybrid derivatives. At the diploid level, Badaeva et al. (1996a,b) have examined the distribution of various repetitive DNA sequences and used C-banding to characterize the chromosomes of the major *Aegilops* species, including recent accessions of the tetraploid and hexaploid species *Ae. crassa* (Badaeva et al. 1998).

In the work presented here, we investigated the distribution of DNA sequences along the chromosomes of *Ae. ventricosa* using in situ hybridization. We aimed to identify a series of markers which can be used for

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chromosome identification and to examine chromosomal rearrangements in this important alien gene source.

Materials and methods

Root-tip preparations were made from seedlings of *Aegilops ventricosa* lines 2270001, 10 and 11 (also known as John Innes accession 2270003), which have been used extensively for the production of wheat breeding lines (Delibes et al. 1997). Lines 2270001 and 11 were taken from the collections maintained at the John Innes Centre, Norwich. The Plant Breeding Institute, Trumpington, Cambridge, originally obtained line 2270001 from Reading University during the 1950s. The Institut National de la Recherche Agronomique (INRA), Rennes, France originally supplied lines 10 and 11. Line 11 was used in the breeding of the wheat line VPM1.

The following labelled probes were used:

– dpTa1, containing a 340-bp tandem repeated sequence unit of DNA isolated from *T. aestivum* (Vershinin et al. 1994). The DNA was labelled by nick translation with either digoxigenin-11-dUTP (Amersham) or biotin-11-dUTP (Sigma).
– pTa71, containing a 9-kb *Eco*RI fragment of the 18S-25S rDNA isolated from *T. aestivum* (Gerlach and Bedbrook 1979) and recloned in pUC19. The DNA was directly labelled with Fluorored, rhodamine-14-dUTP (Amersham), by nick translation.
– pTa794, containing a 410-bp *Bam*HI fragment of the 5S rDNA isolated from the embryos of *T. aestivum* (Gerlach and Dyer 1980) and cloned in pBR322. The DNA was amplified and labelled by polymerase chain reaction (PCR) with either digoxigenin-11-dUTP or biotin-11-dUTP.
– pSc119.2, containing a 120-bp tandem repeated sequence unit of DNA isolated from rye, *Secale cereale* (Bedbrook et al. 1980), subcloned by McIntyre et al. (1988). The DNA was labelled by nick translation with either digoxigenin-11-dUTP or biotin-11-dUTP.
– $(AAC)_5$ synthetic oligonucleotide. The DNA was labelled with biotin-11-dUTP by random primed DNA labelling.
– $(ACG)_{10}$ and $(CGT)_{10}$ synthetic oligonucleotides. The DNA was labelled with biotin-11-dUTP or digoxigenin-11-dUTP by terminal transferase labelling. These probes were used together and separately.
– Genomic *Ae. ventricosa* DNA labelled by nick translation with digoxigenin-11-dUTP.

Techniques for metaphase preparation and in situ hybridization followed Schwarzacher et al. (1992) and Heslop-Harrison (1991). Briefly, seedling root tips were ice-water treated, fixed and digested in enzyme, and chromosome preparations were made on a glass slide. Except for $(ACG)_{10}$/$(CGT)_{10}$, the hybridization mixture [50 ng per slide^{-1} of each probe, 50% (v/v) formamide, 10% (w/v) dextran sulphate, 0.1% (w/v) SDS (sodium dodecyl sulphate), 5 ng μl^{-1} of autoclaved salmon sperm DNA and 2 × SSC; hybridization stringency of 76%] was denatured and applied to slides, and preparations were denatured for 8 min at 80°C before hybridization overnight at 37°C. The most stringent post-hybridization washes were in 20% formamide in 0.1 × SSC at 42°C, enabling probe-target combinations with more than 85% similarity over extended regions to remain stably hybridized. Hybridization sites of digoxigenin- and biotin-labelled probes were detected using anti-digoxigenin conjugated to FITC (Fab fragment, Boehringer) and streptavidin conjugated to Cy3 (Sigma), respectively. For the $(ACG)_{10}$/$(CGT)_{10}$ oligonucleotides, the procedures of Cuadrado and Schwarzacher (1998) were followed; no formamide was used and the high-stringency wash was in 6 × SSC for 2 min at 45°C.

DAPI-stained preparations were examined on a Leitz epifluorescent microscope, and photographs on Fujicolor Super HG 400 and Fuji Superia 400 colour print film were digitized to CD-ROM and printed from Adobe Photoshop using only processing functions that affect all pixels equally.

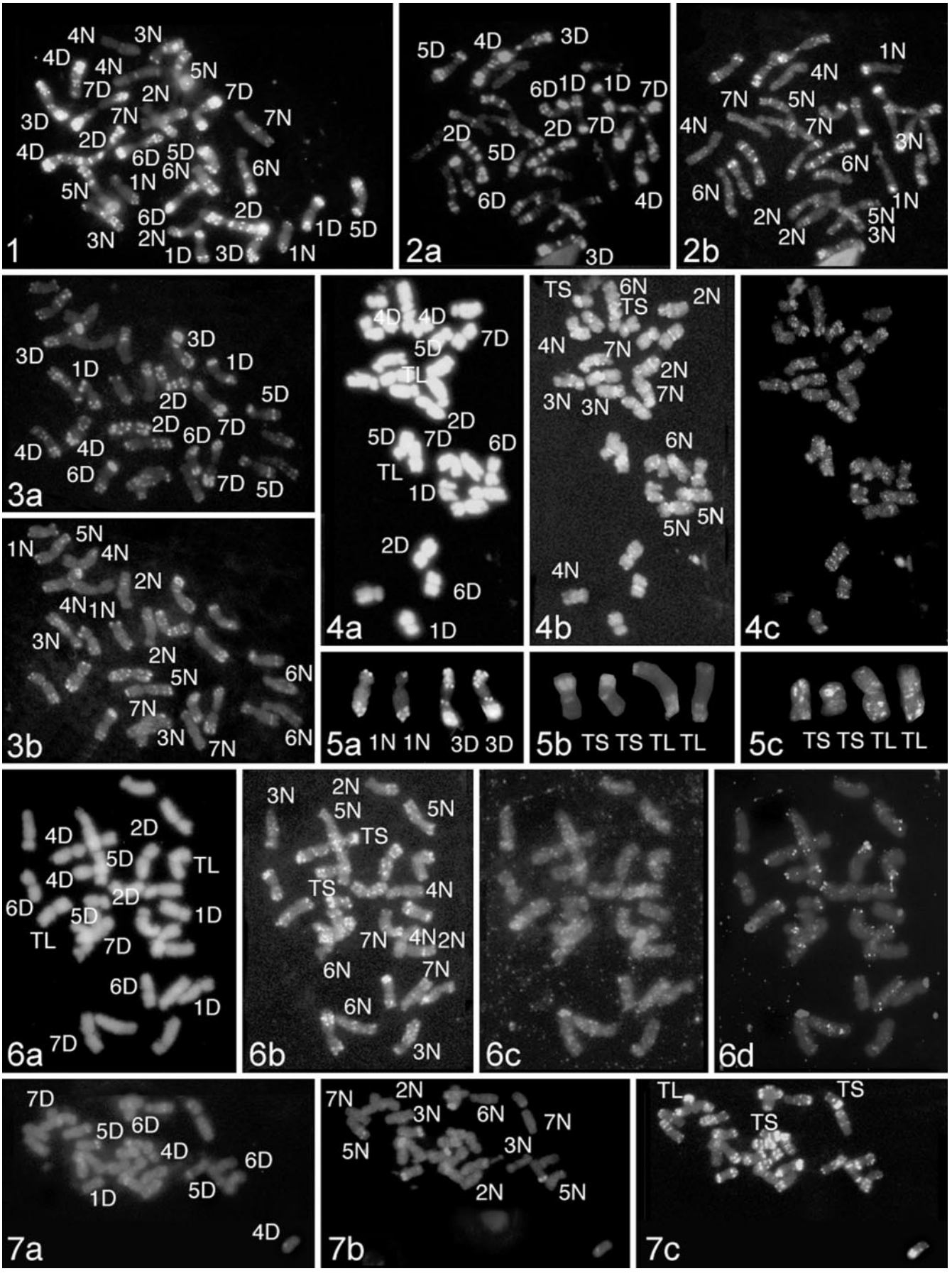
Results and discussion

In situ hybridization of the genomic, repetitive and low-copy DNA sequences revealed characteristic hybridization sites on the chromosomes of *Ae. ventricosa* (Figs. 1–7). Multiple-target hybridization experiments enabled all chromosome types to be identified individually, and the suggested homoeologies are given here. However, confirmation of homoeologous group assignments within the N genome and the extent of conserved synteny will require genetic experiments. In general, there are similarities between the D- and N-genome hybridization patterns.

A 1NL.3DL, 1NS.3DS translocation involving the whole arm of 1NL was found in lines 10 and 2270001 (Fig. 5b,c) when compared with line 11 where 3D is similar to that in *Ae. tauschii* (Fig. 5a). (We use the nomenclature of Koebner and Miller 1986 to describe the translocation.) There were other small polymorphisms between the three lines.

A major 18S-25S rDNA site was located on a single chromosome pair, 5N, in agreement with the presence of one pair of satellited chromosomes (Chennaveeraiah 1960). Up to five chromosome pairs carried minor 18S-25S rDNA sites, with one chromosome pair, 7N, having two sites (Figs. 1, 8). From the D genome, only an intercalary site on 7DS was detected (arm designations of 7D follow Friebe et al. 1996). As in hexaploid wheat, where major loci are present in

Figs. 1–7 Multiple target in situ hybridization to root-tip metaphases of the tetraploid wheat *Ae. ventricosa*. *Grey* DAPI staining shows chromosome morphology, while *bright* (*red* and *green*) *fluorescence* shows sites of probe hybridization. Chromosome homologies are identified in each metaphase. *TS* 1NS.3DS, *TL* 1NL.3DL. Micrographs use single-bandpass filters except **Fig. 1**, and digital overlays are shown in **Figs. 4c** and **6d.** Magnification: × 825 except Fig. 7, × 700. **Fig. 1** A micrograph of a line-11 metaphase using a triple-bandpass filter showing the single major and six minor pairs of 18S-25S rDNA sites (*red* or *yellow* on chromosome 7D where it overlaps dpTa1) and the multiple sites of dpTa1 (*green*). **Fig. 2a, b** Line 11 probed with: **a** dpTa1 (*red*) and **b** the 5S rDNA probe (*green*), hybridizing to four pairs of sites. **Fig. 3a, b** Line 11 showing the multiple sites of the two tandemly repeated probes: **a** dpTa1 (*red*) and **b** pSc119.2 (*green*). **Fig. 4a–c** A metaphase of accession 2270001 (**a,** DAPI staining) probed with: **b** genomic *Ae. ventricosa* (*green*) and **c** 5S rDNA (*red*) with an overlay of the reprobing with dpTa1 (*green*). The *lower* chromosome 1D has been moved up vertically on the print. **Fig. 5a–c** Chromosomes involved in the 1N.3D translocation probed with dpTa1. **a** Chromosomes 1N and 3D from line 11. 1NS.3DS (*TS*) and 1NL.3DL (*TL*) chromosomes from line 10 **b** and accession 2270001 **c**. **Fig. 6a–d** Accession 2270001, stained with DAPI **a**, showing hybridization sites of **b** dpTa1, **c** the simple-sequence repeat $(AAC)_5$ (*red*) and **d** 5s rDNA (*red*) and pSc119.2 (*green*). $(AAC)_5$ shows primarily dispersed hybridization to the chromosomes with a few terminal and centromeric sites of amplification. **Fig. 7** Accession 2270001: **a** probed with the simple-sequence repeat $(ACG)_{10}$ (*red*); **b** reprobed with 5S rDNA (*red*) and **c** dpTa1 (*green*). The *lower* chromosome 4D has been moved up. Colour version available from http://www.jic.bpsrc.ac.uk, search ventricosa


1
4N 3N 4D 4N 5N 7D 2N 7D 3D 7N 7N 2D 4D 6D 5D 6N 6N 1N 5N 6D 2D 1D 5D 3N 2N 1D 3D 1N
2a
5D 4D 3D 6D 1D 1D 7D 2D 2D 7D 5D 6D 4D 3D
2b
1N 4N 7N 5N 4N 7N 3N 6N 6N 1N 2N 5N 2N 3N
3a
3D 3D 1D 1D 5D 2D 2D 6D 7D 4D 4D 6D 7D 5D
3b
1N 5N 4N 2N 4N 1N 3N 2N 5N 6N 7N 3N 7N 6N
4a
4D 4D 5D 7D TL 2D 5D 7D 6D TL 1D 2D 6D 1D
4b
TS 6N TS 2N 4N 7N 2N 3N 3N 7N 6N 5N 5N 4N
4c
5a 1N 1N 3D 3D
5b TS TS TL TL
5c TS TS TL TL
6a
4D 2D 5D TL 4D 2D 6D 5D 1D TL 7D 6D 1D 7D
6b
3N 2N 5N 5N TS TS 4N 7N 4N 2N 6N 7N 6N 3N
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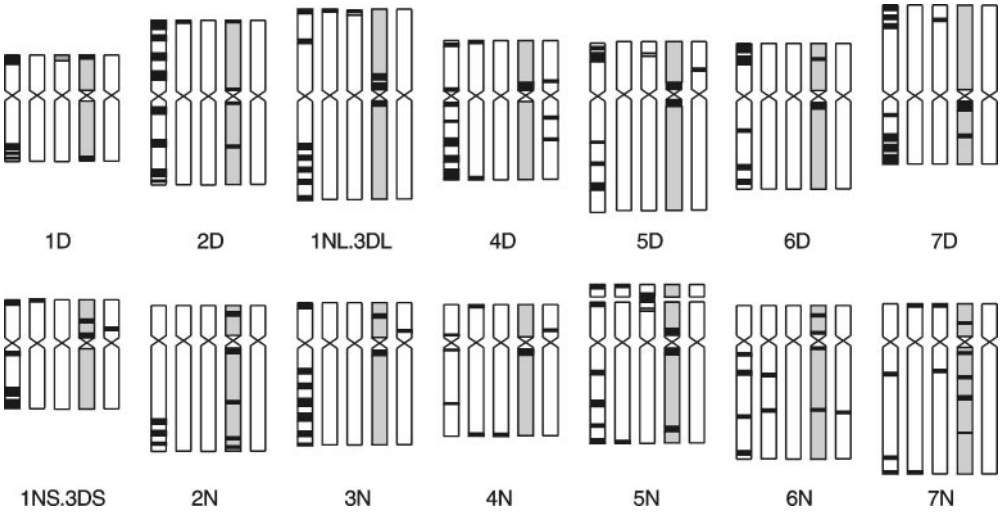

Fig. 8 The distribution of six DNA probes on the 14 chromosomes in *Ae. ventricosa* line 2270001. For each chromosome, drawings show chromosomal locations of, from *left* to *right*, *1* dpTa1, *2* pSc119.2, *3* the 18S-25S rDNA (pTa71) in *black* and 5S rDNA (pTa794) in *grey*, *4* genomic *Ae. ventricosa* DNA, and *5* the simple-sequence repeat $(ACG)_{10}/(CGT)_{10}$

chromosomes 1B and 6B, these data show the rapid loss of 18S-25S rDNA copies that can occur in the evolution of polyploid Triticeae species from the ancestral diploid species. The presence of minor 18S-25S rDNA sites has been reported in most homoeologous groups in the Triticeae (Mukai et al. 1993), and as in our material (e.g. chromosome 6N), Badaeva et al. (1996b) have found variable sites. We detected four pairs of 5S rDNA sites in *Ae. ventricosa* (Figs. 2, 8).

The tandemly repeated sequences dpTa1 and pSc119.2 labelled multiple sites in both the D and N genomes (Figs. 1–8). In general, the N-genome patterns agree with those for the diploid *Ae. uniaristata* (Badaeva et al. 1996a,b). pSc119.2 was located in subterminal sites on half the chromosomes and at intercalary sites on the chromosome identified as 6N. Minor polymorphisms were found in addition to those resulting from the translocation, but there were greater polymorphisms between the tetraploid (Fig. 8) and diploid N genomes (Badaeva et al. 1996a, using the probes pAs1, which is homologous to dpTa1, and pSc119, which is a chimera including pSc119.2). dpTa1 was located towards the end and at intercalary sites of all chromosomes except 4N, which displayed at most faint intercalary and pericentromeric bands. Strongest banding was seen on the D-genome chromosomes. dpTa1 polymorphisms resulted from the 1NL.3DL, 1NS.3DS translocation. In combination with the examination of chromosome morphology, this probe enabled the identification of all chromosomes.

The simple-sequence repeat (SSR) $(AAC)_5$ gave mainly pericentromeric bands on several chromosomes (Figs. 6, 8), in agreement with Cuadrado and Schwarzacher (1998) in which several SSR probes gave pericentromeric bands in wheat. The strongest signal was seen on chromosome 4N. The other SSR, $(ACG)_{10}$ and $(CGT)_{10}$, gave fainter signals, but pericentromeric or intercalary signals could be detected on two D-genome and four N-genome chromosome pairs (Figs. 7a, 8).

Total genomic *Ae. ventricosa* DNA labelled the chromosomes throughout their lengths, although most chromosomes showed enhanced signals in pericentromeric bands and at a few sub-terminal sites; there was no discrimination of the N- and D-genome chromosomes. In many studies of cereal hybrids using genomic in situ hybridization, chromosomes closely related to the probe origin have been shown to hybridize uniformly along their length, perhaps with slightly enhanced or reduced hybridization to major regions of heterochromatin (Schwarzacher et al. 1992). The pattern found here may indicate the presence of major classes of tandemly repeated DNA in the pericentromeric and sub-terminal regions, and the labelling and hybridization conditions enable enhanced hybridization of these sequences.

Conclusions

The repetitive DNA probes described above are of considerable value in identifying each *Ae. ventricosa* chromosome. Within three *Ae. ventricosa* lines which have been used in hybridization programmes, a 1NL.3DL, 1NS.3DS reciprocal translocation was detected. Cytogenetic stocks involving recombination of many chromosome arms are available, and a 5BL.7BL, 5BS.7BS reciprocal translocation is widespread in wheats, particularly of French origin. However intergenomic translocations are unusual in the Triticeae, and 1.3 translocations are not often reported within accessions of wild wheats. *Ae. ventricosa* is a well-characterized source of numerous disease resistance genes and hence of interest to wheat breeders. Use of the probes above is likely to assist with characterization of the chromosome constitution and recombination in wheat breeding lines involving the wild species.

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