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Molecular cytogenetic characterization of novel wheat-*Thinopyrum bessarabicum* recombinant lines carrying intercalary translocations

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Abstract:

Thinopyrum bessarabicum (2*n*=2*x*=14, JJ or E^bE^b) is a valuable source of genes for bread wheat (2*n* = 6*x*=42) improvement because of its salinity tolerance and disease resistance. Development of wheat–*Th. bessarabicum* translocation lines by backcrossing the amphiploid in the absence of the *Ph1* gene (allowing intergenomic recombination) can assist its utilization in wheat improvement. In this study, six novel wheat–*Th. bessarabicum* translocation lines involving different chromosome segments (T4BS.4BL-4JL, T6BS.6BL-6JL, T5AS.5AL-5JL, T5DL.5DS-5JS, T2BS.2BL-2JL, and the whole arm translocation T1JS.1AL) were identified and characterized using genomic *in situ* hybridization (GISH) and fluorescent *in situ* hybridization (FISH). No background translocations between wheat genomes were observed. The involvement of 5 of the 7 chromosomes, and small terminal segments of *Th. bessarabicum* chromosome arm were important, contributing to both reduced linkage drag of the derived lines by minimizing agronomically deleterious genes from the alien species, and high stability including transmission of the alien segment. All three wheat genomes were involved in the translocations with the alien chromosome, and GISH showed the *Th. bessarabicum* genome was more closely related to the D genome in wheat. All the introgression lines were disomic, stable and with good morphological characters.

Keyword: FISH, cytogenetics, wheat/alien introgression, background translocation, recombinant chromosome, linkage drag, salt tolerance.

Introduction:

Domestication, cultivation and thousands of years of selection have led to limited genetic variability in bread wheat, *Triticum aestivum* L. (2*n*=6*x*=42) compared to its wild relatives (Borlaug 1983; Heslop-Harrison and Schwarzacher 2012; Kishii et al. 2010). There is enormous diversity in wheat landraces and within diploid, tetraploid and hexaploid wild relatives: Able and Langridge (2006) noted that as little 10–15% of the available gene pool has been exploited in cultivars. Simmonds (1993), Gale et al. (1989) and Schwarzacher et al. (1992) have discussed how alien introgression of chromosomes by hybridization with wild relatives can introduce desirable characters by crossing and backcrossing into cultivated species, and such chromosome engineering is important for crop breeders to broaden the genetic base of wheat (Friebe et al. 1996; Jiang et al. 1993; Lukaszewski 1990; Sears 1956; Wang 2011).

Species belonging to the *Thinopyrum* Dewey genus (also placed in genera including *Agropyron*, *Elytrigia* and *Lophopyrum*) have been described as potential sources of both biotic-stress disease resistance (Friebe et al. 1993; Knott 1968) and abiotic-stress tolerance (King et al. 1997). Since 1930 breeders have exploited different *Thinopyrum* species after NV Tsitsin and his colleagues first showed that these species readily hybridized with various *Triticum* species (Chen 2005). Breeders have developed wheat-*Thinopyrum* hybrids to transfer these resistances into bread wheat (Friebe et al. 1992a; Friebe et al. 1992b; Graybosch et al. 2009: Wang et al. 2003a, b). *Th. bessarabicum* (2*n*=2*x*=14, JJ; elsewhere the designation E^bE^b is used) is a perennial, rhizomatous maritime wheatgrass distributed in the Black Sea and Mediterranean region. It possesses salinity tolerance and resistance to several diseases (Gorham et al. 1985; King et al. 1997; William and Mujeeb-Kazi 1993). The development of wheat–*Th. bessarabicum* alien chromosome addition lines is providing germplasm for further utilization in wheat improvement (William and Mujeeb-Kazi 1993) including the lines studied here. Several different salt tolerant wheat–*Th. bessarabicum* translocation lines T5AS·5JL where the translocation involved wheat chromosome arm 5AS and *Th. bessarabicum* chromosome arm 5JL were developed through homoeologous pairing induction in the absence of *Ph1* and identified using DNA markers (King et al. 1993). A translocation line T2JS-2BS·2BL involving chromosome 2J of *Th. bessarabicum* was developed and characterized by FISH (Qi et al. 2010).

Fluorescent *in situ* hybridization of repetitive DNA sequences to chromosomes can result in chromosome specific banding patterns for studying chromosome behaviour, phylogenetic relationships and tracing chromosome rearrangements. The *dpTa1* repetitive DNA probe (Bardsley et al. 1999; Rayburn and Gill 1986) identifies wheat D-genome chromosomes. The *pSc119.2* from rye (*Secale cereale* L.) (McIntyre et al. 1990) generates a specific banding pattern on wheat B-genome chromosomes. The GAA microsatellite gives unique hybridization patterns on many chromosome arms (Cuadrado et al. 2008; Cuadrado and Jouve 2002). Together, these probes can identify chromosome arms and translocations (Pedersen and Langridge 1997). The aim of present study was the detailed identification, cytological and molecular identification of chromosomes involved in translocations in a set of wheat-*Th. bessarabicum* translocation lines. Precise characterization of wheat-alien recombinant chromosome is essential for effective utilization of novel traits in wheat breeding and subsequent

tracking with DNA markers.

Materials and Methods

Plant material

The wheat-*Th. bessarabicum* translocation lines used in this study were developed at CIMMYT (International Institute for Wheat and Maize Improvement, Mexico), using the manipulation of the *Ph1* genetic control mechanism (Sears 1977). The lines were made in a Prinia spring wheat background (pedigrees shown in table 1) and were aimed at generating salt tolerant varieties (Muzeeb-Kazi et al. 2013).

Chromosome preparation

Chromosome preparations were made from seedling root tips using standard techniques (Schwarzacher and Heslop-Harrison 2000). Briefly, root tips were incubated in ice-cold water for 24 h at 0̊C before fixing in fresh 3:1 absolute ethanol: glacial acetic acid. Chromosome preparations were made in 60% acetic acid on a glass slide under a cover slip following enzyme digestion with pectinase and cellulase. Selected slides with good metaphase index were dehydrated, dried, and stored at -20 °C until hybridization.

Probes

For *in situ* hybridization probes, total genomic DNA from *Th. bessarabicum* and *T. monococcum* was sheared to 500 to 2kb by autoclaving at 110ºC for 3 min The following repetitive sequences were used: *pSc119.2*, a 120bp tandemly repeated DNA sequence isolated from rye, *Secale cereale* (McIntyre et al. 1990); *pTa71*, a 9 kb *Eco*RI fragment of the repeat unit of 25S-5.8S-18S rDNA isolated from *T. aestivum* (Bedbrook et al. 1980); *dpTa1*, a tandem repeat with a monomeric length of 340bp isolated from *T. aestivum*, subcloned by Vershinin et al. (1994) and homologous to *pAs1* (Rayburn and Gill 1986), *pHcKB6* from *Hordeum chilense* (Anamthawat-Jonsson and Heslop-Harrison 1993) and the *Afa* family (Nagaki et al. 1998); *pTa794* containing a 410 bp fragment of 5S rDNA of *T. aestivum* (Gerlach and Dyer 1980); and a GAA microsatellite amplified from the genomic DNA of *Hordeum vulgare* (Vrána et al. 2000). PCR amplified inserts using M13 primers, or for *pTa71* linearized plasmid, were labelled with biotin-16-dUTP or digoxigenin-16-dUTP (Roche diagnostics) by random priming kit (Bioprime DNA labelling system, Invitrogen).

Fluorescent *in situ* hybridization

DNA *in situ* hybridization followed the method described by Schwarzacher and Heslop-Harrison (2000) with minor modifications. The probe mixture contained 50% (v/v) formamide, 20% (w/v) dextran sulfate, 2 x SSC (pH 7.0), 25-100 ng probe, 20 μg sheared salmon sperm DNA and 0.3% (w/v) SDS (sodium dodecyl sulfate) as well as 0.12 mM EDTA (ethylene-diamine-tetraacetic acid) and autoclaved total genomic DNA from wheat 'Chinese Spring' as blocking DNA at 4-20 times the probe concentration. Probe and chromosomal DNA were denatured together on a heated thermal block (Hybaid Omniblock) for 7 minutes at 71°C and slowly cooled to the hybridization temperature of 37°C. The most stringent post-hybridization washes were in 0.1 x SSC at 42°C, equivalent to 80% stringency. Hybridization sites were detected with streptavidin conjugated to Alexa594 (Life Technologies-Molecular Probes) and antidigoxigenin conjugated to FITC (fluorescein isothiocyanate) (Roche

Diagnostics). Chromosomes were counterstained with DAPI (4′, 6-diamidino-2-phenylindole, 4µg/ml in McIlvaine's pH7 buffer) and mounted in antifade (AF2) solution (Citifluor). Preparations were analysed with a Nikon Eclipse 80i epifluorescence microscope with single band pass filters equipped with a CMOS camera (Nikon DS-Qi1Mc). Individual images were captured using Nikon NIS 3.2 software and prepared for publication using Adobe Photoshop CC with only those functions that treat all pixels of the image equally. For clarity in figure 2, some probe colours were reversed so the genomic *Th. bessarabicum* probe is always shown in red. Between 10 and 20 metaphases were analyzed for each line. To obtain measures of total karyotypic lengths (in pixels), karyotypes from different metaphases were prepared and measured in Nikon NIS 3.2 software.

Results

The *Thinopyrum bessarabicum*–wheat translocation lines with the pedigrees shown in Table 1 were stable and included characteristics of the *Th. bessarabicum* ancestor. In metaphase chromosome preparations, the labeled genomic DNA of *Th. bessarabicum* revealed the alien chromosome segments in the lines, and the repetitive DNA probes allowed identification of wheat and recombinant chromosomes. The hybridization patterns of the repetitive DNA probes were largely consistent with published karyotypes (Danilova et al. 2012, Kubaláková et al. 2005; Fig. 3), allowing identification of most of the chromosomes. All lines had 2*n*=42 with 21 pairs of homologous chromosomes and alien derivatives were always disomic. There was no evidence for inter- or intra-genomic recombination in any of the lines despite the *ph1b* background.

Line 1160: Recombinant chromosome T4BS.4BL-4JL

A small, terminal segment labeled with *Th. bessarabicum* genomic DNA represented 10% of the long arm of a wheat chromosome and 1.2% of the total karyotype length (Fig. 1a). There was no *dpTa1* signal on the translocated chromosome, and *pSc119.2* showed two strong intercalary sites (Fig. 2a), proximal to the alien chromatin on the long arm, and one distal site on the short arm of the wheat chromosome. The GAA microsatellite showed several strong signals at the centromere and two minor bands on the long arm of the recombinant wheat chromosome (Fig. 2a). The probe patterns determine the translocation T4BS.4BL-4JL (Fig. 2a).

Line 1164: **Recombinant chromosome T6BS.6BL-6JL**

A terminal *Th. bessarabicum* segment represented 35% of the long arm (3.6% of the total karyotype length) of the recombinant chromosome (Fig. 1b). The recombinant wheat chromosome carried a major 45S rDNA (*pTa71*) site on the short arm that was also visible as a constriction with DAPI, and *pSc119.2* revealed one strong intercalary and one strong terminal site on the long arm (Fig. 2b); multiple GAA bands were present around the centromere (Fig. 1b). The translocated chromosome was thus identified as T6BS.6BL-6JL (Fig. 2b).

Line 1168: **Recombinant chromosome T5AS.5AL-5JL**

The *Th. bessarabicum* chromosome segment was revealed to represent 25% of a long wheat chromosome arm (2.8% of the total karyotype length; Fig. 1c). The genomic DNA probe labeled the

introgressed segment strongly, and also the D genome chromosomes throughout their lengths with stronger hybridization to repetitive sequences as sites corresponding to the repetitive satellite *dpTa1* (Fig. 1c). Neither *dpTa1* nor *pTa71* signal was detected on this recombinant wheat chromosome but it exhibited a strong terminal *pSc119.2* signal on the short arm and on the alien chromatin on the long arm (Fig. 2c). The *T. monococcum* genomic DNA as a probe identified the recombinant chromosome as one of the seven pairs of A-genome chromosomes (Fig. 2c). The GAA microsatellite showed a minor band at the centromere and on the long arm of the *Th. bessarabicum* chromatin (Fig. 2c). The recombinant chromosome was thus identified as T5AS.5AL-5JL (Fig. 2c).

Line 1172: Recombinant chromosome T5DL.5DS-5JS

The terminal *Th. bessarabicum* segment was revealed on the small arm of a medium sized wheat chromosome (35% of the arm and 2.5% of the total karyotype length), with a minor 45S rDNA (*pTa71*) site at the junction between the alien chromatin and the wheat chromosome (Fig. 1d). Two large *pTa71* sites were visible on chromosome 1B and 6B and so the minor 45S rDNA site identified the chromosome as 5D (Fig. 2d). The *dpTa1* showed five distinctive bands on the long arm and two on the short arm of the recombinant chromosome (Fig. 1d), confirming that it is a T5DL.5DS-5JS translocation (Fig. 2d).

Line 1176: Recombinant chromosome T2BS.2BL-2JL

A *Thinopyrum* segment (45% of the arm and 3.5% of the total karyotype length) was revealed on the short arm of one wheat chromosome pair. A distinctive constriction or gap, not associated with an NOR, was also visible along the *Th. bessarabicum* labelled arm (Fig. 1e, 2e). The long arm of the wheat chromosome had minor *pSc119.2* sites (Fig. 1e) and a strong GAA signal at the centromere and two minor signals on the short arm (Fig. 2e). The translocated chromosome was identified as T2BS.2BL-2JL (Fig. 2e).

Line 1180: Recombinant chromosome T1JS.1AL

The *Th. bessarabicum* probe revealed the presence of a whole arm of *Th. bessarabicum* chromosome (representing 2.5% of the total karyotype length) fused with a complete wheat chromosome arm, together forming a Robertsonian translocation. Both the wheat and *Thinopyrum* chromosome ends of the rearranged chromosome carried a minor *pSc119.2* site (Fig. 1f, 2f). Notably there is a heteromorphism with only one of the two long (wheat-origin) arms of the translocated chromosome carrying a minor *pSc119.2* site. The 45S rDNA probe (*pTa71*) showed the expected major sites on chromosomes 1B and 6B, and minor sites on 5D (Fig. 2f). A single minor site was visible on the *Thinopyrum* labeled chromosome arm. Genomic probe from *T. monococcum* weakly labeled the wheat arm involved in the rearrangement (Fig. 2f) revealing that the translocated wheat arm belongs to the A genome of wheat. This finding together with the fact that the minor *pTa71* site, characteristic to the 1AS, was missing from any of the remaining A-genome chromosomes suggested that the whole short arm of wheat chromosome 1A had been replaced by the *Th. bessarabicum* arm, so the translocation was assigned as T1JS.1AL (Fig. 2f). No *pSc119.2* site was detected on 1AL in Prinia (Fig. 3).

Repetitive sequence locations in Prinia wheat

As a reference, in situ hybridization with GAA and *pSc119.2* was carried out to Prinia chromosomes (Fig. 3). Probes showed characteristic hybridization patterns on all B genome and some A and D genome chromosomes, and could be identified by comparison with Kubaláková et al. (2005) in a tetraploid wheat and Danilova et al. (2012) in the Chinese Spring and Canthach hexaploid wheats, showing minor variations. The DAPI staining of terminal heterochromatin (shown in red in Fig. 3) and *pSc119.2* hybridization pattern identified the 1RS chromosome arm in Prinia.

Discussion

We have identified and characterized six novel wheat-*Th. bessarabicum* translocation lines originating from a programme involving crosses of ('Chinese Spring' wheat x *Th. bessarabicum*) amphiploid and the Chinese Spring *Ph1* mutant. The progenies were backcrossed up to four times to the CIMMYT wheat 'Prinia' (Table 1). Notably, five of the six translocations involved distal alien chromosome segments representing less than half the chromosome arm, and between 1.2 and 3.5% of the whole genome (Fig. 2a-f). These terminal translocations involved four different homoeologous groups on all three genomes (4B, 6B, 2B, 5A and 5D). The sixth line analyzed here was a whole arm 1AL.1JS translocation (Fig. 1f, 2f).

The repetitive DNA probes enabled identification of the wheat chromosomes involved in the translocations. To assist with chromosome identification and show relationships of the genomes, no blocking DNA was used in some of the experiments with *Thinopyrum* genomic DNA probe. The D-genome chromosomes all hybridized weakly throughout their length, showing the close relationship of the dispersed sequences between the J and D genomes. It was also notable that the regions of the D-genome chromosomes homologous to the *dpTa1*/*pHcKB6/Afa* sites were labeled with the J genomic DNA probe when no blocking was used (Fig. 1a), indicating that this sequence family is the primary, highly abundant tandemly-repeated DNA family in the *Thinopyrum* genome. When wheat-blocking DNA was used (Fig. 1c) hybridization to the D-genome chromosomes was substantially reduced, and the *dpTa1* sites were not labeled (hybridization sites would have also been competed for by the *pTa71* probe used in this slide).

Alien chromosome introgression can involve substitution of whole chromosomes. Lines are obtained relatively easy by backcrossing an amphiploid hybrid derivative to the wheat parent, and such lines are available as cytogenetic stocks for a number of alien species; addition lines may also be selected from these crosses (Molnár-Láng et al. 2000, 2012). As noted in the introduction, linkage drag means such lines are not normally grown as varieties (Falke et al. 2009; Feuillet et al. 2008; Gill et al. 2011), and further crosses are needed to reduce the size of the alien chromosome fragment. Spontaneous whole-arm translocations can occur, such as the 1RS.1BL translocation found in many biscuit or feed wheat varieties (Heslop-Harrison et al. 1990, and including the Prinia variety studied here) and occasional recombinants with small alien chromosome segments have been reported e.g. the Danish wheat variety 'Viking' (Schlegel et al. 1993) or the Portuguese wheat landrace 'Barbela' (Ribeiro-Carvalho et al. 1997) including a small terminal rye chromosome segments on chromosome 4B and 2D respectively. These occurrences are rare and unpredictable, and more directed approaches are needed.

There has been interest in generating small alien translocations in introgression lines since the 1950s, first using ionizing radiation (Sears 1956) and then the homoeologous pairing *Ph* mutation, which allows intergenomic pairing of homoeologous chromosomes, as used here, or the 5BL deletion lines (Riley and Chapman 1958, 1968a, b). However, because of background translocations and deletions (Comai 2000), relatively few of these lines have been exploitable in breeding programmes. In some cases, the alien segment may initially be detected only by plant morphology and subsequently proven by molecular cytogenetic methods (e.g. Barbela), while no alien segment may be seen with *in situ* hybridization but only DNA markers indicate its presence (e.g. transfer to wheat of *Lr57* and *Yr40* from *Ae. geniculata*, Kuraparthy et al. 2007). Background wheat translocations and deletions of chromosome segments, as well as aneuploidy, are also found in wheat hybrid genetic stocks. These may be falsely considered as candidates for including alien translocations because of their exceptional morphology and because they are missing wheat DNA markers or be present in addition to the alien segment causing instability. Intercalary recombinant chromosomes will normally carry the wheat centromere sequences, and hence may be more stable than alien addition chromosomes. Here we noted a constriction in the alien segment in line 1176 (Fig.1e, 2e), and it may be that this can act as a neocentromere like the regions described by Kishii et al. (2001) in *Leymus racemosus*-wheat addition lines. Early generations of wide hybrid-derivatives carrying desirable traits may not reveal deleterious characters (Castilho et al. 1996), but performance penalties will become obvious to breeders in trials (Sepsi et al. 2008). Small terminal chromosome segments in wheat may carry a disproportionately high number of the genes on a chromosome (Heslop-Harrison 1991).

Introgression of chromosome segments from the genus *Thinopyrum* has been of considerable recent interest, with reports from hexaploid (Graybosch et al., 2012; Liu et al. 2013; Wang 2003a) and diploid (2x) species such as *Th. elongatum* (Fu et al. 2012; Hu et al. 2012) but work with *Th. bessarabicum* (2x) has been more limited (King et al. 1997; William and Mujeeb-Kazi 1993). Here, each characterized translocation line carries a *Th. bessarabicum* chromosome segment transferred to its homoeologous wheat chromosome (Fig. 2a-f). Studies have revealed about 18 novel disease resistance genes introgressed from *Thinopyrum* species to bread wheat using both irradiation treatment and homoeologous recombination (Fedak and Han 2005). Irradiation treatment was used by Knott (1961) and Sharma and Knott (1966) to transfer the stripe resistance gene *Sr26* from the long arm of group 6 chromosomes of *Th. elongatum* to the long arm of wheat chromosome 6A. Sears (1973, 1977) transferred the *Lr19* leaf rust resistance gene from *Th. elongatum* to wheat by homoeologous recombination. A wheat streak mosaic virus resistance gene *Wsm1* was transferred to wheat from *Th. intermedium* (Graybosch et al. 2009; Mutti et al. 2011, Liang et al. 1979) and has been characterized cytogenetically (Ali 2012). The genomic constitution of the leaf rust resistant wheat-*Th. ponticum* partial amphiploid BE-1 has been characterized using FISH and GISH (Sepsi et al. 2008). An advanced approach by Ayala-Navarrete et al. (2007, 2013) involved pyramiding two desirable resistance genes (*Sr25* and *Lr19*) from *Th. intermedium* and *Th. ponticum* to combine both alien genes on the distal positions on chromosome arm 7DL, giving a trigenomic recombinant chromosome (pontin lines). The *Thinopyrum* genus also has genes of interest for abiotic stress resistance including salinity tolerance (Wang et al. 2003a, b), the target character in the lines studied here. The lines will also carry novel

biotic resistances: for example, the T4BS.4BL-4JL line (Fig. 1a, 2a) has a similar translocation position to the wheat-rye translocation line T4BS.4BL-4RL (An et al. 2013) carrying powdery mildew resistance.

Alien introgression lines are usually selected in the field based on their differences from each other (chromosome additions and substitutions) and for the trait (in particular biotic or abiotic stress resistance) that are desirable in breeding lines. Here, early generations were selected for high fertility plants with 42 chromosomes rather than any particular trait. This will select against background and intra- or inter-genomic translocations within and between the wheat A, B and D genomes, which were not seen in the lines since all non-translocation chromosomes showed *in situ* hybridization patterns expected. However, the strong selection for fertility might have been expected to favor particular chromosome-segment substitutions, so it is interesting that the five lines obtained here involve four homoeologous groups. Yield and drought trials of the lines are now underway, but as yet no resistance has been noted to the fungal diseases FHB (Fusarium Head Blight) or Septoria (Kishii, unpublished). Where there is strong selection for introgression of traits located on particular known chromosome arms, substitution lines can be used in the parentage: for example, Castilho et al. (1997) used lines based on a 1U(1B) substitution line that was crossed to the *ph1b* mutant to introgress chromosome segments carrying high molecular weight glutenin genes into wheat (Brown et al. 1979; Islam-Faridi 1988). Despite several crossing strategies and six independent crosses giving the required transfer, only two types of intercalary wheat-*Ae. umbellulata* recombination events were detected in these lines.

Conclusion

The present study showed that *in situ* hybridization is an accurate method to identify and characterize alien chromosome segments present in hybrid derivatives. In early generations, *in situ* hybridization can be used to modify selection pressure, ensuring the alien chromosome is maintained during selection that includes agronomic traits and fertility, and in later generations can allow directed use of DNA markers based on chromosome knowledge. The lines here have significant potential to allow exploitation of genes from *Th. bessarabicum* in the background of Prinia, a widely grown modern wheat variety, and other wheats.

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Conflict of interest

The authors declare no conflict of interest.

Ethical standards

"This article does not contain any studies with human participants or animals performed by any of the authors."

Informed consent

"Informed consent was obtained from all individual participants included in the study."

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Legends to figures

Fig. 1 Root tip metaphase chromosomes (fluorescing blue with DAPI) of the wheat-*Thinopyrum bessarabicum* introgression lines (2*n*=6*x*=42) carrying disomic recombinant chromosomes after *in situ* hybridization (red and green signals overlaid). (a) Line 1160 probed with genomic *Th. bessarabicum* DNA (red, labeling the introgressed segment strongly, and D-genome chromosomes more weakly) and the repetitive sequence *pSc119.2* (green bands on chromosomes); inset part of another metaphase showing the two translocated chromosomes. (b) Line 1164 probed with the microsatellite GAA (red, labeling B genome chromosomes predominantly at centromeres and telomeres) and genomic *Th. bessarabicum* DNA (green, labeling the introgressed segment). (c) Line 1168 and (d) Line 1172 both probed with genomic *Th. bessarabicum* DNA (red) and the repetitive sequence *dpTa1* (green). (e) Line 1176 and (f) Line 1180 probed with genomic *Th. bessarabicum* DNA (red) and the repetitive sequence *pSc119.2* (green). In all parts of figure, arrows indicate alien chromatin segments. Scale bars =10µm.

Fig. 2 Identification of the six wheat chromosomes involved in the (a-e) five intercalary and (f) one whole-arm wheat-*Th. bessarabicum* recombinant lines. False colour processed images of recombinant chromosome pairs after *in situ* hybridization (centromere position indicated by grey line) show *Th.*

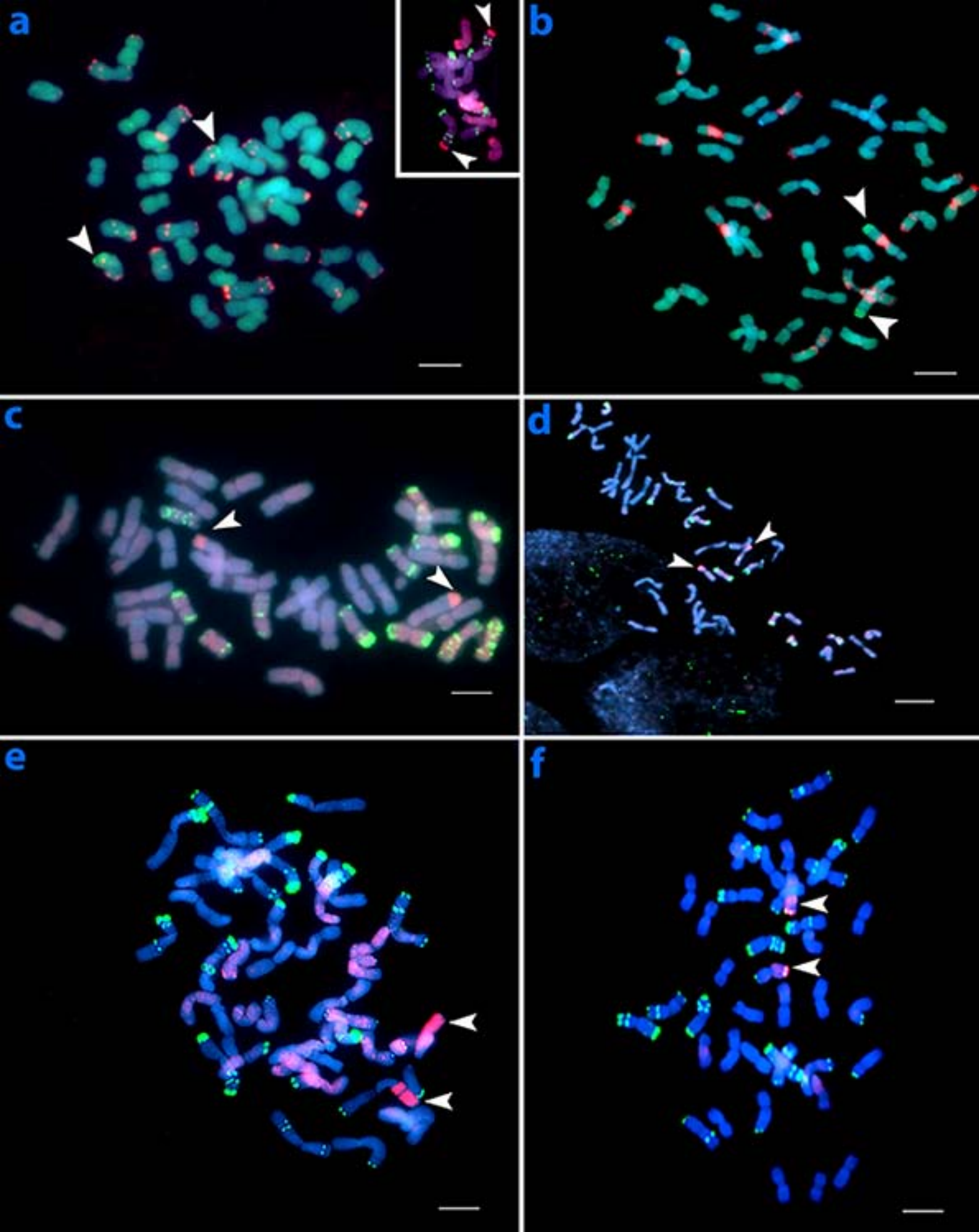
bessarabicum probe in red (overlay on blue chromosomes shows some colour variation from pink-magenta through orange) with other probes with green label (displayed from yellow to cyan). Probes sites (indicated by colours) are shown on diagrammatic chromosomes (outer left) with the corresponding non-translocated wheat chromosome (outer right). (a) Line 1160, GAA (left chromosome image on black background) and *pSc119.2* (right) probes on chromosomes. (b) Line 1164, probes 45S rDNA (left), *pSc119.2* (center) and GAA (right). (c) Line 1168, probes *pSc119.2* (left), GAA (center) and *T. monococcum* genomic DNA (right). (d) Line 1172, probes 45S rDNA (left) and *dpTa1* (right). (e) Line 1176, probes GAA (left) and *pSc119.2* (right). (f) Line 1180, probes *pSc119.2* (left), 45S rDNA (center) and *T. monococcum* genomic DNA (right). Each chromosome pair comes from a single metaphase; one pair in each part is also shown with scale bar in the complete metaphase, with real colour, in Fig. 1.

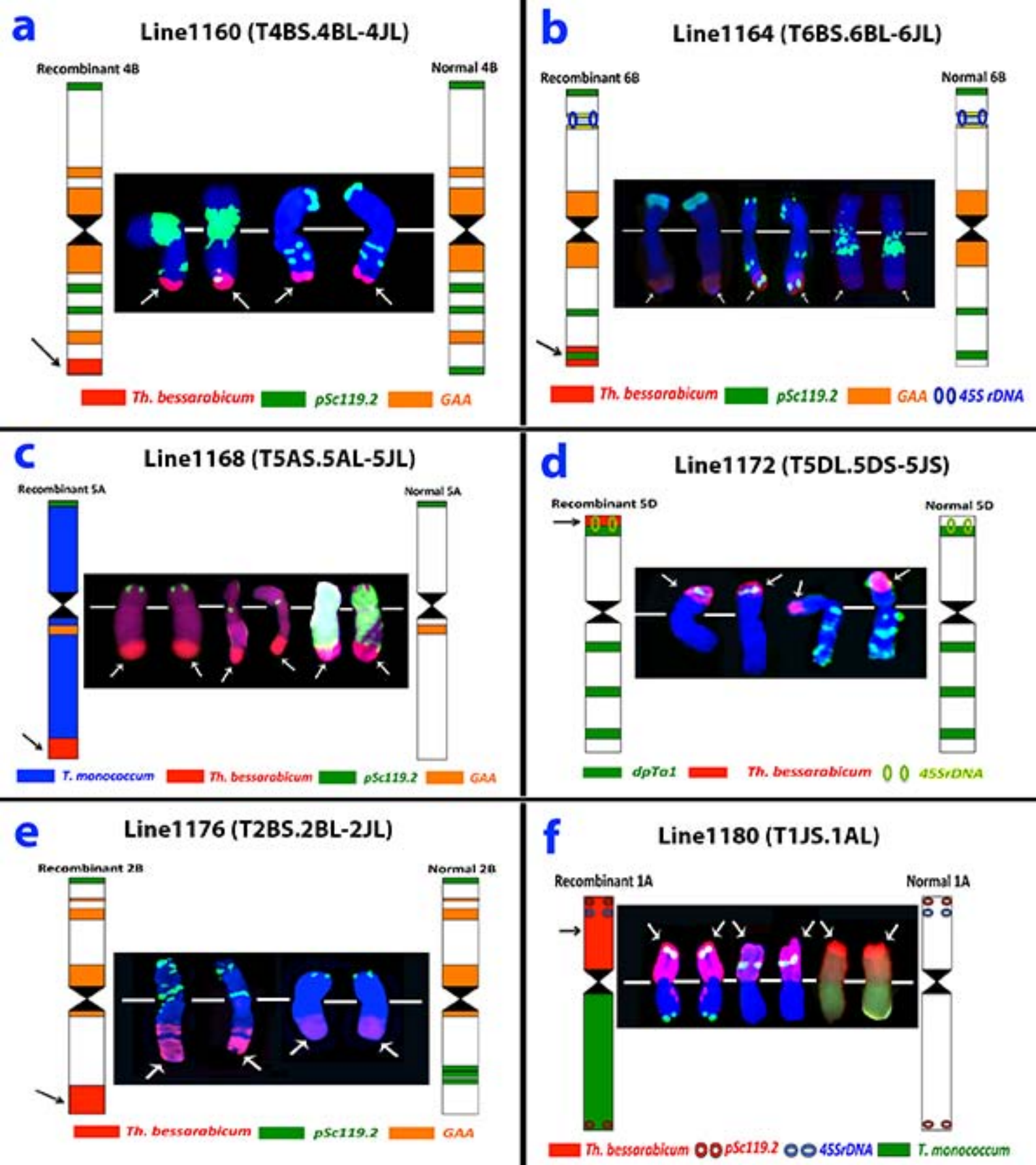
Fig. 3. Chromosomes from Prinia wheat showing sites of *in situ* hybridization with (top) GAA and (lower panel) *pSc119.2* in green. The DAPI staining showing chromosome morphology is displayed in red to improve contrast. The 1RS chromosome arm has a characteristic *pSc119.2* hybridization pattern and bright DAPI fluorescence shows the terminal heterochromatin.

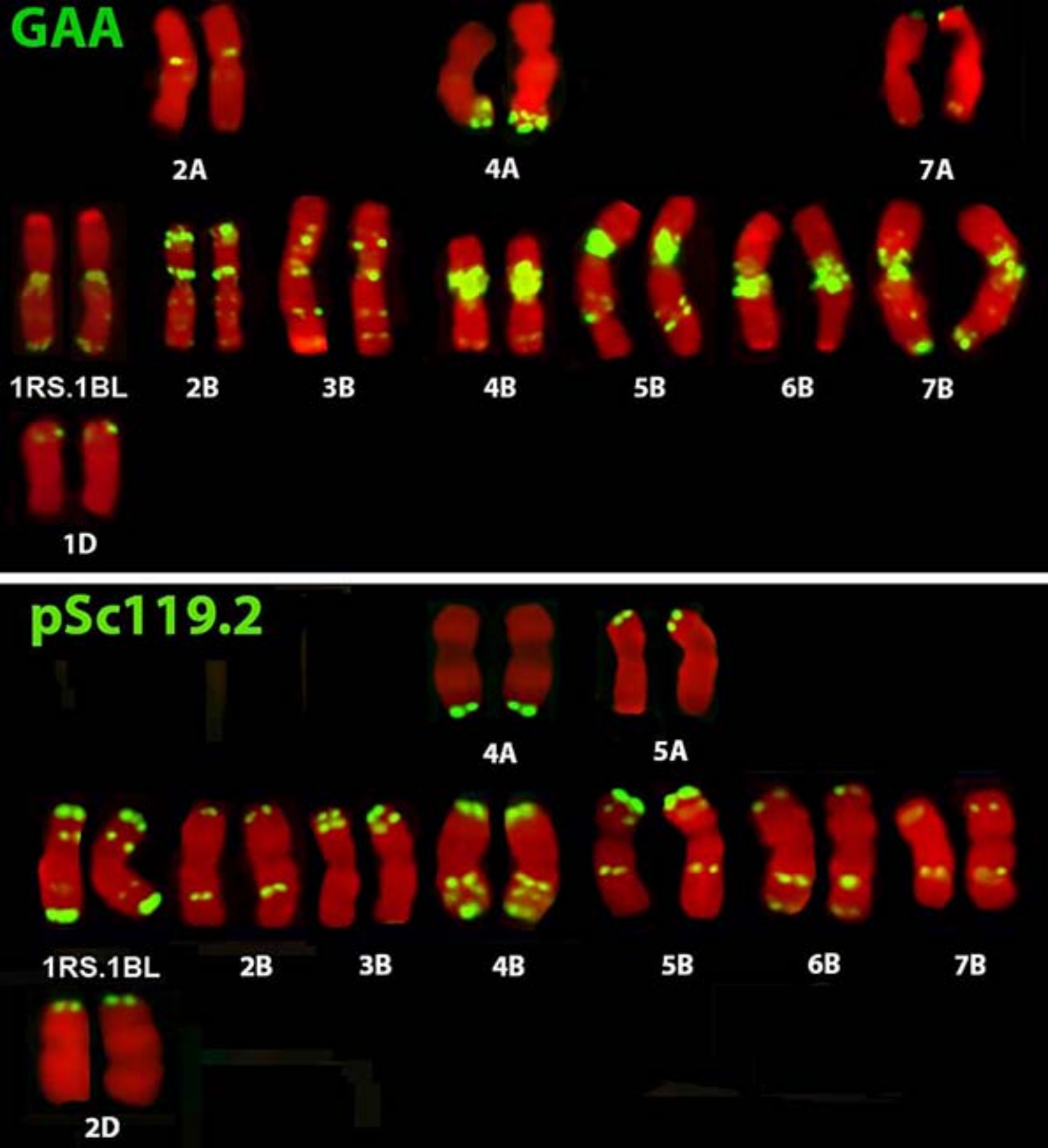
Table 1: Wheat, *Th. bessarabicum* and translocation lines in a Prinia[1] wheat background used in the study			
Line	Entry/Source	Description/Cross	Chromosome Number 2n
CS	Sears, Missouri	Chinese spring (bread wheat)	42
Th. bessarabicum	Genebank, USDA-ARS PI 531711 (France)	*Thinopyrum bessarabicum*	14
CSPh1b	CIMMYT	Chinese Spring *ph1b* mutant line	42
1160	CIMMYT	CS/TH.BESS//CSph/3/4*PRINIA[2]	42
1164	CIMMYT	CS/TH.BESS//CSph/3/4*PRINIA	42
1168	CIMMYT	CS/TH.BESS//CSph/3/4*PRINIA	42
1172	CIMMYT	CS/TH.BESS//CSph/3/3*PRINIA	42
1176	CIMMYT	CS/TH.BESS//CSph/3/2*PRINIA	42
1180	CIMMYT	CS/TH.BESS//CSph/3/3*PRINIA	42
Ph- pairing homeologous gene; Prinia- CIMMYT bread wheat			

1) The pedigree of Prinia is PARULA/VEERY-6//MYNA/VULTURE; 'Veery' carries the 1RS.1BL translocation (from varieties Bezostaya through Kavkaz); Prinia also carries the rye translocation (Fig. 3), which is a widespread translocation in CIMMYT wheats with 'Veery' in their pedigree.

2) In full form, the pedigree of 1160 is [(CS x *Th. bessarabicum*) x CSph] x Prinia backcrossed three more times to Prinia; subsequent generations have been selfed and bulked.


a
b
c
d
e
f


a
Line1160 (T4BS.4BL-4JL)
Recombinant 4B
Normal 4B
Th. bessarabicum
pSc119.2
GAA
b
Line1164 (T6BS.6BL-6JL)
Recombinant 6B
Normal 6B
Th. bessarabicum
pSc119.2
GAA
45S rDNA
c
Line1168 (T5AS.5AL-5JL)
Recombinant 5A
Normal 5A
T. monococcum
Th. bessarabicum
pSc119.2
GAA
d
Line1172 (T5DL.5DS-5JS)
Recombinant 5D
Normal 5D
dpTa1
Th. bessarabicum
45SrDNA
e
Line1176 (T2BS.2BL-2JL)
Recombinant 2B
Normal 2B
Th. bessarabicum
pSc119.2
GAA
f
Line1180 (T1JS.1AL)
Recombinant 1A
Normal 1A
Th. bessarabicum
pSc119.2
45SrDNA
T. monococcum


GAA
2A
4A
7A
1RS.1BL
2B
3B
4B
5B
6B
7B
1D
pSc119.2
4A
5A
1RS.1BL
2B
3B
4B
5B
6B
7B
2D