

INRA Cytogenetical and molecular analysis of *Aegilops variabilis* chromosomes and translocations carrying resistance to nematodes in wheat



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Introduction

Aegilops variabilis is an allotetraploid species ($2n=4x=28$, UUS^vS^v). It is thought to have been formed from hybridization between the diploid species *Ae. umbellulata* (genome U) and *Ae. longissima* (genome S1) (Fig.1). It is a south Mediterranean species occupying the south-western part of the distribution of the genus *Aegilops*, from South Turkey, Iraq Syria to Algeria and Morocco.

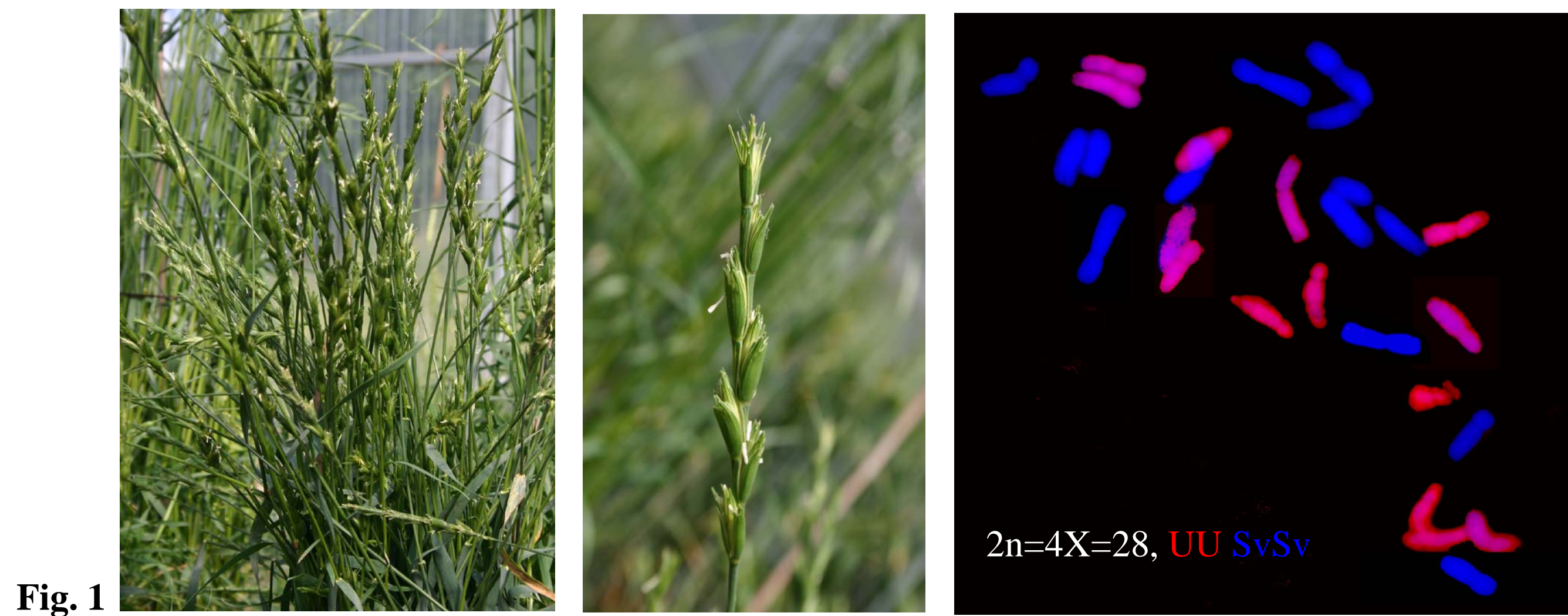


Fig. 1

One accession, *Ae. variabilis* n°1, was found to be resistant to the cereal cyst nematode (CCN) and the root-knot nematode (RKN) (Person-Dedryver and Jahier, 1985; Rivoal et al. 1986). In the progeny of its hybrid with wheat, two disomic addition lines were extracted (Jahier et al. 1998): the **line X35** carrying resistance to both nematodes (genes *CreY* and *Rkn2*) and the **line N** resistant to CCN (gene *CreX*). Even if homoeologous pairing was not induced, two introgressed lines (**X8** and **D3**) were recovered.

Results

Genomic assignment of the *Ae. variabilis* n°1 chromatin in the two addition lines

Tricolour GISH was performed to confirm that the added chromosomes in LX and N lines belong to S^v genome. In both lines, 14 A-genome chromosomes labelled by Texas-Red fluoresced red, 14 chromosomes of D-genome labelled by FITC fluoresced green while the remaining 16 chromosomes blocked by *Ae. speltoides* DNA were blue. Thus, the blue fluorescence detected the 14 chromosomes belonging to the B-genome of wheat and the 2 added chromosomes indicating that these latter in LX and N lines are S^v chromosomes (Fig. 2).

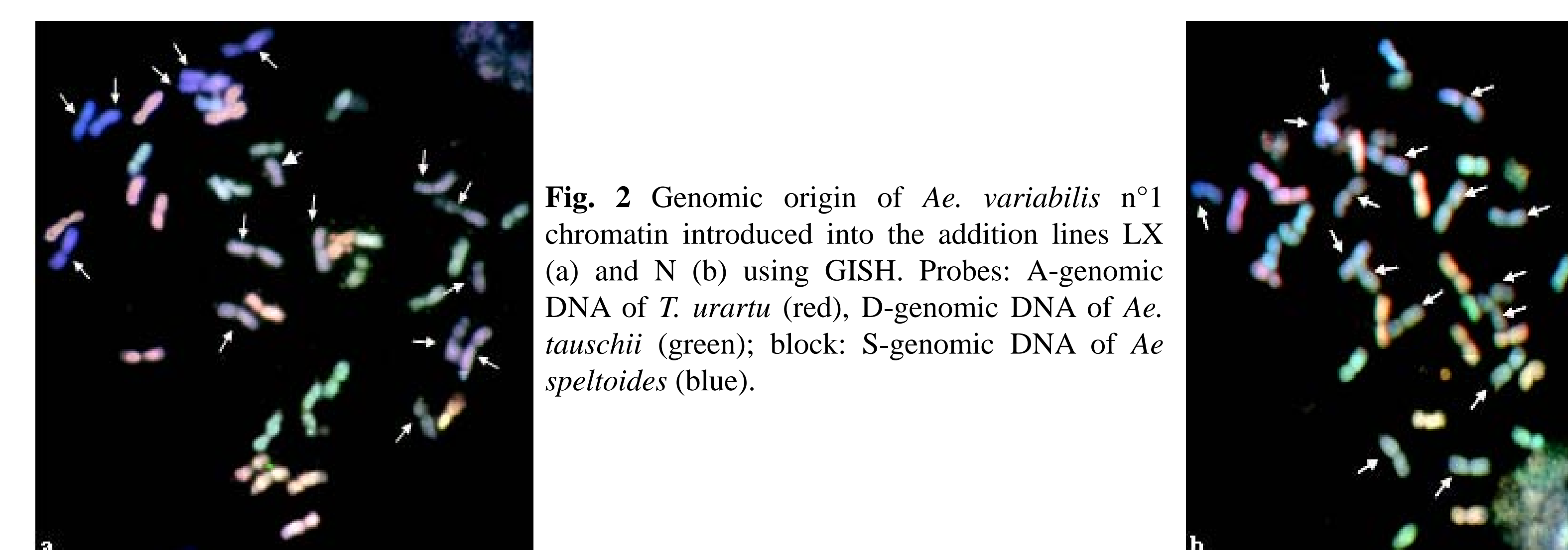


Fig. 2 Genomic origin of *Ae. variabilis* n°1 chromatin introduced into the addition lines LX (a) and N (b) using GISH. Probes: A-genomic DNA of *T. urartu* (red), D-genomic DNA of *Ae. tauschii* (green); block: S-genomic DNA of *Ae. speltoides* (blue).

Identification of the *Ae. variabilis* chromosome carrying *CreX* gene in the addition line N

The BAC 752A31 (or pSC119.2) probe specific of the B genome (red) associated with the pTa71 probe (45S rDNA) (green) permitted to identify three chromosome pairs in the wheat: 1B, 6B and 5D. In the line N, we observed four chromosome pairs carrying a 45S rDNA signal, the unknown chromosome pair revealed a specific pattern with a double red signal in telomeric position and a green interstitial signal. This chromosome was likely to be the *Ae. variabilis* added chromosome (Fig. 3). As the added chromosome has similar hybridization pattern as 6S^v (Badaeva et al. 2004), our tentative conclusion was

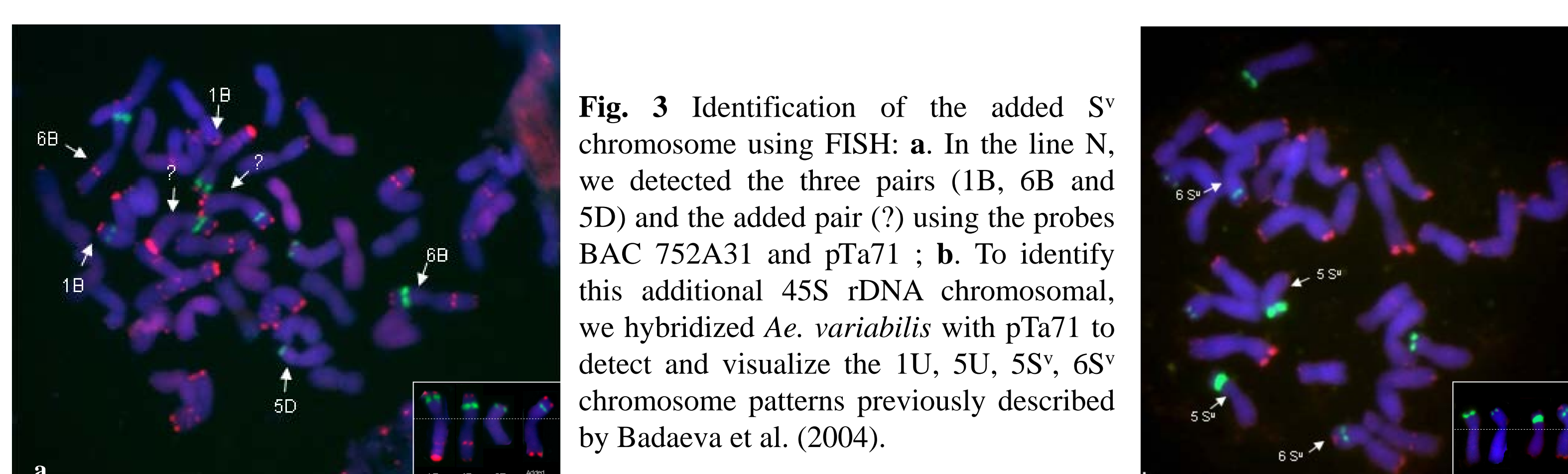


Fig. 3 Identification of the added S^v chromosome using FISH: a. In the line N, we detected the three pairs (1B, 6B and 5D) and the added pair (?) using the probes BAC 752A31 and pTa71; b. To identify this additional 45S rDNA chromosomal, we hybridized *Ae. variabilis* with pTa71 to detect and visualize the 1U, 5U, 5S^v, 6S^v chromosome patterns previously described by Badaeva et al. (2004).

that the added chromosome carrying the gene *CreX* might be designated as being 6S^v.

To confirm this hypothesis, 26 wheat microsatellites of group 6 were investigated to define more precisely the added chromosome in line N. None of them were found on the added chromosome. Therefore, 103 microsatellites of 7 other wheat groups were used. Thus the added chromosome designated provisionally as being 6S^v carries segments homoeologous of the wheat groups 1, 2, 4 and 6 (Fig. 4a).

Characterization of the introgressed segment carrying *CreX* gene in D3

In a first step, it was found that the translocation line D3 had retained the three markers of group 1 and the marker of group 4 of the added chromosome in line N. In a second step, analysis of a F2:F3 population derived from the cross D3 × X8 showed that recombination events occurred at the distal part of wheat 1BL in the vicinity of *Xgwm818* SSR. The *CreX*-carrying shortest alien segment within the F3 introgression lines was tagged by the group 1 SSR markers *Xbarc80* and *Xgwm793*.

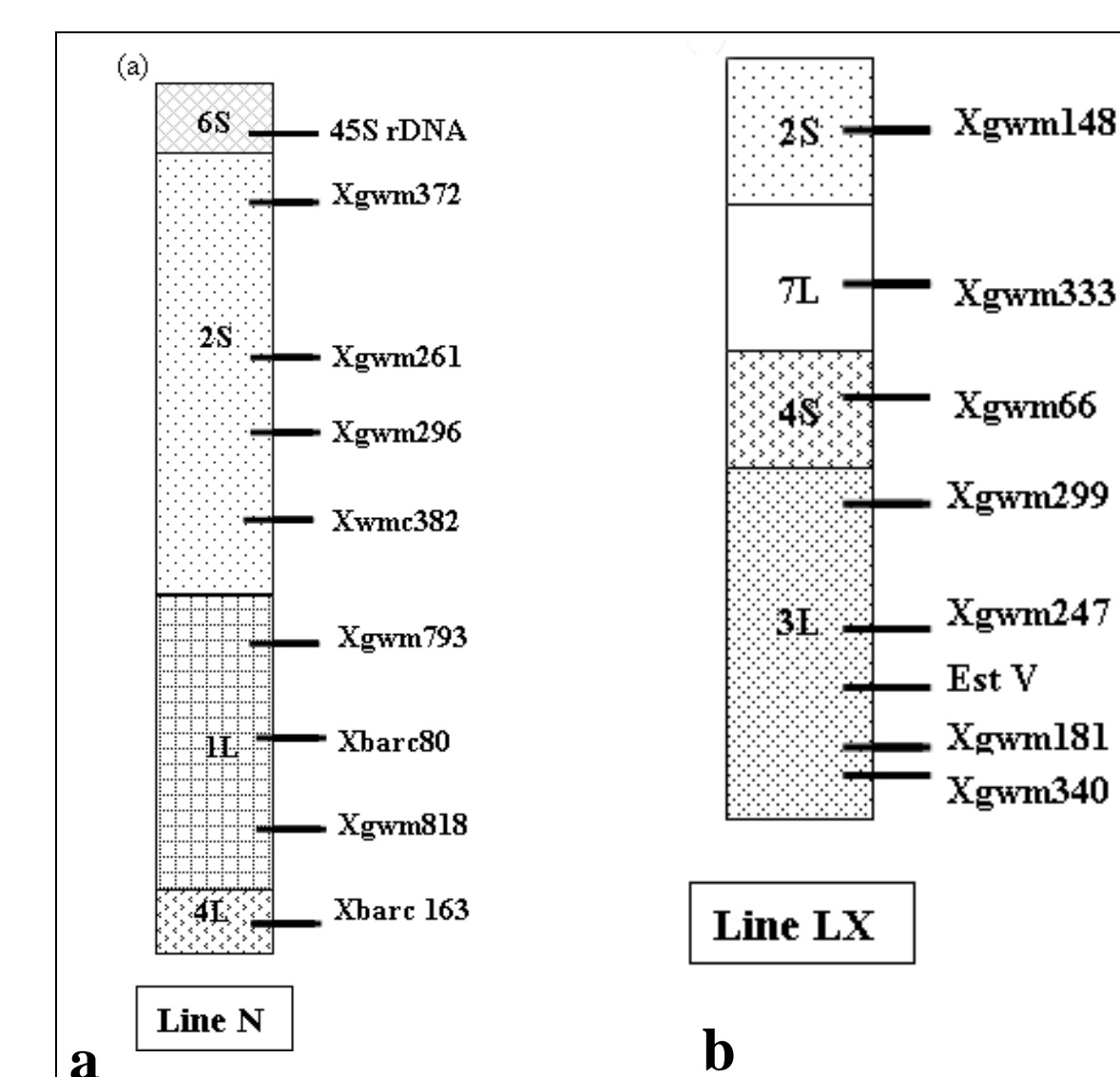


Fig. 4 Putative structure of the added *Ae. variabilis* chromosomes in line N (a), and line LX (b). Positions of SSR were deduced from the wheat SSR mapped position.

Molecular identification of the added chromosome carrying genes *CreY* and *Rkn2* in the addition line LX and of the segment transferred in the line X8

The genomic origin of the added chromosome in line LX which carried the *CreY* and *Rkn2* genes was postulated to be 3S^v based on the presence of the 3BL-specific *Est5* gene on the added chromosome and the translocated segment in line X8 (Yu 1991). Among the 18 polymorphic SSR markers of 3B, four of them were found in *Ae. variabilis* and in the addition line LX. Therefore, 112 microsatellites of other groups were used. Four mapped on 3BL, 3 on 7BL, 1 on 2BS and 1 on 4BS were identified both in *Ae. variabilis* and in the added LX chromosome (Fig. 4b). In the recombination line X8, all homoeologous 3BL SSR markers amplified in *Ae. variabilis* and *Est5* were found, whereas all wheat homoeoloci were absent. This result confirmed that a recombination event between the added chromosome of LX line and wheat took place within the distal part of the 3BL.

Discussion

We demonstrate that there are multiple rearrangements in the S^v chromosomes. The *Ae. variabilis* chromosome carrying the gene *CreX* for resistance to CCN combined segments with homoeology to wheat groups 1, 2, 4 and 6. The *CreX* gene belongs to the group 1 part and it was likely to have been introduced into chromosome 1B at a similar location as the previously found *QTL QCre.srd-1B for CCN resistance*. The second *Ae. variabilis* chromosome carrying *CreY* and *Rkn2* combined segments with homoeology to wheat groups 2, 4 and 7 on its short arm and 3 on its long arm. It was designated as 3S^v. The two genes for resistance are carried by its long arm and have been transferred to wheat chromosome 3B through homoeologous and genetically balanced recombination. Different SSR markers present in the introgressed segments could be used in marker-assisted selection.

References

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