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Karyotype characterization of wheat breeding lines carrying resistance genes from Aegilops ventricosa.

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We have used in situ hybridization combining genomic and repeated DNA fluorescent probes to determine the karyotype composition of two bread wheat introgression lines: H-93-33, which carries the gene *H27* for resistance to the Hessian fly *M. destructor* (Delibes et al. 1997); and H-93-8, carrying the gene *Cre2* which confers resistance to the cereal cyst nematode *H. avenae* (Delibes et al. 1993). Both introgression lines had been derived from an earlier cross between *T. aestivum* subsp. *aestivum* (2n=42; genome composition AABBDD) and a semi-fertile hybrid between *T. turgidum* subsp. *turgidum* (2n=28; genome composition AABB) and the wild grass *Ae. ventricosa* (2n=28; genome constitution $D^vD^vN^vN^v$). We also have examined several resistant advanced lines that were obtained from H-93-33 (lines ID-2151, ID-2193, Ma-1612-a and Ma-1612-b) or H-93-8 (line ID-2150) after 3 to 5 backcrosses with commercial wheats.

The ISH protocol was essentially as described in Sánchez-Morán et al. (2001). Three different DNA probe combinations were separately hybridized on mitotic slides from each of those breeding lines. The first mix contained differentially labelled A- and S-genome DNA probes, and D-genome DNA blocking. A second mix contained differentially labelled A- and D-genome DNA probes, and S-genome DNA blocking. These two probe combinations revealed the number of chromosomes belonging to the A and B genomes of wheat and to the D genome from either wheat or *Ae. ventricosa*. The third mix was primarily designed to reveal the suspected presence of N^v-genome chromosomes in those lines, which contained chromosome pairs that had been blocked by any of the two former probe combinations. This mix contained differentially labelled D- and N-genome DNA probes with durum wheat (AB) DNA was added as blocking. This mix also included the ribosomal DNA probe pTa71 and the repeated DNA probe pAs1 (Rayburn and Gill 1987). The latter probe provides a distinctive ISH pattern for individual D-genome chromosomes in wheat (Pedersen and Langridge 1997) and *Ae. ventricosa* (Badaeva et al. 2002). A summary of the karyotype findings in the lines examined is described here (Tables 1 and 2).

Table 1. Chromosome constitution of the bread wheat breeding lines. The D genomes of wheat and *Ae. ventricosa* are pooled in column D. An * indicates the genome includes a D-N translocation).

	Genome			
Line	A	B	D	N
H-93-33	14	14	12	2
ID-2151	14	14	14	0
ID-2193	14	14	14*	2*
Ma-1612-a	14	14	12	2
Ma-1612-b	14	14	14	0
H-93-8	12	14	12	4
ID-2150	14	14	14	0

Table 2. Identification of individual chromosomes in the breeding lines. A + indicates presence and a – indicates absence; T^1 is the translocation 4DS-4NS.4NL and T^2 is the translocation 5DS.5DL-$5D^vL$.

	Wheat							*Ae. ventricosa*	
Line	1D	2D	3D	4D	5D	6D	7D	D^v	N^v
H-93-33	+	+	+	-	-	+	-	$3D^v$, $5D^v$	$4N^v$
ID-2151	+	+	+	+	+	+	+	0	0
ID-2193	+	+	+	T^1	+	+	+	0	T^1
Ma-1612-a	+	+	+	-	+	+	+	0	$4N^v$
Ma-1612-b	+	+	+	+	+	+	+	0	0
H-93-8	+	+	-	-	T^2	+	-	$3D^v$, $4D^v$, T^2	$5N^v$, $7N^v$
ID-2150	+	+	+	+	+	+	+	0	0

H-93-33 and derived lines. The ISH analysis confirmed the existence of a $4N^v$(4D) substitution in H-93-33, which had been proposed from earlier biochemical and cytological analy-

ses (Mena et al. 1989). Comparison between the ISH patterns of pAs1 found in this line and those reported by Pedersen and Langridge (1997) and Badaeva et al. (2002) undoubtedly demonstrated the presence of additional *Ae. ventricosa* introgressed chromosomes, i. e., a 5D^{v}(5D) substitution and the replacement of wheat 7D by its nonhomoeologous 3D^{v}. None of these D^{v} genome introgressions is maintained in any of the Hessian fly resistant lines derived from H-93-33 that were checked. However, the 4N^{v}(4D) substitution has been transmitted to line Ma-1612-a, and a large part of the long arm of this alien chromosome is still present in a 4D-4N^{v} translocation detected in line ID-2193. These findings confirm former data indicating that gene *H27* is linked to Acph-N^{v}1, a molecular marker located on 4N^{v} (Delibes et al. 1997).

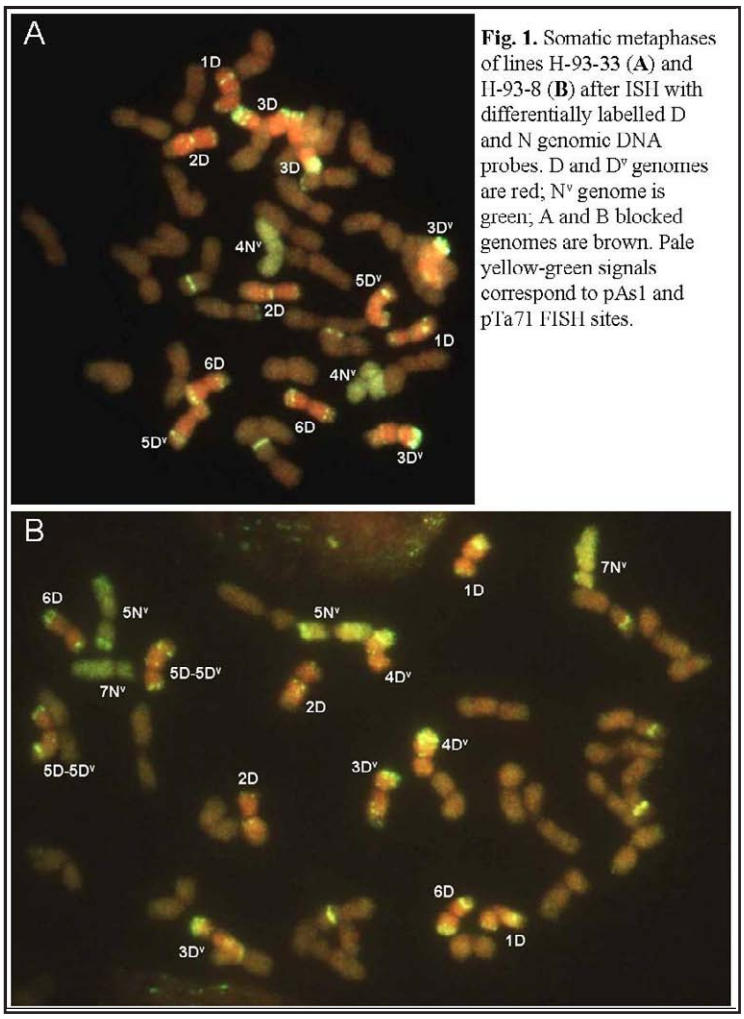

Fig. 1. Somatic metaphases of lines H-93-33 (**A**) and H-93-8 (**B**) after ISH with differentially labelled D and N genomic DNA probes. D and D^{v} genomes are red; N^{v} genome is green; A and B blocked genomes are brown. Pale yellow-green signals correspond to pAs1 and pTa71 FISH sites.

H-93-8 and derived lines. Previous results had proposed a double substitution in line H-93-8: 5N^{v}(5A) and 7N^{v}(7D) (Mena et al. 1993). The ISH analysis has demonstrate the presence of 5N^{v} and 7N^{v} and the absence of 7D in this introgression line, although it could not be confirmed that 5A is the A-genome pair absent in this line. Two additional substitutions (3D^{v}(3D) and 4D^{v}(4D)) and a 5D-5D^{v} translocation that were not previously detected by molecular marker approaches have been also cytologically evidenced (Fig. 1B). None of these alien chromosomes or translocations appears in the advanced line ID-2150, whose ISH karyotype is indistinguishable from that of bread wheat.

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