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Comparative RFLP Analysis of Chromosome 2M of *Aegilops comosa* Sibth et Sm. Relative to Wheat (*T. aestivum* L.)

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ABSTRACT

Based on the co-linearity in the Triticeae, comparative RFLP analysis of 2M chromosome of *Ae. comosa* Sibth et Sm. was performed with 2MS and 2M additional lines of *Triticum aestivum* L. cv. Chinese Spring. Among the wheat RFLP probes conserved in the short arms of wheat chromosome 2, those above psr912 were located on the long arms of 2M in *Aegilops comosa*. The rest probes on the short arm and all the probe sequences on the long arm of group 2 chromosome in wheat were conserved on the equivalent chromosomal position in *Aegilops comosa*. So, it is apparent that some chromosomal segment from the short arm had been transferred to long arm while reconstructing 2M chromosome relative to wheat chromosomes. The breakpoint was located between psr912 and psr131 of the short arm. This rearrangement of chromosome 2M might be a molecular evidence of the M genome speciation from an ancestral type.

Key word : *Aegilops comosa*, chromosomal rearrangement, RFLPs.

Genomes in the Triticeae including those of cultivated wheat are considered to evolved from an ancestral genome. This identity has been applied in the comparative genome analysis and alien chromosome identification in wheat, barley, rye, and even in their recombinants from inter or intra generic crosses (Miller & Reader., 1987; Sharp et al., 1989).

By the homoeology analysis of each chromosome of wild relatives, 4/5 translocation of *Ae. umbellulata, Triticum urartu,* and 4/7 translocation of *Secale montanum* (Koller & Zeller, 1976; King et al., 1994), 2/7 translocation in *Secale cereale* (Liu et al., 1992; Devos & Gale, 1993; Devos et al., 1993) and 4/7 translocation of *Ae. longissima* (Friebe et al., 1993; Naranjo, 1995) have been described in the Triticeae.

Cytogenetic stocks including alien chromosome additional lines in wheat have been used for understanding genomic organizations (Jiang & Gill, 1994; Badaeva, 1995) and improving cultivated wheat in both qualities and disease resistances (Sear, 1966; Feldman & Sear, 1981; Dhaliwal et al., 1993). It is an unsolved problem that undesirable characters were involved in wheat translocations developed (Koebner & Shepherd, 1986; Gustafson, 1988). These come from the depression of the recombinations, caused by the partial or serious structural differences of chromosomes between segment or whole chromosome. The integration of the genome information of wild relatives might increase their usefulness in wheat breeding.

This research aimed to analyze chromosome 2M of *Ae. comosa* relative to wheat. Also, the mechanism of chromosomal rearrangement would be discussed with RFLP results.

MATERIALS AND METHODS

Plant materials

T. aestivum L. cv Chinese Spring (CS) and *Ae. comosa* Sibth. et Sm were provided by Cambridge Laboratory in the United Kingdom. As for the genetic stocks (Miller et al., 1988), two wheat/*Ae. comosa* additional lines (CS +2MS and CS+2M from Chines Spring) were used for comparative RFLP analysis. Also, the ditelosomic lines with 2AS (DT2AS), 2BL (DT2BL) and 2DS (DT2DS) chromosomes were used as reference materials.

RFLP analysis

RFLP analysis procedures including DNA extraction, digestion with restriction enzymes, southern blot and hybridization were described by Devos et al. (1992). The genomic DNAs were digested with three different restriction enzymes (*Hind*Ⅲ *Eco*RⅠ, and *Eco*RⅤ). Twenty-one wheat RFLP probes on the homoeologous group 2 were selected on the basis of polymorphisms of *Ae. comosa* (Table 1).

RESULTS AND DISCUSSION

Southern hybridization of wheat RFLP probes in the linkage group 2 to 2M additional line (CS+2M) and 2MS additional line (CS+2MS) which were cytologically evaluated by Miller et al. (1988) provided that all the probes were well conserved in the genome of *Ae. comosa* (Park et al., unpublished). The origin of probe sequences could be analyzed by their polymorphisms. Also, the

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Table 1. RFLP probes of wheat genomic DNAs used for the comparative analysis of chromosome 2M of *Ae. comosa*.

Arms of group 2 chromosome in wheat	Probes selected by polymorphisms			
Short arm	psr100,	psr107,	psr137,	psr126,
	psr143,	psr912,	psr131,	psr900,
	psr108,	psr109,	psr928,	psr566,
	psr908,	psr649		
Long arm	psr388,	psr151,	psr102,	psr331,
	psr540,	psr934,	psr609	
Total	21			

ditelosomic lines (DT2AS, DT2BL and DT2DS) were used for allocation of each wheat chromosome (Devey & Hart, 1993). The probe sequences, psr928 on the short arms of the group 2 chromosomes in wheat was detected on the long arm of chromosome 2M (Fig. 1). The comparative RFLP analysis was conducted using the 21 RFLP probes which showed the specific polymorphisms to chromosome 2M of *Ae. comosa*. Based on the wheat chromosomal arm map, 8 probe sequences located on above psr912 were not detected in 2MS additional wheat line (CS+2MS). Whereas, the probes on the near centromeric region of short arms and whole long arm were detected only on 2M additional line (CS+2M). Therefore, the RFLP loci above psr912 locus which was mapped on the interstitial region of the short arms, were transferred to the long arm of same chromosome 2M during the speciation of *Ae. comosa*. The rest of RFLP loci on the short arm and all loci on the long arm were retained as expected by the co-linearity in the Triticeae. So, this is a clear evidence for the transfer of some chromosomal segment from the short arm to the long arm, and the breakpoint on the short arm during this process was located between psr131 and psr912.

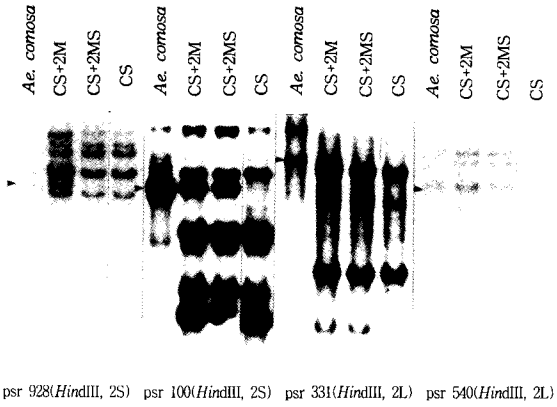

Fig. 1. Examples of RFLP analysis of *Ae. comosa*, the additional lines (CS+2M and CS+2MS) and wheat (Chinese Spring, CS) with wheat RFLP probes on linkage group 2. Solid triangles indicate 2M specific polymorphisms of corresponding probes.

The RFLP map of the homoeologous group 2 was constructed with the large number of molecular probes (Devos et al., 1993; Nelson et al., 1995). As for the wheat/*Ae. comosa* translocations, Park et al. (unpublished) figured these translocations as 2MS-2ML.2AS or 2MS-2ML.2DS using genomic *in situ* hybridization and RFLPs. It is noticeable that the only one copy of probe sequences whether it is from wheat or *Ae. comosa*, was detected in all the translocations and the probe sequences could be deduced with the translocations. Therefore, it is concluded that the probe sequences which were transferred to the long arm from the short arm during the differentiation of chromosome 2M, was turned to be reverse in the order, compared with those of wheat.

The comparative organization of chromosome 2M based on the wheat RFLP map is shown as Fig. 2. The RFLP data of this study were combined with the physical map of the group 2 chromosomes of wheat (Delaney et

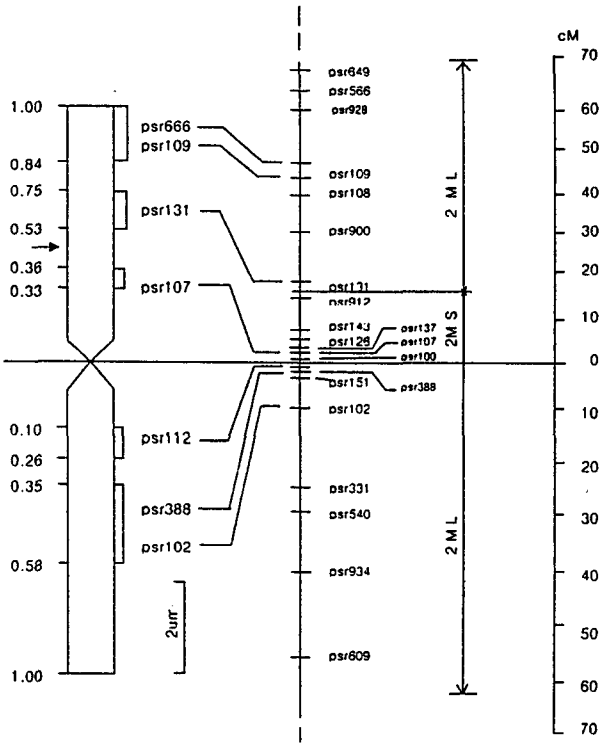

Fig. 2. Comparative RFLP mapping of chromosome 2M to the homoeologous group 2 of wheat. Some segment of short arm was transferred to long arm through structural rearrangement of chromosomes. Physical map is referred from the report of Delaney et al. (1995).

al., 1995) and *Ae. comosa* (Park et al., 1997). Homoeoloci of RFLPs on chromosome 2A, 2B and 2D are well conserved and there is 2BS/6BS translocation in wheat (Sharp et al., 1989; Devos et al., 1993). Some irregular recombination around a locus, psr131 was found in the segregating progenies of the cross between 'Timgalen' and 'RL4137' (Devos et al., 1993). So, it is likely that the chromosomal region around psr131 on the short arm of homoeologous group 2 had been changed during the genomic differentiation in the Triticeae. It is known that 4A of wheat is reverse relative to its homoeologous chromosomes, 4B and 4D through a pericentric inversion (Naranjo et al., 1987). A similar process of chromosomal rearrangement in chromosome 2M is well explained by a pericentric inversion, and this is one evidence contributing the M genome differentiation of *Ae. comosa*. The short arms of homoeologous group 2 involved in the speciation of several genome such as 2B of wheat, 2R of rye (Devos et al., 1993), and M of *Ae. comosa*. This inversion in chromosome 2M of *Ae. comosa* may reduce the frequency of homoeologous pairing due to the partial homology. This will be a logical reason for the difficulty in getting desirable recombinants while introducing rust resistant gene from chromosome 2M to wheat (Miller et al., 1988). The information of each genome will be useful in a trial to utilize genes in the wild relatives of crops; the possibility of recombinants with only target characters by homoeologus pairing and the marker based selection in wheat breeding.

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