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C-banding analysis on wild Emmer (*Triticum dicoccoides* Körn) strains with and without spontaneous reciprocal translocations

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Abstract C-banding polymorphism was analyzed in eight strains of wild Emmer, *Triticum dicoccoides* Körn, which included six translocation homozygotes reported previously. Polymorphisms were detected in all of the strains examined, and the breakpoints of five spontaneous translocations were successfully identified by C-bands. Of the eight breakpoints that could be precisely identified, one was located in the centromeric region while the remaining seven were located in proximal to distal euchromatic regions. The two breakpoints of one translocation could only be approximately localized to proximal regions due to the scarcity of C-bands. The present results are in contrast with those observed on *T. araraticum*, another wild tetraploid wheat belonging to the Timopheevi group, in which most of the breakpoints were located in centromeric regions. In *T. dicoccoides*, the six translocation chromosome types were derived from the standard karyotype primarily by a mechanism other than centric breakage-fusion.

Key words *Triticum dicoccoides* · Reciprocal translocation · Translocation breakpoint · C-banding

Introduction

Triticum dicoccoides Körn (2n = 4x = 28; AABB genome) is a wild species belonging to the Emmer group of the tetraploid wheats. It is the ancestral species of cultivated Emmer wheats (*T. dicoccum* Schübl., *T. durum* Desf. etc.) and hence is the progenitor of all hexaploid common wheats. This species is distributed in the Near East along the Fertile Crescent, in western Iran, northern Iraq, eastern Turkey, Syria, Lebanon, Jordan and Israel. *T. dicoccoides* is characterized by a high level of polymorphism of morphological characteristics, proteins and disease resistance (Nevo 1988 for review). It can form hybrids with cultivated tetraploid wheats, and its genes can be easily transferred to cultivated hexaploid wheats through a partially fertile pentaploid hybrid.

Several authors have shown that hybrids between different strains of *T. dicoccoides* form quadrivalents at meiosis (Rao and Smith 1968; Dagan and Zohary 1970). This suggests that spontaneous reciprocal translocations are common in this species. Recent findings (Kawahara and Nevo unpublished) indicate that translocations occur at a rate of 0.205 per strain in Israeli populations. Information on chromosomal polymorphism is indispensable for utilizing the genetic resources of the wild gene pool in wheat breeding programs. However, to date only one translocation has been characterized by C-banding for both translocated chromosomes and breakpoints (Lukaszewski and Curtis 1993). Kawahara (1984) found one minor and five major reciprocal translocations relative to the standard chromosme type. Although chromosomes involved in these translocations were recently identified by telocentric analysis (Nishikawa et al. 1994), their breakpoints have not yet been identified.

In the study presented here, the translocation tester strains of *T. dicoccoides* were analyzed by the C-banding technique to obtain information regarding the degree of C-band polymorphism and the translocation breakpoints.

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Materials and methods

Genetic stocks

Eight strains of *T. dicoccoides* representing seven chromosome types differing with respect to reciprocal translocations were used in our study (Table 1). KU-8817 and 8935 have the standard chromosome

Table 1 Eight strains of *Triticum dicoccoides* used in this study

Translocalation type[a]	Strain (KU no.)	Origin
E_{1a}	8817	Iraq
E_{1a}	8935	Turkey
E_{1b}	108–2	Syria
E_2	109	Israel
E_3	195	Israel
E_4	8915A	Turkey
E_5	1945	Turkey
E_6	1952	Turkey

[a] Kawahara (1987)

structure, E_{1a}, of this species, whereas each of the other six strains is homozygous for different translocations relative to the standard (Kawahara 1984, 1987). Nishikawa et al. (1994) identified the translocated chromosomes in types E_{1b} and E_2–E_6 as 2A–2B, 2B–3B, 5B–7B, 3B–4B, 6B–7B and 1A–5A, respectively, using telocentric lines of *T. durum* cv 'LD222'. All these strains are maintained by controlled selfing at the Plant Germ-plasm Institute, Faculty of Agriculture, Kyoto University.

Cytological methods

The C-banding procedure followed that of Giraldez et al. (1979) except that Wright's eosin methylene was used for staining. C-banding patterns and karyotypes were compared between E_1a and types E_{1b} and E_2–E_6.

Results

Pattern of variation

The two E_{1a} testers (KU-8817 and 8935) showed basically an identical C-banding pattern, and only minor differences were noted between them. Figures 1a and 2 show the banded chromosomes of KU-8817. When compared with KU-8817, KU-8935 had additional bands in the ends of the long arm of chromosome 7A and the short arm of 4B while the long arm of 3B had fewer bands (data not shown). These two strains had additional bands that were not observed in the A- or, especially, in the B-genome chromosomes of *T. aestivum* L. cv 'Chinese Spring' (Gill et al. 1991); they were more similar with respect to banding pattern to *T. durum* cv 'LD222' (Taketa unpublished).

In the remaining strains, the chromosomes which could be shown by telocentric analysis to be interchanged (Nishikawa et al. 1994) showed different C-banding patterns compared with those of the E_{1a} testers. Hereafter, chromosomes known to be translocated are called "critical" chromosomes, while the others are referred to as "non-critical". In the E_{1b} tester, KU-108-2, one critical chromosome, 2A, had a prominent terminal band in the long arm, while the other critical chromosome, 2B, showed no major change (data not shown). As a result, it was impossible to detect this translocation. In the following text, differences in the C-banding patterns in critical chromosomes are described first and the breakpoints of translocations are estimated. Subsequently, C-band polymorphisms in non-critical chromosomes are summarized.

Positions of the translocation breakpoints

KU-109 (Type E_2)

Two modified metacentric chromosomes, one short and the other long, were observed; these were considered to be the products of a reciprocal translocation between chromosomes 2B and 3B (Figs. 1b, 2). We estimated that the translocation breakpoints are located in the interstitial region of the long arm of chromosome 2B and in the subterminal region of the short arm of 3B (Fig. 3). An additional interstitial band in the long arm of 3B was considered to be a polymorphic band.

KU-195 (E_3)

A 5B-like chromosome with a modified short arm and a 7B-like chromosome with a modified short arm were observed in KU-195 (Figs. 1c, 2). As shown in Fig. 3, we estimated that the short arms of 5B and 7B were interchanged with the breakpoints in the centromeric region of 5B and in the proximal region of the short arm of 7B. The lack of a terminal band in the short arm of 5B translocated to 7B was regarded to be due to polymorphism.

KU-8915A (E_4)

A 3B-like short metacentric chromosome and a 4B-like chromosome with an elongated long arm were observed (Figs. 1d, 2). We considered the translocation breakpoints to be located in the proximal region of the long arm of 3B and in the interstitial region of the long arm of 4B (Fig. 3). The subterminal band of the short arm of 3B of this strain was fainter than that of the other strains.

KU-1945 (E_5)

A 6B-like chromosome with three additional distal bands in the long arm and a 7B-like chromosome which had lost three distal bands in the long arm could be recognized in KU-1945 (Figs. 1e, 2). This clearly indicates that the reciprocal translocation occurred between chromosomes 6B and 7B with breakpoints in the subterminal region of the long arm of 6B and in the interstitial region of the long arm of 7B (Fig. 3). A prominent interstitial C-band in the short arm of 6B was considered to be a polymorphic band.

KU-1952 (E_6)

The C-banding patterns of the short arms of chromosomes 1A and 5A of this strain were reversed in comparison with those of the standards (Figs. 1f, 2). This indicated that the reciprocal translocation occurred between chromosomes 1A and 5A. However, we could

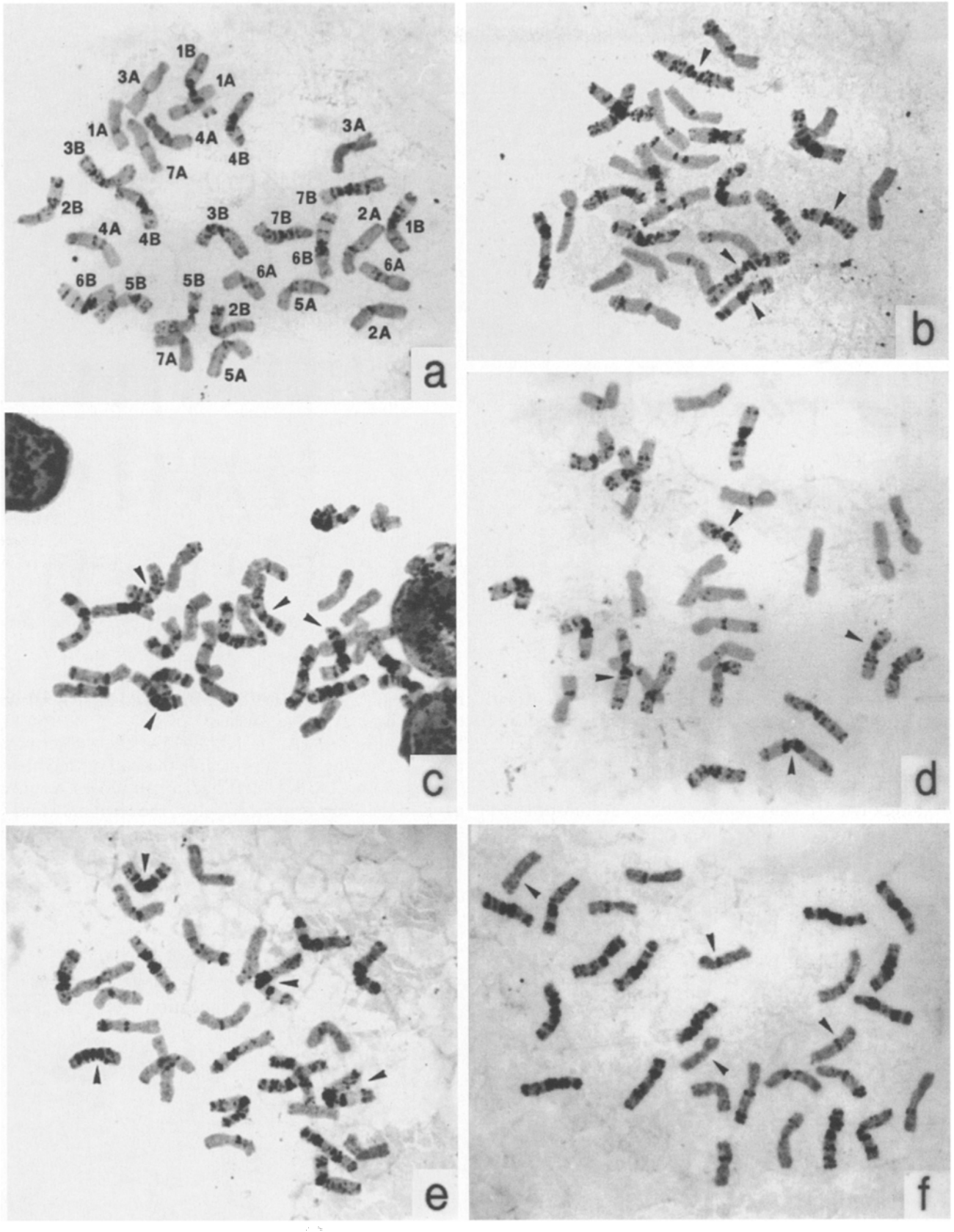

Fig. 1a–f C-banded mitotic metaphases of *T. dicoccoides*. **a** KU-8817 (E_{1a}), **b** KU-109 (E_2), **c** KU-195 (E_3), **d** KU-8915A (E_4), **e** KU-1945 (E_5), **f** KU-1952 (E_6). *Arrowheads* indicate translocated chromosomes

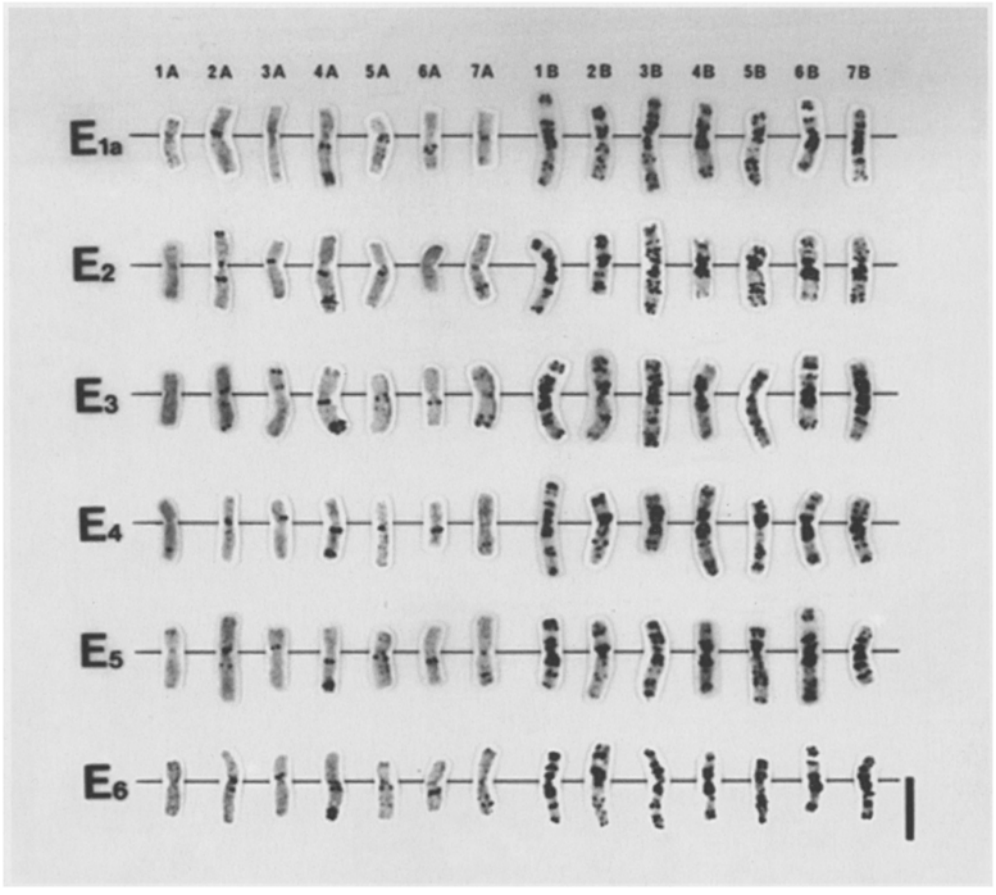

Fig. 2 C-banded chromosomes of six *T. dicoccoides* strains. Type E_{1a} carries no reciprocal translocation. In types E_2–E_6, translocated chromosomes are placed in the column of their normal counterparts. *Bar* = 10 μm

only approximately estimate that the breakpoints are in the proximal regions of both chromosomes because of the scarcity of C-bands in these chromosomes (Fig. 3).

C-band polymorphism in non-critical chromosomes

C-band polymorphisms in non-critical chromosomes included the presence/absence or size differences of a band. C-band polymorphisms were apparent in chromosomes 1A, 2A, 3A, 6A, 7A, 3B, 4B and 6B (Fig. 2) and can be summarized as follows:

Chromosome 1A: in KU-8915A, the subterminal band in the long arm was clearer than all of the others. Chromosome 2A: KU-109 had a prominent terminal band in the short arm; in KU-195, the terminal band in the long arm was clearer. Chromosome 3A: a prominent subterminal band in the short arm was found in KU-195. Chromosome 6A: interstitial bands of the long arm were faint in KU-109.

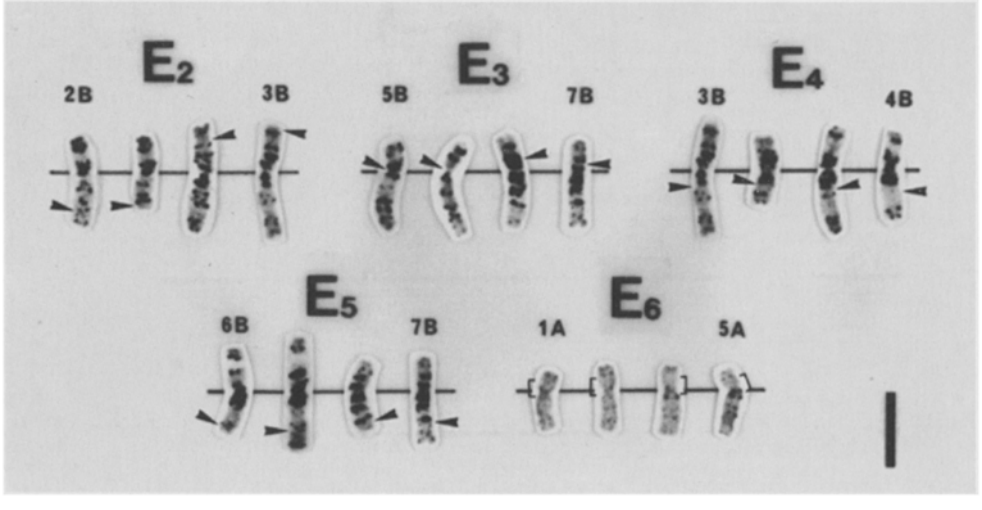

Fig. 3 C-banded translocation (*central two*) and normal (*outer two*) chromosomes. Normal chromosomes are from KU-8817 (E_{1a}). *Arrowheads* indicate translocation breakpoints. In type E_6, regions where the translocation is estimated to have occurred are marked by *brackets*. *Bar* = 10 μm

Chromosome 7A: the number of C-bands in the distal regions of the long arm vaired from one to three among the strains examined. Chromosome 3B: KU-1945 and 8935 lost one of the two interstitial bands in the long arm. Chromosome 4B: KU-108-2 and 8935 had a terminal band in the short arm, and KU-195 lacked a subterminal band in this arm; an interstitial band was consistently observed in the long arm in KU-1945 and 1952. Chromosome 6B: KU-108-2 had a thick interstitial band next to the proximal heterochromatin block in the short arm.

Discussion

The non-critical chromosomes of the E_2–E_6 testers of *T. dicoccoides* were similar in C-banding pattern to the corresponding chromosomes of the E_{1a} testers, but the degrees of C-band polymorphism were higher in the former than those observed in the A- and B-genome chromosomes of bread wheat and triticale (Seal 1982). The present results show that the major reciprocal translocations are simple exchanges between two non-homologous chromosomes of the standard E_{1a} testers. Thus, the C-banding patterns observed in the present materials can be considered to occur only within the species *T. dicoccoides* and to be clearly different from those of *T. araraticum* (Hutchinson et al. 1982). We conclude that the E_2–E_6 types were derived from the E_{1a} type through spontaneous reciprocal translocations in their distribution site, as suggested by Kawahara (1987). Similar results have been reported by Noda and Koulin (1989) and Badaeva et al. (1995) in *T. araraticum*, who studied translocations by N- and C-banding, respectively. Thus, the banding polymorphism observed in the two wild tetraploid wheats belonging to different groups, Emmer and Timopheevi, can primarily be explained by translocations.

The present C-banding analysis revealed that the critical chromosomes in the E_2–E_6 testers show major differences in C-banding pattern (Fig. 2). On the basis of these differences, translocation breakpoints of the E_2–E_6 testers were successfully estimated, as shown in Fig. 3. The C-banding technique is powerful in detecting major translocations in *T. dicoccoides*. On the other hand, the minor reciprocal translocation in the E_{1b} tester (Kawahara 1984) and that suggested to exist in all of the testers along with the major one (Nishikawa et al. 1994) could not be detected. This is probably because minor translocations with short chromosomal segments cause little change in the C-banding pattern.

Recently, in situ hybridization using total genomic DNA as probes was used successfully to localize the precise breakpoints of translocations between chromosomes from different genomes. Mukai et al. (1993), for example, demonstrated that in 'Chinese Spring' the distal 32% of the long arm of 4A was derived from the B-genome by translocation. However, this method is inappropriate for analysis of the major reciprocal translocations detected here because all of these are intragenomic.

The findings regarding chromosome pairing and the results of the present C-banding analysis gave different estimates of the lengths of the interchanged chromosome segments in the E_2 and E_5 testers. Nishikawa et al. (1994) estimated that fairly long segments were interchanged based on the high frequencies of a ring quadrivalent. However, the present results of C-banding analysis indicate that in both testers one of the interchanged segments was very small. It is possible that the frequency of ring quadrivalents does not reflect the physical length of interchanged chromosome segments as recombination has been shown to occur preferentially in the distal regions of chromosomes in Emmer wheat (Lukaszewski and Curtis 1993).

Many spontaneous translocations have been reported in *T. araraticum* and their breakpoints have mostly been localized to centromeric regions. Of eight breakpoints studied by Noda and Koulin (1989), six were in the centromeric regions. Badaeva et al. (1995) also found that the most widely distributed translocation type in *T. araraticum* was the centric breakage-fusion translocation. In *T. dicoccoides*, Lukaszewski and Curtis (1993) reported a reciprocal centromeric translocation, 1BS.5BL 5BS.1BL, in accession TTD 126.

In the present study, however, among the eight breakpoints that could be identified precisely, seven were located outside the centromeric regions, i.e. proximal to the distal regions of the chromosomes involved (Fig. 3). The causes of this discrepancy are not clear. Reciprocal centromeric translocations are most likely to have originated from the misdivision of univalents and subsequent fusion of resulting telocentric chromosomes (Morrison 1956). On the othe hand, reciprocal non-centromeric translocations must have been generated by other mechanisms (Gupta and Gupta 1991 for review). Due to the intragenomic nature of the present translocations, homoeologous pairing can be ruled out as the mechanism of their generation.

The present study clearly shows that the C-banding technique is effective in determining the breakpoints of reciprocal translocations, as well as in clarifying intraspecific polymorphism of chromosome structure. For the detection of translocations involving short segments and/or segments with little C-banding, we should enrich chromosome markers by employing other techniques with higher resolution, such as in situ hybridization using specific DNA probes.

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