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Amplification of repeated DNA sequences in wheat × rye hybrids regenerated from tissue culture*

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Summary. Previous C-banding analysis of wheat (*Triticum aestivum*) × rye (*Secale cereale*) hybrids regenerated from tissue culture revealed enlarged C-bands in some rye chromosomes, but the molecular nature of the change was not determined. In situ hybridization using two DNA probes containing repeated sequences from rye telomeric heterochromatin was conducted on these wheat × rye hybrids and their progeny to investigate the occurrence of amplification in repeated sequences. Clones pSC 74 and pSC 119, which contain sequences from the 480-bp and 120-bp repeated DNA families of rye, respectively, were used as probes. Amplification of 480-bp repeated sequences in the short arm telomere of chromosome 7R was detected in three wheatxrye hybrids and their progeny. The amplified 480-bp sequences were detected by an enlarged hybridization site for pSC 74 at the 7RS telomere, and by the appearance at this same telomeric site of an unlabeled, blue chromosome segment in an otherwise completely brown chromosome hybridizing entirely to the biotin-labeled pSC 119 probe. This variant form of chromosome 7R was not observed in several 'Chaupon' plants, or in the other hybrids derived from the same embryos, indicating the origin of the change in tissue culture. The amplified sequences were inherited up to at least three generations. Deletions and translocations were also observed.

Key words: Amplification – Repeated DNA sequences – Wheat × rye hybrids – Tissue culture

Introduction

The heritable phenotypic variation exhibited by plants regenerated from tissue cultures (Larkin and Scowcroft 1981) is an indication that the tissue culture process induces modifications in the genome. Changes occurring at the cytological level have been shown for almost all plant species that are amenable to regeneration in tissue culture. The changes observed range from gross changes, such as aneuploidy, to those involving small chromosome segments, such as translocations, inversions, and deletions (Armstrong et al. 1983; Cummings et al. 1980; D'Amato 1978; Gill et al. 1985, 1986, 1987; Karp and Maddock 1984; Lapitan et al. 1984; Larkin and Scowcroft 1981; McCoy et al. 1982; Novak 1980; Orton 1980; Sacristan 1971). In many cases, however, phenotypic variation cannot be attributed to cytologically observable changes, suggesting the occurrence of changes at the molecular level.

In flax, it has been shown that suboptimum environments, such as nutrient stress can induce quantitative changes in repeated DNA sequences (Cullis 1981, 1983). Amplification and deletion in ribosomal RNA (rRNA), 5SRNA, and other highly repeated sequences were observed. Recent studies have shown that similar changes also occur in plant tissue cultures. In triticale and *Solanum tuberosum,* reduction in the copy number of rRNA genes was observed in plants regenerated from immature embryo and protoplast, respectively (Brettell et al. 1986; Landsmann and Uhrig 1985). Evidence of amplification in repeated sequences was shown in doubled haploid plants of *Nicotiana sylvestris* Speg. & Comes. and *N. tabacum* L. derived from anther cultures using DNA renaturation kinetics (DePaepe et al. 1982/83; Dhillon et al. 1983). Because of the limited evidence available so far, it is not known how widely

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these changes are occurring in different tissue culture systems, or whether particular sequences or chromosomal regions are susceptible to such changes.

In a recent study of hybrids between wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) regenerated from tissue culture, we have observed the appearance of enlarged C-bands in two rye chromosomes 2R and 7R (Lapitan et al. 1984). Since heterochromatic C-bands consist of repeated sequences (Appels et al. 1981; Bedbrook et al. 1980), the observed change may have reflected amplification of repeated sequences, but this could not be determined by the C-banding technique employed.

By the in situ hybridization technique, the telomeric C-bands of rye chromosomes were determined to consist of four families of highly repeated sequences, with repeating units of 120-bp, 480-bp, 610-bp, and 630-bp (Jones and Flavell 1982a). It has been hypothesized that these families are arranged in separate blocks and at specific positions relative to one another, based on the pattern of deletion of repeated sequences from the telomere of rye chromosomes. For example, in some lines, all of the 480-bp repeated sequences were observed to be deleted without affecting the amount of other families of repeated sequences. It was thus suggested that the 480-bp sequences occupy the most distal portion of the telomere. Other observations lead to the suggestion that the 120-bp sequences occupy the most proximal position, while the 610- and 630-bp sequences are located between the 120-bp and 480-bp sequences.

The level of in situ hybridization has been shown to reflect the copy number of repeated sequences detected by blot hybridization in different species of *Secale* (Jones and Flavell 1982b). Because of this, in situ hybridization was employed in this study to determine whether amplification of repeated sequences occurred in the wheat×rye hybrids regenerated from tissue culture and to identify the families of repeated sequences involved. This paper reports the detection of amplification in the 480-bp repeated sequence family in chromosome 7R by the change in the in situ hybridization patterns to two rye probes containing repeated sequences from rye heterochromatin. Other chromosomal changes such as deletions and translocations are also described.

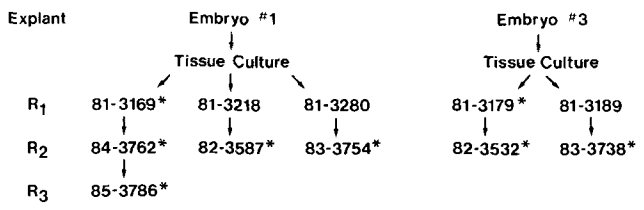

Fig. 1. Wheat×rye hybrids regenerated from tissue culture and selfed to give R_1, R_2, and R_3 generation plants. *Asterisks* indicate plants included in the study

Materials and methods

Eight wheat×rye hybrids (AABBDDRR) that were regenerated from tissue culture were analyzed in this study (Fig. 1). These hybrid regenerants were produced from crosses between wheat (*T. aestivum*) cv. ND7532 and one plant of rye (*S. cereale*) cv. Chaupon as the male parent. As described in Lapitan et al. (1984), haploid R_0 plants were regenerated from two immature hybrid embryos (Nos. 1 and 3), treated with colchicine, and selfed for 1, 2, or 3 subsequent generations to give R_1, R_2, and R_3 plants, respectively. Previous analysis of R_1 plants by C-banding revealed that 81-3169, 81-3218, and 81-3179 possessed an enlarged C-band in one telomere of chromosome 7R (Lapitan et al. 1984). These hybrids and their progeny were analyzed by in situ hybridization. Hybrids originating from the same embryo sources, but not showing the enlarged C-band in chromosome 7R, were included for comparison. Seeds of these materials were germinated. Root tips 1 to 2 cm long were pretreated with ice-water kept at 0 °C for 24 h and fixed in 3 : 1 ethanol acetic acid. Root tip squashes prepared according to Endo and Gill (1984) were then used for in situ hybridization.

Clones pSC 119 and pSC 74, obtained from Dr. R. B. Flavell, Plant Breeding Institute, Cambridge, England, were used as probes. pSC 74 and pSC 119 contain rye DNA sequences from the 480-bp and 120-bp repeated sequence families, respectively (Bedbrook et al. 1980). The probes were labeled with biotin and used for in situ hybridization. The details of the procedure for nick translation, in situ hybridization, and detection of hybridization were as described by Rayburn and Gill (1985).

The in situ hybridization sites were detected by the appearance of a brown-colored precipitate over blue (Giemsa-stained) chromosomes (Lapitan et al. 1986; Rayburn and Gill 1985). The in situ hybridization patterns of each rye chromosome in wheat-rye addition lines ('Chinese Spring'/'Imperial' 1R to 7R) were first observed. The karyotype of rye cv. Chaupon was then constructed based on similarity of in situ hybridization patterns between Chaupon chromosomes and rye chromosomes in the wheat-rye addition lines. Variation in the number and size of hybridization sites for pSC 119 and pSC 74 was examined in twenty plants of Chaupon. Hybridization sites for pSC 74 were measured in twenty cells, and their relative sizes are depicted in Fig. 2b.

The rye chromosomes in the wheat×rye hybrids were analyzed for homozygous or heterozygous changes in in situ hybridization patterns to pSC 74 and pSC 119 and in size of hybridization sites by comparison with those of Chaupon. A heterozygous change would denote an origin from the sexual process or during development of the plant as a result of somatic mutation, whereas a homozygous change would indicate an origin from the haploid primary regenerant that was treated with colchicine. The hybridization sites of chromosome 7R were measured in a total of twenty cells from four different plants of each hybrid.

Measurements were taken from photographic prints with a plastic ruler and average values expressed in micrometers (μm). Photographs were taken with Kodak film Tech Pan 2415, using a Zeiss III photomicroscope.

Results

Distribution of 480-bp and 120-bp repeated sequences in rye

The individual chromosomes of rye cv. Chaupon were readily identified by their in situ hybridization patterns

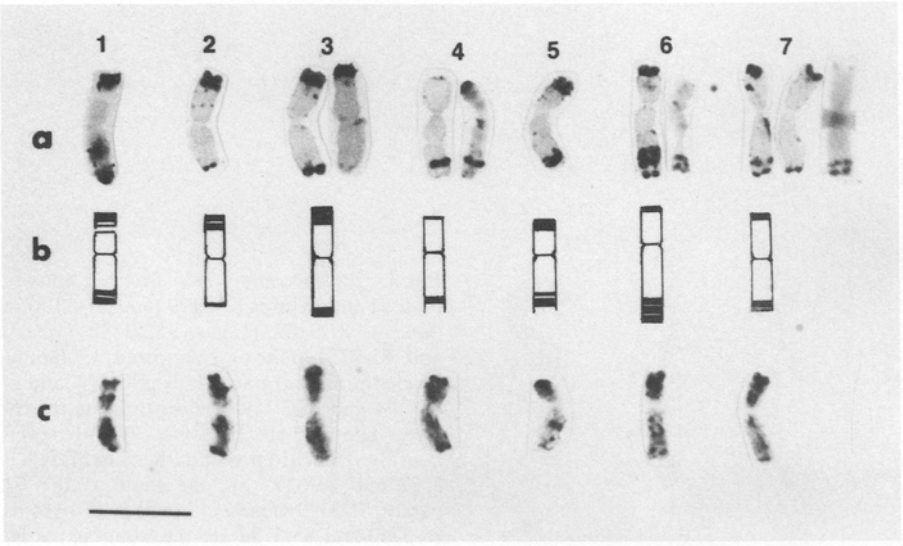

Fig. 2. Chromosomes 1R to 7R of *Secale cereale* cv. Chaupon hybridized to biotin-labeled probes *a*) pSC 74 (480-bp) and *c*) pSC 119 (120-bp). Heteromorphism in chromosomes 3R, 4R, 6R, and 7R is shown in *a*. The diagram in *b* shows the 20 hybridization sites for pSC 74 and the relative size of each site. Note the overall hybridization of pSC 119 to the seven chromosomes in *c*. Bar = 10 μm

to pSC 74, which were similar to those of rye chromosomes in wheat-rye addition lines (Jones and Flavell 1982a; this study). The 480-bp sequences detected by pSC 74 were clustered at 18 to 20 sites distributed over the seven chromosomes (Fig. 2a, b). Of these sites, eight were interstitial and the rest were telomeric. The quantitative distribution of 480-bp sequences, as judged by differences in the size of hybridization sites, varied among these sites (Fig. 2b). The differential distribution of the 480-bp repeated sequences among the different chromosomal sites was consistent from cell to cell and from one plant to another. The largest hybridization site was 1.2 μm and was observed in the short arm (S) telomere of chromosome 3R. The smallest hybridization site was located in the long arm (L) telomere of chromosome 2R, and was approximately 0.3 μm in length.

Variation for the presence of some hybridization sites was observed in chromosomes 3R, 4R, 6R, and 7R (Fig. 2a). One of the 10 plants was heterozygous for the presence of the hybridization site in the long arm telomere of chromosome 3R. Nine plants were homozygous for the absence of the 4RS site, whereas one plant showed hybridization at this site in one of the homologues. Heterozygosity for the 6RS site was observed in one plant. Chromosome 7R showed heteromorphism in both arms. In the long arm, the interstitial site close to the telomere was absent in one of the homologues of eight plants. One plant was homozygous for the absence of this interstitial site. Another plant was homozygous for the absence of the hybridization site in the short arm telomere of chromosome 7R. A high frequency of heteromorphism for 480-bp sequences has already been noted in other cultivars of *S. cereale* and in other *Secale* species (Jones and Flavell 1982a, b).

In situ hybridization with biotin-labeled pSC 119 has been shown to produce a brown appearance in the entire length of all rye chromosomes, indicating the dispersed distribution of the 120-bp sequences in the rye chromosomes (Jones and Flavell 1982a; Lapitan et al. 1986). Individual chromosomes of rye were thus indistinguishable by their in situ hybridization patterns to pSC 119, and were identified by arm ratios and chromosome lengths corresponding with chromosomes identified by means of in situ hybridization patterns to pSC 74 (Fig. 2c). Chromosomes 5R and 6R were both heterobrachial and easy to recognize, with chromosome 5R having the largest arm ratio in the complement. Chromosomes 1R and 4R were both submetacentric, but 1R was smaller than 4R and was distinguished by the hybridization site on the nucleolus organizer region (NOR) in the short arm. The three nearly metacentric chromosomes 2R, 3R, and 7R, were distinguished from each other by their chromosome lengths, 3R being the longest, and 2R, the smallest of the three. No variation for the distribution of the 120-bp repeated sequences was detected in the 10 plants of Chaupon.

Variation in in situ hybridization patterns of rye chromosomes in wheat × rye hybrid regenerants

The rye chromosomes were readily distinguished from the wheat chromosomes of the wheat × rye hybrid regenerants by the in situ hybridization patterns to pSC 74 and pSC 119 probes. In contrast to the hybridization patterns of rye chromosomes, wheat chromosomes do not hybridize to pSC 74 and 11 wheat chromosomes hybridize to pSC 119 only at a few localized sites (Lapitan et al. 1986; Rayburn and Gill 1985).

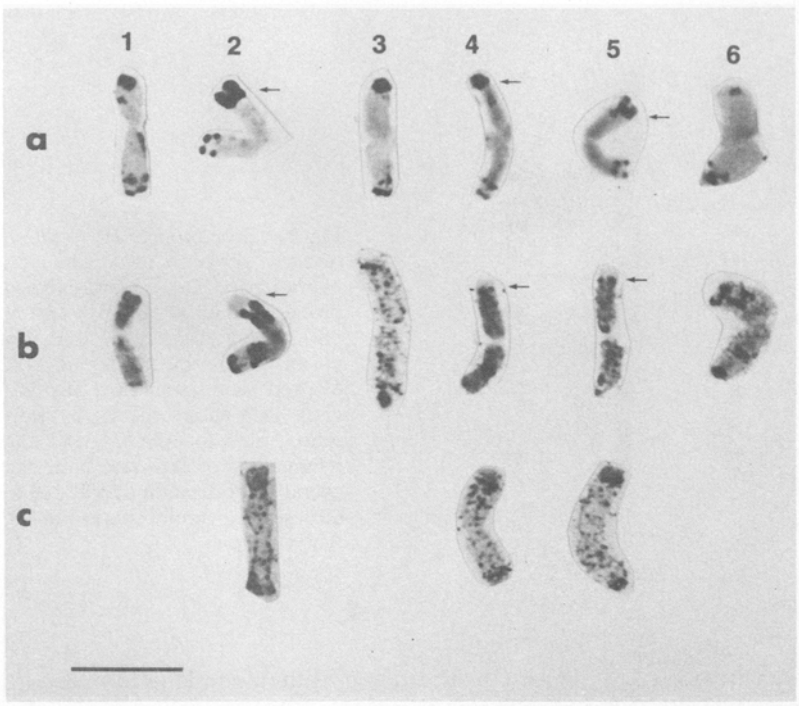

Fig. 3. Chromosome 7R from Chaupon (*lane 1*) and hybrids 81-3179 (*lane 2*), 83-3738 (*lane 3*), 81-3169 (*lane 4*), 82-3587 (*lane 5*) and 83-3754 (*lane 6*) hybridized to biotin-labeled probes *a*) pSC 74, *b*) pSC 119, and *c*) pSC 74 and pSC 119. *Arrows* indicate the altered site in chromosome 7R of seven hybrids. The chromosome 7R of hybrids 82-3532 and 85-3593 are the same as the R_1 parent 81-3179 (*lane 2*), and that of hybrids 84-3762 and 85-3786 are the same as the R_1 parent 81-3169 (*lane 4*) and are not shown here. Note the increased hybridization for pSC 74 at the 7RS telomere (*a*). This same site is unlabeled with pSC 119 (*b*) but labeled in the presence of both pSC 74 and pSC 119 probes (*c*). Bar = 10 μm

Analysis of the rye chromosomes in the regenerants revealed that the in situ hybridization patterns of chromosome 7R in three R1 plants and/or their progeny differed from that observed in Chaupon. The hybrids exhibiting the variation included 81-3179, 82-3532, 81-3169, 84-3762, 85-3786, and 82-3587.

In the six wheat×rye hybrid regenerants, the homologous pair of chromosome 7R showed an increased hybridization for pSC 74 in the short arm telomere (Fig. 3a, Table 1). In 81-3179 (Fig. 3a, lane 2) and its progeny 82-3532 (Table 1), the 7RS hybridization site was approximately three times larger than that in Chaupon. In 81-3169 (Fig. 3a, lane 4) and its progeny 84-3762 and 85-3786, and in 82-3586 (Fig. 3a, lane 5), the 7RS site was observed to be about two times that of Chaupon. In contrast, such increased hybridization for pSC 74 in the short arm telomere of chromosome 7R was not observed in other plants derived from the same embryo sources. In 83-3738 (Fig. 3a, lane 3), this site was observed to be of the same size as in Chaupon. In 83-3754 (Fig. 3a, lane 6), the 7RS site was much smaller than that observed in 81-3169 and 82-3587.

A very unusual in situ hybridization pattern to pSC 119 was observed in chromosome 7R of the same hybrids which showed increased hybridization to pSC 74 at the 7RS site. An unlabeled segment, seen as a blue segment in an entirely brown chromosome, appeared in one telomere of chromosome 7R (Fig. 3b, lanes 2, 4, and 5 and Fig. 4, Table 1). In contrast, no similar unlabeled segment was observed in chromosome 7R of Chaupon (Fig. 3b, lane 1) or in the hybrids 83-3738 and 83-3754 (Fig. 3b, lanes 3 and 6).

Table 1. Average measurements (N = 20) of hybridization sites on the telomere of the short arm of chromosome 7R in Chaupon and the regenerated wheat-rye amphiploids

Plant	Mean length (μm) of pSC 74 site on 7RS	SE[a]	Mean length (μm) of 7RS unlabeled with pSC 119	SE
Chaupon	0.81	0.09	–	–
81-3179	2.38	0.07	1.78	0.22
82-3532	2.19	0.11	1.36	0.14
83-3738	1.0	0.10	–	–
81-3169	1.31	0.08	1.20	0.20
84-3762	1.56	0.30	1.32	0.34
85-3786	1.25	0.00	1.25	0.00
82-3587	1.44	0.09	1.48	0.15
83-3754	0.60	0.10	–	–

[a] SE = Standard error of the mean

The appearance of an unlabeled, blue chromosome segment together with a heavily labeled, brown chromosome segment after in situ hybridization with biotin-labeled pSC 119 was previously observed in translocated chromosomes containing chromatin from both wheat and rye (Lapitan et al. 1986). To verify the source of the unlabeled telomere in chromosome 7R, pSC 74 and pSC 119 were simultaneously hybridized to the chromosomes of the hybrids. Chromosome 7R, in the hybrids

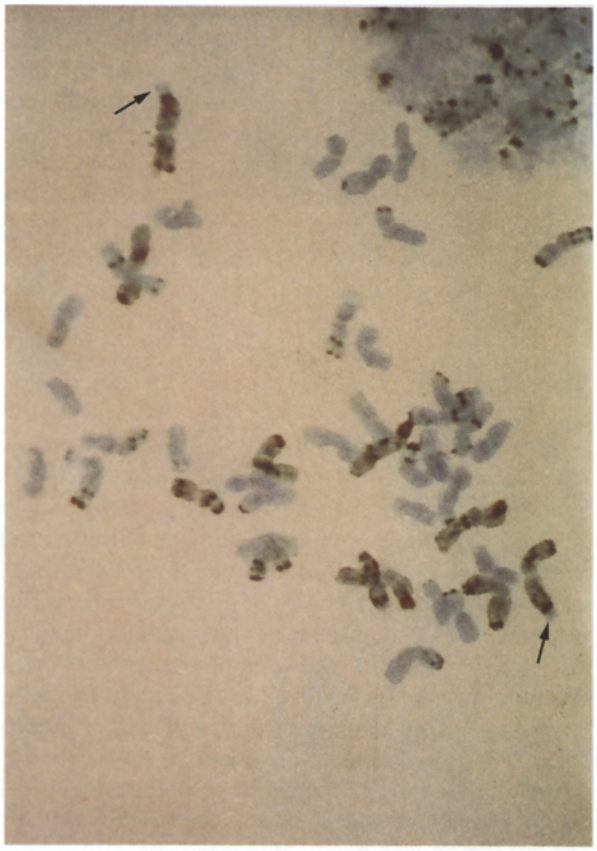

Fig. 4. In situ hybridization of biotin-labeled pSC 119 probe to chromosomes of R_1 plant 81-3179. Note the unusual in situ hybridization patterns of the homologous pair of chromosome 7R (indicated by *arrows*). An unlabeled, blue chromosome segment was present at the telomere of a brown chromosome hybridizing entirely to biotin-labeled pSC 119 probe

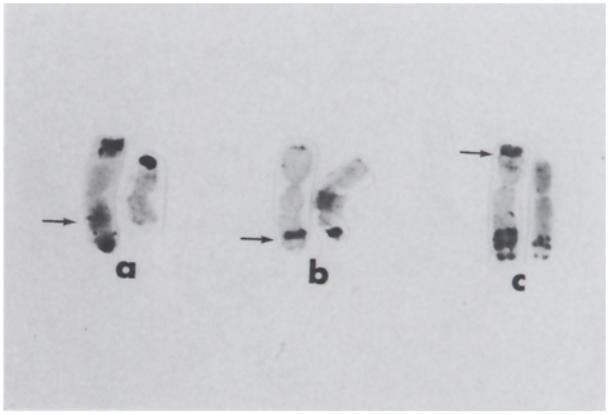

Fig. 5. In situ hybridization to biotin-labeled pSC 74 of chromosomes with deletions in the wheat × rye hybrid regenerants. Deletion of *a*) half of 1RL, *b*) 4RL telomeric segment, *c*) 6RS telomere. Each pair shows the normal chromosome on the *left*, chromosome with deletion on the *right*. (*Arrows* indicate breakpoints in normal chromosomes)

that possessed the unlabeled telomere, was heavily labeled (Fig. 3c) indicating the presence of rye chromatin in the whole chromosome including the telomeres.

These results indicate that additional chromatin, without 120-bp repeated sequences, was present in the short arm telomere of chromosome 7R in the hybrids 81-3179 and 82-3532 that originated from embryo no. 1 (see Fig. 1). Based on the observation that hybridization for pSC 74 site was increased in these hybrids, it can be concluded that the additional chromatin in the telomere contained increased copies of 480-bp repeated sequences. This interpretation is in agreement with the proposed arrangement of the 480-bp and 120-bp repeated sequence families in the telomere of rye chromosomes (Jones and Flavell 1982a). The appearance of an unlabeled chromosome segment after in situ hybridization with pSC 119 at the same site of the chromosome that showed increased hybridization to pSC 74 can only be explained by the separate arrangement of these two repeated sequence families in the telomere, with the 480-bp repeated sequence family occupying the most distal end.

In hybrids (81-3169, 81-3762, 85-3786, and 82-3587) that originated from embryo no. 1 (Fig. 1), although the length of the pSC 74 labelled segment was almost twice the original Chaupon telomeric length, the length of the unlabelled segment with pSC 119 was about the same as the pSC 74 labelled segment (Table 1). This means that the sequence pSC 74 was considerably reduced in size before it became exclusively amplified to about twice the size of the average Chaupon telomere. Thus, several amplification and deamplification cycles of the pSC 74 sequence probably occurred during tissue culture.

C-banding has shown that deletions of rye telomeric heterochromatin frequently occur in tissue culture (Lapitan et al. 1984). The possibility that additional 480-bp sequences in 7RS came from these deleted segments through translocations was investigated by observation of deletions in other rye chromosomes. In situ hybridization revealed more deletions that were not detected by C-banding or were inadvertently identified as other types of chromosome structural changes. In 81-3179 and 82-3543, homozygous deletions of one-half of 1RL and a small telomeric segment in 4RL were observed (Fig. 5a, b). These changes were previously identified to be translocations involving 1R and 4D (Lapitan et al. 1984). In 81-3169, 84-3762, and 85-3786, the hybridization site for pSC 74 was deleted from the short arm telomere of both 6R chromosomes (Fig. 5c).

The only possible source of the additional 480-bp sequences in the R_1 hybrid 81-3179 was the deleted 1RL segment, but the size of this segment was much larger than the observed increase in chromosome 7R. In the

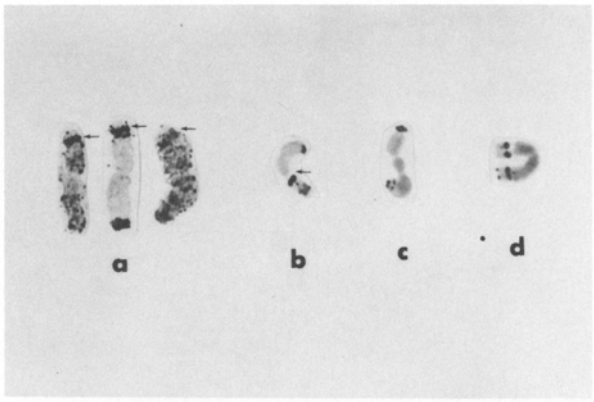

Fig. 6. Chromosome translocations in the wheat×rye hybrid regenerants: *a*) 3R with translocated wheat chromosome segment hybridized to biotin-labeled probes pSC 119 (*left*), pSC 74 (*middle*), and pSC 74 + pSC 119 (*right*). In situ hybridization to biotin-labeled pSC 74 of translocations involving *b*) 5R, *c*) 7R, and *d*) 7R. Other rye chromosomes involved in the translocations in *b, c* and *d* were not determined. (*Arrows* indicate breakpoints, where identifiable)

R_1 hybrid 81-3169, the similarity in size between the deleted pSC 74 site in 6RS and the increase in pSC 74 site in 7RS indicated the possibility of the 6RS segment being translocated to the 7RS telomere. However, the fact that hybrid 82-3587, which was derived from the same embryo, did not show the 6RS deletion but also possessed additional chromatin on 7RS suggests that this possibility is unlikely. Hence, the observed increase of 480-bp sequences in the 7RS telomere did not merely involve rearrangement of repeated sequence-containing chromosome segments but apparently involved an amplification event resulting in increased copies of 480-bp sequences at one chromosomal site.

In situ hybridization also revealed translocations between wheat and rye chromosomes and between rye chromosomes (Fig. 6). In 83-3738, the homologous pair of chromosome 3R was observed to contain a translocated wheat chromosome segment at the telomere of the short arm, consistent with previous C-banding results (19). The translocated wheat chromosome appeared as an unlabeled segment at the tip of a chromosome heavily labeled with pSC 119 (Fig. 6a), similar to the pattern of chromosome 7R with pSC 119 described above. But unlike the latter, the telomeric segment in chromosome 3R remained unlabeled even after in situ hybridization with pSC 74 alone or with both pSC 74 and pSC 119 (Fig. 6a). This indicated that either the unlabelled segment is a small translocation from a wheat chromosome or yet another rye repeated DNA segment has become amplified to the exclusion of the pSC 74 and pSC 119 DNA sequences.

Heterozygous translocations occurring during the sexual cycle were observed in some of the R_2 and R_3 hybrids. In one plant of the R_3 hybrid 85-3786, a translocation involving rye chromosome 5R and another unidentified rye chromosome was observed (Fig. 6b). One R_2 plant 82-3587 was highly mosaic. Two kinds of translocations involving chromosome 7R were observed in two different cells of the same plant (Fig. 6c, d). The other rye chromosomes involved in the translocations were not determined.

Discussion

In previous studies, quantitative changes in repeated sequences were detected by the change in the level of hybridization of a radioactively-labeled probe to known amounts of DNA immobilized on paper (Brettell et al. 1986; Cullis 1983; Landsmann and Uhrig 1985). In this study, increase in the copies of one family of repeated sequences was demonstrated cytologically by means of in situ hybridization. With this technique, the specific chromosomal region involved in the amplification was determined. The telomere in the short arm of chromosome 7R was determined to contain increased copies of the 480-bp repeated sequences by increased hybridization to pSC 74, which detects the 480-bp sequence, and by the appearance at this same site of a segment unlabeled with pSC 119, which detects the 120-bp sequence. The change in the hybridization pattern of the chromosome to biotin-labeled pSC 119 was particularly striking because of its difference from the expected overall labeling pattern in rye chromosomes. The amplification in the 480-bp repeated sequence family gave rise to the enlarged C-band in chromosome 7R previously observed in the wheat×rye hybrids regenerated from tissue culture (Lapitan et al. 1984).

The homozygous condition of the variant chromosome 7R in the seven wheat×rye hybrids indicates its initial presence in the haploid R_0 regenerant that was treated with colchicine, but does not discriminate between the possibility of its preexistence in the rye parent used or its origin from a change occurring during tissue culture. However, several observations indicate that the preexistence of a chromosome 7R with increased copies of 480-bp sequences in the rye pollen was unlikely. First, although heteromorphism for repeated sequences exists, the variant form of chromosome 7R present in the hybrids was not observed in 20 Chaupon plants analyzed by in situ hybridization to pSC 74 and pSC 119. Second, none of the 50 Chaupon plants previously analyzed by C-banding showed the enlarged C-band (Lapitan et al. 1984) observed in the chromosome 7R of the wheat×rye regenerants. Third, not all plants regenerated from the same embryos contained increased

copies of the 480-bp repeated sequences. Finally, the quantity of 480-bp sequences in 7RS of hybrids derived from embryo No. 1 differed from those derived from embryo No. 3, manifesting two different amplification events in tissue culture.

The reason for the occurrence of two independent amplification events at the same chromosomal site is not known. Because of the small number of plants examined, it is not possible to conclude whether certain chromosomal regions affect the predisposition of sequences to changes. During evolution, however, amplification of sequences apparently originates from the telomeres of rye chromosomes and these sequences are then transferred to interstitial sites, based on the distribution of ancient repeats at telomeric as well as interstitial sites and of recent, species-specific repeats mainly at the telomeres of rye chromosomes (Jones and Flavell 1982b). Whether repeated sequences in other rye telomeres also underwent quantitative changes will have to be investigated further on a larger number of plants.

The present study provides additional evidence for the occurrence of quantitative changes in repeated DNA sequences during tissue culture, and is the first report of DNA sequence amplification in cereals regenerated from immature embryos. The change observed in the wheat×rye hybrid regenerants have features in common with previously reported DNA changes in other tissue culture-regenerated plants and in flax plants that have undergone nutrient stress (Brettell et al. 1986; Cullis 1981, 1983; Landsmann and Uhrig 1985; Walbot and Cullis 1985). The 480-bp sequences, like all the other repeated DNA reported to have undergone a change in copy number, are in tandem arrays (Bedbrook et al. 1980). The quantitative DNA changes in Triticale (Brettell et al. 1986) and in flax (Cullis 1981, 1983) were observed to be transmitted stably to subsequent generations. Likewise, the amplified 480-bp repeated sequences in chromosome 7R were inherited up to at least three generations.

This study demonstrated two levels of genome modification in plants regenerated from tissue culture. At the cytological level, translocations and deletions were observed, and at the molecular level, amplification in one family of repeated sequence was shown. All the available evidence so far, therefore, indicates that the observed phenotypic variation among plants regenerated from tissue culture emanates from various kinds of genome alterations as a result of the tissue culture process.

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