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Translocations and other karyotypic structural changes in wheat × rye hybrids regenerated from tissue culture *

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Summary. The spontaneous occurrence of chromosome breaks, deletions, and translocations in plant tissue cultures is well documented. This study investigated the usefulness of tissue culture as a method of introgressing alien genes into wheat. Wheat × rye hybrids were regenerated from embryo scutellar calli maintained in culture for 222 days. The regenerated seedlings then were treated with colchicine to produce amphidiploids (AABBDDRR). The karyotypes of ten amphidiploids were analyzed by C-banding to determine chromosome structural changes that occurred during tissue culture. Three wheat/rye and one wheat/wheat chromosome translocations, seven deletions, and five amplifications of heterochromatin bands of rye chromosomes were identified. One amphidiploid contained a reciprocal translocation between wheat chromosome 4D and rye chromosome 1R. Non-reciprocal translocations between 2B and 3R, and between an unidentified wheat chromosome and 2R, were found independently in two amphidiploids. An additional plant had a translocation between wheat chromosomes 6B and 5A. All deletions involving rye chromosomes were noted in all 10 amphidiploids. Twelve of the 13 breakpoints in chromosomes involved in translocations and deletions occurred in heterochromatin. Amplification of heterochromatin bands on 2RL and 7RL chromosome arms also was observed in five plants. These results indicate a high degree of chromosome structural change induced by tissue culture. Therefore, tissue culture may be a useful tool in alien gene introgression and manipulation of heterochromatin in triticale improvement.

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Key words: Wheat × rye hybrids – Translocations – Introgression – Tissue culture – C-banding

Introduction

Common wheat, *Triticum aestivum* (2n = 6x = 42), is a hexaploid consisting of three genomes, A, B, and D. The polyploidy has resulted in a highly buffered genome so that genes from unrelated genomes of alien genera have been successfully incorporated and exploited in commercial wheat production. For example, genes for leaf and stem rust resistance were transferred into wheat from *Aegilops* (Sears 1956; Dvořák 1977), *Agropyron* (Knott 1961; Sharma and Knott 1966; Knott et al. 1977) and *Secale* (Joshi and Singh 1979). Other examples of useful genes transferred from related species were reviewed recently by Sharma and Gill (1983). Three methods were used for these genetic transfers: (i) use of ionizing radiations, (ii) inducing homoeologous chromosome pairing, and (iii) exploiting the tendency of univalents to misdivide and rejoin (Sears 1972). Supplemental methods are desirable that may further facilitate interspecific genetic transfer in exploiting the gene pool of wheat relatives.

Tissue cultures create chromosomal instability, which is manifested as chromosome breaks, deletions, translocations, and changes in chromosome number among regenerated plants (Sunderland 1977; D'Amato 1978; Cummings et al. 1980; Orton 1980a; McCoy et al. 1982). Because of this, plant tissue cultures are considered useful for generating beneficial genetic variability. Recently, the potential usefulness of tissue culture as a method for introgressing alien genes into a cultivated crop was recognized when isozyme patterns

of regenerated *Hordeum vulgare* × *H. jubatum* hybrids indicated exchange of chromosome segments between the two species (Orton 1980 b). Unfortunately, however, the physical exchange was not demonstrated due to lack of chromosome identification techniques for this plant material.

The wheat-rye tissue culture system may be ideal for investigating the potential usefulness of tissue culture as a method of introgressing alien genes into wheat. The necessary protocols for regeneration of plants from wheat and rye embryo scutellar calli are available (Sears and Deckard 1982). Plant regeneration from the winter wheat cultivar 'ND 7532', in particular, has been shown to be predictable and stable. Furthermore, C-banding can allow the identification of chromosome translocations between wheat and rye through distinct heterochromatin patterns in the long arm (L), and short arm (S) of each chromosome in wheat and rye (Bennett and Smith 1975; Gill and Kimber 1977; Lukaszewski and Gustafson 1983).

The objective of this study was to determine chromosomal structural changes, particularly, translocations between wheat and rye chromosomes in hybrids regenerated from tissue cultures. This paper reports the occurrence of translocations, deletions, and heterochromatin amplification in chromosomes of wheat and rye. The possible applications of tissue culture for wheat and triticale improvement are discussed.

Materials and methods

The materials used in this study came from a cross between *Triticum aestivum* cv. 'ND 7532' (Froid × Centurk) and *Secale cereale* cv. 'Chaupon'. Six immature (11–13 days after anthesis) embryos of the hybrid were placed scutellar side up, on a modified Murashige Skoog (M.S.) medium containing 1 mg/L 2,4-D, to induce callus formation from the scutellum (Sears and Deckard 1982). Calli originating from two embryos (nos. 1 and 3) were maintained in culture for 222 days by monthly subculturing in media with 0.5 mg/L 2,4-D. During the maintenance period, each callus was subdivided into approximately 5 mm-diameter pieces and a culture pedigree was recorded (Fig. 1). Plant regeneration from callus and subsequent transplanting was done according to Sears and Deckard (1982). The individual regenerated plants were tiller-cloned and grown as amphihaploids (R_0), backcrossed to ND 7532, or treated with colchicine to induce amphidiploidy. The colchicine treatment was done by immersion of roots in 0.05% colchicine and 1.5% DMSO for 5 h as described by Winkle and Kimber (1976). With this method, a total of 183 amphidiploids were obtained. All plants were grown to maturity in the greenhouse.

Ten amphidiploids were analyzed for chromosomal changes. Five amphidiploids were chosen at random from each of the two embryo explants that formed calli. The explant origin and relationships of the ten amphidiploids are shown in Fig. 1.

Seeds of the amphidiploids and of 'ND 7532' and 'Chaupon' were germinated in petri dishes with moistened filter paper. Root tips 1 to 2 cm long were collected and pretreated with ice-cold water kept at 1 °C for 24 h, then fixed in 3:1 alcohol:acetic acid and kept overnight before use in squash preparations.

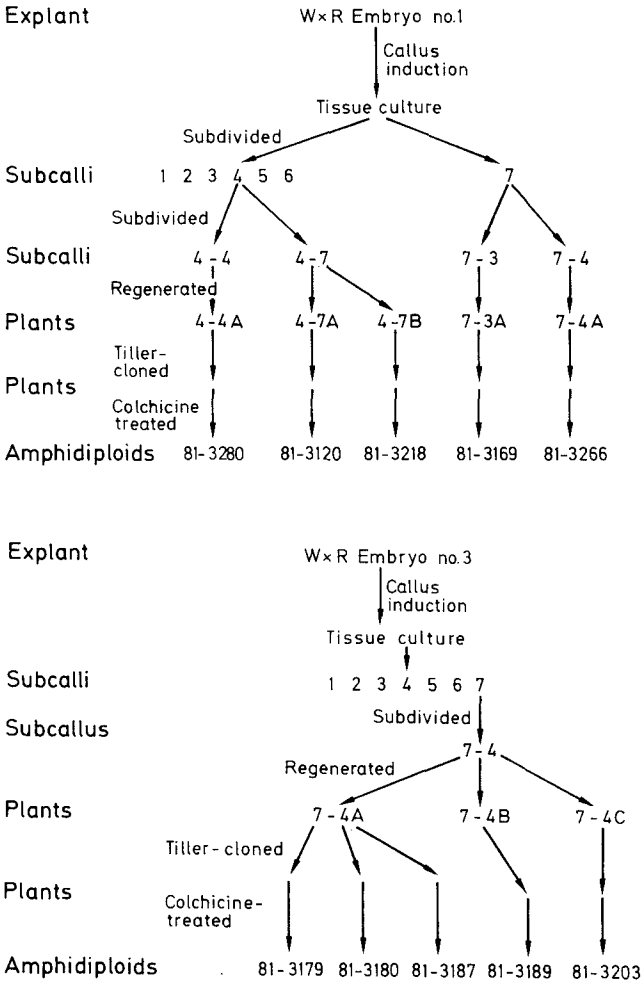

Fig. 1. Tissue culture origin and lineage of the ten amphidiploids analyzed for chromosomal changes (*W* wheat; *R* rye)

The C-banding technique employed in this study followed the procedure of Lukaszewski and Gustafson (1983), except that the buffer used here was made by mixing equal parts of 0.01 M Na_2HPO_4 and 0.01 M KH_2PO_4 (adjusted to pH 6.8), as a substitute for the prepared Canadian phosphate buffer they used (Lukaszewski and Gustafson personal communication).

The C-banded karyotypes of 'ND 7532' and 'Chaupon' were constructed using similarities between their C-banding patterns and those of wheat cv. 'Chinese Spring' and rye cv. 'Dankowskie Zlote' (Lukaszewski and Gustafson 1983), respectively. Ideograms showing the arm ratios and position of the C-bands were drawn, based on measurements taken from 10 intact cells from photographic prints of same magnification. In addition, 50 'Chaupon' plants were examined for variation in their C-banding patterns. The karyotypes of wheat × rye amphidiploids were analyzed and compared with the standard karyotypes of wheat and rye. The C-bands of rye chromosomes in the amphidiploids were measured (average 10 cells) and compared with the standard 'Chaupon' rye karyotype and expressed as percent change in Table 2.

Photographs were taken on high contrast Kodak film (Tech Pan 2415) with a green filter, using a Zeiss III photomicroscope with planopochromatic 100X lens.

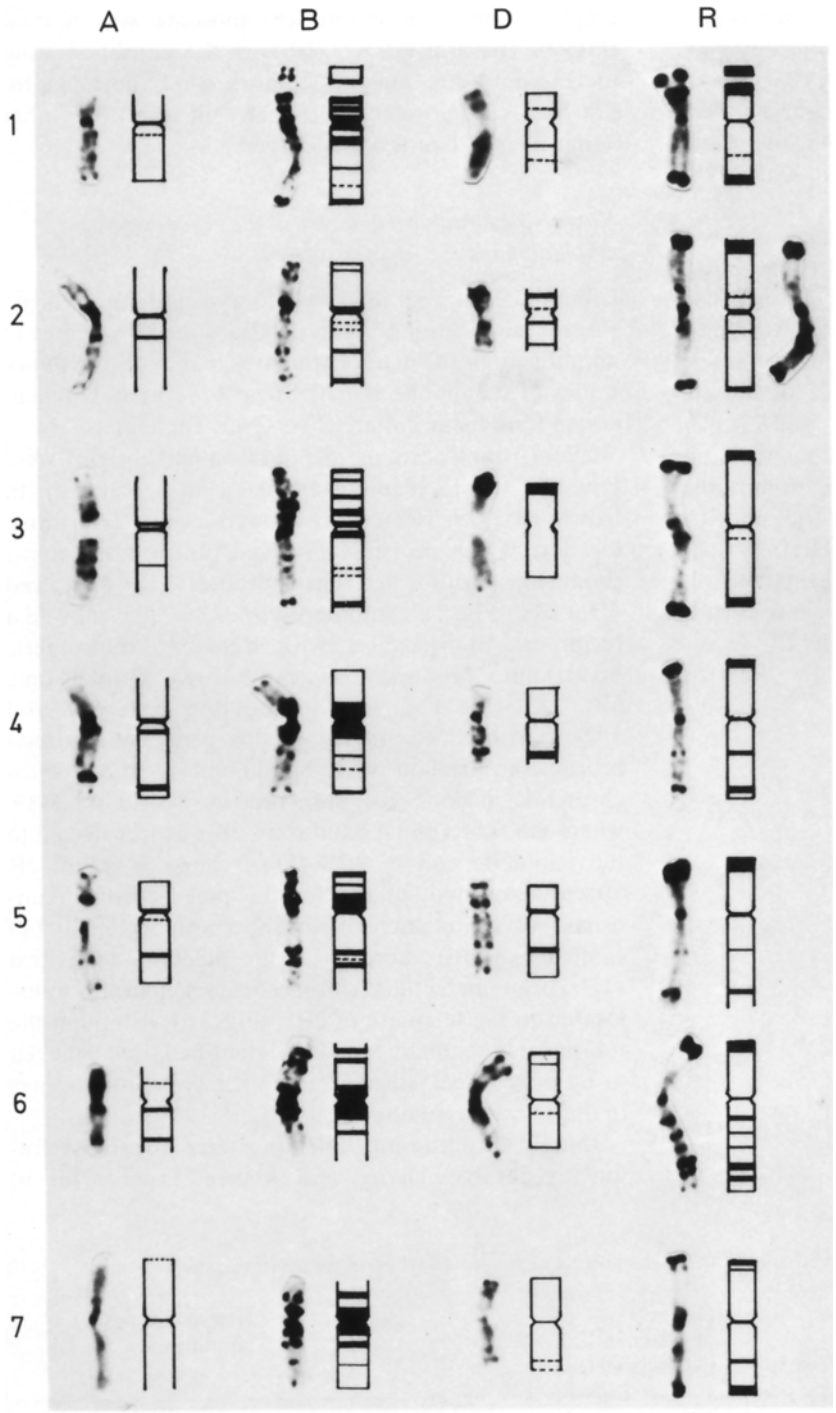

Fig. 2. C-banded karyotypes of *T. aestivum* cv. 'ND 7532' in columns *1, 2,* and *3* and *S. cereale* cv. 'Chaupon' in column *4.* C-banding heteromorphism for 2R is shown. *Solid lines* on ideograms represent frequently observed bands and *dotted lines,* rarely observed bands

Results

Karyotypes of wheat and rye

The 21 chromosomes of 'ND 7532' were identified by their C-banding patterns (Fig. 2) and were similar to 'Chinese Spring' chromosomes (Lukaszewski and Gustafson 1983), except 7A, 7D, 2B and 3B. Chromosomes 7A and 7D possessed only faint bands in 'ND 7532', in comparison to distinct telomeric and interstitial bands in 'Chinese Spring'. The long arms of both chromosomes 2B and 3B showed distinct subterminal bands in 'ND 7532' that are not present in 'Chinese Spring'. All chromosomes of 'ND 7532' were submeta-

centric (Table 1). The group 1 and five chromosomes were the most heterobrachial, and chromosomes 2D, 7A and 7D were nearly metacentric.

The wheat chromosomes formerly assigned as "4A" and "4B" were designated here as 4B and 4A, respectively, according to studies by Chen and Gill (1983) and Dvořák (1983).

The C-banding patterns of chromosomes of 'Chaupon' were similar to 'Dankowskie Zlote' (Lukaszewski and Gustafson 1983) and all seven rye chromosomes were identified (Fig. 2). Chromosomes of 'Chaupon' were characterized by the presence of highly diagnostic, large telomeric bands on the short arms of all chromosomes, and on the long arms of 1R, 3R, and 7R. The long arms of 2R, 4R, and 6R had small telomeric bands whereas 5RL did not have this band. Chromosomes 2R, 3R and 7R were nearly metacentric and the remaining ones were submetacentric (Table 1).

Among the 50 'Chaupon' plants examined for heterochromatin variation, three plants showed a C-banding heteromorphism for chromosome 2R. In one chromosome 2R, the interstitial band on 2RL was amplified and the second chromosome was normal (Fig. 2). The arm ratio of 2R with the amplified band increased to 1.6, making it the second most heterobrachial chromosome (after 5R) in rye. No other variation in C-bands was observed.

Table 1. Arm ratio (long/short) of the A, B, and D, genome chromosomes of 'ND 7532' and R genome chromosomes of 'Chaupon'

Chromosome no.	Genome			
	A	B	D	R
1	1.8	1.9	1.7	1.4
2	1.3	1.3	1.1	1.2
3	1.3	1.4	1.3	1.1
4	1.4	1.4	1.5	1.4
5	1.7	1.7	2.0	1.7
6	1.5	1.2	1.2	1.6
7	1.1	1.6	1.1	1.1

Karyotypic structural changes in the regenerated amphidiploids

C-banding showed three wheat/rye and one wheat/wheat translocations, seven deletions, and five cases of amplification of heterochromatin bands in chromosomes of rye among the 10 wheat × rye hybrids regenerated from tissue culture (Figs. 3–5, Table 2).

Four translocations of independent origin were found in the 10 plants examined (Fig. 3, Table 2). In hybrid 81-3179, 1R and 4D showed a reciprocal translocation, which probably involved centric breakpoints producing translocated chromosomes 1RS/4DL and 4DS/1RL (Fig. 3 a). Another hybrid, 81-3203, showed a reciprocal translocation with apparent centromeric breakpoint, producing a translocated chromosome 6BL/5AL (Fig. 3 b). The chromosome arms 6BS and 5AS were both eliminated in this plant. A non-reciprocal translocation with breakpoint in the heterochromatic region was observed in hybrid 81-3189, where the subterminal band from 2B was transferred to the telomeric end of 3RS (Fig. 3 c). As a result, 3R appeared normal, except for the presence of an unusual-looking telomere in its short arm while 2B became metacentric because of the deletion. In hybrid 81-3180, an unidentified chromosome segment was translocated to the telomere of 2RL (Fig. 3 d). Although the translocated segment cannot be identified, it is believed to be from wheat, since all the other rye chromosomes in this hybrid were normal.

Small chromosome deletions were the most frequently observed change and occurred in all of the 10

Table 2. Chromosome structural changes observed among the tissue culture-regenerated wheat × rye amphidiploids

Embryo source	Hybrid no.	Translocation	Deletion (amount, in % of C-band deleted)	Heterochromatin amplification
No. 1	81-3280	–	4RL (100%)	proximal band on 2RL
No. 1	81-3120	–	5RS (100%)	proximal band on 2RL
No. 1	81-3218	–	4RS telomere (70%)	telomeric band on 7RL
No. 1	81-3169	–	4RS telomere (70%)	telomeric band on 7RL
No. 1	81-3266	–	4RS telomere (70%)	telomeric band on 7RL
No. 3	81-3179	1RS/4DL; 4DS/1RL		telomeric band on 7RL
No. 3	81-3180	2R/unidentified wheat	4RS telomere (70%)	–
No. 3	81-3187	–	4RS telomere (70%)	–
No. 3	81-3189	3R/2BL segment	1 RL (100%)	–
No. 3	81-3203	6BL/5AL	6RS telomere (80%)	–

[a] Brackets indicate that plants containing the change originated from the same subcallus, and thus involved only one change

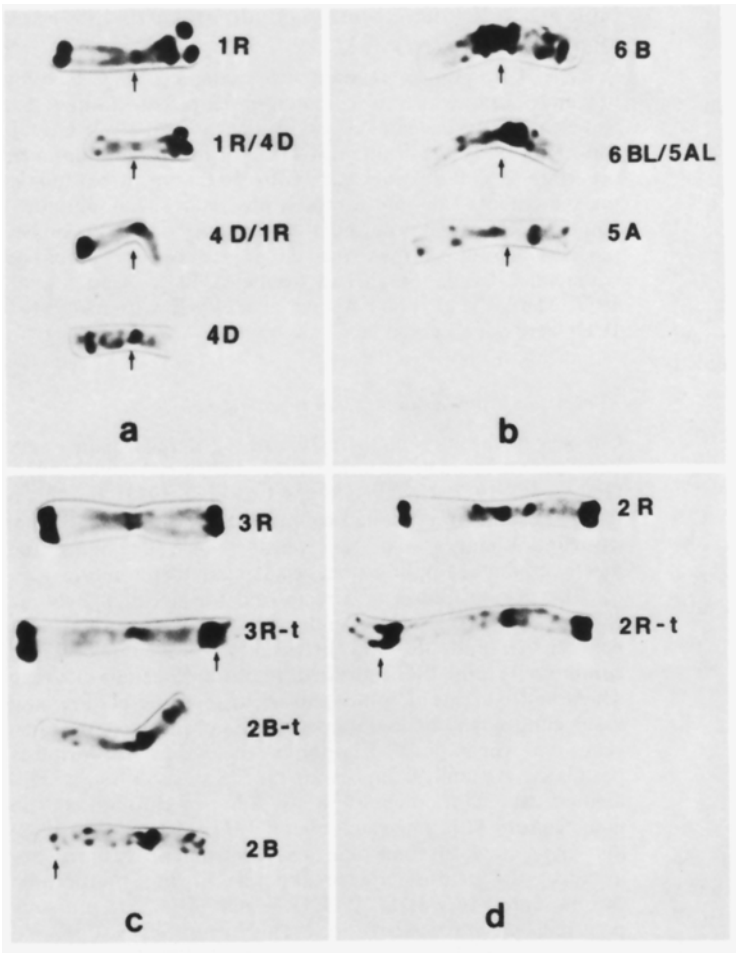

Fig. 3a–d. Chromosome translocations in regenerated wheat×rye amphidiploids: **a** normal 1R, 1R/4D and 4D/1R translocations, normal 4D; **b** normal 6B, 6BL/5AL translocation, normal 5A; **c** normal 3R, 3R with translocated 2BL segment on short arm telomere, 2B with deletion in long arm, normal 2B; **d** normal 2R, 2R with unidentified segment in long arm (*arrows* indicate breakpoints in chromosomes)

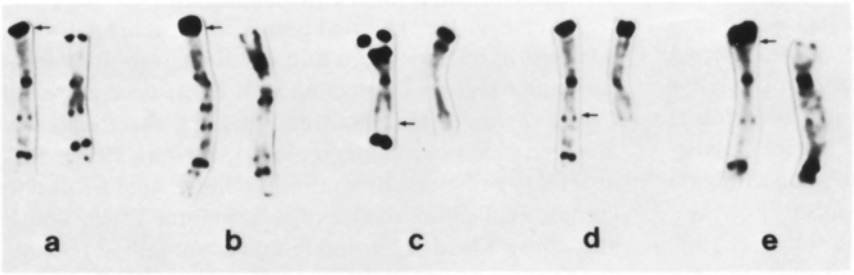

Fig. 4. Chromosome deletions in regenerated wheat×rye amphidiploids: **a** 4RS; **b** 6RS; **c** 1RL; **d** 4RL; **e** 5RS. Each pair shows a normal chromosome on the *left,* chromosome with deletion on the *right* (*arrows* indicate breakpoints in normal chromosomes)

amphidiploids analyzed (Fig. 4, Table 2). Seven deletions of independent origin were observed: three in 4RS, one each in 5RS, 6RS, 4RL and 1RL. A deletion of approximately two-thirds of the telomeric heterochromatin band of 4RS was observed in eight hybrids derived from three calli (Fig. 4 a, Table 2). Deletions observed in 5RS, 6RS and 4RL also involved breakpoints in heterochromatin bands, the first two being terminal, and the third, interstitial (Figs. 4 e, b, d). The breakpoint in 1RL was interstitial and the only one that occurred in a euchromatic region (Fig. 4 c).

Amplified C-bands involving chromosomes 2R and 7R were observed in five hybrids (Fig. 5, Table 2). The proximal band in 2RL was amplified in two hybrids derived from the same embryo but different subcalli (Fig. 5 a). This band was very similar to the naturally occurring amplified band in 2R observed in three 'Chaupon' plants. Three hybrids regenerated from

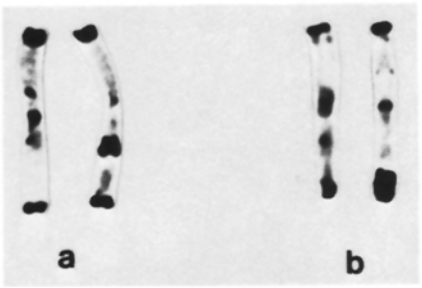

Fig. 5. Amplified C-bands in regenerated wheat × rye amphidiploids: **a** normal 2R on the *left*, 2R with amplified interstitial band on the *right;* **b** normal 7R on the *left*, 7R with amplified telomeric band on the *right*

different calli showed an amplified telomeric band on 7RL (Fig. 5 b).

Elimination of some chromosomes also was observed, though at a very low frequency. Hybrid 81-3187 was nullisomic for both chromosomes 6B and 7B, and 81-3203 for 1R, 6BS, and 5AS.

Discussion

Karyotypes of wheat and rye

The general similarity in C-banding patterns of 'ND 7532' and 'Chaupon' chromosomes with 'Chinese Spring' and 'Dankowskie Zlote' chromosomes, respectively (Lukaszewski and Gustafson 1983), allowed identification of all 21 chromosomes of 'ND 7532' and seven chromosomes of 'Chaupon'. The differences between 'ND 7532' and 'Chinese Spring' in C-banding of chromosomes 7A, 7D, 2B and 3B probably are due to intervarietal variation. Faint telomeric bands in chromosome 7A, as observed in 'ND 7532', were reported for the wheat cultivars 'Cappelle-Desprez' and 'Hairy-necked Viking' (Seal 1982). The distinct subterminal bands in 2BL and 3BL, found only in 'ND 7532', also are present in the wheat cultivar 'Hope', and cultivars 'Cheyenne', 'Timstein', and 'Wichita' also show the band in 2B (Endo and Gill 1984). Alternatively, differences in phosphate buffer or length of staining time used may have caused some variations in C-banding of 'ND 7532' and 'Chinese Spring' chromosomes.

The arm ratios of 'ND 7532' were similar to 'Chinese Spring' chromosomes (Endo and Gill 1984), except for 4A, 4B, 1B and 5B, which were 1.4, 1.4, 1.9, and 1.7 in 'ND 7532', and 1.7, 1.1, 1.7, and 2.0, respectively, in 'Chinese Spring'. These discrepancies may be explained by structural changes in the chromosomes, such as pericentric inversion believed to have occurred in 4B (4A in Endo and Gill 1984) of 'Chinese Spring', or probably by measurements being taken prior to banding in 'Chinese Spring' (Endo and Gill 1984) and after banding in 'ND 7532'.

The 'Chaupon' population was examined for C-banding polymorphism, which is common in *S. cereale* (Singh and Robelen 1975; Miazga et al. 1981; Ataeva et al. 1982; Gustafson et al. 1983), to distinguish changes in rye chromosomes occurring in culture from variations that were preexistent in the rye parent. The only variation observed was an amplified band in 2RL, which occurred at a frequency of 6%. Previously reported variations involving either reduced or amplified telomeric C-bands (Singh and Robbelen 1975; Bennett et al. 1977; Miazga et al. 1981; Ataeva et al. 1982; Gustafson et al. 1983) were not observed in 'Chaupon'.

Karyotypic structural changes in regenerated hybrids

Changes in chromosomal structure of regenerated plants have been shown through meiotic pairing behavior (Bayliss 1975; Green et al. 1977; Novak 1980; McCoy et al. 1982), but this is the first report of a detailed characterization of chromosomal structural changes, such as translocations, deletions, and heterochromatin amplification, occurring in tissue culture.

The demonstration of a reciprocal translocation between chromosomes 4D and 1R, and non-reciprocal translocations between 2B and 3R, and between an unidentified wheat chromosome and 2R, indicated that translocation between wheat and rye chromosomes can occur in tissue culture, and tissue culture may be useful as a method of introgressing alien genes into wheat, or other important food crops, as previously postulated (Orton 1980a; Larkin and Scowcroft 1981). This method exploits the increasing instability of chromosomes in tissue culture with time (Sunderland 1977; McCoy et al. 1982) that may result in breakage and centromeric fusions. For instance, the centromeric breakpoints in the translocated chromosomes 1RS/4DL, 4DS/1RL and 6BL/5AL probably occurred by centromeric fusions. This hypothesis is supported by our earlier observations (unpublished data) that the frequency of telocentric chromosomes is rather high in plants regenerated from cultures that were at least six months old. McCoy et al. (1982) also observed a high frequency of chromosome breakage in oats regenerated from calli that had been maintained in culture for at least four months.

Twelve of the 13 breakpoints in the chromosomes involved in translocations and deletions were in heterochromatic regions (Figs. 3 and 4). In tissue cultures of *Crepis capillaris*, chromosome breakage also frequently involved heterochromatic regions (Sacristan 1971). This instability of heterochromatin in culture may be due to the late replicating nature of its repetitive DNA, which may cause a bridge formation and eventually, breakage at anaphase (Bennett 1977; McCoy at al. 1982). Breakage at heterochromatic regions appears to be at random, occurring in frequencies of 5/12 in telomeric, 4/12 in centromeric, and 3/12 in interstitial C-bands. The actual frequencies of breakpoints in tissue culture may be different, however, since cells with rearranged chromosomes may be selected against during regeneration (Orton 1980a).

It has been shown previously that removal of large blocks of telomeric heterochromatin in rye chromosomes results in improved endosperm development, kernel characteristics, and yield of the hybrid (Bennett

and Gustafson 1982; Gustafson and Bennett 1982). The high susceptibility to breakage of telomeric heterochromatin in rye chromosomes in tissue culture also may be exploited for regenerating rye plants with chromosomes having reduced telomeric heterochromatin, which may be used as parents in the production of improved triticales.

The amplified interstitial band in chromosome 2R of hybrids 81-3280 and 81-3120 is believed to have originated in tissue culture (even though a similar band was found to exist as a form of heteromorphism in the 'Chaupon' population) since the other three hybrids 81-3218, 81-3169, 81-3266, coming from the same embryo (No. 1) did not possess the band. The mechanism for the amplification of the C-band in 2R and in 7R telomere is not known. It is possible, however, that the amplified telomeric heterochromatin in 7R came from deleted C-bands in 4R through a non-reciprocal translocation.

The translocations, deletions, and heterochromatin amplification found in the 10 regenerated amphidiploids could have arisen only in tissue culture, since the plants were homozygous for all the changes as a result of colchicine treatment after regeneration. By tracing the culture pedigree of each amphidiploid and comparing with others regenerated from the same embryo it can be seen that all chromosome structural changes occurred after the initial callus induction step. For example, hybrids 81-3179, 81-3180, 81-3189 and 81-3203 can be traced back to a common embryo and initial callus and subcallus origin (Fig. 1) but contained

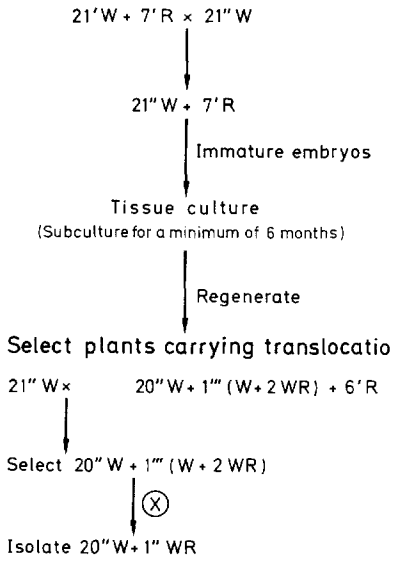

Fig. 6. Proposed scheme for use of tissue culture in generating and recovering chromosome translocations between wheat and rye (*W* wheat; *R* rye; *WR* chromosome with translocation between wheat and rye)

different chromosomal translocations, indicating the possible occurrence of changes during subculturing. Furthermore, the different karyotypes in hybrids 81-3179, 81-3180 and 81-3187 indicate that the original R_0 plant 7–4A probably was composed of 3 R_0 plants. Simultaneous germination of embryoids in close proximity could easily fuse and appear like one individual somaclone instead of several. Segregation from one somaclone should therefore be interpreted carefully since different R_1 plants may actually be from fused embryoids and thus different R_0 plants. Head rowing progeny of R_0 plants would be most accurate in assessing somaclonal variation from tissue culture.

The results presented indicate that chromosomes are unstable in tissue culture and can give rise to different chromosomal structural changes. The phenotypic effects of such changes have not been determined. However, a wide variability among regenerated plants can be expected. Based on observed translocations between wheat and rye chromosomes in vitro, it is concluded that tissue culture may be used as a method of introgressing alien genes into wheat, or into any other crop species that can be grown in culture and can be regenerated.

It should be noted that, although tissue culture can facilitate translocations between wheat and rye chromosomes, many intact rye chromosomes remain that need to be eliminated. Therefore, it may be necessary to resort to tissue culture of BC_1 embryos (21″W + 7′R) and then plants regenerated from such embryos should be used as male parents in crosses with the recurrent parent (Fig. 6). The wheat/rye translocated chromosomes will be transmitted preferentially in competition with intact rye chromosomes. This also will provide a quick method for the recovery of translocated chromosomes.

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