TRANSLOCATIONS AND OTHER KARYOTYPIC STRUCTURAL CHANGES IN WHEAT X RYE HYBRID PLANTS REGENERATED FROM TISSUE CULTURE

by

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REVIEW OF LITERATURE

Common wheat, Triticum aestivum L.(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;Dvorak,1977), Agropyron (Knott, 1961;Sharma and Knott,1966;Knott et al.,1977), and Secale (Joshi and Singh, 1979). The transfer of short arm of rye chromosome 1R in wheat gave resistance to mildew and yellow rust (Mettin et al.,1973;Zeller, 1973), as well as to wheat curl mite (Martin et al.,1976). A translocation between wheat chromosome 2A and rye chromosome 2R results in increased kernel protein content (May and Appels, 1980). Other examples of gene transfers from related species to wheat were recently reviewed by Sharma and Gill (1983).

Presently, there are three ways by which transfer of genetic materials can be induced: (1) use of ionizing radiations, (2) induction of homoeologous chromosome pairing, and (3) exploitation of the tendency of univalents to misdivide (Sears,1972). Recently, Lukaszewski and Gustafson (1983) exploited the tendencies of univalents to misdivide and rejoin to obtain 195 different wheat / rye translocations from F_4 progenies of triticale (AABBRR) x wheat (AABBDD) crosses. Supplemental methods are desirable that may further facilitate interspecific genetic transfer in exploiting the gene pool of wheat's relatives.

Tissue culture is considered useful in the area of crop improvement because of the wide variability generated among somaclones, or regenerated plants (Bajaj, 1974; Bhojwani et al., 1977; Green,1977; Kleinhofs and Behki, 1977; Skirvin, 1978; Larkin and Scowcroft, 1981). In regenerated oats for

example, Cummings et al.(1980) observed altered phenotypes in plant height, heading date, awn morphology, and fertility.

The spontaneous occurrence of changes in chromosome number and structure are common in plant tissue cultures (Sunderland, 1977; D'Amato, 1978). Many of the observed phenotypic variations may be due to changes in chromosome structure (Orton, 1980a; Edallo et al., 1981; Larkin and Scowcroft,1981; McCoy et al., 1982). Sears et al. (1982) reported association of significant differences in plant height, vigor, and yield among wheat somaclones with chromosome instability. Chromosome fragmentation, deletions, and translocations were observed by analysis of meiotic behavior in regenerated plants of ryegrass (Ahloowahlia, 1978), oats (McCoy et al., 1982), maize (Green et al.,1977), carrots (Bayliss,1975), and garlic (Novak, 1980). In Crepis and Haplopappus, these changes were easily demonstrated in mitotic chromosomes because of their distinct karyotypes and low chromosome numbers (Sacristan, 1971; Sunderland, 1977).

Why chromosomes become unstable in tissue culture is presently not understood. However, it is known that this instability increases with time in culture (Sunderland, 1977; McCoy et al., 1982).

Since chromosome breaks and translocations can be induced by prolonged culture of cells in vitro, tissue culture may be potentially useful for transferring alien genes into the genome of a commercial crop species (Orton, 1980a and b; Larkin and Scowcroft,1981; McCoy et al., 1982). Such chromosome transfer was postulated to occur in tissue culture-regenerated Hordeum hybrids (Orton, 1980b). Esterase (EST) and glutamateoxaloacetate transaminase (GOT) isozyme patterns indicated the transfer of chromosome segments from H. jubatum to H. vulgare. Unfortunately however, the physical exchange was not shown due to lack of chromosome identification techniques for this plant material.

Chromosome banding techniques are very useful for identifying individual chromosomes of a species, and translocation between different species. Two techniques namely, C-banding and N-banding, are used to identify individual chromosomes in rye, wheat, and other species of the Triticinae (Gill and Kimber, 1974a and b; Gerlach, 1977; Seal, 1982; Lukaszewski and Gustafson, 1983). The construction of C-banded karyotypes for these species has allowed the recognition of translocations between wheat and its relatives. Bennett and Smith (1975) showed the presence of a wheat/rye translocation between 1B and 1R in the wheat cultivars 'Aurora' and 'Kavkaz'. Gill and Kimber (1977) showed translocations involving rye chromosome 1R and Triticum umbellulatum chromosome 6 with wheat chromosomes 1D and 6B, respectively. Lukaszewski and Gustafson (1983) also used C-banding to show the occurrence of wheat/rye translocations in offsprings of Triticale x wheat crosses.

The possible occurrence of chromosome translocations between two species in tissue culture can therefore be demonstrated with the use of plant materials whose chromosomes can be identified with C- or N-banding. These plants must also be amenable to growth in tissue culture and regeneration. Wheat and rye are examples of such plants. Several wheat cultivars have been shown to be regenerable from tissue cultures initiated from embryos (Shimada and Yamada, 1979; Sears and Deckard, 1982), young inflorescences (Ozias-Akins and Vasil,1982), anthers (Henry and Buyser, 1981; Liang et al.,1982), and leaf base (Ahuja et al.,1982). Hexaploid triticales can also be regenerated from immature embryos (Nakamura and Keller,1982). Sears and Deckard (1982) showed that regeneration from wheat embryo cultures is predictable and stable when the appropriate genotype is used. The cultivar 'ND 7532' was identified to have the highest frequency of callus induction and regenerable callus formation among thirty-nine genotypes of wheat.

The demonstration of chromosome translocations between wheat and rye in hybrid plants regenerated from tissue culture will be an indication that tissue culture may be used as a method of introgressing alien genes into wheat. This method of introgression may also be applied to any commercial crop that can be grown in tissue culture and regenerated.

Demonstration of such wheat/rye translocations occurring in vitro is thus necessary before tissue culture can be applied in the improvement of wheat and other food crops.

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INTRODUCTION

Common wheat, *Triticum aestivum* L. (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; Dvorak,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 recently reviewed 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's relatives.

Tissue cultures create chromosomal instability that 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* x *H. jubatum* hybrids indicated exchange of chromosome segments between the two species (Orton,1980b). 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, is 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 x 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 M.S. media containing 1 mg/l 2,4-D to induce callus formation from the scutellum (Sears and Deckard, 1982). Calli originating from two embryos (numbers 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 hours 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 which 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 on a petri dish with moistened filter paper. Root tips 1 to 2 cm long were collected and pretreated with ice-cold water kept at 1oC for 24 hours, then fixed in 3:1 alcohol: acetic acid and kept overnight before use in squash preparations.

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 0.01M Na_{2HPO4} and 0.01M KH_2PO_4 (adjusted to pH 6.8), as a substitute for the prepared Canadian phosphate buffer they used (Lukaszewski and Gustafson, pers. commun.).

The C-banded karyotypes of ND 7532 and Chaupon were constructed based on similarities of their C-banding patterns with 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. In addition, 50 Chaupon

plants were examined for variation in their C-banding patterns. The karyotypes of wheat x rye amphidiploids were analyzed and compared with the standard karyotypes of wheat and rye.

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

RESULTS

Karyotypes of wheat and rye

The twenty-one 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 submetacentric (Table 1). The group 1 and 5 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 Dvorak (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 were submetacentric (Table 1).

Among the fifty 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 after 5R, the most heterobrachial chromosome in rye. No other variation in C-bands was observed.

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 ten wheat x rye hybrids regenerated from tissue culture(Figs.3,4,5,Table 2).

Four translocations of independent origin were found in the ten 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. 3a). Another hybrid, 81-3203, showed a reciprocal translocation with apparent centromeric breakpoint producing a translocated chromosome 6BL/5AL (Fig. 3b). 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. 3c). 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. 3d). 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 ten 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. 4a, 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. 4b,c,d). The breakpoint in 1RL was interstitial and the only one that occurred in a euchromatic region (Fig. 4e).

Amplified C-bands involving chromosomes 2R and 7R were observed in five hybrids (Fig. 5, Table 2). The proximal band in 2RL was amplified (Fig. 5b) in two hybrids derived from the same embryo but different subcalli (Fig. 1). This band was very similar to the naturally occurring amplified band in 2R observed in three Chaupon plants. Three hybrids regenerated from different calli (Fig. 1) showed an amplified telomeric band on 7RL (Fig. 5b).

Elimination of some chromosomes was also 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 twenty-one 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 are probably due to intervarietal variation. Faint telomeric bands in chromosome 7A as 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 , are also present in the wheat cultivar 'Hope", and cultivars 'Cheyenne', 'Timstein', and 'Wichita' also show the band in 2B (Endo and Gill, 1983). 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, 1983), 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, 1983) of Chinese Spring, or probably because measurements were taken prior to banding in Chinese Spring (Endo and Gill, 1983) and after banding in ND 7532.

The Chaupon population was examined for C-banding polymorphism, which is common in S. cereale (Singh and Robbelen, 1975; Miazga et al., 1981; Ataeva et al., 1982; Gustafson et al.,1983), to distinguish changes in rye chromosomes that occurred 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 have been maintained in culture for at least four months.

Twelve of the thirteen 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 (McCoy et 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 previously shown 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 may also 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 ten 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 different chromosomal translocations, indicating the possible occurrence of changes during subculturing.

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.

However, it should be noted that although tissue culture can facilitate translocations between wheat and rye rye chromosomes, yet 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 preferably transmmitted in competition with intact rye chromosomes. This will also provide a quick method for the recovery of translocated chromosomes.

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

Chromosome Number	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

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

Embryo Source	Hybrid Number	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	-	{	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	-	{	-
No.3	81-3189	3R/2BL segment	1RL(100%) {	-
No.3	81-3203	6BL/5AL	6RS telomere (80%)	-

* Brackets indicate that plants containing the change originated from the same subcallus, and thus involved only one change.

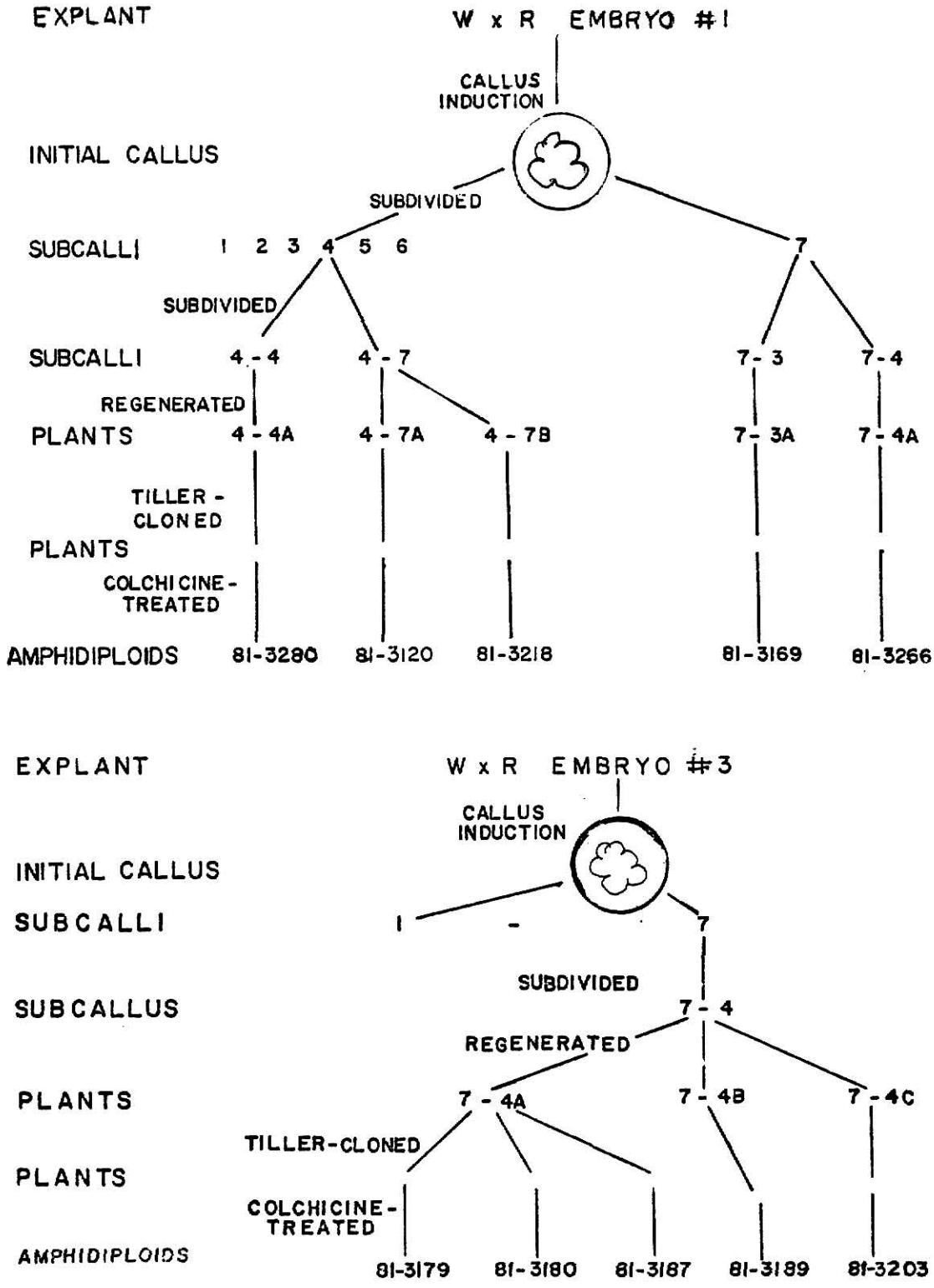

Fig.1. Tissue culture origin and lineage of the ten amphidiploids analyzed for chromosomal changes (W-wheat; R-rye)

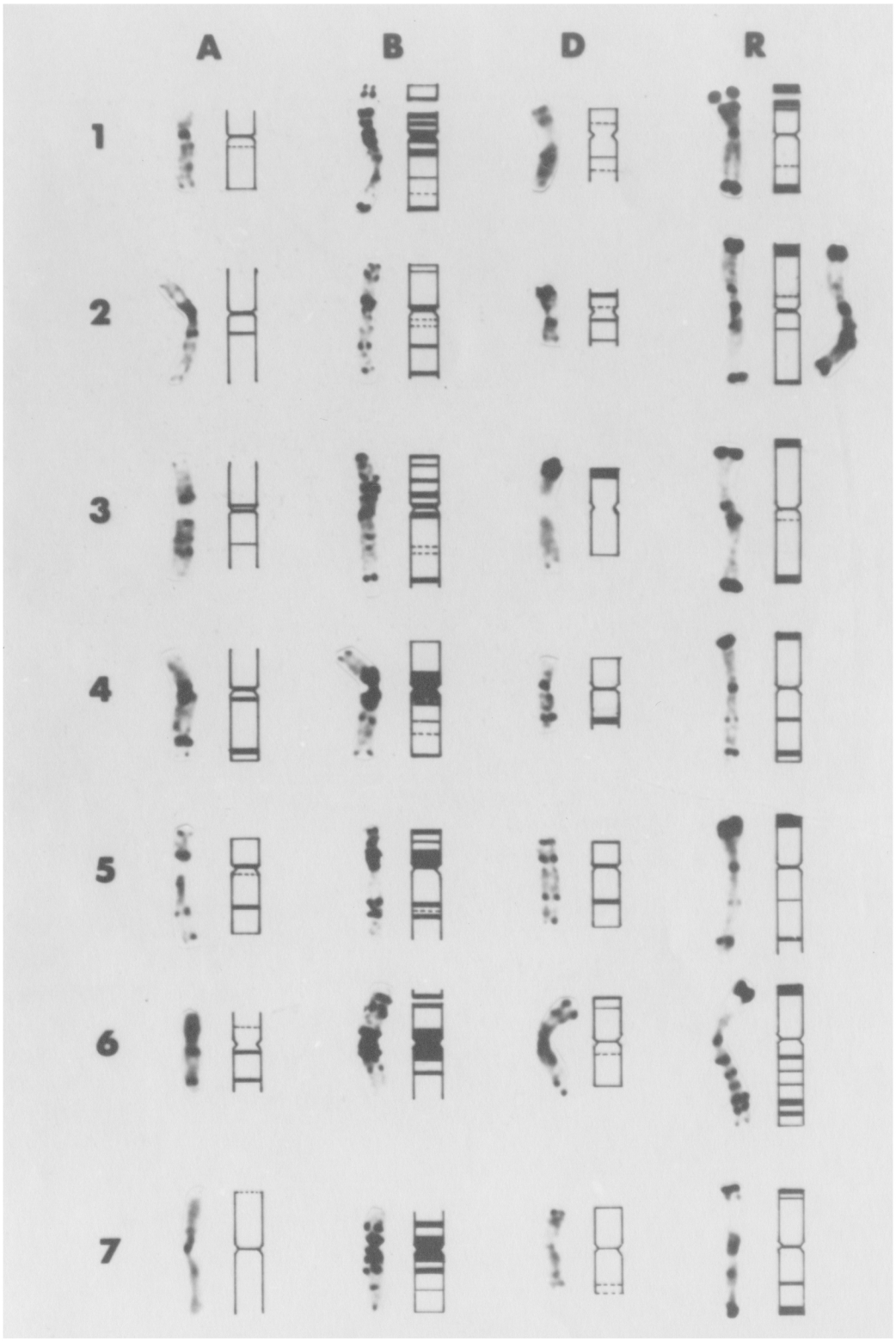

Fig. 2. C-banded karyotypes of T. aestivum cv. ND 7532 in columns 1.2, and 3 and S. Cereale cv. Chaupon in column 4. C-banding herteromorphism for 2R is shown. Ideo grams show arm ratios and positions of C-bands; solid lines represent frequently ovserved bands., dotted lines, rarely observed bands.

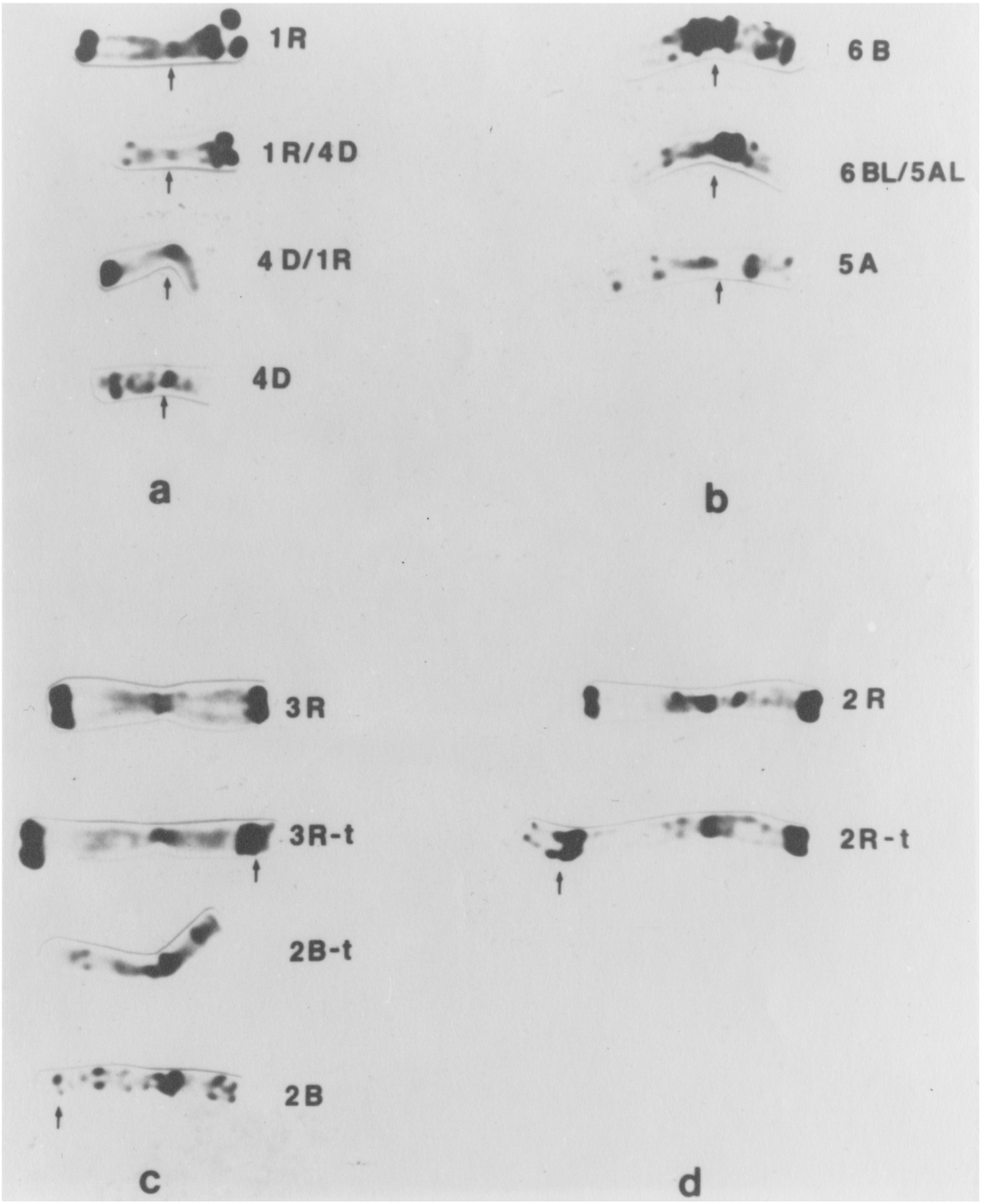

Fig.3. Chromosome translocations in regenerated wheat x rye amphidiploids: a.) Normal 1R, 1R/4D and 4D/1R translocations, normal 4D; b.) Normal 5B, 6BL/4AL translocation, normal 5A; c.) Normal 3R, 3R with translocated 2BL segment on short arm telomere, 2B with deletion on long arm, normal 2B; d.) Normal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

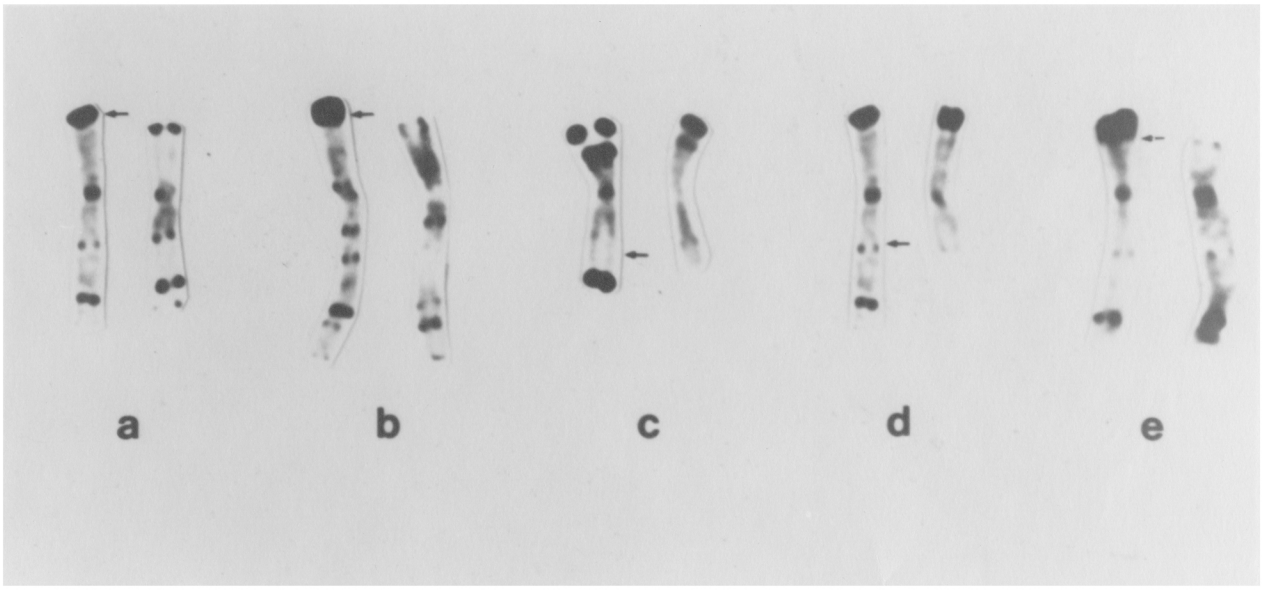

Fig. 4. Chromosome deletions in regenerated wheat x rye amphidiploids: a.) 4RS; b.) 5RS; c.) 6RS; d.) 4RL; e.) 1RL. Each pair shows a normal chromosome on the left, chromosome with deletion on the right. (Arrows indicate breakpoints in normal chromosomes)

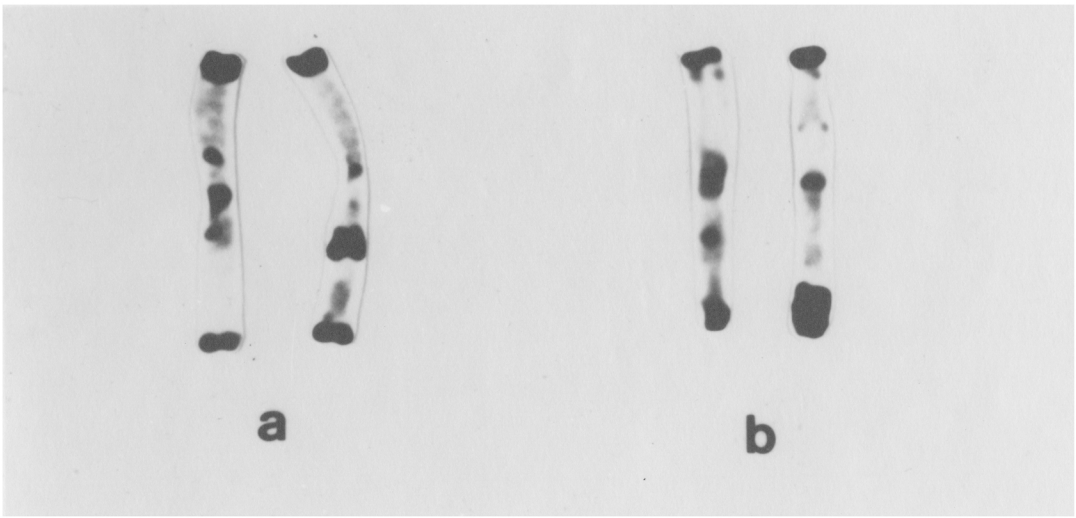

Fig. 5. Amplified C-bands in regenerated wheat x rye amphidiploies: a.) Normal 2R on the left, 2R with amplified interstitial band on the right; b.) Normal 7R on the left, 7R with amplified telomeric band on the right

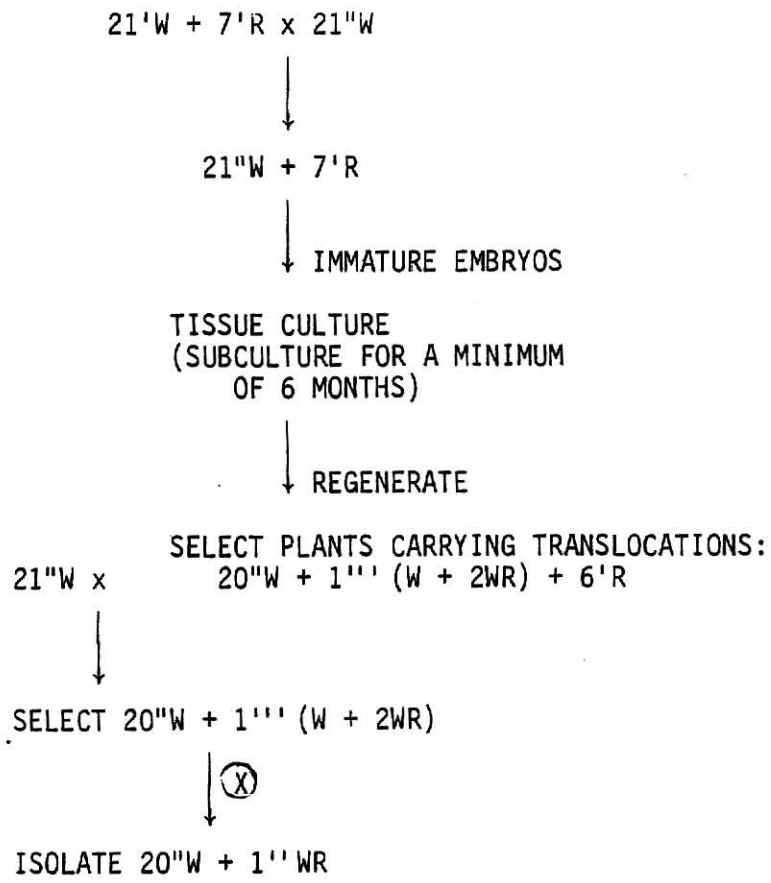

Fig.6. Proposed sheme for use of tissue culture in generating and recovering chromosome translocations between wheat and rye (W-wheat; R-rye; WR-chromosome with translocation between wheat and rye)

TRANSLOCATIONS AND OTHER KARYOTYPIC STRUCTURAL CHANGES IN WHEAT X RYE HYBRID PLANTS REGENERATED FROM TISSUE CULTURE

by

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ABSTRACT

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 x rye hybrids were regenerated from embryo scutellar calli maintained in culture for 222 days. The regenerated seedlings were then 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 ten amphidiploids. Twelve of the thirteen breakpoints in chromosomes involved in translocations and deletions occurred in heterochromatin. Amplification of heterochromatin bands on 2RL and 7RL chromosome arms was also 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.