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TETRAPLOID TRITICALE AS A POTENTIAL SOURCE OF NEW VARIATION FOR RYE

ABSTRACT

High crossability of tetraploid triticale (X *Triticosecale* Wittmack) with rye (*Secale cereale* L.) and relatively high fertility of the resulting hybrids make the triticale an attractive bridge species for introduction of wheat genes into rye breeding populations. It was found, with the use of *in situ* hybridization technique, that some 4x triticale materials bred in Radzików contain small wheat translocations, of both distal and intercalary type, into the rye 5R chromosome. The distal wheat translocation occupying less than 5% of the long arm was transferred into diploid rye, but a disomic line has not been established yet. Other wheat translocations of the chromosomes *1R* and *5R* were found in hybrids of 4x rye with 4x triticale. Besides the intergenomic crossing–over, at least one another mechanism of DNA rearrangements operated. Small intercalary two–dot signals of the wheat fluorescent probe were also found in one line on a rye chromosome different than *5R*. Most puzzling was the „invisible" migration of wheat DNA to rye chromosomes, detectable on southern dot–blots, but not on the *in situ* slides. The wheat probe dot–blot signals were recorded for more than 1/3 of rye plants from the first back–cross of the 4x triticale × 2x rye hybrids to rye.

Thousands of 2x rye plants and hundreds of lines were derived from numerous 4x triticale × 2x rye crosses. The lines with resistance to brown rust and powdery mildew were selected, as well as the lines with reduced content of anti–nutritive non–starch polysaccharides. No link between disease resistance and wheat DNA migration was proved, in spite of attempts.

There exists a remarkable variation in crossability, hybrid sterility, homoeologous crossing–over frequency and in transmission of wheat chromosomes to rye. It makes a basis for selection of 4x triticale and rye materials with better suitability for chromosome engineering in rye. The recurrent intergeneric crossing method was used for pre–breeding of such materials.

Key words: chromosome translocation, introgression, rye, tetraploid triticale

INTRODUCTION

Rye has been used much successfully as a source of new variation in wheat breeding, but gene flow in the opposite direction, from wheat to rye, was almost none. Only one case of successful chromosomal engineering, conducted in hexaploid triticale, was reported by Łukaszewski *et al.* (1999) who transferred the *Glu–D1* gene alleles 5+10, the most potent ones controlling gluten quality in wheat, into the 1R chromosome of rye. The hybrid chromosome was introduced into a 2x rye population

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using repeated back–crosses and a remarkable improvement of rheo–logical properties was recorded. Unfortunately, there was also a nega–tive side effect of the translocation on seed plumpness. Besides grain quality, wheat may contribute to rye breeding with a large pool of dis–ease and pest resistance genes, which is constantly growing as the new variation from the related genera is incorporated. The 4x triticale seems to be a possible facilitation in the introgression process as a bridge spe–cies and a new, simplified system for chromosomal engineering.

The first cases of transfer of wheat chromosomes to diploid rye, de–scribed by Schlegel (1982), did not involve the tetraploid triticale medi–ation. A few years later the 4x triticale × 2x rye crosses were recognized as the most convenient source of rye forms, which may carry wheat ad–ditions or introgressions (Bernard and Bernard 1985, Melz and Thiele 1990, Baum 1991, Łapiński 1999). However no stable introgression was reported in these papers. The German scientists found relatively large translocations in chronmosomes 5B (Schlegel, 1988) and 3B (Melz et al, 1990) using chromosome banding and enzyme markers. Unfotunately, the translocations could not be transferred to the next generation. In the Polish programme an interesting variation appeared and stable lines were drawn, but the attempts to proove influence of wheat genes, with the use of genomic probes, failed. In spite of the rather frustrating sci–entific results of this period the work in Poland is continued and new at–titudes are being tested. Apolinarska (1996) took advantage of better buffering of chromosomal changes on the 4x ploidy level, in comparison to the 2x level.. She reported stable additions, substitutions and translocations of wheat chromosomes in tetraploid rye derived from tetraploid rye x tetraploid triticale crosses. The present paper author's attitude has been concentrated rather on pre–breeding of rye and triticale materials suitable for the chromosomal manipulations and successful wheat DNA introgression to the diploid rye.

MATERIALS AND METHODS

The winter tetraploid triticales used in the programme came from a breeding population derived from a single primary 4x triticale line, which was crossed with numerous 6x triticale lines (Łapiński *et al.* 1996). The population maintained significant karyotype features of the 4x parent. The wheat mixogenome was composed mainly from the A ge–nome, with possible B chromosome substitutions in groups 2 and 3, and fixed homozygosity of *1A, 4A, 6A, 7B* and a *5AS.AL/RL* translocated chromosome. The presence of this translocation was related to expres–sion of winter growth habit (Łapiński, Schwarzacher 1998).

The diploid rye source lines were self–fertile and showed good toler–ance of inbreeding. They represented materials used in hybrid rye breeding in IHAR–Radzików. The short–strawed varieties Napocal and Teku, of Romanian origin, were used as the tetraploid rye components.

The triticale × rye crosses (and back–crosses) were performed in both directions, which made possible production of both auto– and alloplasmic rye recombinants. A part of F_1 seed (dried) was irradiated with 70 Gy of gamma rays.

A Southern blot technique variant, known as dot–blot, was used for detection of wheat DNA in the recombinant rye DNA extracts. The probe was prepared from the genomic DNA of *Triticum durum* Desf. fragmented to ca 10 kbp and random priming labelled with digoxigenin. Hybridization, immunological detection and color development were performed according to the Boehringer Mannheim Kit (Cat. No 1093657) instructions. Rye total genomic DNA sheared to fragments 200–400 bp long was added as a block to the hybridization mixture in concentrations 25–50 times higher than the probe DNA.

The cytogenetic preparations were made according to Schwarzacher (1994) or Łukaszewski (1995). The genomic in situ hybridization (GISH) method based on the procedure of Leitch *et al.* (1994). The durum wheat probe was labelled with digoxigenin or immediately with FITC. Counterstaining was with DAPI or propidium iodide.

RESULTS AND DISCUSSION

Crossability and sterility barriers

The tetraploid triticale was known earlier as a species easily crossable with rye. Krolow (1974) reported 11.2% of flowers set germinable seed after diploid rye pollination. The series of 60 crosses between tetraploid triticale and diploid rye, made in IHAR in 1993, confirmed relatively high seed set and good germination. The percentage of flowers setting germinable seed reached the average values of 5.0% in field and 24.5% in greenhouse. The field results for single spikes varied between 0 and 35.0%. The highly significant influence on crossability was stated for both the mother form (triticale) and for the father one (rye) genotypes. Fertility of the F_1 hybrids was also relatively high, average 5.4 grains per spike at open pollination with rye and 4x triticale. It was also much variable among the F_1 clones, e.g. one clone with the best female fertility was completely male sterile, while a majority of F_1–s formed a proportion of viable gametes of both female and male type. About 50% of clones showed dehiscence of anthers, so the self–pollination was possible. Still this character was not a good indicator of pollen functionality. The clones derived from the 4x triticale line C76 formed the normally bursting pollen sacks, but the most successful pollen for fertilization of emasculated rye spikes was obtained from artificially opened pollen sacks of another clones family. The certation type back–cross was important for vigour and fertility of the next generation rye type plants. They were much more vigorous on the rye cytoplasm. Generally, the alloplasmic forms (with wheat cytoplasm transferred from the 4x triticale) showed much reduced germination, slow growth and fertility reduced by ca.

70%. However, among thousands of alloplasmic plants a number of individuals could be selected with almost normal vigour. In the more recent crosses the use of rye–cytoplasmic forms of tetraploid triticale set basis for elimination of this problem.

The relatively high crossability and hybrid fertility of the 4x triticale × 2x rye hybrids is a remarkable advantage of the 4x triticale considered as a possible system for chromosomal manipulations causing wheat introgression to rye. The work on rye chromosomes in the competitive 6x wheat–triticale system requires passing through the much sterile back–cross generations at the final phase of transfer to the diploid level. The 4x triticale × rye F1 hybrids are much vigorous and particularly with help of cloning are able to produce hundreds of spikes and thousands of viable seeds, giving much opportunity to select wheat–introgressed rye plants.

However, the easiness of overcoming the crossability and hybrid sterility barriers is not the only requirement for successful introgression.

Mechanisms of DNA rearrangement

The ability of chromosomes to form homoeologous bivalents is a necessary requirement for intergenomic crossing–over. In the 4x triticale × 2x rye F_1 hybrids the conditions for such conjugation seemed rather difficult, because each rye chromosome had its homologous rye partner and a wheat relative chromosome could be discriminated in the competition. In spite of this Baum (1991) reported the numbers of wheat–rye bivalents per pollen mother cell ranging between 0.03 and 1.00, among five F_1 plants investigated. It seems sufficient to create a chance for intergenomic crossing–over, at least in certain plants.

The first field observations and breeding selection results seemed to be a realization of this chance. Some B_1F_1 families (derived from single F_1 clones) showed particularly high morphological variation and they were a source of rye plants and lines with triticale glume characters, improved resistance to brown rust or mildew and lines with reduced content of antinutritive non–starch polysaccharides in grain. However, the C–banding cytogenetic investigation of the rye plants karyotypes produced no confirmation for introgression of a wheat chromosome fragment. On the other hand, the molecular screening on Southern dot–blots with the wheat genomic probe revealed an abundat flow of wheat DNA to the recombinant rye (Łapiński *et al.*, 1999). The frequencies of rye plants showing the wheat genomic probe hybridization signal are shown in Table 1 for the B_1F_1 plants and for the next generation derived from the selected plants with distinct positive dot–blot result. In the group of equation crosses (F_1 × rye) a number of positive results (most probably 6 cases) could be attributed to presence of whole wheat chromosome additions, which were earlier found in ca. 18% of the back–crosses progeny. In the certation back–cross progeny (rye × F_1) the influence of single additions of wheat chromosomes may be neglected,

because the whole wheat chromosomes are seldom transmitted through the rye type male gametes of the F_1 hybrids. The precise estimation of frequencies of introgressed plants in $B_1F_1$was not possible because of a numerous class of uncertain results of dot–blot signals, only slightly more intense than those for the normal rye control. Anyway, the results of both kinds of the first back–cross indicated intense migration of wheat DNA to rye. High male transmission of the DNA suggested, that the size of the introgressed chromosome fragments was small. The investigation was continued on progeny of selfed and free pollinated (with rye) B_1F_1 plants showing distinct hybridization with the wheat probe. High transmission rate was confirmed. The segregation ratios in B_1F_2 suggested the action of one or two Mendelian factors. The role of three or more loci could be excluded. However, the participation of non–Mendelian factors is probable too. The extensive colonization of one genome by transposable elements from the other one was proved recently in cotton (Hanson *et al.,* 1999).

Table 1

Results of Southern dot–blot hybridization of the wheat genomic DNA probe to DNA from rye type recombinants of the F_1 hybrids between 4x triticale and 2x rye back–crossed to 2x rye.

Generation and parents	Positive	Uncertain	Negative
B_1F_1 : F_1 × rye (equation back–cross)	13	5	12
B_1F_1 : rye × F1 (certation back–cross)	10	14	12
B_1F_2 : B_1F_1 selfed	13	2	1
B_2F_1 (+B_1F_2?): B_1F_1 free pollination with rye	23	2	11

The genomic *in situ* hybridization (GISH) method of molecular cytogenetics was used in attempts at localization of the migrant fragments on chromosomes. Unfortunately, no wheat signals were found with the use of this method in progeny of rye plants showing even the most conspicuous wheat characters and the most intense dot–blot signals. The same cytogenetic procedure applied to the tetraploid triticale parents yielded clear–cut results on translocations in the homoeology group 5 (Łapiński, Schwarzacher 1998a). The *5AL/RL* translocation, which resulted from replacement of 28% of the wheat *5A* chromosome long arm with the corresponding rye fragment, was present in almost all triticale plants (Fig 1a,b). The more detailed analysis suggested, that the translocated chromosome was a source of cytogenetic instability in some lines. The rye terminal fragment of the translocated chromosome initiated conjugation with its *5R* rye homoeologue and the secondary crossing–over events, in points located proximally to the primary translocation point, produced small intercalary wheat inserts to rye chromosomes (Fig 1c,d). This kind of translocation could be a good explanation for the previous results, but there was no cytogenetic proof of its transfer to rye. It is a possibility, that size of such secondary

translocations could be below the susceptibility level of the GISH method. In experiments of Łukaszewski (personal communication) a large part of primary translocations (more than 10%), detectable with C–banding owing to replacement of rye terminal heterochromatic blocks, could not be confirmed by GISH.

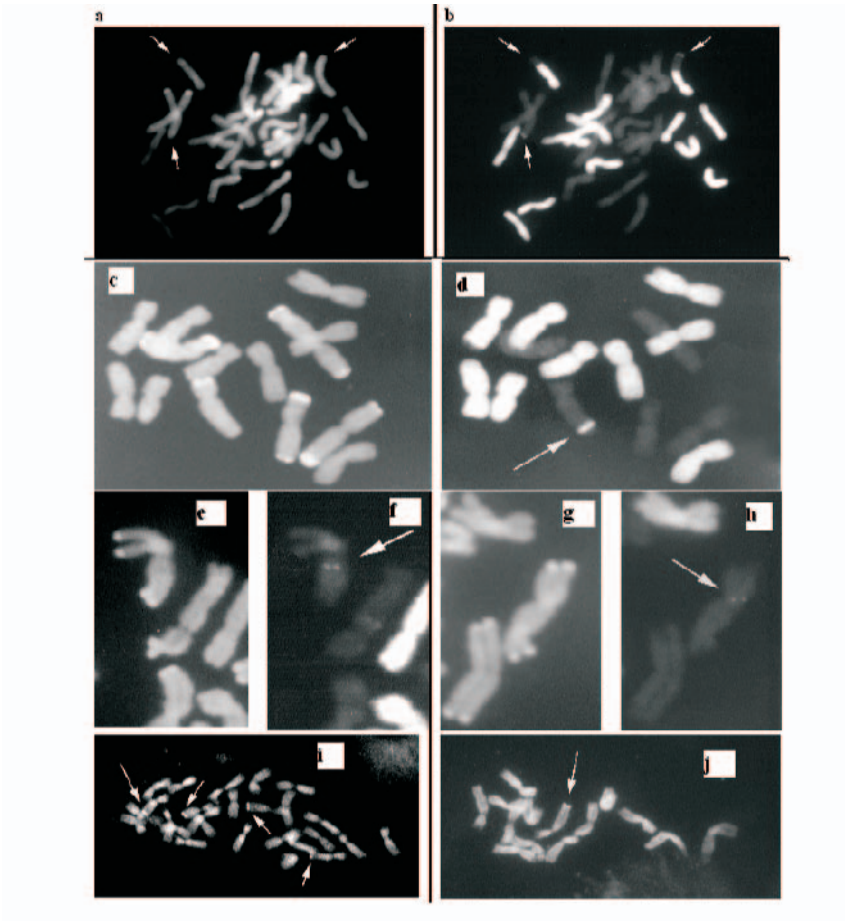

Fig. 1 The *in situ* hybridysation evidence for the translocations and migrations of wheat DNA to rye chromosomes in tetraploid triticale (b, d, f, h) and in its rye type progeny on tetraploid (i) and the diploid (j) level). The DAPI staining of the same metaphase plates (a, c, e, g) is helpful in identification of the *5RL* translocated arms (a–d and i, j) and chromosomes, which were involved in the DNA migration (e–h)

On the verge of the method susceptibility were also two–dot signals found only in a few cells in the late metaphase stage of two plants from the *C521* unstable line of 4x triticale (Fig 1e,f and 1g,h). They were located near centromeres of submetacentric rye chromosomes and had no relation to the other translocations, which were detected exclusively in the homeology group 5. Considering both the location and size of the

signals, it is highly unprobable, that these small DNA rearrangements resulted from two independent homoeologous translocations with close crossing–over points. They seem to originate from an action of a sepa–rate, unknown mechanism. A relationship of these DNA migrations with the dot–blot results seems probable, if to consider that they could not be detected on chromosomes because of weaker responsiveness of rye to the GISH procedures, in comparison with the 4x triticale.

Another way to make proof for wheat origin of the improved rye char–acters could be co–segregation of a character with a dot–blot hybridiza–tion signal. The appropriate experiments were arranged for wide glumes and resistances to brown rust and mildew. No co–segregation was found for any of these characters, so their origin remains uncertain.

Toleration of alien genetic material

The level of toleration of alien genetic material is important both for easiness of initial manipulations with whole chromosomes and for per–formance of final products carrying a small desirable translocation. In wheat, chromosomal engineering starts usually from additions or sub–stitutions of whole chromosomes or their large fragments, and gener–ally, these changes do not restrict dramatically the vigor and fertility of plants. In diploid rye, the wheat addition plants show usually more symptoms of weakness and infertility, since no stable disomic addition lines have been produced yet. Beginning such manipulations on the 4x ploidy level, in hybrids between 4x triticale and 4x rye, seems a good measure for the preliminary phase, until the size of an alien fragment is reduced to a small extent tolerable on the 2x ploidy level.

Propagation of fertile recombinants of our ten crosses of this type, made in 1993 and 1994, led to 28 lines, which were investigated in 2000 with the *in situ* method. Six of the lines, derived from different F_2 indi–viduals, were modified tetraploid ryes carrying wheat additions, substi–tutions or translocations. One stable line was vigorous enough to carry two disomic substitutions, one of a complete wheat chromosome and one of the translocated *5AS*. *AL*/*RL*/*AL* one. Another line contained the vigorous plant with four small terminal wheat translocations (Fig 1i), most probably a tetrasomic of *5RS.RL*/*WL*. Probably this kind of translocation was transferred from the 4x triticale parent; it was de–tected in two from eight secondary 4x triticale lines analysed earlier (Łapiński, Schwarzacher, 1998). It is double represented on Fig. 1b, oc–cupying the end of one *5R* chromosome and the rye end of one of the translocated chromosomes.

Majority of the 4x triticale × 4x rye crosses produced rather non–in–teresting 4x triticale lines with stable karyotypes of parental type. The abundance of new secondary translocations was found only in six 4x triticale lines from three combinations, two of them sharing the same 4x triticale component. The situation resembled that in 4x triticale, where the karyotypically stable genotypes prevailed over the distinct group of

unstable ones (Łapiński, Schwarzacher 1998a, Łapiński, unpublished data).

Transfer of a wheat translocation from the tetraploid to the diploid background should be done as early as possible, because the low ploidy level makes more provocative conditions for further elimination of non–coadapted linked alien genes. The natural selection pressure facilitates spontaneous reduction of a foreign insert size, as it occurred in the 2x rye materials produced by Łukaszewski *et al.*(2000).

The diploid chromosome complement of rye was not expected to provide a plant with the level of chromosomal changes tolerance comparable to any polyploid, like wheat, where even whole alien chromosome arm substitutions are quickly assimilated in the highest yielding varieties. However, the vigor and fertility in majority of single wheat additions to diploid rye indicates also a level of buffering capacity of the rye genome. Thus, even in case of the existing side effects, some hope is justified for development of a good tolerance of small alien chromosomal inserts in rye. If the tolerance of a new gene is insufficient or size reduction of an alien DNA fragment does not occur, there is still no reason for rejection of an insert as useless. Such translocation may require some extra breeding effort for compensation of adverse side effects. Such breeding is usually difficult only at the beginning, as it requires a combination of reproductive isolation and genetic diversity for creation of an initial breeding population of translocation homozygotes. Once established, the isolated but genetically rich population of this kind almost guarantees a progress, especially when the high dynamism of cross and selection cycles is enforced by the self–incompatibility system. However, a success may require time.

Hybrid rye varieties seem to open special possibilities to diminish significance of the adverse side effects, especially for the dominant alien genes. Chromosomal changes frequently show little or no bad influence when present in monosomic condition. In such case, the problem of compensation may be restricted to the vigour of those line components, in which the translocation is controlled and preserved.

Wheat and rye chromosomes in triticale reached a high level of intergenomic co–adaptation, which may be relevant for wheat fragments transferred into rye. In order to capture this hypothetical effect and to accumulate posible introgressions, as well as any genetic variation important for successful transfer, we conducted pre–breeding of special stocks of 4x triticale and 2x rye for chromosomal engineering in rye. We call the breeding method „recurrent intergeneric crossing", because the rye type and the triticale type segregants of earlier crosses between 4x triticale and 2x rye were used to make new crosses of the same kind. After three cycles of such procedure an increase was noticed in transmission of whole wheat chromosomes from F_1 to B_1F_1 diploid rye type plants (see Table 2). After the first cross, in 1994, the percentage of plants with single additions of wheat chromosomes was ca. 18%, which

corresponded well with the 15.6% result reported by Baum (1991). After the third recurrent cross the respective percentage amounted ca. 29%. However, more interesting was variation among families derived from different F1–combinations. The difference between the families RC413 (showing 50% of wheat addition plants) and RC416 (with 8% of the ad–ditions) was significant (χ^2=5.27, at p=0.05). The plants from the RC420 and RC422 families looked promising either, as cross components useful in further work on increase of ability to maintain alien chromosomes or their fragments.

Table 2

Occurrence of added wheat chromosomes in various families of rye derived in 2000 from triple recurrent crosses with 4x triticale, in comparison with the single cross results from 1994.

Mother form (F1)	14R	14R+1W	14R+3W	Other	Sum	% of additions
RC 413'00	10	10	1	15R	22	50
RC 414'00	12	2	1	–	15	20
RC 416'00	13	–	–	15R+1W	14	7
RC 420'00	–	2	–	–	2	100
RC 422'00	1	1	–	–	2	50
RC 424'00	7	1	–	–	8	13
RC 425'00	15	4	1	–	20	25
Sum'00	58	20	3	2	83	29
Sum'94	74	14	2	–	90	18

14R = 14 chromosomes of rye, 1W = one chromosome of wheat

The terminal 5RL/WL translocation (Fig 1j) was detected recently, with the *in situ* hybridization method, in the 2x rye material from the last cycle of recurrent crossing. Four plants of the 53 investigated with the method contained the translocation in monosomic condition. The occurence of this introgression could be attributed to one intergeneric F_1 combination, which gave rise to a family with average frequency of wheat additions. It makes no proof for relevance of the recurrent proce–dure and should be ascribed rather to the random. The translocation is probably the same as the one shown on the Fig 1b and was transferred from the 4x triticale parent.

Another advantage of the pre–breeding was selection of rye genotypes with better toleration of wheat cytoplasm coming from the 4x triticale mother parents. At the beginning, majority of alloplasmic rye plants showed remarkable reduction in vigour. There were also disturbances in extrusion and dehiscence of anthers. The most vigorous rye plants with the best pollen shed were selected to next crosses with 4x triticale. It caused a level of compensation of the alien cytoplasm effects and the present population of alloplasmic rye looks more normal. No easily de–

tectable improvements in crossability and fertility of intergeneric F_1 hybrids were noticed yet in the recurrent crossing derived materials.

We hope, that further pre–breeding work will produce the more useful materials with combination of high crossability, high intergenomic crossing–over frequency on the 4x level and high toleration of alien introgession on the diploid level.

CONCLUSIONS

1. Tetraploid triticale is a carrier of wheat introgressed rye chromosomes.
2. The relatively large wheat segments in rye chromosomes, originating from homoeologous crossing–over, constitute only a part of the introgressions. The most frequent are small DNA migrations, undetectable by the GISH (genomic in situ hybridization) method, but detectable by Southern blotting. The mechanism of their origin seems different.
3. Rye lines with various wheat characters were selected in the 4x triticale–2x rye crossing programme, but no link could be confirmed between the wheat DNA introgressions and the characters.
4. A remarkable variation in triticale–rye crossability, hybrid sterility, intergenomic crossing– over frequency and toleration of whole wheat chromosones in rye has been stated.

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