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Male transmission of the translocated chromosome in a tertiary trisomic of rye: genetic variation and relation to the rate of development of aneuploid pollen grains

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Summary. Variation in male and female transmission of the translocated extra chromosome ($5R^{3R}$) was studied in a tertiary trisomic of rye (*Secale cereale* L.). In two F_5 lines derived from a single F_4 line, female transmission was lower than in five others derived from another F_4 line. This could be caused by genetic factors or by the strong inbreeding depression in these lines, leading to low viability of trisomic progeny. After selfing, male transmission was estimated as very low, but this was primarily based on the occurrence of tetrasomics that probably have a very poor viability. In testcrosses with disomic female parents, male transmission was much higher (up to 27%), without variation within F_5 lines. One F_5 line showed significantly higher male transmission than any of the seven tested, including a sister line from the same F_4. This was consistent in the F_6. Apparently high male transmission is genetically determined. There was a positive correlation with recombination of the marker *ti* (tigrina) on the extra chromosome and the normal 5R chromosomes. At the first meiotic metaphase, trivalents and quinquivalents were frequent in the trisomics. Assuming loss of univalents, 40% of the microspores should carry the translocated extra chromosome. In most lines, more than 40% were found at pollen mitosis. Observations on timing of pollen mitosis showed a delayed development in aneuploid spores, with clear differences between plants, but no correlation with male transmission. The cause of reduced male transmission and the expression of genetic variation therein can, therefore, not be found in meiotic behaviour or delayed microspore development. Pollen germination and tube growth may be more important.

Key words: *Secale cereale L.* – Tertiary trisomic – Male transmission – Genetic variation – Aneuploid microspores

Introduction

In rye, *Secale cereale L.*, optimal use of heterosis can be made by breeding hybrid varieties. Also, hybrids are more uniform than open pollinated varieties and the introduction of specific characters is easier.

Self-fertile inbred lines can be developed but to produce hybrid seed, selfing of the seed parent must be prevented. One method for this uses genetic male sterility (*ms*) but then the propagation of the male sterile seed parent is a problem. A possible solution is the use of the "Balanced Tertiary Trisomic" (BTT) system, as proposed by Ramage (1965) for barley. In this system, the extra, translocated chromosome in a tertiary trisomic carries the dominant *Ms* allele and the dominant allele *A* of a selective marker gene. The two normal chromosomes carry the recessive alleles *ms* and *a*. If the extra chromosome is not transmitted through the male gametes, all functional pollen will be homogeneous for the recessive alleles *m* and *a*. If the extra chromosome can be transmitted through the eggs, the progeny after selfing will consist of parental male fertile tertiary trisomics and male sterile disomics. As the latter will express the recessive marker, selection is possible. When the tertiary trisomic is used to pollinate male sterile plants, all progeny will be male sterile disomics again. Thus, the tertiary trisomic can be used as a maintainer line for male sterile stocks that serve as the seed parent in the production of hybrids. The BTT itself is maintained by selfing (Ramage 1965; Wiebe and Ramage 1971).

As the system works for barley, although not yet in practice, Sybenga (1982) proposed it for rye. A number of aspects of its construction and application for rye hybrid breeding was studied by De Vries (1984a).

Several meiotically stable tertiary trisomics could be isolated (De Vries 1984 b). A number of morphological selective markers has been located on their respective chromosomes. Male sterile mutants were more difficult to isolate and localize with the translocation tester set (De Vries and Sybenga 1984).

As mentioned, the maintenance of male sterile lines using pollination with BTTs requires no male transmission of the translocated chromosome. For efficient maintenance of the BTT, female transmission should be as high as possible.

De Vries (1984 b) found considerable variation in male and female transmission in the selfed progenies of different tertiary trisomics. In some cases, additional variation was detected between different lines of the same trisomic. Part of this variation might be determined by genetic factors. Male transmission varied between 0% and 7%, whereas female transmission ranged from 14% to 51%. In selfed progenies, trisomics can result from male and from female transmission. As tetrasomics tend to have a strongly reduced viability, but are the basis for distinction between the two transmission types, male transmission could have been underestimated.

Several studies have been carried out on male and female transmission in primary trisomics. In some reports it was shown that transmission rates estimated on the basis of selfed progenies differed considerably from those found in testcrosses $2n \times (2n+1)$ and $(2n+1) \times 2n$, which define male and female transmission separately.

In primary trisomics of *Nicotiana sylvestris* Goodspeed and Avery (1939) found a male transmission rate of 0%–34% and a female transmission of 16%–29%, both in testcrosses. In some trisomics the total transmission after selfing, which ranged from 20% to 39%, was smaller than male and female transmission separately. In other cases, it was even greater than male and female transmission together. Even when male transmission was high, tetrasomic individuals were very rare in selfed progenies of trisomics. The same phenomenon was observed in primary trisomics of *Lotus peduncu-latus* (Chen and Grant 1968). This is also the only species studied whose trisomics showed an equal transmission through male and female (9% on average). For primary trisomics of *Hordeum vulgare* it was shown that male transmission was zero in all testcrosses $2n \times (2n+1)$. Still, for some trisomics the selfed progeny showed a transmission rate that was either significantly higher or lower than the female transmission in testcrosses (Tsuchiya 1960). In most studies, data have been given for only one or a few plants per trisomic type. No attention has been paid to the possibility of (genetic) variation between plants carrying the same extra chromosome.

Studies on transmission rates in tertiary trisomics are scarce. In his proposal for the use of BTTs for hybrid barley breeding, Ramage (1965) stated that the aneuploid microspores in tertiary trisomics developed more slowly, causing absence of male transmission. Experimental results were not given. In contrast, Lehmann (1972) found in the same tertiary trisomics that male transmission could vary from 0% to 13% in testcrosses. Again, no tetrasomics were found in the selfed progeny.

Apparently, good estimates for male and female transmission are difficult to obtain from a selfed progeny. Not only the viability of tetrasomic individuals, but also that of trisomics can play a role. Usually, trisomic seeds are smaller and have a lower weight and a poorer germination than disomic seeds (Blakeslee and Avery 1938; Tsuchiya 1960; Kasha and McLennan 1967).

Thus, the first aim of the present study was to further examine the variation in male and female transmission in a rye tertiary trisomic. In view of its importance for the successful application of BTTs for hybrid breeding, attention was focussed on male transmission. Progenies obtained by selfing and progenies from the testcross $2n \times (2n+1)$ were analyzed for trisomic plants in several F_5s and F_6s. Attempts have been made to assess genetic factors involved in variation in male transmission.

The second aim of this study was to establish the causes of low male transmission. Theoretically, a transmission rate of 50% is expected. This percentage can be reduced due to:

a) elimination of the extra chromosome in meiosis.
b) subnormal or delayed development of aneuploid microspores.
c) reduced fertility of aneuploid pollen grains.
d) competition between euploid and aneuploid pollen grains during pollen germination and tube growth.
e) subnormal development of aneuploid zygotes, embryos or endosperm.
f) reduced or delayed germination of $2n+1$ seeds.
g) reduced vigour of $2n+1$ seedlings (cf. Khush 1973).

Similar factors play a role in reducing female transmission. The reasons for the differential transmission rates through the male and female should be sought in the differential morphology and development of the respective gametophytes. Therefore, it may be expected that the most important factors in reducing male transmission are those concerning development and functioning of the aneuploid pollen grains (b–d).

In meiosis, the resulting percentages of n, $n+1$ or aberrant gametes depend on: the extent of pairing of the translocated chromosome with its homologous parts of the normal chromosomes, the number and location of

chiasmata, the orientation of the resulting multivalents and the behaviour of any univalents (Sybenga 1972).

In barley tertiary trisomics, up to 37% of the pollen mother cells in metaphase I contained a univalent (Lehmann 1972). In tertiary trisomics of pearl millet, 35% univalents and a high frequency of adjacent orientation of multivalents were found. As a consequence, the progeny after selfing contained only 3% tertiary trisomics but also 5% primary trisomics (Singh et al. 1982). However, in four rye tertiary trisomics De Vries (1984b) noted that meiotic multivalents and alternate orientations were frequent. Thus, a high transmission rate of the translocated chromosome through male meiosis is expected.

Few reports are available on the development of aneuploid microspores to mature pollen grains. According to Gülcan and Sybenga (1967), aneuploid microspores produced in autotetraploids reached their first mitotic division later than euploid microspores. In a previous paper (Janse 1985) it was shown that the same applies to the aneuploid microspores in a rye tertiary trisomic. A linear correlation was found between the percentage of dividing microspores containing the extra chromosome and the percentage of binucleate microspores, the latter expressing the stage of development. The total percentage of aneuploid microspores passing through pollen mitosis was high (42%) and equalled the number expected as a result of regular segregation of alternate multivalents in meiosis, assuming loss of univalents. It could not be concluded whether the delayed development of aneuploid microspores directly causes the reduction in male transmission.

Therefore, a possible correlation between the delay in development of aneuploid microspores and the male transmission rate of the extra chromosome was investigated here. If this correlation is strong, the delay may be one of the causes of reduced male transmission, for instance because the aneuploid microspores will frequently not be able to reach maturity in time for anthesis. For several tertiary trisomic plants from different lines, the percentage of aneuploid microspores expected after meiosis was estimated from meiotic configuration frequencies. Then, the extent of delay in development was determined as described before (Janse 1985) and finally male transmission rates were obtained from testcrosses.

Material and methods

Plant material

The tertiary trisomic (2n = 15) used in this study carries the short translocation chromosome of translocation 240, that is a member of the translocation tester set established at our laboratory (Sybenga and Wolters 1972). The extra chromosome contains the translocated segment from the short arm of chromosome 3R and the short arm plus a part of the long arm of 5R (Fig. 1) (Nomenclature according to Sybenga et al. 1985). The same trisomic was used in a previous study on the rate of development of aneuploid microspores (Janse 1985).

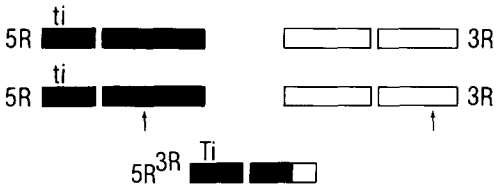

Fig. 1. Tertiary trisomic 240: the chromosomes involved. Nomenclature according to Sybenga et al. (1985). Arm length ratios derived from Sybenga and Wolters (1972). *Ti* and *ti* alleles of the tigrina locus (De Vries and Sybenga 1984). *Arrows* show the translocation breakpoints

The marker gene *Ti/ti* is used, located on the short arm of 5R (De Vries and Sybenga 1984). The normal chromosomes have the recessive allele whereas the extra translocated chromosome carries the dominant allele (Fig. 1). Homozygous recessive plants show the "tigrina" feature: coiling of leaves with yellow transverse striping. When grown under field conditions and in normal stands, these plants cannot compete with the wild types. Therefore, in the progeny of this BTT after selfing the parental tertiary trisomics are automatically selected. Here, all plants were grown in a greenhouse at 20 °C during the spring of 1984 (F_5) and 1986 (F_6). Then, *titi* genotypes are usually viable.

The experiments were carried out on seven different F_5 lines. Lines 1 and 2 were derived from the same F_4 line, whereas lines 3, 4, 5, 6 and 7 descended from another. Both F_4 lines were part of the study of De Vries (1984b).

In the F_5 lines, segregation for *ti* and trisomy was determined to estimate male and female transmission rate and recombination. In every line two or three tertiary trisomics with the *Ti* allele were selected. These plants were used as pollen parents for the testcrosses $2n \times (2n+1)$. One of these plants was also used for analysis of pollen mitosis, to determine the delay in development of aneuploid microspores. Other ears from it were selfed to obtain F_6 lines. Meiotic analysis was carried out on other tertiary trisomic plants of every F_5 line, as production of ears was not sufficient to make all observations on the same plants. In the F_6 lines, two or three tertiary trisomics were selected again and used as pollen parents in new testcrosses.

Segregation in progenies after selfing and testcross

Approximately 12 days after sowing the F_5 plants were scored for the *ti* marker. In the first two lines all plants were also karyotyped. The other lines contained many more plants and so in these cases only the plants carrying the *Ti* allele were scored for the presence of the extra, translocated chromosome. Root tips were pretreated in a saturated solution of α-bromonaphtalene for 1,5–2,5 h at 25 °C, macerated in 1 *N* HCL for 12 min at 59 °–60 °C and stained with Feulgen reagens. Squash preparations were made in 45% acetic acid.

For testcrosses tertiary trisomics with the *Ti* allele were used as a father. Unless recombination had occurred, the *Ti* allele was located on the translocated chromosome, while the normal chromosomes carried the *ti* alleles, as in the parental trisomics. The female parents in the testcrosses had a normal karyotype (2n = 14) and were homozygous recessive for *ti*. They were emasculated and pollinated when the stigmas were

receptive (approx. 8 days after emasculation). Plants in the progenies of the testcrosses were scored for *ti*. Wild types were karyotyped as they were expected to be tertiary trisomics (due to male transmission) unless recombination had occurred. From the frequencies of trisomics and disomics in this group, the actual male transmission rate and the recombination could be calculated.

Estimates of transmission and recombination

Male and female transmission frequencies are expressed as m and f respectively, while r is the recombination fraction between *ti* and the translocation breakpoint. De Vries (1984b) deduced that the expected frequencies of the different types after selfing can be given as:

	Wild type	tigrina
2n+2	$mf(1-r^2)$	mfr^2
2n+1	$[m(1-f)+f(1-m)](1-r+r^2)$	$[m(1-f)+f(1-m)](r-r^2)$
2n	$(1-m)(1-f)(2r-r^2)$	$(1-m)(1-f)(1-r)^2$

In this model it is assumed that multivalent orientation in meiotic metaphase is alternate, which is common in rye (Sybenga 1972; De Vries 1984b). Further, the "tigrina" phenotype is not supposed to affect viability. For the first two F_5 lines, in which all plants had been karyotyped, maximum likelihood methods had to be used to estimate m, f and r on the basis of these formulae. For the other lines, they were directly calculated from the frequencies of di-, tri- and tetrasomics among the wild types. With the same assumptions, it can be deduced that the frequencies of the possible types in the progeny of testcrosses can be expressed as:

	Wild type	tigrina
2n+1	m(1−r)	mr
2n	r(1−m)	(1−m)(1−r)

From the frequencies of disomic and trisomic plants among the wild types, m and r were calculated.

Meiosis

Anthers containing pollen mother cells (PMCs) in first meiotic metaphase were fixed in acetic alcohol (1:3) and stored at −10 °C. After staining in 2% acetocarmine for 24 h, the anther was squashed in 45% acetic acid and mounted in Euparal. A random sample of 200 PMCs was taken to study meiotic configurations.

Pollen mitosis

Temporary preparations of anthers containing microspores in first pollen mitosis were made in acetocarmine. Three random samples of 100 microspores were taken to determine the percentage of binucleate cells. Chromosomes were counted in all microspores in mitotic metaphase. This was done in several preparations for every plant. The percentage of aneuploids among the dividing microspores was plotted against the percentage of binucleates. The surface covered by the graph was calculated to determine the total percentage of aneuploid microspores passing first pollen mitosis. This procedure is described in Janse (1985).

Results and discussion

Transmission and recombination

Segregation for *ti* and the extra chromosome in the seven F_5 lines are given in Table 1, in addition to estimates for male (m) and female (f) transmission and recombination fraction (r). For lines 1 and 2, where more equations than variables had been formed, maximum likelihood methods did not provide solutions for m, f and r. When m and f were deduced from total frequencies of tetra-, tri- and disomics, however, different values for r could be obtained from the different equations. Minimum and maximum values are given in Table 1. The failure to obtain maximum likelihood estimates can be explained by assuming that the presence of an extra chromosome or the "tigrina" phenotype or both reduce viability of the plant to different extents. It can be seen that for these lines only a small percentage of the sown seeds were eventually scored. Germination was poor and many seedlings died. In the other F_5 lines m, f and r could be estimated only from segregation for chromosome number among the wild types. Interaction between genotype or karyotype and viability could therefore not be detected. However, germination was better in these lines and most seedlings survived.

Although total numbers were small in lines 1 and 2, there were indications that female transmission was lower here than in the other lines. This could have been caused by reduced viability and vigour of trisomic seeds and seedlings. Apart from the low germination percentage and the high seedling mortality, all plants from these two lines looked less vigourous than the others. They obviously suffered from severe inbreeding depression, which could have reduced the appearance of trisomics in the progeny. Another possible explanation for the observed differences in female transmission is the involvement of genetic factors, which influence female transmission directly. This will also apply to the male transmission, estimated as very low in all progenies studied, due to the low frequencies of tetrasomics. Reduced viability and vigour of tetrasomic seeds and seedlings was probably even more important here.

For the parental F_4 line of lines 1 and 2, studied by De Vries (unpublished), f and m were estimated as 0.54 and 0.09, respectively, whereas for the parental line of the other F_5 lines f and m were 0.48 and 0.04. Although it may be expected that transmission rate decreases in subsequent inbreeding generations, it is not clear why this decrease is so strong in the first group. However, there were few plants in the F_4 progenies.

Segregation for *ti* and the extra chromosome in the progenies of testcrosses $2n \times (2n+1)$ and estimates for m and r are shown in Table 1 (columns at right). The male transmission rate in almost all progenies was

Table 1. Segregation for *ti* and the extra chromosome in seven F_5 lines of balanced tertiary trisomic 240 and in progenies of testcrosses $2n \times 2n+1$ with trisomic F_5 plants used as a pollinator. Estimates of male (m) and female (f) transmission rates and recombination fraction (r). Chromosome numbers in parentheses

Line	F_5=(15) Ti ×											(14) ti × (15) Ti							
	Ti			ti			Total (% of sown)	f	m	r	Deviant	Parent	Ti		ti	Total (% of sown)		m	r
	(16)	(15)	(14)	(16)	(15)	(14)							(15)	(14)					
1	0	7	1	0	1	21	30 (39)	0.27	0.0	0.021	(16)[a] Ti	1a	5	5	83	93	(98)	0.06	0.06
										–		1b	0	2	60	62	(98)	0.0	0.03
										0.17		1c	15	2	193	210	(92)	0.07	0.01
2	0	5	2	0	0	19	26 (35)	0.19	0.0	0.0	–	2a	12	11	69	92	(94)	0.15	0.14
										–									
										0.051		2b	9[c]	6	27	42	(100)	0.27	0.19
3	0	42	2		75		119 (80)	0.36	0.0	0.020	–	3a	8[c]	3	144	155	(98)	0.05	0.02
												3b	5	3	91	99	(95)	0.05	0.03
4	1	42	2		71		116 (73)	0.36	0.02	0.014	–	4a	6[c]	3	35	44	(98)	0.15	0.08
												4b	9	6	99	114	(93)	0.08	0.06
5	1	58	0		79		138 (90)	0.42	0.02	0.0	–	5a	0	0	39	39	(91)	0.0	0.0
												5b	5	1	67	73	(94)	0.07	0.02
6	1	70	3		111		185 (80)	0.38	0.01	0.013	(23)[b] Ti	6a	13	7	98	118	(98)	0.12	0.07
												6b	3	10	103	116	(93)	0.03	0.08
7	0	56	1		99		156 (77)	0.36	0.0	0.010	–	7a	4	1	69	74	(94)	0.05	0.01
												7b	4	0	67	71	(96)	0.06	0.0

[a] 15 normal and 1 translocation chromosome

[b] Probably resulting from second division restitution and fertilization with a normal sperm cell (16 + 7 = 23)

[c] Including one plant with a telocentric chromosome instead of the translocation chromosome; probably resulting from centromere misdivision of the univalent translocated chromosome in meiosis. As plants are of wild type, 5 RS must be involved. As the telocentric chromosome must have come from the male, transmission must have occurred

Table 2. Segregation for *ti* and the extra chromosome, male transmission rate (m) and recombination fraction (r) in progenies of testcrosses 2n×2n+1, with tertiary trisomics from different F_5 lines used as pollinators (pooled from Table 1). Significance of differences between lines

Line	Ti		ti	Total (% of sown)	m	r	Significantly different from line:
	(15)	(14)					
1	20	9	336	365 (95)	0.06	0.03	2 ($P<0.005$) 6 ($0.02<P<0.05$)
2	21[a]	17	96	134 (96)	0.19	0.16	all other nos.
3	13[a]	6	235	254 (97)	0.05	0.03	2 ($P<0.005$) 6 ($0.02<P<0.05$)
4	15[a]	9	134	158 (94)	0.10	0.06	2 ($0.02<P<0.05$) 7 ($0.02<P<0.05$)
5	5	1	106	112 (93)	0.05	0.01	2 ($P<0.005$)
6	16	17	201	234 (96)	0.07	0.08	2 ($0.005<P<0.01$) 7 ($0.02<P<0.05$) 1,3
7	8	1	136	145 (95)	0.06	0.01	2 ($P<0.05$) 4,6

[a] Including one plant with an extra telocentric instead of a translocation chromosome

much higher than estimated on the basis of the F_5 progeny to which the trisomic pollinator belonged. As mentioned above, this phenomenon had also been shown for primary trisomics of several species and for barley tertiary trisomics. It also emphasizes that the reduced viability of tetrasomics causes an underestimation of male transmission in progenies after selfing compared to progenies after testcross. This will be stronger after subsequent inbreeding, as appears from the transmission rates in F_4 and F_5 lines.

In heterogeneity tests, no significant differences were found between tertiary trisomics of the same F_5 line with respect to m and r. However, it should be noted that in some cases the numbers in the euploid *Ti* category were small, which reduces the reliability of the test.

The data for each line were pooled and m and r calculated again (Table 2); tests of heterogeneity were carried out for every pair of F_5 lines. The most striking differences were between line 2 and all others. The male transmission rate and the recombination fraction in line 2 were exceptionally high.

Other significant differences were also found. Line 6 deviated from 1, 3 and 7. As the male transmission did not differ much, this was probably due to the recombination fraction being little higher in line 6. Line 7 differed from 4 and 6, because it had a lower recombination fraction; line 4 also showed a higher transmission rate.

From every F_5 line, one F_6 line was obtained by selfing a wild type tertiary trisomic. As the same plant had also been used for testcrosses and analysis in pollen mitosis, it was not possible to obtain enough F_6 seeds to study segregation, as was done for F_5 lines. However, testcrosses (2n×(2n+1)) with F_6 tertiary trisomics were made, from which segregation data and estimates for m and r are given in Table 3. In one case (4a) the number of F_6 seeds was so small and germination so poor, that only a few plants were obtained, none of which appeared to be a tertiary trisomic.

Again, male transmission of the translocated chromosome had occurred in almost all crosses. In one case (line 2A) it became clear, that one of the F_6 plants was a recombinant, as it gave an improbable number of "recombinant" euploid *Ti* plants in the testcross progeny. This plant must have carried a *Ti* allele instead of *ti* on one of its normal chromosomes. The translocated chromosome still carried a *Ti* allele. Using adjusted formulae for the frequencies of wild type di- and trisomics in the testcross progeny (see Table 3, note a), m and r could also be estimated.

Tests of heterogeneity showed no significant differences between the m and r of tertiary trisomics of the same F_6 line in a testcross. The test is not very reliable for small expected numbers in one or more categories. In line 2A (Table 3) it could not be carried out in the usual way. In this case the harmonic means of m and r were calculated, then expected numbers for the categories in the testcross progeny were determined for the two trisomics using the null hypothesis (i.e. no difference between the two trisomics with respect to m and r); the adapted formulae were used for the second trisomic. The expected and observed numbers were used in the χ^2 test.

Table 3. Segregation for *ti* and the extra chromosome and estimates for male transmission rate (m) and recombination fraction (r) in progenies of testcrosses 2n×2n+1, with tertiary trisomics from different F_6 lines used as a pollinator. Pooled data for each F_6 line and significance of differences between lines

F_6 line	F_5 plant selfed	2n×2n+1						Pooled						Significantly differing from line:
		Ti		ti	Total (% of sown)	m	r	Ti		ti	Total (% of sown)	m	r	
		(15)	(14)					(15)	(14)					
1C	1c	3	1	57	61 (82)	0.05	0.02	11	1	147	159 (85)	0.07	0.01	2 ($0.01 < P < 0.02$)
														6 ($P < 0.01$)
		8	0	90	98 (88)	0.08	0.0							7 ($0.02 < P < 0.05$)
2A	2a	6	5	23	34 (89)	0.22	0.19	6	5	23	34 (89)	0.15[b]	0.09[b]	all others
		14	52	46	112 (88)	0.13[a]	0.06[a]	14	52	46	112 (88)			
3A	3a	4	3	46	53 (91)	0.08	0.06	6	6	108	120 (89)	0.05	0.05	2 ($P < 0.05$)
		2	3	62	67 (88)	0.03	0.05							
4A	4a	no trisomics			– –	–	–	–	–	–	– –	–	–	
5A	5a	1	1	40	42 (86)	0.02	0.02							
		5	6	88	99 (95)	0.05	0.06	8	7	162	177 (91)	0.05	0.04	2 ($0.01 < P < 0.02$)
		2	0	34	36 (88)	0.06	0.0							
6A	6a	4	10	77	91 (90)	0.05	0.12	6	13	148	167 (89)	0.04	0.08	2 ($0.01 < P < 0.02$)
		2	3	71	76 (88)	0.03	0.04							1
7A	7a	0	0	23	23 (64)	0.0	0.0	1	4	114	119 (82)	0.01	0.03	2 ($P < 0.005$)
		1	4	91	96 (88)	0.01	0.04							1

[a] Frequency of recombinant types was too high to solve m and r. The parental trisomic probably carried a *Ti* allele on one of the normal chromosomes as well. The frequency of (15) *Ti* and (14) *Ti* can then be expressed as m and ½(1−m) (1+r) respectively, then m and r can be calculated

[b] Result cannot be pooled here, but m and r for this line were calculated as the harmonic mean of m and r found for the two trisomics

Pooled testcross segregation data for every F_6 line and estimates for m and r based on them are given in Table 3 (right column). The harmonic means of m and r are given for line 2A. Again, heterogeneity tests were carried out for every combination of two lines. For line 2A, calculation was modified as described above. It differed considerably from all other F_6 lines, due to its high male transmission rate and high recombination fraction. Two more differences were found: line 1C differed from 6A and 7A, probably due to the higher recombination fraction in 6A and the lower transmission rate in 7A. Finally, no significant differences were found between the segregation in testcrosses of F_6 lines and of their parental F_5 lines.

Genetic factors determining male transmission and recombination seem to be present. High values were found in F_5 line 2, consistent with its derived F_6 line 2A. Evidence for genetic segregation comes from the differential behaviour of F_5 lines 1 and 2 which originate from the same F_4 line. Both showed strong inbreeding depression and, perhaps as a result, a low female and no male transmission after selfing. However, in testcrosses one line showed a consistent high male transmission and recombination, while the other showed moderate rates comparable to those in the other group of F_5 lines. Segregation for one or more genetic factors, even in F_4, seems to be the only explanation for these differences.

If genetic factors determine male transmission and recombination, selection should be possible. As only high or moderate transmission rates were found, it is not yet clear whether selection can lead to the development of lines without male transmission of the translocated chromosome and without recombination in testcross. For the successful application of the BTTs in hybrid rye breeding, these lines should also show a high female transmission after selfing. As can be concluded from this study, a good viability of zygotes, seeds and seedlings (i.e. a good tolerance to inbreeding) is one of the prerequisites for female transmission.

Although it seems that male transmission and recombination showed a positive correlation in this study, it is not clear whether they are determined by the same factor(s). This apparent correlation is an interesting phenomenon for which no ready explanation could be found.

Meiosis

Frequencies of first metaphase configurations in tertiary trisomics of the different F_5 lines are given in Table 4. All configurations showed an alternate orientation, as is common for rye. Chain trivalents were most

Table 4. Frequency of MI configurations involving the translocated chromosome in 200 PMCs of tertiary trisomics 240 from different F_5 lines. Expected percentages of viable aneuploid gametes resulting after meiosis, assuming loss of univalents

Line	Plant	Quinquivalents			Quadri- + univalents			Trivalents			Univalents	Total	Expected aneuploids
			 Total			 Total				 Total			
1	1b	0.0	0.230	0.230	0.025	0.0	0.025	0.0	0.0	0.465 0.465	0.280	1.000	35.2
2	2c	0.033	0.367	0.400	0.033	0.0	0.033	0.067	0.0	0.417 0.484	0.083	1.000	44.9[a]
3	3c	0.005	0.150	0.155	0.030	0.005	0.035	0.005	0.0	0.585 0.590	0.220	1.000	37.9
	3d	0.005	0.275	0.280	0.030	0.0	0.030	0.0	0.0	0.575 0.575	0.115	1.000	43.4
4	4c	0.0	0.240	0.240	0.075	0.0	0.075	0.010	0.0	0.465 0.475	0.210	1.000	37.1
	4d	0.0	0.220	0.220	0.045	0.0	0.045	0.0	0.005	0.485 0.490	0.245	1.000	36.3
	4e	0.0	0.230	0.230	0.050	0.0	0.050	0.005	0.0	0.565 0.570	0.150	1.000	41.0
5	5c	0.0	0.245	0.245	0.090	0.0	0.090	0.0	0.0	0.475 0.475	0.190	1.000	37.7
6	6c	0.005	0.285	0.290	0.075	0.0	0.075	0.005	0.0	0.480 0.485	0.150	1.000	40.3
7	7c	0.005	0.295	0.300	0.070	0.0	0.070	0.010	0.0	0.500 0.510	0.120	1.000	42.0
	7d	0.0	0.310	0.310	0.055	0.0	0.055	0.005	0.005	0.495 0.505	0.130	1.000	41.9
Mean		0.005	0.259	0.264	0.053	0.0	0.053	0.010	0.001	0.501 0.511	0.172	1.000	39.8

[a] As a result of poor quality of flowers only 60 PMCs could be scored in this case

frequent, occurring in about 50% of all pollen mother cells. Also, chain quinquivalents were regularly found; in most remaining cases the translocated chromosome was univalent and the normal chromosomes 5R and 3R formed bivalents.

The number of aneuploid microspores after meiosis could be calculated from these frequencies, as described before (Janse 1985). Univalents are assumed to stay behind or be lost in meiosis, as is known to occur in rye (Sybenga 1972). Quinqui- and trivalents are expected to produce 50% euploid and 50% aneuploid microspores. Configurations consisting of a quadrivalent plus an univalent will give rise to 50% unbalanced (not viable) and 50% normal gametes, but are rare.

On average, almost 40% of the microspores formed in these tertiary trisomics were expected to carry the translocated chromosome in addition to the normal complement (Table 4). Thus, causes of reduced male transmission should be sought in processes occurring after meiosis.

However, it is remarkable that the highest percentage of expected aneuploid microspores (44.9%) was found in the F_5 line that showed the highest male transmission rate in testcrosses (Tables 1 and 2). The high expected percentage was the result of a relatively high frequency of quinquivalents and a low frequency of univalents. On the other hand, the lowest percentage of aneuploid gametes was expected in line 1 which had a relatively high frequency of univalents, but its male transmission frequency did not differ from lines 3 to 7. Probably, not only the reduction in male transmission, but also the genetic variation in this reduction will be expressed in processes after meiosis.

Causes for differences in recombination fraction, however, must be sought in meiosis. The high frequency of quinquivalents found in line 2 could be the cause of the high recombination in the testcrosses with trisomics from this line. In all quinquivalents and also in quadri-plus univalents both arms of the translocated chromosome are bound. Pairing must have taken place between the unchanged arm of the translocated chromosome (5R) and the short arm of a normal 5R chromosome, but also between the exchanged segment and its homologous part in the short arm of a normal 3R chromosome. In addition, chiasmata must have been formed in the paired segments, a prerequisite for recombination between *ti* and the centromere. Trivalents can be of two types. The first is association of the translocated chromosome with the normal 5R chromosomes, where the unchanged arm 5R is bound and recombination is possible. The other association consists of the translocation chromosome and both 3R chromosomes, where the other arm is bound and recombination is impossible. These types can not be distinguished in acetocarmine stained preparations, but De Vries (1984b) found them in equal frequencies in C-banded preparations. However, trivalent frequencies in line 2 did not deviate from those found in other lines, so they are not expected to contribute to variation in recombination fraction.

Pollen mitosis

The relation between the percentage of binucleate microspores (i.e. the stage of development) and the percentage of aneuploid microspores in first pollen

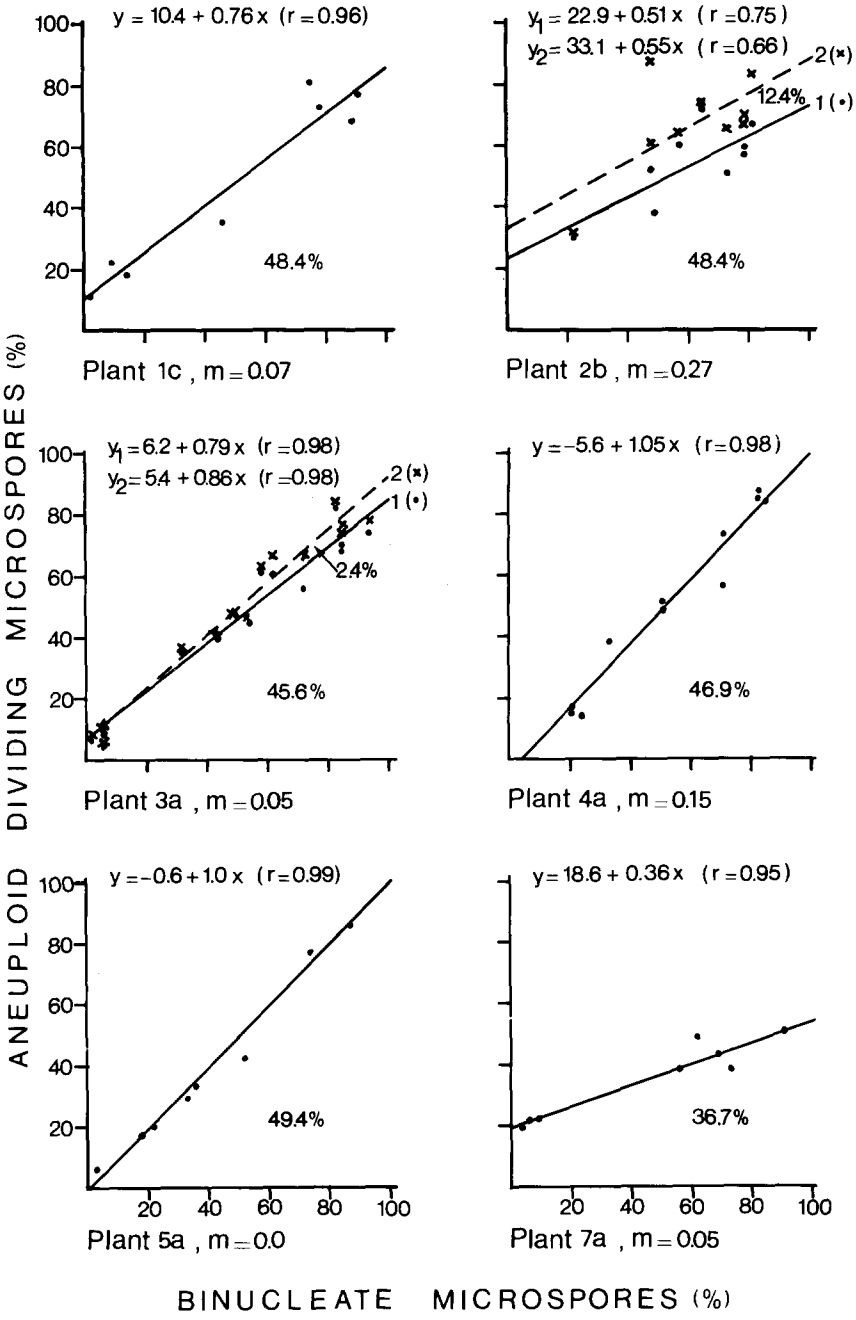

Fig. 2. Percentage of dividing microspores with aneuploid chromosome number during pollen mitosis in six tertiary trisomic plants of different F_5 lines. Ordinate: percentage of binucleate cells, representing the stage of development. Abscissa: percentage of microspores carrying the extra, translocated chromosome (*1*) and, if different, the total percentage of aneuploid microspores (*2*), including aberrant aneuploids. Equations of regression lines ($y = a + bx$), regression correlation coefficients (r) and the surface covered by the graph (in %) are shown. Male transmission rate in testcross (m) is given for tertiary trisomics involved

mitosis is shown in Fig. 2 for tertiary trisomics of six F_5 lines.

In most plants, the translocated chromosome could be easily recognized in dividing microspores with eight chromosomes. However, sometimes aberrant microspores were observed in pollen mitosis (Fig. 3). In some cases, a telocentric instead of a translocated chromosome occurred. It probably originated from centromere misdivision of a univalent translocation chromosome in meiosis. In later stages, microspores with 14, 15 or 16 chromosomes were observed. They could have resulted from first or second division restitution in meiosis. Finally, there were microspores with nine chromosomes, one of which was usually recognized as the translocated chromosome. These aberrant microspores can arise from adjacent segregation of chromosomes in anaphase I.

In anthers of all plants investigated, the percentage of aneuploid microspores increased with the percentage of binucleate cells, i.e. with the stage of development.

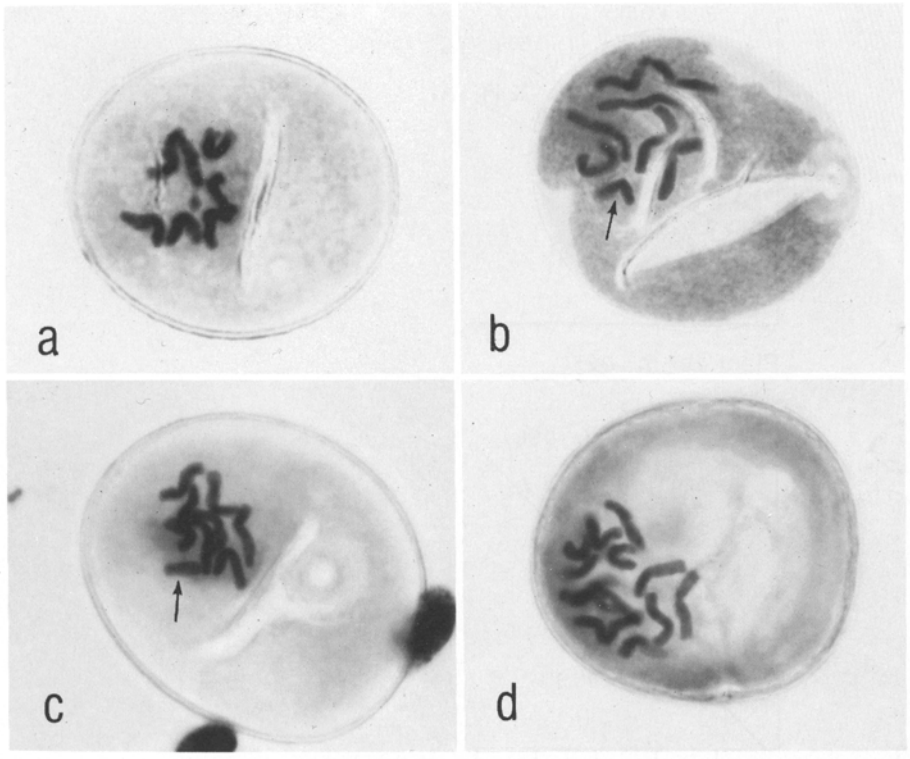

Fig. 3a–d. Microspores formed in anthers of tertiary trisomics 240, in first pollen mitosis: **a** normal microspore with seven chromosomes (including one satellite chromosome); **b** aneuploid microspore with the translocated chromosome (*arrow*) in addition to the normal haploid complement; **c** aberrant aneuploid microspore with nine chromosomes, including one small (probably the translocated) chromosome (*arrow*); **d** aberrant aneuploid microspore with 15 chromosomes (not all visible at this depth of field)

This means that the aneuploid microspores always showed a slower rate of development than the normal ones, as was described before (Janse 1985).

It can easily be seen that the slope of the regression line gives a measure for the extent to which the aneuploid microspores are retarded, while the surface covered by the graph is a measure for the total number of aneuploid microspores that have passed pollen mitosis (Fig. 2). For the F_5 plants, these data can be compared with the values for male transmission in testcrosses and with the percentages of aneuploid microspores expected from meiotic configurations (Table 4). With the exception of plant 7a, total percentages of aneuploid microspores were higher than expected from meiotic analysis. Probably, univalents were not always lost in meiosis, as was assumed, but were frequently able to reach the daughter nuclei. Even in line 1, where only 35% aneuploids were expected, 48.4% of the microspores passing pollen mitosis were aneuploid, which equalled the percentage found in line 2. However, it should be kept in mind that meiotic analysis was carried out on other plants, although from the same line in the same generation and the same season, as the analysis of pollen mitosis.

In line 2, a high percentage (12.4%) of aberrant aneuploid microspores were found in pollen mitosis. More than half of them contained nine chromosomes, including one translocated chromosome. Most other aberrations consisted of the presence of a telocentric instead of a translocated chromosome. It is not clear why centromere mis-division was more frequent in this line, particularly when univalent frequency was low. These results indicate again that the observed differences in male transmission rates are not expected to originate from differential meiotic behaviour.

When the slopes of the regression lines for pollen mitosis were compared with the transmission rates in testcrosses, no clear correlation could be found. Plants 4a and 5a, for example, showed the strongest delay in development of aneuploid microspores. In the first trisomic, male transmission rate reached 0.15 whereas in the second plant it was zero. In plant 7a the weakest delay was observed. Still, male transmission was not high (0.05) although in this case the lower total number of aneuploids might have played a role as well. In plant 2b, the delay was still considerable, but male transmission was very high (0.27). It should be noted that, for plant 2b, only one aberrant microspore contributed to fertilization and produced a telocentric trisomic after testcross. In two other testcross progenies a telocentric trisomic occurred also (Table 1).

From the analysis of meiosis and pollen mitosis two important conclusions can be drawn. Firstly, from the high percentages of aneuploid microspores resulting after meiosis and going through pollen mitosis, it is obvious that the causes of reduced male transmission

and the genetic variation can not be found in the meiotic behaviour of the translocated chromosome. Also, the aneuploid microspores apparently all show a normal development, at least up to the young binucleate stage.

Secondly, the extent to which aneuploid microspores showed a slower development, as shown by the timing of pollen mitosis, is not directly related to the male transmission rate. Therefore, it is likely that, despite this delay, most aneuploid microspores are able to reach the mature pollen stage. Then, the reduction in male transmission and the expression of genetic variation must have its basis in reduced pollen germination or pollen tube growth, or in disturbances at even later stages.

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