Cytogenetic approaches to breeding and propagation of male sterile parent lines for hybrid varieties of rye

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Promotor: dr.ir. J. Sybenga, hoogleraar in de erfelijkheidsleer.

STELLINGEN

1. Hybride veredeling van rogge, gebruikmakend van genetische mannelijke steriliteit en gebalanceerde chromosoomsystemen, is technisch mogelijk; uit een oogpunt van economische haalbaarheid en van kwekersrecht is nader onderzoek wenselijk.
   Dit proefschrift.

2. Interstitiële chiasmata bij reciproke translocaties tussen (sub-)-metacentrische chromosomen geven aanleiding tot een alternate-1 : adjacent-1 centromeeroriëntatie verhouding van 1 : 1.
   Dit proefschrift.

3. Khush' bewering, dat een verschil in frequentie van de twee mogelijke tertiaire trisomen in de nakomelingschap van een translocatie trisoom wordt bepaald door de keuze van het extra chromosoom, wordt ondersteund noch door waarnemingen noch door theoretische overwegingen.
   G.S. Khush, 1973. Cytogenetics of aneuploids. Academic Press, New York and London.
   Dit proefschrift.

4. Evenals de oorspronkelijke weigering te aanvaarden dat de aarde rond is, vindt het fictieve onderscheid tussen altenate-1 en alternate-2 centromeeroriëntatie bij ringquadrivalenten van reciproke translocaties zijn wortels in een tekortschietende interpretatie van een tweedimensionele waarneming aan een driedimensioneel systeem.
   I.A. Boussy, 1982. Genetics 100: 505-509.
   D.G. Cochran, 1983. Genetics 104: 215-217.

5. Indien Koornneef en van der Veen veronderstellen dat een lage transmissie van het telocentrische chromosoom karakteristiek is voor telocentrische trisomen, houden zij onvoldoende rekening met het verloop van de meiose bij deze trisomen.
   M. Koornneef and J.H. van der Veen, 1983. Genetica 61: 41-46.
   M. Koornneef, 1982. Proefschrift, Landbouwhogeschool, Wageningen.

6. De rol van de meiose bij het tot stand komen van landbouwkundig interessante combinaties van erfelijke eigenschappen verdient hernieuwde aandacht.

7. Het nog steeds door veel agronomen veronderstelde traditionalisme van de kleine boer in ontwikkelingslanden getuigt van een zelfde instelling bij eerstgenoemden.

8. Een verplichte landelijke registratie van erfelijkheidsgegevens is niet bezwaarlijk, als deze zich beperkt tot genetische gegevens en indien er ten hoogste adviezen aan mogen worden ontleend.

9. Het ontbreken van uitroeptekens in wetenschappelijke publicaties betekent geenszins dat daarmee geen emoties gepaard gaan.

10. Het kostenbesparend effect van individuele warmteverbruiksmeting voor bewoners van goed geïsoleerde, centraal verwarmde nieuwbouwflats is niet afdoende bewezen; dit systeem werkt kostenverhogend voor niet-buitenshuis werkenden en landgenoten met een tropische oorsprong.

11. 'No-nonsense' als typering voor het beleid van de huidige regering is beledigend ten aanzien van vorige kabinetten.

12. Creationisme is geen wetenschap, maar bijgeloof.

13. Ongeloof in bijgeloof is ook geloof.

Proefschrift van J.N. de Vries
Cytogenetic approaches to breeding and propagation of male sterile parent lines for hybrid varieties of rye
Wageningen, 13 januari 1984.

J.N. de Vries

Cytogenetic approaches to breeding and propagation of male sterile parent lines for hybrid varieties of rye

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
hoogleraar in de veeteeltwetenschap,
in het openbaar te verdedigen
op vrijdag 13 januari 1984
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen.

ISN : 200196

Omslag:
het iso-7RS telo-7RL compenserend trisoom, hier in een translocatie 300 (5RL, 7RS) heterozygote achtergrond, hangt de heer B. Weijman weliswaar boven het hoofd, maar het is nog geen hybride rogge die hij oogst.

Woord vooraf

Het spreekt vanzelf, dat niemand een lezer kan verbieden, meteen maar het eerste hoofdstuk op te slaan om zijn of haar honger naar nieuwe kennis te stillen. Bovendien: de bladzijden hierna liggen te ritselen van ongeduld. Eén misverstand zou dan licht post kunnen vatten, namelijk dat dit boekje tot stand is gekomen door de inspanningen van een enkel persoon. Het onmogelijke is echter nu eenmaal niet mogelijk.

Professor J. Sybenga ben ik zeer dankbaar voor de voortreffelijke manier waarop hij mij tijdens het onderzoek heeft begeleid. Jaap, jouw ideeën, en je interesse voor alles wat ik met het door jou ontwikkelde basis-materiaal aan het uitspoken was, hebben hun stimulerende werking niet gemist, getuige onder meer de hoeveelheid half onderzocht en heel interessant materiaal dat nog 'op zolder' ligt. Dat het wat lang heeft geduurd voor ik achter de schrijftafel ben gaan zitten, heeft niet alleen te maken met mijn geringe weerstand tegen de verleiding, de mogelijkheden van het materiaal zoveel mogelijk uit te buiten, ook een zekere drempelvrees voor het eerste artikel speelde een rol. Jouw motto "Als iets echt moet, dan kan het ook" heeft wat dat betreft de nodige wonderen verricht, en voor je kritische kanttekeningen bij mijn vaak wat wijdlopige betogen ben ik je blijvend zeer erkentelijk.

Professor J.H. van der Veen, U als beheerder van de vakgroep Erfelijkheidsleer zeg ik dank voor de gastvrijheid die mij de afgelopen vier jaar is geboden.

De 'Barak' mag dan zijn langste tijd hebben gehad, de herinneringen aan de bevolking ervan zijn onuitwisbaar. Threes van Cruchten, jij hebt er niet slechts toe bijgedragen dat ik me snel op de vakgroep thuisvoelde, je hebt ook een belangrijk aandeel gehad in het combineren van allerlei chromosoomafwijkingen waaruit in latere generaties veel interessants tevoorschijn kwam. Mijn dank gaat ook uit naar Sonja van der Schaaf voor haar werk, op de vakgroep, maar ook thuis waar de microscoop een plekje had gevonden naast de kinderkamer. Henny Verhaar, jouw aandeel in het onderzoek dat aan dit proefschrift ten grondslag ligt, is zeer groot. Dank ook voor alle discussies over de meest uiteenlopende onderwerpen. Het soms gepeperde gehalte ervan is ongetwijfeld te danken aan onze uiteenlopende standpunten, ofschoon ik me ook wel eens heb afgevraagd of er een verband is met die 'héérlijke soep', die jij altijd heter eet dan 'ie ooit opgediend wordt. Hans (met een 'M') de Vries ben ik zeer erkentelijk voor zijn bijdragen, zowel op cytogenetisch als op fotografisch vlak. Het besef, dat jouw achternaam in ons land nogal eens voorkomt, begint in het buitenland steeds verder door te dringen. Hilbert Booij, ook jou bedank ik voor het fotowerk dat

je 'tussendoor' hebt willen doen.

De wereld houdt buiten de barak niet op te bestaan. Ramon Giraldez dank ik langs deze weg nogmaals voor zijn inbreng in de ontwikkeling van de Giemsa-kleuringstechniek, waarvan hier nog steeds met overgave gebruik wordt gemaakt. Om wat dichter bij huis te blijven: Piet Stam heeft mij, door het schrijven van een listig computerprogramma, voor heel wat rekenwerk behoed, waarvoor ik hem zeer erkentelijk ben. Erg veel werk heb ik Trees Makkes juist bezorgd. Het uitmuntende typewerk op de paginaas hierna is van haar. Dat er een aardig net boekje moest komen, heeft me altijd wel voor ogen gestaan. Dat het uiteindelijk prachtig is geworden, is geheel te danken aan jouw inzet. De bijdragen van Aafke Sieswerda en Marion van Hunnik mogen hier uiteraard niet onvermeld blijven, evenals de bereidheid van Cees Bos, zolang wat 'hard-ware' ter beschikking te stellen. De verzorging van de planten, ook van de 'zwakke soorten', was bij de heren Weijman, van IJmeren, van Blijderveen en Arends in vertrouwde handen. Dit gold ook ten aanzien van het verwijderen ervan - op verzoek uiteraard.

De hulp van een groot aantal stagiaires van de STOVA en doctoraalstudenten van de LH heeft de grote 'omzet' aan worteltoppen en meeldraden, die nu eenmaal onontbeerlijk is voor het doen van kwantitatieve uitspraken, mede mogelijk gemaakt. Met plezier denk ik terug aan de samenwerking met Jolanda ter Brugge, Ronald Eijlander, Francesco Holtus, Joke Janse, Eric Jongedijk, Christa Kievit, Sandra Miller, Joke Mouris, Paul Sales, Gerard Schaafsma, Aart van Voorst, Eppie van der Wal en Arnold Willems. Zo'n rijtje is wat onpersoonlijk, maar de tekst zou van het papier lopen wanneer ik ieders specifieke bijdrage zou beschrijven. Daarom op deze plek alleen de welgemeende opmerking dat het werk van ieder van jullie onmisbaar is geweest en dat ik er veel van heb kunnen opsteken. Van harte hoop ik dat jullie er ook wat aan hebben gehad.

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Pa en Moe, bedankt dat jullie me hebben laten studeren. En Marleen bedankt voor de hulp bij het tillen van de laatste loodjes.

Contents

General introduction

A major objective of plant breeding is the exploitation of the heterotic potential of crop plants. For generatively propagated crops, this is most effectively achieved by breeding hybrid varieties. Hybrid varieties have the additional advantage of being uniform.

To make hybrid breeding economically attractive, the extra benefit of growing a hybrid should amply cover the extra cost of the sowing seed. Part of the extra cost is the result of special techniques and systems involved in the prevention of self-fertilization of the female parent (or: 'seed parent') of the hybrid, and the maintenance and propagation of this parent for renewed cycles of hybrid seed production.

Prevention of self-fertilization of the seed parent is required to accomplish that each plant of the crop is a hybrid. To prevent selfing, use can be made of emasculation, by hand (tomato) or mechanically (maize), chemicals (*Cucumis* species), incompatibility (*Brassica* species), cytoplasmic male sterility ('CMS'; sugar beet, maize) or the segregation of male sterile plants in the progeny of fertile heterozygotes for a male sterility allele (genetic male sterility, 'GMS').

In cultivated rye (*Secale cereale* L.), several combinations of CMS and corresponding fertility restoring genes have become available during the last decade, offering the opportunity to breed and propagate male sterile parent lines with relative ease (Geiger, 1982). As yet, however, successful use in hybrid rye breeding programs is being made only of one type of cytoplasm, named 'Pampa' (Morgenstern and Geiger, 1982) implicating that this cytoplasm probably will be shared by all hybrid varieties released. The southern corn leaf blight epidemic of maize in 1970 (Tatum, 1971), caused by the specific interaction between the T-race of *Helminthosporium maydis* and the 'Texas' cytoplasm extensively used as the source of male sterility of the seed parents in hybrid seed production, is a dramatic illustration of the potential danger of such narrow-based CMS-approach to hybrid breeding. Therefore, although the use of CMS is advantageous with regard to operationality, it is, in view of the vulnerable genetic base of the hybrids, useful to develop alternative systems to breed and propagate male sterile seed parents for hybrid rye.

A number of systems have been worked out for other crop plants, making use of genetic male sterility (GMS) and extra chromosomal material which usually is structurally modified. In these systems, male sterility is monogenic recessively inherited. The extra genetic material carries the dominant male sterility allele, while the normal chromosome complement carries the recessives. Owing to certation, the extra material carrying the dominant allele is only transmitted through the female haplophase. Consequently, all pollen taking part in reproduction contains a normal, haploid set of chromosomes. This pollen exclusively carries the recessive male sterility allele, provided that no crossing-over takes place resulting in the transfer of the dominant or recessive male sterility alleles, respectively, to the normal chromosome complement or the extra genetic material, respectively. The propagation of an all male sterile seed parent is accomplished by repeated cycles of pollinating male sterile disomics by fertile plants having the chromosomal and allelic properties mentioned. The latter are maintained by selfing (see below).

For barley hybrid breeding, Ramage (1965) proposed to use balanced tertiary trisomics for the propagation of the male sterile seed parent. These trisomics carry an extra translocation chromosome on which the dominant male sterility allele is located, as close to the translocation breakpoint as possible to reduce crossing-over between allele and breakpoint. The normal chromosomes carry the recessives. Certation prevents pollen to take part in reproduction, so that disomic male sterile offspring will be produced by pollination of male sterile disomics by fertile tertiary trisomics. The pollinator is maintained by selfing. Since *female* transmission of the extra chromosome is permitted, the selfed progeny of the pollinator will contain both fertile tertiary trisomics and karyotypically normal sterile disomics. Elimination of the latter requires extensive karyotyping of the progeny, unless di- and trisomics can be distinguished morphologically, in which case hand elimination of the disomics before flowering is still necessary. To avoid these laborious and time consuming procedures, a self-reproducing system to maintain the pollinator can be developed, making use of a monogenic recessively inherited lethality marker, of which the dominant allele is again closely linked with the translocation breakpoint, whereas recessive alleles are carried by the normal chromosomes (Wiebe and Ramage, 1971).

The use of duplication(-deficiency) heterozygous disomics, in which one translocation chromosome is present instead of a corresponding normal chromosome, has been proposed by Patterson (1973) for hybrid breeding of maize. The recessive male sterility allele is carried by the normal complement, while the dominant allele and the translocation breakpoint are closely linked. When the duplication is relatively large and the deficiency small, only pollen with a normal chromosome make up carrying the recessive allele is expected to be reproductive, at the same time permitting female transmission of the duplication-deficiency karyotype with the dominant allele. Similarily, also for maize, Phillips and Springer (1972) suggested to use a disomic double duplication heterozygote in which the absence of two normal non-homologous chromosomes is compensated by two translocation chromosomes, originating from two different interchanges in which both absent chromosomes are involved. In this system, plants have no deficiencies, and owing to certation caused by the three-fold presence of the two "between breakpoint" regions, only karyotypically normal pollen is reproductive. For rye, Sybenga (1982) proposed the use of compensating trisomics in which the absence of one normal chromosome is compensated by the presence of two modified chromosomes. In the XYZ-system designed by Driscoll (1972) for wheat, the extra genetic material carrying the dominant male sterility allele is obtained from related species or genera like rye, barley, *Aegilops*, *Agropyron* and *Triticum monococcum* (Driscoll, 1981).

Thus, the basic idea behind all GMS-based systems for hybrid breeding is to prevent that pollen serves as a vector for the dominant allele of a male sterility gene (and, if desired, a lethality gene), at the same time permitting female transmission. Therefore, pollen transmission of the modified extra genetic material carrying this allele, as well as recombination with the recessives on the normal chromosome complement should be absent. The eggs, on the other hand, should be able to transmit the extra material in as high a frequency as possible for an efficient maintenance of the deviant karyotype upon selfing. The prevention of male transmission, however, requires extra chromosomal material of sufficiently large size, which conflicts with the requirement of high female transmission, and with the fact that the viability of plants carrying the extra material should not be affected too drastically. Another conflict arises from the necessity of frequent formation of chiasmata between the extra genetic material and homologous segments of the normal chromosome complement, offering the

opportunity for recombination between the dominant alleles on the extra and the recessives on the normal chromosomes.

Subject of the present thesis are a number of aspects related with the construction of modified chromosomal systems, which, in combination with GMS, may be of use in the breeding and propagation of male sterile parent lines of hybrid rye (*Secale cereale* L.).

Chapter 1 contains a description of 17 monogenically inherited morphological markers, of which a number are lethal or conditionally lethal, whereas one causes male sterility. Thirteen of these markers are devided over six linkage groups which are assigned to six of the seven chromosomes of rye. Of nine markers, the arm location is established. Four markers could not be localized. The first linkage maps of chromosomes 2R and 5R are presented.

Chapter 2 deals with recombination in the segment between the centromere and the breakpoint of a translocation chromosome. Chiasma formation in this 'interstitial' segment is usually reduced, offering a potentially favourable location for male sterility and marker genes in GMS-based hybrid breeding. The translocation described in this chapter represents a clear exception to the rule of reduced interstitial chiasma formation.

Basic requirements for an efficient construction of two balanced chromosomal systems are discussed in chapters 3 and 5. Chapter 3 describes the origin of four tertiary trisomics, in each of which an extra translocation chromosome is present. Chapter 5 deals with the origin of four telotertiary compensating trisomics, in which one normal chromosome is replaced by a translocation and a telocentric chromosome.

Chapter 4 reports about the behaviour of the four tertiary trisomics of which the isolation is described in chapter 3. The extra translocation chromosome in all four includes a segment of varying length of chromosome 5R, on which the dominant allele of a conditionally lethal marker ('tigrina') is located, whereas the normal chromosomes carry the recessive alleles. The suitability of the four 'balanced' tertiary trisomics (BTTs) for use in hybrid breeding programs is evaluated, taking into account meiotic behaviour, male and female transmission of the extra material, recombination between the tigrina gene and the translocation breakpoint, and the occurrence in selfed BTT-progenies of deviant karyotypes which may disturb the system. In relation to the telotertiary compensating trisomics isolated (chapter 5), some of these aspects are shortly elucidated in the 'GENERAL DISCUSSION'.

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1. Chromosomal location of 17 monogenically inherited morphological markers in rye (*Secale cereale* L.) using the translocation tester set.

by

J.N. de Vries and J. Sybenga

Department of Genetics, Agricultural University,
Gen. Foulkesweg 53,
6703 BM Wageningen.
The Netherlands.

ABSTRACT

The combined segregation of translocations and markers is investigated in backcrosses and F2s involving 17 monogenically inherited morphological markers and 8 reciprocal translocations in rye. Chromosomal location is established for *an* and *ct1* (chromosome I), *dw3* (chromosome II), *dw2* , *mo* and *Ps* (chromosome III), *lg1* (chromosome IV), *wh* (chromosome V) and *br*, *ct2*, *gr*, *ti* and *wi1* (chromosome VI). (Chromosome nomenclature follows de Vries and Sybenga, 1976). Arm location is determined for *an* and *ct1* (Ib), *lg1* (IVb), *wh* (Va), *br* and *ti* (VIa) and *ct2*, *gr* and *wi1* (VIb), using a number of translocation derived trisomics. No markers could be localized on the satellite chromosome (VII). Four markers remain unlocalized: *lg2* is not located in VIa, *sr* is not linked with *dw2*, and *wi2* shows independent segregation from *wi1* and translocation 240, involving chromosomes II and VI. Reduced fertility of translocation homozygotes prevented the localization of *ms*. The correspondence between these findings and the linkage groups presented by Schlegel and Mettin (1982), as well as the relationship between our chromosome nomenclature and that of the *Triticinae* are discussed.

Keywords: *Secale cereale* L. - gene localization - linkage groups - translocations - trisomics.

INTRODUCTION

In recent years, important progress has been made with the localization of genes on the chromosomes of rye (*Secale cereale* L.) in studies which involved wheat-rye chromosome substitutions, additions and translocations. In these investigations the location of genes for resistance to several wheat pathogens, as well as a number of biochemical and molecular markers such as isozymes, endosperm proteins and repetitive DNA sequences has been established. Descriptions of simply inherited morphological traits within the species *S. cereale* have been available for many years, but reports concerning their linkage relationships and chromosomal location are relatively scarce (reviews by Schlegel and Mettin (1982) and Appels (1982)). The development of a variety of tester stocks for international use would considerably accelerate the progress in this field. Important first steps in this respect are the proposals and intentions of the 'International workshop on rye chromosome nomenclature and homoeology relationships' (Sybenga, 1983), including an inventory of all available material and the choice of the addition set of 'Imperial' rye to 'Chinese Spring' wheat as the standard set.

In their review, Schlegel and Mettin (1982) have described the arrangement of 12 genes in four linkage groups, of which two groups are assigned to specific chromosomes.

The present article deals with the linkage relationships and the chromosomal location of 17 monogenically inherited markers in rye, some of which are probably identical with genes mentioned by Schlegel and Mettin (1982). For chromosomal location, use was made of the Wagingen translocation tester set (Sybenga and Wolters, 1972; Sybenga, 1983) covering the complete genome.

In studies involving telocentric-, tertiary- and telocentric tertiary compensating trisomics, the arm location of six markers could be established. This article only provides preliminary data from these investigations. More extensive reports will be published later.

MATERIALS AND METHODS

Marker genes (table 1)

According to the recommendations of the 'International workshop on rye chromosome nomenclature and homoeology relationships' (Sybenga, 1983), two-letter gene symbols are used, referring to the main fenotypic characteristic. A dominant allele is indicated by a capital letter. Genes having phenotypically similar effects are designated by the same symbol, followed by an Arabic numeral differentiating between the genes. New symbols (table 1) are proposed for a number of genes previously described by Sybenga and Prakken (1962).

For several markers, identity with genes described in other studies was proven or may be considered plausible. The genetic identity of *c* ('canary grass; Sybenga and Prakken, 1962), and *m* ('monstrosum'; obtained from Fedorov, Vavilov Inst., Leningrad in 1967, code number '*gc5*') was proven by a cross between an anthocyaninless (*an an*) 'canary grass' mother and a 'monstrosum' plant with anthocyanin. The F1 having anthocyanin, showed the characteristic strongly branched ears. The new gene symbol *mo* will be used to replace both *c* and *m*. The mutant *ct2* was obtained from Fedorov also in 1967 (code number '*gc8*'), who named it 'compactum'. Fedorov, Smirnov and Sosnikhina (table 1) symbolized the compactum gene as *ct*. In view of the origin of the material, it may be assumed that *ct2* and *ct* are identical. The material provided by Kobyljanski in 1974 (Vavilov Inst., Leningrad, code number 'WIR 10415')was phenotypically similar to compactum. Crosses between this material and that obtained from Fedorov yielded compactum F1 plants which proved the presence of *ct2* in Kobyljanski's material.

A cross between the compactum genes *ct1* ('awnless', *al*; Sybenga and Prakken, 1962) and *ct2* segregated normal and compactum plants in the F2 in a 9:7 ratio (Sybenga, unpublished), showing that *ct1* and *ct2* are unidentical and unlinked genes with complementary action.

Next to *ct2*, 'WIR 10415' contained a gene for male sterility (*ms*) and a factor determining winter or spring habit (*wi1*). *ms* may well be similar to Kobyljanksi's *rf2* (table 1). With regard to their source, also identity of *wi1* and the winter type gene *ae* is not excluded, but allelism test are still lacking.

Table 1. Marker genes investigated in the present study. All markers have a monogenic recessive inheritance, except *Ps*, which is inherited dominantly

Gene symbol	Name	Main phenotypical characteristics	Scoring stage	Source (code)	References and gene symbols used previously (see text)
an	anthocyaninless	all plant parts without anthocyanin	seeds (aleuron: xenia); coleoptiles of two day old seedlings	W	1,8,10,14-*a** 2,3,4,5-*vi* 11,12,13-*a* **
br	brittle	all plant parts brittle, stems easily breaking with clear sound	from heading onward	W	11,12,13-*b*
ct1	compactum 1	compact head; dwarf habit; spikelets closely packed; awns practically lacking; leaves relatively broad	from heading onward	W	12-*a1*
ct2	compactum 2	like *ct1*; packing of spikelets somewhat less close	from heading onward	Le (gc 8 and WIR 10415)	3,4,5,7,9-*ct*
dw2	dwarf 2	dwarf habit; small ears with awns; plants becoming lighter green at maturity; highly tillering; narrow leaves	from heading onward	W	12-*d2*
dw3	dwarf 3	dwarf habit; plant colour and tillering normal; relatively broad leaves	from heading onward	W	
gr	grass type	narrow, tapering and curled leaves; stems very short and bent; ears often rudimental	four weeks old seedlings	W	
lg1	light green 1	leaves, stems and ears light green; dwarf habit; tillering higher than normal; at maturity resembling *dw2*	three day old seedlings	W	

Table 1. (continued)

lg2	light green 2	leaves, stems and ears light green; dwarf habit; ears and tillering normal; relatively broad leaves; except colour resembling *dw3*	three day old seedlings	W	
mo	monstrosum	ears strongly branched with many practically awnless florets	from heading onward	Le (gc 5) Lu	3,5,7,10–*m* 12–*c*
ms	male sterile	no formation of fuctional pollen	at flowering	Le (WIR 10415)	6–*rf2*
Ps	purple seed	seed coat deep violet (no xenia)	from seed set onward	Le (gc 45)	
sr	secondary roots defective	plants fall over after tillering	from tillering onward	W	12–*sr*
ti	tigrina	leaves coiling with yellow transverse striping; dwarf habit	two week old seedlings and later	W	13–*t*
wh	white	chlorophyll almost completely lacking	two day old seedlings	W	
wi1	winter type 1	cold period required before flowering	from heading of spring type plants within same population onward	Le (WIR 10415)	4,10–*ae*
wi2	winter type 2	like *wi1* (complementary genes)	like *wi1*	W	

Le = N.I. Vavilov All Union Institute of Plant Industry, Leningrad (USSR)
W = Department of Genetics, Agricultural University, Wageningen, The Netherlands
Lu = University of Lund (Sweden)
1: Dumon (1938); 2: Fedorov and Smirnov (1967); 3: Fedorov et al. (1970a); 4: Fedorov et al. (1970b); 5: Fedorov et al. (1970c); 6: Kobyljanski and Katerova (1973); 7: Smirnov and Sosnikhina (1981); 8: Sturm et al. (1981); 9: Sturm et al. (1982); 10: Surikov and Romanova (1978); 11: Sybenga and Mastenbroek (1980); 12: Sybenga and Prakken (1962); 13: Sybenga and Wolters (1972); 14: Watkins and White (1964).
* no direct evidence for identity of *a* and *an* available
** *a* and *an* are identical

Although the descriptions from various authors show a high degree of resemblance, direct evidence for the similarity of the anthocyanin genes *an*, *a* and *vi* (table 1) is not available yet. (The gene *an* is identical with *a* described in references 11, 12 and 13 of table 1). All crosses, except those involving *ct2*, *wil* and *ms* from 'WIR 10415', were made between plants from self compatible spring type lines, mainly of Petkus rye origin. Plants were grown in a greenhouse at 18-20 ^{o}C; from populations which segregated for both *ct2* and *wil*, winter type plants were grown to maturity in the field. When available F2s of crosses between markers were grown when evidence for linkage had been obtained by gene localization using reciprocal translocations. Data concerning the F2 between *dw2* and *mo* (table 5) are from Sybenga and Prakken (1962).

Translocations (table 2)

Use was made of a selection from the tester set of reciprocal translocations (Sybenga, 1983) described by Sybenga and Wolters (1972) and de Vries and Sybenga (1976). In this article the chromosome nomenclature of these authors will be applied. In table 2 the proposals concerning the correspondence with the standard nomenclature of the *Triticinae* (Sybenga, 1983) are provisional. The suggestion of Schlegel and Mettin (1982) results from an extensive literature survey, while the proposal of Sybenga (in prep.) is based on preliminary data from crosses between the translocation tester set and the'Imperial-Chinese Spring'standard addition set. In general, all translocation heterozygotes of table 2 when selfed give rise to an offspring with translocation homozygotes (TT), -heterozygotes (TN) and normal (NN) plants, segregating 1:2:1. Exceptions are TN 303 and TN 242. From TN 303, homozygotes have never been recovered, although both female and male translocation (T)-gametes have proven to be transmitted. In TN 242 one duplication gamete is fertile on the female as well as on the male side, which results in viable duplication heterozygotes (Sybenga and Verhaar, 1980) and even homozygotes. Most TT and TN plants are viable and fertile, although male fertility of TT indivuals may in a few instances be somewhat reduced.

Table 2. Reciprocal translocations of rye used in this study.
a and S: short arm; b and L: long arm

Code number	de Vries and Sybenga (1976)	Schlegel and Mettin (1982)	Sybenga (in prep.)
240	IIa-VIb	2RS-5RL	3RS-5RL
242[a]	III-Vb	7R -6RL	2R -6RL
248	Va-VIIa	6RS-1RS	6RS-1RS
273	VIa-VIIb	5RS-1RL	5RS-1RL
282	Ia-VIb	3RS-5RL	7RS-5RL
303	Ib-VIb	3RL-5RL	7RL-5RL
305[a]	III-VIa	7R -5RS	2R -5RS
501	IVb- VIb	4RL-5RL	4RL-5RL

[a]: Recent observations have shown that the breakpoints of translocations 242 and 305 are located in different arms of chromosome III.

Table 3. Expected frequencies of the four backcross- and the six F2 classes in terms of the recombination fraction r. Between brackets: designations for numbers observed

Backcross data

Phenotype	Karyotype TN	Karyotype NN	Segregation of marker
Dominant	$\frac{1-r}{2}$ (n_1)	$\frac{r}{2}$ (n_2)	1/2
Recessive	$\frac{r}{2}$ (n_3)	$\frac{1-r}{2}$ (n_4)	1/2
Segregation of translocation	1/2	1/2	1 (n)

F2 data

Phenotype	Karyotype TT	Karyotype TN	Karyotype NN	Segregation of marker
Dominant	$\frac{1-r^2}{4}$ (n_1)	$\frac{1-r+r^2}{2}$ (n_2)	$\frac{2r-r^2}{4}$ (n_3)	3/4
Recessive	$\frac{r^2}{4}$ (n_4)	$\frac{r-r^2}{2}$ (n_5)	$\frac{1-2r+r^2}{4}$ (n_6)	1/4
Segregation of translocation	1/4	1/2	1/4	1 (n)

Gene localization

Crosses were made between plants with normal (NN) karyotype, homozygous for the marker gene, and wild type translocation homozygotes (TT) as far as available. F1 seedlings were karyotyped for safety and selfed and/-or backcrossed with homozygous recessive NN plants, and the offspring karyotyped and scored for the marker.

Since gametes which carry a duplication for one chromosome segment and a deficiency for another are not functional (except with TN 242), the frequencies of F1 gametes taking part in the reproduction may be written as:

gamete	*TA*	*NA*	*Ta*	*Na*
frequency	$\frac{1-r}{2}$	$\frac{1-r}{2}$	$\frac{r}{2}$	$\frac{r}{2}$

in which r is the recombination fraction between a locus *A-a* and a translocation breakpoint. Table 3 contains the expected frequencies of the four backcross and the six F2 classes in terms of r, based on the assumption that r is the same in both female and male gametogenesis. To detect linkage, contingency tests were carried out in the case of backcrosses, while r was estimated from $(n_2+n_3).n^{-1}$ (table 3). In the case of F2 data, linkage was detected using a log-likelihood ratio test, and estimates of r were obtained by maximization of

$$\frac{n!}{n_1!n_2!n_3!n_4!n_5!n_6!} \cdot \frac{(1-r^2)^{n_1}}{4} \cdot \frac{(1-r+r^2)^{n_2}}{2} \cdot \frac{(2r-r^2)^{n_3}}{4} \cdot \frac{(r^2)^{n_4}}{4} \cdot \frac{(r-r^2)^{n_5}}{2} \cdot \frac{(1-2r+r^2)^{n_6}}{4} \cdot$$

(table 3)

When segregations of the marker and/or the translocation do not fit their expected 3:1 and 1:2:1 ratios respectively, this procedure leads to an underestimation of r.

Kramer (1954), applying principally the same procedure for barley, presents a different likelihood term. With regard to the translocation only two classes, semi-sterile and fertile were distinguished, corresponding to TN and (TT+NN) respectively. In rye, however, semi-sterility is no adequate marker, and the translocations used can be scored readily in root tips.

Backcross and F2 data involving translocation 242 are compiled in the same way, disregarding all plants carrying a duplication. To obtain comparable estimates of r this is legitimate, as 'normal' translocation heterozygotes eliminate their duplication offspring also, but at an earlier stage. The duplication class did not yield additional information.

Karyotypes were classified in root tip mitoses after pretreatment in saturated alpha bromonaphthalene for 2 hours at 24 °C, fixation-maceration in 1 N HCl for 12 min. at 60 °C and Feulgen staining. For any specific combination of a marker gene and a translocation all available backcross or F2 data were pooled, as heterogeneity was not significant. Backcross data of *an* and *br* involving translocation 282 (table 4A) are from Sybenga and Mastenbroek (1980).

RESULTS

Segregation data on backcrosses and F2s between markers and translocations are found in tables 4A and 4B. Table 4A lists the data from populations in which linkage between the marker and the translocation could be detected, whereas the markers and translocations of table 4B showed independent segregation.

Table 5 contains F2 data from crosses between five pairs of marker genes of which location on the same chromosome for each pair was evident from their linkage with translocations.

DISCUSSION

The correspondence between our chromosome nomenclature and that of the *Triticinae* (between brackets) is based on the proposal of Sybenga (see table 2).

Chromosome I (7R)

Chromosomes I and VI are involved in translocations 282 and 303. Without providing segregation data, Sybenga and Wolters (1972) already mentioned linkage between *an* and translocation 282 while no linkage was found with translocations involving chromosomes VI and II (240) and VI and VII (273) respectively. They concluded that *an* is located on chromosome I. Sybenga and Mastenbroek (1980) investigated the meiotic behaviour of translocation 282 and obtained evidence for the location of *an* in arm Ib. The difference between the recombination fractions of *an* with translocations 282 and 303 (table 4A) is discussed extensively by de Vries (1983).

Linkage between *an* and *ct1* (table 5) is very close, because in an F2 (repulsion phase) of 662 plants no recombinants were found. It may be safely concluded that *an* and *ct1* are located on the same arm of chromosome I.

Chromosome II (3R)

Translocation 240 involves chromosomes II and VI. Tables 4A and 4B shows a close linkage of *dw3* with the translocation breakpoint of translocation 240, and independent segregation from translocations 273 and 282 involving chromosome VI and VII, and I and VI respectively, and consequently *dw3* is located on chromosome II.

Table 4A. Linkage between 12 marker genes and 8 reciprocal translocations in rye. B: backcross, n: population size, r: estimated recombination fraction, s_r : standard deviation of the estimated recombination fraction; r is significantly different from 0.5, and single factor ratios fit their expectations at the 5% level, unless indicated

Gene/ translocation	Chromosomes involved	B or F2; notes	n	Dominant TT	Dominant TN	Dominant NN	Recessive TT	Recessive TN	Recessive NN	$r \pm s_r$
an -282	I-VI	B;*a*	320	7	160	-	144	9	-	0.050±0.012
an -303	I-VI	B;*b*	429	-	45	187	-	149	48	0.217±0.020
ct1-282	I-VI	B;*c*	349	-	168	10	-	5	166	0.043±0.011
dw3-240	II-VI	F2	55	16	26	0	0	2	11	0.041±0.027
mo -242	III-V	B	58	-	23	12	-	6	17	0.310±0.061
mo -242	III-V	F2	52	16	16	6	2	6	6	0.326±0.076
mo -305	III-VI	B	60	-	33	1	-	1	25	0.033±0.023
mo -305	III-VI	F2	71	18	34	0	0	1	18	0.014±0.014
Ps -242	III-V	B	34	-	5	9	-	17	3	0.235±0.073
Ps -305	III-VI	F2;*d*	58	3	25	9	8	10	3	0.327±0.072
dw2-305	III-VI	B	68	-	26	5	-	4	33	0.132±0.041
lg1-501	IV-VI	F2;*e*	244	-	-	-	0	11	26	0.149±0.041
wh -242	V-III	F2	51	5	27	6	1	3	9	0.216±0.064
wh -248	V-VII	F2	50	10	28	0	0	0	12	0.0
br -282	VI-I	B;*a*	320	7	158	-	144	11	-	0.056±0.013
ti -282	VI-I	B;*c*	349	-	171	1	-	2	175	0.009±0.005
ti -273	VI-VII	B	184	-	76	5	-	9	94	0.076±0.020
ct2-240	VI-II	F2;*f*	95	22	32	18	0	7	16	0.264±0.052
wil-240	VI-II	F2;*f*	95	22	36	13	0	3	21	0.166±0.041
wil-501	VI-IV	F2;*g*	105	10	47	17	3	11	17	0.323±0.054

a : from Sybenga and Mastenbroek (1980)
b : segregation of TN 303 disturbed ($0.02<P<0.05$)
c : same backcross
d : segregation of *Ps* disturbed ($0.02<P<0.05$)
e : only recessives karyotyped ($n = 37$); segregation of TN 501 1:2:1 in random sample of 30 F2 plants (see text)
f : same F2s ; see also table 7a
g : segregation of TN 501 disturbed ($P<0.01$); same F2 as *ct2*-501 in table 4B; see also table 7b.

Table 4B. Independent segregation of 6 markers and 7 reciprocal interchanges in rye. r is not significantly different from 0.5 and single factor ratios fit their expectations at the 5% level, unless indicated. For further legend see table 4A.

Gene/ translocation	Chromosomes involved	B or F2; notes	n	Dominant TT	Dominant TN	Dominant NN	Recessive TT	Recessive TN	Recessive NN	$r \pm s_r$
dw3-273	VI-VII	F2	54	12	16	15	1	5	5	0.428±0.082
dw3-282	I-VI	F2;*a*	96	7	43	23	2	14	7	0.507±0.062
mo -240	II-VI	F2	73	7	29	18	3	11	5	0.536±0.071
mo -273	VI-VII	B	92	-	17	29	-	22	24	0.554±0.052
mo -282	I-VI	F2	105	21	38	21	6	11	8	0.472±0.060
Ps -240	II-VI	B	55	-	10	14	-	14	17	0.491±0.067
Ps -273	VI-VII	B;*b*	72	-	14	10	-	24	24	0.528±0.059
lg1-240	II-VI	F2;*c*	341	-	-	-	9	25	16	0.430±0.050
lg1-305	III-VI	F2;*c*	108	-	-	-	1	5	3	0.389±0.115
lg1-242	III-V	B	35	-	7	10	-	7	11	0.486±0.084
lg1-273	VI-VII	B	24	-	5	7	-	5	7	0.500±0.102
ct2-501	IV-VI	F2;*d*	105	9	45	21	4	13	13	0.423±0.059
ct2-248	V-VII	F2;*e*	96	7	39	24	7	9	10	0.544±0.062
wi1-248	V-VII	F2;*e*,*f*	96	5	38	26	9	10	8	0.629±0.059

a: segregation of TN 282 disturbed (P<0.01)
b: segregation of *Ps* disturbed (P<0.01)
c: only recessives karyotyped (see text)
d: segregation of TN 501 disturbed (P<0.01); same F2 as *wi1*-501 in table 4A; see also table 7b
e: segregation of TN 248 disturbed (0.01<P<0.02); same F2s
f: r is significantly different from 0.5 (P=0.04) due to fortuitous disturbance of *wi1*-segregation among TT-individuals

Table 5. Phenotype segregation in F2s derived from crosses between five pairs of marker genes. C or R: coupling or repulsion phase. +,+: dominant alleles of both markers expressed, +,2: expression of dominant allele of marker 1, recessive of marker 2; 1,+: expression of recessive allele of marker 1, dominant of marker 2; 1,2: recessive alleles of both markers expressed. r estimated according to Stevens (1940). r differs significantly from 0.5, and single factor ratios fit 3:1 expectation at the 5% level in all instances

Markers 1 - 2	C or R	Segregation +,+	+,2	1,+	1,2	$r \pm s_r$
an - *ct1*	R	343	144	175	0	0.0
Ps - *mo*	C	173	28	36	28	0.297±0.035
Ps - *dw2*	C	92	3	0	40	0.022±0.013
mo - *dw2*	R[a]	219	94	107		0.315±0.123
ct2 - *wi1*	C[b]	64	6	5	21	0.117±0.035

[a]: from Sybenga and Prakken (1962). Owing to cryptomeric masking of the *Dw2*-*dw2* segregation by *mo mo*, double recessives could not be recognized
[b]: TN 248 segregated in the same F2, independent from *ct2* and *wi1* (see table 4B)

Chromosome III (2R)

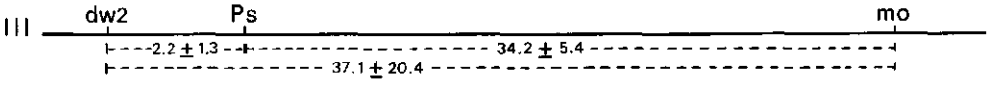
Translocations 242 (between III and V) and 305 (between III and VI) both involve chromosome III. Linkage between these translocations and *mo* was established in backcrosses as well as in F2s (table 4A), while no linkage was observed with translocations not involving chromosome III (table 4B). Evidently, *mo* is located on chromosome III.

Backcross data of *Ps* and translocation 242 as well as F2 data of *Ps* and translocation 305 revealed linkage (table 4A), although in the latter case the significance was marginal ($\chi^2_2 = 5.069$; $P \approx 0.025$) due to a shortage of *Ps*-individuals. The expression of *Ps* is influenced by both genetic background and environmental factors, and the *Ps*-shortage may well be attributed to the circumstance that this F2 was grown during winter. It is concluded safely however, that *Ps* is located on chromosome III. This conclusion is confirmed by the linkage between *Ps* and *mo* in an F2 (coupling phase) of 265 plants (table 5), and is also in agreement with the independent segregation of *Ps* from translocations 240 and 273 (table 4B), which both do not involve chromosome III.

Translocation 305 and *dw2* appeared to be linked also (table 4A). Moreover close linkage between *dw2* and *Ps* was found in an F2 (coupling phase) of 135 plants (table 5), while Sybenga and Prakken (1962) already presented data on the linkage between *dw2* and *mo*, symbolized as *d2* and *c* in their report (table 5). It is evident that *dw2* is also located on chromosome III.

The most probable arrangement of *dw2*, *Ps* and *mo* on chromosome III is given in fig. 1. The relatively large standard deviations of the recombination fraction (table 5) and the map distance (fig. 1) between *dw2* and *mo* are due to cryptomeric masking of the *Dw2-dw2* segregation by *mo mo*, so that only the classes *MoDw2* and *Modw2* are available for the estimation of *r*. Because three point tests in which *dw2*, *Ps* and *mo* are involved simultaneously have not yet been carried out, an alternative arrangement, with *dw2* between *Ps* and *mo* remains possible. The position of the genes relative to the centromere, as well as their arm location are not known yet.

Fig. 1. Most probable arrangement of *dw2*, *Ps* and *mo* on chromosome III. Position of centromere unknown. Map distance and standard deviations in cM, corrected according to Kosambi (1944). See also table 5.



Chromosome IV (4R)

Table 6 shows the segregation of translocation 501 (involving chromosomes IV and VI) among 37 out of 39 *lg1 lg1* seedlings from an F2 of 244 individuals. The segregation of the marker does not fit its 3:1 expectation due to a clear shortage of *lg1 lg1* plants (table 4A). To avoid extensive karyotyping, only root tips of light green plants were examined. Translocation 501 segregated 1:2:1 in a random sample of 30 F2 seedlings, of which two were *lg1 lg1*.

In table 6 the deviation from the 1:2:1 segregation of translocation 501 in the *lg1 lg1*-class, which is expected under the assumption of r = 0.5, is highly significant, whereas in F2s between *lg1* and translocations 240 and 305 in which chromosome IV is not involved, no deviations from random segregation in the *lg1 lg1*-class were found (table 4B). Also, no linkage could be detected in backcrosses between *lg1* and translocations 242 (III-V) and 273 (VI-VII). It is therefore concluded that *lg1* is located on chromosome IV.

In all F2s in which *lg1* segregated (table 4A and 4B), a significant shortage of *lg1 lg1* plants was found, while in backcrosses between comparable translocation heterozygous F1 mother plants and *lg1 lg1* plants with a genetic background different from the mother (table 4B), the observed segregation was in good agreement with 1:1. Apparently, there is certation against pollen with genotype *lg1*. In addition, embryolethality may play a role, because F2s with lower germination rates tended to show a greater *lg1* shortage. As in the backcrosses no *lg1* shortage was observed, an effect of inbreeding on embryolethality must also be assumed.

Table 6. Karyotype distribution among 37 out of 39 *lg1 lg1* seedlings from an F2 (n = 244) between *lg1* and translocation 501 (IV-VI); 247 seeds were sown.

Karyotype	TT	TN	NN	Total	
observed	0	11	26	37	
expected (r = 0.5)	9.25	18.5	9.25	37.0	χ^2 = 42.62 (P<0.01)

Chromosome V (6R)

Chromosome V is involved in translocations 242 and 248. Linkage was established in F2s between both translocations and *wh* (table 4A), which must be located on chromosome V. No recombinants were found in the F2 with translocation 248.

Chromosome VI (5R)

Chromosome VI is involved in translocations 240 (II-VI), 273 (VI-VII), 282 (I-VI) and 501 (IV-VI).

Sybenga and Wolters (1972) already found linkage between *br* and translocations 240, 282 and 273, and between *ti* and translocations 240 and 282, and they concluded that *br* and *ti* are located on chromosome VI. Table 4A lists the recombination fractions between *ti* and translocations 273 and 282 found in the present study in addition to the data from Sybenga and Mastenbroek (1980) concerning *br* and translocation 282. Using a telocentric trisomic for the short arm of VI, the latter authors demonstrated the location of *br* in this arm. In F2s (repulsion phase) between *br* and *ti* of several thousands of plants, no recombinants were found, and F3 progeny testing did not reveal recombination either (Sybenga, unpublished). It is, therefore, concluded that *br* and *ti* are very closely linked, and both located in the short arm of VI.

Independent segregation of translocation 248 (V-VII) from *ct2* and *wi1* respectively (table 4B) was found in the F2 of the cross TT *Wi1 Wi1 Ct2 Ct2* x NN *wi1 wi1 ct2 ct2*. The same F2 however revealed linkage between *ct2* and *wi1*, (table 5), suggesting the location of both markers in one chromosome, different from V or VII. Linkage between *ct2* and *wi1* was established also in two F2s which involved translocations 240 (II-VI) and 501 (IV-VI) respectively (table 7a-b). Moreover, linkage between *wi1* and both these translocations appeared to be significant (table 4A), even in spite of a disturbed segregation of translocation 501. In addition, *ct2* is linked with translocation 240 (table 4A), but no sufficient support for weak linkage with translocation 501 was found (table 4B). Nevertheless it is evident that *ct2* and *wi1* are both located on chromosome VI. F2s with *br* and *ti* were not available.

Table 7. Segregation in the F2 from *Wi1 Wi1 Ct2 Ct2* TT x *wi1 wi1 ct2 ct2* NN, involving translocations 240(a) and 501 (b), respectively. r is significantly different from 0.5 at the 5% level, except with *ct2*-501. For fit of single factor ratios with their expectations, see tables 4A and 4B

(a) Karyotype	Phenotype				Total	Linkage between:	$r \pm s_r$
	+,+	+,*ct2*	*wi1*,+	*wi1*,*ct2*			
TT	22	0	0	0	22	*wi1*-*ct2*	0.148±0.040
TN	32	4	0	3	39	*wi1*-240	0.166±0.041
NN	11	2	7	14	34	*ct2*-240	0.264±0.052
Total	65	6	7	17	95		

(b) Karyotype	Phenotype				Total	Linkage between:	$r \pm s_r$
	+,+	+,*ct2*	*wi1*,+	*wi1*,*ct2*			
TT	7	3	2	1	13	*wi1*-*ct2*	0.210±0.046
TN	41	6	4	7	58	*wi1*-501	0.323±0.054
NN	16	1	5	12	34	*ct2*-501	0.423±0.059
Total	64	10	11	20	105		

Table 8. (a) segregation observed in an F2 involving translocation 240 and the complementary genes *wi1* and *wi2*; (b) expected frequencies in terms of the recombination fraction r between *wi1* and TN 240, assuming independent segregation of *wi2* from *wi1* and TN 240 respectively; (c) expected segregation when r = 0.152 (cf. table 4A)

(a) Phenotype	Karyotype			Total
	TT	TN	NN	
Spring type	10	40	23	73
Winter type	18	27	7	52
Total	28	67	30	125

(b) Phenotype	Karyotype			Total
	TT	TN	NN	
Spring type	$\frac{6-6r-3r^2}{16}$	$\frac{6-6r+6r^2}{16}$	$\frac{3-3r^2}{16}$	9/16
Winter type	$\frac{4-6r+3r^2}{16}$	$\frac{2+6r-6r^2}{16}$	$\frac{1+3r^2}{16}$	7/16
Total	1/4	1/2	1/4	1

(c) Phenotype	Karyotype			Total
	TT	TN	NN	
Spring type	6.58	40.83	22.90	70.31
Winter type	24.67	21.67	8.35	54.69
Total	31.25	62.50	31.25	125.00

The observed segregation (a) fits the expectation (c): $\chi^2 = 5.13$; d.f. = 4

gr is also located on chromosome VI. Its localization will be discussed in the section concerning the arm location of several markers.

Chromosome VII (1R)

None of the markers investigated in this study could be localized on the satellite chromosome.

Four markers *lg2*, *ms*, *sr* and *wi2* have remained unlocalized. From the segregation of *lg2* in the offspring of a compensating trisomic involving the telocentric of VIa, it was concluded that this marker is not located on the short arm of chromosome VI. Due to scoring difficulties, *sr* could not be localized. In an F2 between *sr* and *dw2* - in which *sr* could be scored unequivocally - the markers segregated independently from each other.

Correct scoring of *ms* was hampered by male sterility of translocation homozygotes, which occurred in several F2s.

Sybenga (pers. comm.) observed segregations of spring vs. winter types in several F2s, indicating the action of two complementary winter type genes with independent segregation. Surikov and Romanova (1978) also assume participation of two factors in the control of winter or spring habit. In the present study, one F2 involving *wi1* and TN 240 showed a segregation between spring and winter types of 73:52 (table 8a), deviating significantly from the expected 3:1 ratio (χ^2 = 18.37), but fitting a 9:7 ratio (χ^2 = 0.23), so that we suppose that there is a second winter type gene *wi2*, complementary with and segregating independently from *wi1*. Table 8b contains the frequency expectations of the six F2 classes under the assumption that *wi2* is not linked with translocation 240 either, while *r* is the recombination fraction between *wi1* and TN 240. When 0.152 is substituted for *r* (table 4A), the expected numbers of table 8c are found. The expectation fits the observed segregation (χ^2 = 5.13; P ≈ 0.25) which supports the assumption that there is no linkage between *wi2* and TN 240, indicating that *wi2* is not located on IIa or VIb.

Linkage groups

Linkage group numbering in this article corresponds with the chromosome nomenclature of the *Triticinae*, and follows the proposal of Sybenga

(table 2), which deviates from that given by Schlegel and Mettin (1982). Table 9 summarizes the conclusions of the present study, while also an attempt is made to relate the results with the linkage groups described by Schlegel and Mettin in their review. Except for *an*, *ct2*, *wi1* and *mo* (see 'MATERIALS AND METHODS'), identity of a marker from the present study with any one mentioned by Schlegel and Mettin in their table 8 is, in view of the source of the markers considered improbable.

As for linkage group 1, no marker genes are localized on the satellite chromosome so far.

Linkage group 2 contains the markers *mo*, *Ps* and *dw2*. Smirnov and Sosnikhina (1981) reported linkage between *m* (identical with *mo*, see table 1) and a gene *el* (elymoides), and between *el* and *ct*, from which they concluded that these markers are located on one chromosome. Based on the recombination fractions between *m* and *el* (r = 0.315) and *el* and *ct* (r = 0.435), and the independent segregation of *m* and *ct*, an arrangement *m-el-ct* was proposed. Provided however, that *ct2* is identical with *ct* (see table 1 and the discussion on linkage group 5), the localization of *m* and *ct* in the same chromosome is in disagreement with the findings of the present study. It may be argued whether the very high recombination fraction between *el* and *ct* found by Smirnov and Sosnikhina, provides sufficient evidence to conclude that *el* and *ct* are located in one chromosome. Considering the closer linkage between *el* and *m* (= *mo*), it was decided here to place *el* in linkage group 2, together with *mo*, *Ps* and *dw2* (table 9).

Linkage group 3 only contains *dw3*, and *lg1* is the only marker in linkage group 4.

Table 9. Linkage groups of rye. Numbering of linkage groups provisional, according to the correspondence between our chromosome nomenclature and that of the *Triticinae* as proposed by Sybenga (in prep.). Gene symbols between brackets are from table 8 in the review of Schlegel and Mettin (1982)

Linkage group	Chromosome	Markers
1	VII	
2	III	*mo,Ps,dw2(el)*
3	II	*dw3*
4	IV	*lg1*
5	VI	*br,ct2,gr,ti,wi1(ae,hs)*
6	V	*wh*
7	I	*an,ct1(a,wlb,vi,w)*
-	-	*wi2,sr,ms,lg2(ct,Hl)*

Linkage group 5 contains the markers *br*, *ct2*, *gr*, *ti* and *wil*. The compactum gene *ct2* was provided to our department by Fedorov in 1967 (code number *'gc8'*). Fedorov, Smirnov and Sosnikhina in their publication symbolized this gene as *ct* (table 1). Sturm et al. (1982) referring to these authors, reported the localization of *ct* on their chromosome B. According to Schlegel and Mettin (1982), this chromosome corresponds with 7R, which is our chromosome I (Sybenga, in prep.). Provided that *ct2* and *ct* are identical, Sturm's findings and the localization of *ct2* on chromosome VI (5R) in the present study are in conflict, because the morphological differences between chromosomes 5R and 7R are distinct. 7R is (sub)metacentric and has terminal C-bands in both arms of which the one in the shorter arm is often more pronounced, while 5R is subacrocentric with only one pronounced terminal C-band in the short arm (Schlegel and Mettin, 1982). Crosses between *ct2* and *ct* are required to test their identity. Until then *ct* cannot be assigned to any of the linkage groups established here. The same applies to the dwarf gene *Hl* which has been located on chromosome B also (Sturm and Engel, 1980). A recombination fraction between *Hl* and *ct* of 0.39 is mentioned by Schlegel and Mettin (1982).

Surikov and Romanova (1978) reported linkage between their markers *ae* (winter type) and *hs* (glabrous leaf sheath) ($r = 0.323 \pm 0.024$). With regard to their source, it is not unlikely that *wil* and *ae* are identical (see table 1), although again results of allelism tests are not available. Under this reserve *ae* and *hs* are placed in linkage group 5.

Linkage group 6 only contains *wh*.

Linkage group 7 contains *an* and *ct1*, closely linked. Several authors (see references of table 1) have described one main factor with monogenic recessive inheritance, effecting the anthocyanin colouring of the caryopsis (xenia), coleoptiles and nodes of rye seeds and plants while its expression may be influenced by one or more other genes. Sturm et al. (1981) reported the localization of a similar factor (*a*) on their chromosome C, corresponding with chromosome 3R according to Schlegel and Mettin (1982). Provided that *an* and *a* are identical - which has not been tested yet - and the proposal of Schlegel and Mettin is correct, there is disagreement between the findings of Sturm et al. and the localization of *an* on chromosome I (7R) in the present investigation. However, since the morphological differences between chromosomes 3R and 7R are not as distinct as between 5R and 7R (Schlegel and Mettin, l.c.),identity of chromosome C with I is not excluded, which may be tested by a cross between Sturm's trisomic C and translocation 282, involving chromosomes 5R and 7R.

Arm location

To establish the arm location of a marker, use can be made of telocentric trisomics, which is illustrated by the localization of *ti* and *br* on the short arm of VI. Tertiary and telocentric tertiary compensating trisomics (Khush, 1973) can also be applied, and may even provide accurate details on the markers' position.

Examples are given here of the use of tertiary trisomics in the localization of *lg1* and *ti*, and of telocentric tertiary compensating trisomics in the localization of *ct2*, *gr*, *wi1* and *wh*. The location of *an* on chromosome arm Ib has been established earlier by Sybenga and Mastenbroek (1980).

Table 10 shows the segregation of the gene *lg1* in the selfed offspring of a tertiary trisomic with chromosome VI^{IV} of translocation 501 as the extra chromosome. As discussed earlier, shortage in the *lg1* class is ob served in several F2s, giving segregations significantly deviating from 3:1 (table 4A and 4B). The opposite applies to the *lg1* segregation in table 10, where a significant excess of *lg1* individuals is found (χ^2 = 20.35). This segregation can only be explained, when the dominant allele *Lg1* is in the exchanged segment of chromosome VI^{IV}, with recessive alleles *lg1* on both normal chromosomes IV. (This is a balanced tertiary trisomic, symbolized by $\frac{Lg1}{T}\ \frac{lg1}{N}\ \frac{lg1}{N}$. Since the exchanged segment of translocation chromosome VI^{IV} corresponds with the terminal part of the long arm of chromosome IV (cf. de Vries and Sybenga, 1976), it is concluded that *lg1* is located terminally in IVb.

Based on the low recombination of *br* in a telocentric trisomic for the short arm of chromosome VI, Sybenga and Mastenbroek (1980) supposed that this marker is located fairly close to the centromere. This would also apply to *ti*, due to the very close linkage between *ti* and *br*. The same authors point out however, that the distance between the locus and the centromere might have been underestimated as a result of reduced pairing in the centromere region of telocentric trisomics. The segregation of *ti* in the selfed offspring of a balanced tertiary trisomic with chromosome III^{VI} of translocation 305 as the extra chromosome (genotype $\frac{Ti}{T}\ \frac{ti}{N}\ \frac{ti}{N}$; table 11) revealed that the tigrina locus (*ti*) is located in the small exchanged segment of chromosome III^{VI} which corresponds with the terminal part of VIa, so that *ti* has indeed a more terminal position than previously assumed.

Table 10. *lg1* segregation in the selfed offspring of a balanced tertiary trisomic with an extra translocation chromosome VI^{IV} of 501. VI^{IV} carries the dominant *Lg1* allele, while both copies of IV carry the recessives (see text). Presence of one copy of VI^{IV} indicated by 'T', of IV by 'N'

Phenotype	Karyotype			
	TTNN*	TNN	NN	Total
Lg1	1	18	14	33
lg1	0	5	27	32
Total	1	23	41	65

* VI^{IV} transmitted by pollen and egg

Table 11. *ti* segregation in the selfed offspring of a balanced tertiary trisomic with an extra translocation chromosome III^{VI} of 305. III^{VI} carries the dominant *Ti* allele, while both copies of VI carry the recessives (see text). Presence of one copy of III^{VI} indicated by 'T', of VI by 'N'

Phenotype	Karyotype		
	TNN	NN	Total
Ti	158	2	160
ti	1	202	203
Total	159	204	363

Table 12 contains data concerning the segregation of *ct2*, *gr* and *wi1* in backcross- and selfed offsprings of a telocentric tertiary compensating trisomic, in which one chromosome II is replaced by telocentric IIa and chromosome II^{VI} of translocation 240 ("compensating trisomic 240-IIa"). The two normal homologues of chromosome VI are present in this trisomic, while the exchanged segment of chromosome II^{VI} corresponds with a large part of the long arm of VI (fig. 2).

Table 12. Segregation of *ct2*, *gr* and *wi1* in offsprings of compensating trisomic 240-IIa (fig. 2). 'T' indicates simultaneous presence of translocation chromosome II^{VI} and telocentric IIa, 'N' represents one copy of VI. (a) *ct2* segregation in a cross between compensating trisomic 240-IIa in which one copy of VI carries a dominant, the other a recessive *ct2* allele, and *ct2 ct2* NN male tester parents. (b), (c) and (d) segregation of *gr*, *wi1* and *ct2* respectively in the selfed offspring of a compensating trisomic 240-IIa in which one of the copies of VI carries the dominant, the other the recessive alleles of these genes. In all instances, the dominant allelle is located on the exchanged segment of II^{VI}.

(a)

Phenotype	Karyotype		
	TNN	NN	Total
Ct2	27	63	90
ct2	1	39	40
Total	28	102	130

(b)

Phenotype	Karyotype		
	TNN	NN	Total
Gr	21	73	94
gr	0	15	15
Total	21	88	109

(c)

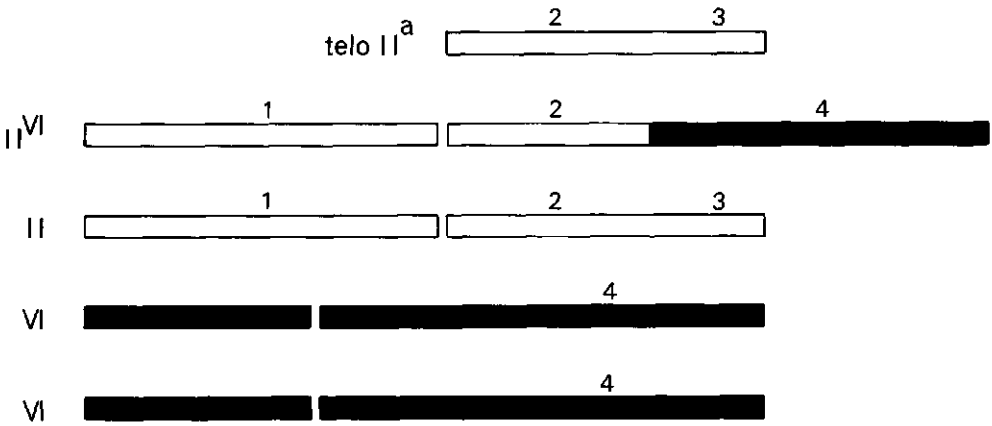
Phenotype	Karyotype		
	TNN	NN	Total
Wi1	21	70	91
wi1	0	13	13
Total	21	83	104*

(d)

Phenotype	Karyotype		
	TNN	NN	Total
Ct2	21	52	73
ct2	0	12	12
Total	21	64	85**

* : no ears observed in 5 *gr* plants (compare table 12b)
**: *ct2* scoring impossible in all 15 *gr* plants and in 9 out of 13 *wi1* plants (compare tables 12b and c)

Fig. 2. Telocentric tertiary compensating trisomic 240-IIa .
Telocentric IIa and chromosome II^{VI} of translocation 240 compensate the absence of one normal chromosome II. Both normal chromosomes VI are present.
Numbers indicate different positions of the gene *gr* (see text): 1 - on IIb; 2 - on IIa and interstitially in II^{VI}; 3 - on IIa but not interstitially in II^{VI}; 4 - on VIb.



The location of *wi1* and *ct2* on chromosome VI has already been discussed, and from tables 12a, c and d it is concluded that both markers are located in the long arm VIb.

Grass type (*gr*) individuals spontaneously segregated in the same F2 population. In table 12b, the *gr* segregation among the 21 trisomics deviates significantly from 3:1 (χ^2 = 7.0), so that evidently this marker is also located on one of the 'compensating' chromosomes, i.e. either in telo IIa, in translocation chromosome II^{VI}, or both (fig. 2). The *gr* segregation among the 88 normal individuals (table 12b) fits a 3:1 ratio (χ^2 = 2.97), indicating that both the dominant and the recessive *gr* allele are present in the normal genome. Since in compensating trisomic 240-IIa the normal chromosome II is present single, it is concluded that the gene *gr* is located on chromosome VI (arm VIb, position 4 in fig. 2), of which both normal homologues are present. Data on linkage of *gr* with *wi1* and *ct2* are not yet available.

A diagram of chromosome VI with the approximate position of its markers is given in fig. 3.

Fig. 3. Arrangement of the markers *br*, *ct2*, *gr*, *ti* and *wi1* on chromosome VI. C is centromere. Arrows indicate breakpoint positions of translocations 240 and 305. Position of *gr* relative to *wi1* and *ct2* is yet unknown. Recombination fractions are from table 7a.

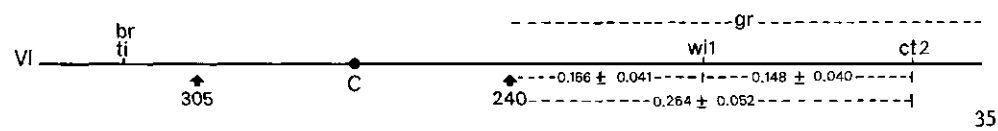


Table 13. *wh* segregation in the selfed offspring of compensating trisomic 248-VIIa. Simultaneous presence of translocation chromosome VII^V and telocentric VIIa indicated by 'T', of normal chromosome V by 'N'. Both copies of V carry the recessive *wh* allele, while VII^V carries the dominant allele on its exchanged segment (see text)

Phenotype	Karyotype			Total
	TTNN*	TNN	NN	
Wh	5	79	3	87
wh	0	0	138	138
Total	5	79	141	225

* : chromosomes VII^V and VIIa transmitted by pollen and egg

The segregation of *wh* in the selfed offspring of a balanced telocentric tertiary compensating trisomic in which one of the satellite chromosomes is replaced by the telocentric of the satellite arm and chromosome VII^V of translocation 248 ("compensating trisomic 248-VIIa, genotype $\frac{Wh}{T}\frac{wh}{N}\frac{wh}{N}$) is given in table 13. The localization of *wh* on chromosome V, which is present two fold in the trisomic, has been discussed in a previous section. Because the small exchanged segment of chromosome VII^V is identical with the terminal part of Va, it is concluded from table 13 that *wh* is located terminally in the short arm of chromosome V.

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Theor Appl Genet (1983) 66:329–339

2. High recombination between the breakpoint of a reciprocal translocation in rye (*Secale cereale* L.) and an interstitially located gene.

J. N. de Vries
Department of Genetics, Agricultural University, Generaal Foulkesweg 53, NL-6703 BM Wageningen, The Netherlands

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Summary. The recombination fraction between the interstitially located gene *an* and interchange 303 of rye was found to be 0.244 ± 0.038 in a test cross using the translocation as the male parent. In first metaphase translocation configurations in pollen mother cells of the same plant, the chiasma frequency between *an* and the translocation breakpoint was found to be significantly more than twice the recombination fraction. Recombination was concluded to be masked by a difference in the alternate frequency between configurations without interstitial chiasmata and configurations with interstitial chiasmata, the effect of the first type being of major importance. Random centromere orientation of translocation multivalents with interstitial chiasmata was concluded to be a realistic assumption. The exceptionally high recombination between *an* and translocation 303 is discussed. Consideration is also given to the use of interchanges in the establishment of a marker's chromosomal position, and to the use of translocation chromosomes in balanced systems for hybrid breeding purposes.

Key words: *Secale cereale* L. – Translocations – Interstitial chiasma formation – Centromere orientation – Recombination

Introduction

In interstitial segments of interchange heterozygotes, chiasma formation and consequently recombination are usually reduced due to disturbed pairing around the translocation breakpoint. In addition, observed recombination is affected by the orientation – alternate or adjacent – of the translocation configuration (Lamm 1948). Kramer and Blander (1961) presented formulae describing the influence of centromere orientation and interstitial chiasma frequency on the observed recombination fraction between interstitially located genes and the translocation breakpoint. In their report the frequencies of alternate orientation and interstitial chiasma formation were based on the level of semisterility, while no further cytological observations were made.

The present article deals with the recombination between a reciprocal translocation of rye (*Secale cereale* L.) and an interstitially located morphological marker with monogenic recessive inheritance. The relation between observed recombination, the observed frequencies of first metaphase (MI) configurations formed by the interchange and their orientation is analyzed. In addition, some consideration is given to the interpretation of recombination fractions between genes and translocations in determining the chromosomal positions of marker genes, and to the use of translocations in balanced systems, set up for the maintenance of genetically male sterile seed stocks for hybrid breeding purposes.

Model: relation between meiotic configuration frequencies and recombination fraction

In karyotypically normal diploids, with maximally one chiasma between two loci, the chiasma frequency is twice the recombination fraction. For a gene located interstitially in an interchange heterozygote (Fig. 1), the degree in which the chiasma frequency between the gene and the breakpoint is reflected in the recombination fraction is determined by the centromere segregation (alternate, adjacent-1 or adjacent-2). Considering

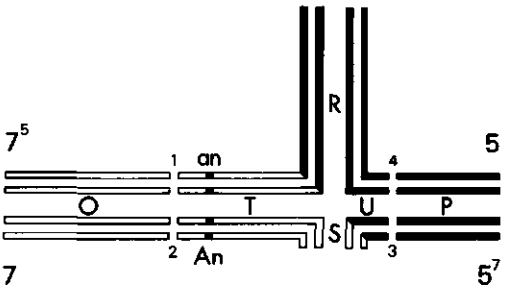

Fig. 1. Pairing diagram of translocation heterozygote 303 between 5RL and 7RL. *O* and *P:* unchanged arms, *R* and *S:* exchanged segments, *T* and *U:* interstitial segments. Locus *An – an* (anthocyanin vs. anthocyaninless) located in T, i.e. on 7RL, not far from centromere. Recessive allele on translocation chromosome. With alternate disjunction, centromeres 1 and 3 move to same pole, 2 and 4 to opposite pole. With adjacent-1 disjunction, centromeres 1 and 4 move to one pole, 2 and 3 to the other pole. With adjacent-2 disjunction, homologous centromeres (1 and 2, and 3 and 4 respectively) move to same poles

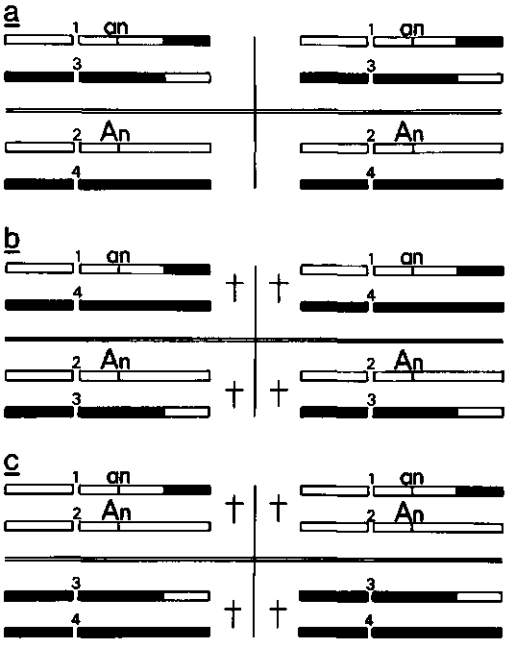

Fig. 2. Tetrads from alternate (**a**), adjacent-1 (**b**) and adjacent-2 (**c**) disjunction after chiasma formation in at least 3 out of 4 end segments *O, P, R* and *S* (Fig. 1). Chromatids are not drawn proportional to length. *Double line:* reductional cell wall, *single line:* equational cell wall. Abortive cells are marked with cross. There is no recombination between marker and translocation breakpoint

the reduced pairing around translocation breakpoints, it is realistic to assume that a maximum of one chaisma is formed in the interstitial segment. Numerical non-disjunction is assumed to be infrequent and will not be considered.

With zig-zag (alternate) disjunction, the two alternate (non-homologous) centromeres move pairwise to the same pole. With adjacent-1 disjunction two adjacent non-homologous centromeres move to the same pole. Homologous adjacent centromeres move to the same pole with adjacent-2 disjunction (Fig. 1). The frequency of alternate orientation among translocation multivalents without interstitial chiasmata (ring-of-4, chain-of-4, chain-of-3 with univalent), is z. For multivalents with interstitial chiasmata (closed-figure-8, frying-pan, O. K.- and Y-shaped multivalents), the alternate orientation frequency is z'. The frequency of multivalents in which either one or both exchanged segments R and S (Fig. 1) have chiasmata, in addition to at least one of the segments O, P, T or U, is e, and $1-e$ is the frequency of configurations without chiasmata in the exchanged segments. The chiasma frequency in interstitial segment T (Fig. 1) is t, that in U is u. The fraction of the chiasmata in segment T formed between interstitial locus *An-an* (Fig. 1) and the translocation breakpoint is f, whereas that between the locus and the centromere is $1-f$.

A formula can be derived which relates the recombination fraction (r) between gene and breakpoint to the chiasma frequency in this chromosome segment ($f \cdot t$) in dependence of the orientation frequencies. Table 1 lists the contribution of each group of translocation configurations to the four classes of reproductive gametes (translocation or normal, recombinant or non-recombinant). Multivalents (frequency e) without interstitial chiasmata (frequency $(1-t)\ (1-u)$) only contribute to the class of non-recombinant spores (Table 1). Since gametes originating from either adjacent-1 or adjacent-2 orientation are unbalanced, this contribution is directly proportional to the frequency of alternate orientation (z), from which four balanced gametes per configuration result (Fig. 2). The contribution to r of multivalents having a chiasma in one interstitial segment depends on whether the chiasma is formed in T or U, on the fraction of the chiasmata in T between marker and translocation breakpoint (f) and on the type of orientation. The assumption is made that adjacent-2 orientation, giving only unbalanced gametes, may be excluded, as co-orientation of homologous centromeres is very unlikely here. Absence of adjacent-2 is a reality in translocations between (sub-)metacentric chromosomes having at least one relatively large interstitial segment (Burnham 1950; Vosselman 1981). Two non-recombinant and two unbalanced gametes result from alternate disjunction (frequency z',

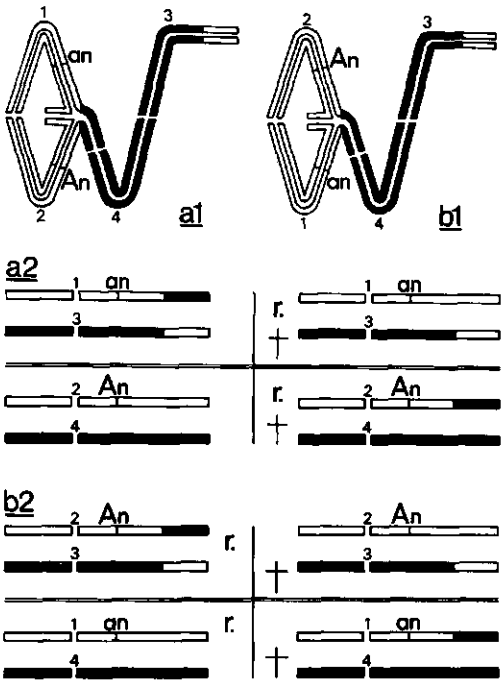

Fig. 3. Diagrams of MI-configurations (**a1, b1**) resulting from chiasma formation in *O, P, R* and in *T* between *An – an* and translocation breakpoint (chromosomes not drawn proportional to length; Fig. 1). Recombinant tetrad cells (**a2, b2**) are indicated with "*r*". For further legend see Fig. 2. *Note:* alternate (**a1**) and adjacent (**b1**) "frying pans" have identical morphology, but in tetrads from alternate disjunction (*a2*) recombinant chromatids are included in abortive cells, while recombinant tetrad cells from adjacent-1 disjunction (**b2**) are viable. Frying pans with chiasma in U (not in T) have smaller ring and larger "handle" than frying pans with chiasma in T (not in U) (Fig. 1)

Fig. 3), when a chiasma is formed between marker and breakpoint (fraction *f*), whereas adjacent-1 disjunction (frequency $1-z'$) gives two recombinant and two unbalanced spores. Irrespective of orientation type, only non-recombinant gametes result from chiasma formation between marker and centromere (fraction $1-f$) as well as from chiasma formation in U (frequency $(1-t)\,u$), 50% of the gametes being unbalanced again. Only as long as $z'=0.5$, equal proportions of recombinant and non-recombinant spores originate from multivalents having a chiasma between the gene and the breakpoint without a chiasma in U (Fig. 3). Irrespective of orientation type, multivalents always give rise to equal proportions of recombinant and non-recombinant gametes when a chiasma in U is formed simultaneously (Fig. 4). When both T and U have chiasmata, the contribution to each class of gametes, therefore, only depends on *f* (Table 1).

In Table 1, the production of viable gametes by cells with multivalents includes both trivalents with one univalent and quadrivalents. However, due to the loss of the univalents in the first meiotic division, the gamete production resulting from trivalents is half of that from quadrivalents. With considerable univalent frequency, this should be taken into account.

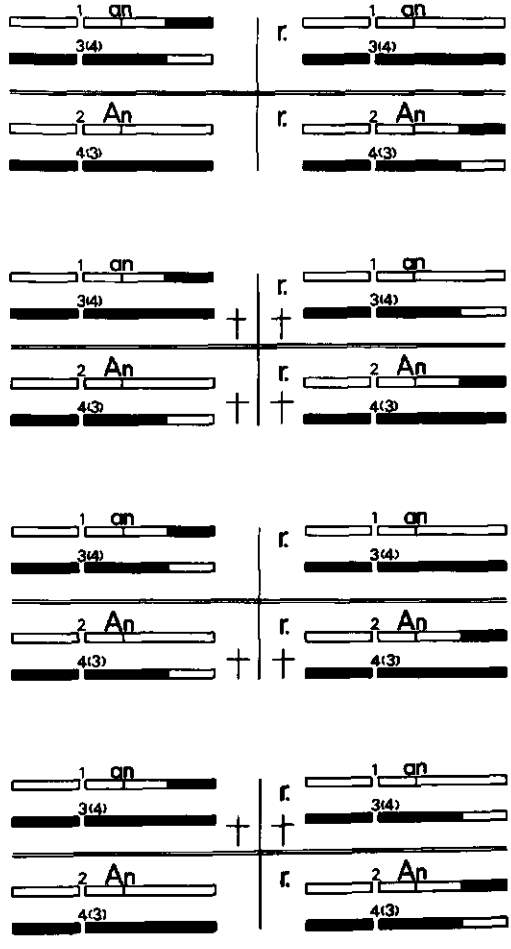

Fig. 4. Tetrads from chiasma formation in T between *An – an* and translocation breakpoint, and in U. For legend, see Figs. 2 and 3. Half of balanced tetrad cells is recombinant, irrespective of orientation

Translocation bivalents are assumed to result from chiasma formation between chromosomes with homologous centromeres. This is true for configurations consisting of two ring bivalents, or one ring- and one open bivalent. Part of the open translocation bivalent pairs, however, may also originate from crossing over in the exchanged segments, linking non-homologous centromeres. Adjacent-2 orientation is the result, and there is no contribution to any of the four gamete classes. Simultaneous absence of chiasmata in both unchanged arms is required, which is highly improbable for the (sub-)metacentric rye chromosomes with frequencies of arms being bound often approaching 1. Therefore, the error introduced by assuming that all translocation bivalents are the result of chiasma formation between chromosomes with homologous centromeres is limited, the more so when the frequency of cells with multivalents is high relative to that of cells without. Due to independent orientation of the translocation bivalents, an alternate to adjacent-1 ratio of 1 : 1 is realized, and $z = z' = 0.5$ is substituted in the terms for the multivalent contributions (Table 1) to obtain the frequencies in which configurations without chiasmata in the exchanged segments (frequency 1-e) contribute to the four gamete classes, since the same rules apply to these configurations and multivalents. The term 1-e includes translocation configurations with two or four univalents, which do not contribute to any class of reproductive gametes. This should be taken into account, when their frequency cannot be neglected.

The fraction of recombinant gametes in the total gamete pool now is (Table 1)

$$r = \frac{1-(1-u)\,e\,(2z'-1)}{2[1+(1-t)\,(1-u)\,e\,(2z-1)]} \cdot f \cdot t \qquad \text{(A)}$$

In formula A, the term $[1-(1-u)\,e\,(2z'-1)] \cdot [2[1+(1-t)\,(1-u)\,e\,(2z-1)]]^{-1}$ determines the degree in which the chiasma frequency between marker and translocation breakpoint ($f \cdot t$) is recovered in the recombination fraction (r). In the following, this term will be referred to as d. As long as alternate and adjacent orientations are equal, no effect on the recombination fraction – other than that due to the overall disturbance of pairing and chiasma formation around the translocation breakpoint – should be observed (Kramer 1954). As in a normal diploid, the chiasma frequency between the two factors under consideration should then be twice their recombination fraction: $d = 0.5$. When $z = z' = 0.5$ is substituted in formula A, it is indeed found that $r = 0.5\,f \cdot t$.

The term d also equals 0.5 when $e = 0$, i.e. when only translocation bivalents are formed between chromosomes with homologous centromeres, giving random orientation of the non-homologous centromeres, or when $u = 1$, i.e. when the interstitial segment without the gene has always a chiasma, resulting in the inclusion of recombinant and non-recombinant chromatids in the abortive spores in equal proportion, irrespective of orientation type (Fig. 4).

With random orientation in open translocation multivalents, the ratio of alternate to adjacent-1+2 is expected to be 1 : 1 (Sybenga 1975). This ratio has been observed by Burnham (1950) in several interchanges of

Table 1. Contribution to the recombinant and non-recombinant gametes for each group of translocation configurations. Maximum of one chiasma per interstitial segment. Chiasma frequency in T (Fig. 1): t; in U: u. Multivalent frequency: e (see text). Fraction of chiasmata in T between gene and breakpoint: f. Alternate frequency among multivalents without interstitial chiasmata: z; with interstitial chiasmata: z'. Half of the gametes are normal, the other half have the translocation karyotype

Configurations	Contributions to non-recombinant spores	Contributions to recombinant spores
Multivalents		
without interstitial chiasmata	$4(1-t)(1-u)e \cdot z$	–
chiasma in one interstitial segment	$2 \cdot f \cdot t(1-u)e \cdot z' + 2(1-f)t(1-u)e + 2(1-t)u \cdot e$	$2 \cdot f \cdot t(1-u)e(1-z')$
chiasma in both interstitial segments	$f \cdot t \cdot u \cdot e + 2(1-f)t \cdot u \cdot e$	$f \cdot t \cdot u \cdot e$
Bivalents		
without interstitial chiasmata	$2(1-t)(1-u)(1-e)$	–
chiasma in one interstitial segment	$f \cdot t\,(1-u)\,(1-e) + 2\,(1-f)t\,(1-u)\,(1-e) + 2(1-t)u(1-e)$	$f \cdot t(1-u)(1-e)$
chiasma in both interstitial segments	$f \cdot t \cdot u(1-e) + 2(1-f)t \cdot u(1-e)$	$f \cdot t \cdot u(1-e)$

maize with two short interstitial segments. In rye, alternate orientation predominates among translocation multivalents without interstitial chiasmata (Sybenga 1968): $z > 0.5$. Apparently chance coordination is not realized here, probably for reasons of centromere reorientation during prometaphase and greater stability of the alternate than of the adjacent orientation (Sybenga 1975). In translocation multivalents having either one or both interstitial segments associated by chiasmata, alternate and adjacent-1 cannot be microscopically distinguished in first metaphase configurations of pollen mother cells (PMCs) without morphological markers in the centromeres. There is, however, no a priori mechanical reason why the frequencies of alternate and adjacent-1 would be different (Fig. 3), so that a 1 : 1 ratio of both orientations ($z' = 0.5$) would be expected. For any translocation, with given values of t, u and e ($u \neq 1, e \neq 0$), the term d becomes smaller with increasing values of either or both z and z'. The denominator of d is at its maximum for $z = 1$, and for $z' = 1$ the numerator reaches its minimum. Thus, with predominant alternate orientation in the open multivalents ($z > 0.5$), and random orientation among closed multivalents ($z' = 0.5$), part of the interstitial chiasma frequency will be "masked" and not expressed in the recombination fraction. When $z' = 0.5$, the degree of masking is limited: d reaches a minimum of value of 0.25 when $z = 1$, $e = 1$, $u = 0$ and t approaches 0, in which case of course hardly any recombination will be observed. With increasing values of t, d becomes larger than 0.25, and will be 0.5 again when $t = 1$: with more configurations having interstitial chiasmata, the contribution to the total pool of reproductive gametes orginating from random centromere orientation ($z' = 0.5$) increases at the cost of that resulting from non-randomness ($z > 0.5$), so that interstitial chiasma formation is more fully expressed. Only when $z' > 0.5$, can d become smaller than 0.25.

Values of e, t, u and z are obtained by meiotic observations, while r is estimated in backcrosses or F'2s segregating for the marker and the translocation (de Vries and Sybenga 1983). Substitution in formula A together with the expected value of 0.5 for z' results in an estimate of f. f can also be estimated from the interstitial chiasma frequency t when the genetic distance between marker and centromere is known. Then, using formula A it can be checked whether the assumption that $z' = 0.5$, is realistic.

When p is substituted for r, x for t, y for u, a' and a for z' and z respectively, and $(1 - P)$ for $(1 - t)(1 - u)$, and when $e = 1$ and $f = 1$, formula IV in the report of Kramer and Blander (1961) is found. The validity of their formula is, therefore, limited to recombination between centromere and translocation breakpoint ($f = 1$), in translocations in which either one or both exchanged segments in addition to at least one of the other segments are practically always bound ($e = 1$).

Materials and methods

The translocation investigated in this study (Fig. 1) carries code number 303 and is part of the Wageningen translocation tester set (Sybenga and Wolters 1972; de Vries and Sybenga 1976; Sybenga 1983). Translocation 303 involves chromosomes 5R and 7R, with the breakpoints proximally in 5RL and distally in 7RL (de Vries and Sybenga 1983; Sybenga, in preparation). Due to the difference in length of the exchanged segments (R and S, Fig. 1), both translocation chromosomes are easily recognized in mitotic metaphase. In the first metaphase of meiosis, the short translocation chromosome 5^T is markedly small when appearing as a univalent. Open bivalents in which chromosome 5^T is involved are identified by their heteromorphic appearance, whereas a ring bivalent formed by chromosomes 5^T and 5 is about half the size of an average normal ring bivalent, showing a large projection which represents the long exchanged segment.

The gene *an*, determining the presence of anthocyanin in the caryopsis (xenia), coleoptile and nodes of rye seeds and plants, is located on chromosome 7RL (Sybenga and Mastenbroek 1980; de Vries and Sybenga 1983), interstitially in chromosome 7^T of translocation 303 (Fig. 1). Sybenga and Mastenbroek (l.c.) established a genetic distance of 5 cM between *an* and the centromere in translocation heterozygote 282, in which the breakpoint is in the other arm of chromosome 7R.

One plant, heterozygous for translocation 303 and for *an*, was used in a reciprocal test cross with karyotypically normal, anthocyaninless (*an an*) plants. In the translocation heterozygote, the dominant allele *An* was located on the normal chromosome 7R (N), while translocation chromosome 7^T (T) carried the recessive allele *an*. The test cross is symbolized as $\frac{an\ An}{T\ N} \times \frac{an\ an}{N\ N}$. A total of 327 seedlings of the test cross progeny was karyotyped and scored for *an*, providing data for the estimation of the recombination fraction r between *an* and the translocation breakpoint. Configuration frequencies at first metaphase were scored in 1,000 PMCs of the plant which was also used in the test crosses. In translocation multivalents without interstitial chiasmata (rings-of-four, chains-of-four, chains-of-three with univalent) the type of orientation – alternate or adjacent – was established simultaneously. The PMCs were in early MI, since a few cells at diakinesis and none at first anaphase were observed. This implies that final orientation had not been reached and the frequency of alternate orientation was underestimated (Sybenga 1968).

All plants were grown in a greenhouse at 18°–20 °C. Karyotypes were classified in root tip mitoses after pretreatment in a saturated aquaeous alpha bromonaphthalene solution for 2 h at 24 °C, fixation-maceration in 1 N HCl at 60 °C for 12 min, and Feulgen staining. Anthers were fixed in 1 : 3 acetic alcohol and stored at −10 °C for two years. First metaphase chromosomes were stained with 2% aceto carmine, and the preparations were mounted in Euparal for cytological analysis.

Results

The segregation of translocation 303 and *an* in the offspring of the test cross $\frac{an\ an}{N\ N} \times \frac{an\ An}{T\ N}$ (TN 303 as ♂)

Table 2. Segregation of translocation 303 and marker gene *an* in the testcross progeny of $\frac{an}{N}\ \frac{an}{N} \times \frac{an}{T}\ \frac{An}{N}$ (a), and of the reciprocal (b); *r*: estimated recombination fraction between *an* and the translation; s_r: standard deviation of *r*

a

Phenotype \ Karyotype	NN	TN	Total	
An	58	16	74	
an	16	41	57	
Total	74	57	131	$r \pm s_r = 0.244 \pm 0.038$

Segregation of TN 303 and *an* fit their 1 : 1-expectation at the 5% level ($\chi^2 = 2.206$)

b

Phenotype \ Karyotype	NN	TN	Total	
An	85	14	99	
an	24	73	97	
Total	109	87	196	$r \pm s_r = 0.194 \pm 0.028$

Segregation of TN 303 ($\chi^2 = 2.469$) and *an* ($\chi^2 = 0.020$) fit their 1 : 1-expectation at the 5%-level

is presented in Table 2 a; the reciprocal cross in Table 2 b.

The Tables 3–6 contain the classification of MI translocation configurations in 1,000 PMCs. In 980 cells, the translocation configurations could be distinguished from the configurations originating from the 5 pairs of normal chromosomes (group A). In 943 of these, multivalents occurred (configurations 1–9, Table 3). No multivalents were observed in 37 cells, but the translocation bivalent formed by chromosomes 5 and 5^7 could be morphologically identified. In addition, 6 ring bivalents were found in these same cells. In 6 of the 37 cells with bivalents only, 7 ring bivalents were observed, of which one was small and had a large projection so that the translocation configuration could be concluded to consist of two ring bivalents (conf. 10). A heteromorphic open bivalent formed by chromosomes 5 and 5^7 next to 6 ring bivalents was found in 30 PMCs, which means that in these cells the chromosome pair $7–7^5$ formed a ring (conf. 11). Finally, 1 cell carried a pair of univalents originating from chromosomes 5 and 5^7 next to 6 rings, and consequently a ring bivalent must have been formed by chromosomes 7 and 7^5 in this cell (conf. 12, Table 3).

To establish the translocation configuration in the remaining 20 PMCs in which no direct distinction

Table 3. First metaphase translocation configurations in 1,000 PMCs of the plant of Table 2. Configuration frequencies of no. 10–15 partly based on Tables 4–6 (see text)

Type no.	Configuration		Orientation	No. of cells	Notes
1	ring-of-4		alternate	10	
2	chain-of-4		alternate	196	
3	ring-of-4		adjacent	2	
4	chain-of-4		adjacent	43	
5	chain-of-3 with univalent		alternate	12	[a]
6	closed-figure-8			1	
7	frying pan quadrivalent			643	
8	Y-shaped quadrivalent			2	
9	frying pan trivalent with univalent			34	[a]
			subtotal	943	
	Bivalents of chromosomes				
	$7–7^5$	$5–5^7$			
10	ring	ring		9	
11	ring	open		33	
12	ring	2 univalents		1	
13	open	ring		7	
14	open	open		6	[b]
15	open	2 univalents		1	[b]
			total	1,000	

[a] Univalent is chromosome 5^7

[b] Configurations 14 and 15 are assumed to have originated from chiasma formation between chromosomes with homologous centromeres (see text)

Table 4. Bivalent configurations for the 5 pairs of chromosomes not involved in the translocation complex, in 980 PMCs in which the translocation configuration can be distinguished directly (group A)

Ring bivalents	Open bivalents	Pairs of univalents	No. of cells
5	0	0	696
4	1	0	254
3	2	0	29
2	3	0	1
			980

Total number of configurations: $5 \times 980 = 4{,}900$
Total number of open bivalents: 315 (6.43%)

could be made between configurations originating from the translocation complex and the normal chromosome pairs (group B), a procedure outlined by Sybenga and Mastenbroek (1980) was followed. Table 4 presents the distribution of bivalent configurations originating from the 5 pairs of normal chromosomes in group A. The configurations found in the 20 PMCs of group B are listed in Table 5. In 8 cells a heteromorphic open bivalent formed by chromosomes 5 and 5^7 was observed, next to 5 ring bivalents and 1 morphologically normal open bivalent (type I, Table 5). Of the $8 \times 5 = 40$ configurations formed by the 5 pairs of normal chromosomes, 6.43% (Table 4) or 3 bivalents are expected to be open bivalents. Chances are small that these were not formed in three different cells. Thus in 3 of the 8 PMCs of type I, a ring must be assumed to have been formed by chromosomes 7 and 7^5 (although not recognized at meiosis), while in the remaining 5 cells these chromosomes must have formed an open bivalent. The expected translocation configurations in the other PMC types of Table 5 are derived in a similar way, leading to the configuration frequencies of Table 6. When added to the configurations of group A, the totals of Table 3 are found.

Table 5. Configurations in the 20 PMCs in which the translocation configuration is not a multivalent and cannot be directly distinguished (group B)

PMC-type	Ring bivalents	Heteromorphic open bivalents (chr. $5-5^7$)	Open bivalents (not heteromorphic)	Heteromorphic pairs of univalents (chr. $5-5^7$)	Pairs of univalents (not heteromorphic)	No. of cells
I	5	1	1	0	0	8
II	5	0	1	1	0	1
III	4	1	2	0	0	1
IV	6	0	1	0	0	6
V	5	0	2	0	0	2
VI	4	0	3	0	0	1
VII	6	0	0	0	1	1
						20

Table 6. Translocation configurations in the 20 cells of group B, estimated from Tables 4 and 5

Type no. of Table 3	Bivalents of chromosomes		Contribution of PMC types listed in Table 5							No. of cells included in Table 3
	$7-7^5$	$5-5^7$	I	II	III	IV	V	VI	VII[a]	
10	ring	ring	–	–	–	2	–	–	1	3
11	ring	open	3	–	–	–	–	–	–	3
13	open	ring	–	–	–	4	2	1	–	7
14	open	open	5	–	1	–	–	–	–	6
15	open	2 univ.	–	1	–	–	–	–	–	1
Total			8	1	1	6	2	1	1	20

[a] The pair of univalents in this PMC is arbitrarily attributed to one of the pairs of normal chromosomes

Discussion

Interstitial chiasmata

Chiasma formation in both interstitial segments simultaneously is scarce among multivalents, as can be concluded from the fact that only one configuration with such origin, a closed-figure-8, was observed (configuration 6, Table 3). The frequency is much higher among the translocation bivalents: 9 cells were found with configuration 10, whereas cells with configurations 11, 13 and 14 may also have originated from chiasma formation in both T and U. The number of these cells cannot be established microscopically, but from Table 7 it is seen that all open bivalents consisting of chromosomes 7 and 7^5 must have originated from chiasma formation in 0 ($o=1$), while of the heteromorphic open bivalents formed by chromosomes 5 and 5^7 a fraction of $\frac{(1-p)\,u}{p\,(1-u)+(1-p)\,u}=0.022$ (Table 7) must have originated from crossing over in U. Consequently, only 1 cell, out of the 33 with configuration 11, can be added to those with 6 or 10 (Table 8).

The remaining 32 cells with configuration 11 have a chiasma in T but not in U, which also applies to cells with configurations 9 or 12 in which 5^7 appeared as a univalent. Configuration 13 has a chiasma in U but not in T, while in cells with configurations 1–5, and 14 and 15 no chiasmata have been formed interstitially (see above).

This leaves the configurations 7 and 8, which may arise from crossing over in either T or U. When the frequency of all configurations (translocation bivalents as well as multivalents) without interstitial chiasmata is $(1-t)\,(1-u)$, and that of configurations with chiasmata in both interstitial segments is $t \cdot u$, it follows from Table 3 that

$(1-t)\,(1-u)=0.270$ (conf. 1–5, 14 and 15), and

$t \cdot u \quad =0.011$ (conf. 6, 10 and 11 (1 cell)).

The largest root of these equations represents the chiasma frequency in the larger segment. Thus, $t=0.725$ and $u=0.015$ (chiasma interference between interstitial segments is not taken into account). A chiasma in U has actually been formed in configurations 6, 10, 11 (1 cell) and 13, i.e. in 18 out of 1,000 PMCs (Table 3). This is close to $u=0.015$ and it is improbable that additional configurations have resulted from chiasma formation in U. Therefore, configurations 7 and 8 are concluded to have a chiasma in T, and not in U. This is supported by the fact that, although multivalent types 7 and 8 originating from chiasma formation in U are expected to be morphologically different from configurations having a chiasma in T (size of ring and "handle" of the frying pan, Fig. 3), no such differences were noted. No frying pans were detected with a ring notably smaller than normal ring bivalents, although ring bivalents formed by chromosomes 5 and 5^7 are easily recognized. The conclusion, therefore, must be that most – if not all – multivalent types 7 and 8 have originated from chiasma formation in T.

Table 8 summarizes these conclusions. It is seen that in 723 of the 1,000 PMCs a chiasma has been formed in T ($t=0.723$), while the chiasma frequency in U (u) is 0.018. For bivalents alone (Table 7), t and u are 0.754 and 0.295 respectively. The difference indicates strong positive interference between exchanged and interstitial segments, for which obviously the short segment U is the most susceptible. Strong positive interference between interstitial and exchanged segments has been frequently observed in rye interchange heterozygotes (Sybenga 1970; Sybenga and Mastenbroek 1980).

Estimates of e, z and r

As seen from Table 3, the multivalent frequency (e) equals 0.943. In 218 of 263 PMCs having multivalents without interstitial chiasmata, alternate orientation was

Table 7. Frequencies of bivalent configurations formed by chromosome pairs 7–7^5 and 5–5^7 respectively among 57 PMCs with configurations 10–15 (Table 3) and estimated frequencies o, p, t and u in which segments O, P, T and U respectively are bound among configurations without chiasmata in R and S (Fig. 1). Chiasma interference is not taken into account

Configuration	Chromosome pair 7–7^5		Chromosome pair 5–5^7	
	Frequency	Observed	Frequency	Observed
Ring bivalents	$o \cdot t$	0.754	$p \cdot u$	0.281
Open bivalents	$o\,(1-t)+(1-o)\,t$	0.246	$p\,(1-u)+(1-p)\,u$	0.684
Pairs of univalents	$(1-o) \cdot (1-t)$	0.0	$(1-p) \cdot (1-u)$	0.035
	$o=1.0$		$p=0.950$	
	$t=0.754$		$u=0.295$	

Table 8. Origin of translocation configurations (Table 3) with respect to interstitial chiasma formation, as concluded from Tables 3 and 7

Configuration type	Chiasma formation		No. of cells
	in T	in U	
6, 10 and 11[a]	+	+	11
7, 8, 9, 11[b] and 12	+	–	712
13	–	+	7
1, 2, 3, 4, 5, 14 and 15	–	–	270
			1,000

[a] 1 of 33 cells
[b] 32 of 33 cells

Total of configurations with chiasma formation in T: 723 ($t=0.723$)
Total of configurations with chiasma formation in U: 18 ($u=0.018$)

observed: $z=0.829$. This is considered a minimum estimate because the PMCs were in early metaphase, while Sybenga (1968) observed an increasing frequency of alternate orientation – up to 95% – with progressing MI. A value of 0.9 for z is considered more realistic and will therefore be used in further calculations.

For the recombination fraction, the value of Table 2a may be taken ($r=0.244\pm0.038$), because the meiotic data of Table 3 were obtained from PMCs of the same male parent.

Estimates of f

Two approaches are given to estimate the fraction of chiasmata in T formed between the *an*-locus and the translocation breakpoint (f). The first is based on the assumption that centromere orientation is random among configurations having either one or both interstitial segments bound by chiasmata ($z'=0.5$), the second makes use of the genetic distance between *an* and the centromere of chromosome 7R, which is 5 cM according to Sybenga and Mastenbroek (1980).

1. By substituting $z'=0.5$, $z=0.9$, $t=0.723$, $u=0.018$, $e=0.943$ and $r=0.244\pm0.038$ in formula A, f becomes 0.813 ± 0.127. Reliable confidence limits for f cannot be given, since the statistical distributions of t, u and e are not known.

Univalent formation causes a reduction in the production of viable gametes. Due to configuration 9 (frequency 0.034, Table 3) the contribution to the class of recombinant spores is reduced by $0.034.f(1-z')$, and the contribution to the non-recombinant class by $0.034.(f\cdot z'+1-f)$, whereas the reduction of the contribution to the latter caused by configuration 5 (frequency 0.012) equals 0.024 z. Introducing these corrections gives $f=0.814\pm0.127$, and it appears that their effect is negligible. No consideration will be given to the effect on the gamete productions of configurations 12 and 15, because their frequencies are extremely low (0.001 for each, Table 3).

Configurations 5 and 9 only contribute to the karyotypically normal spores, since the univalent is always the short translocation chromosome 5^7. It is interesting to note, that the preferential loss of univalent 5^7 results in a shortage of translocation heterozygotes in both test crosses (Table 2a, b), which becomes significant when the two populations are pooled ($\chi^2=4.65$; $P<0.05$).

2. A genetic distance of 5 cM between *an* and the centromere of chromosome 7 corresponds with a chiasma frequency of 10% in this segment, or a chiasma in 100 out of the 1,000 PMCs analyzed. With 723 PMCs having a chiasma in T (Table 8), there would be 623 with a chiasma between *an* and the translocation breakpoint, and f is then estimated as $\frac{623}{723}=0.862$. This is within the limits established for $z'=0.5$ (0.813 ± 0.127, see above) and relatively close to the average value, in spite of the fact that the genetic distance between *an* and the centromere was established in translocation heterozygote 282 (Sybenga and Mastenbroek 1980), in which the gene is located in the unchanged arm and not interstitially like in translocation 303.

Difference between z and z'

With all chiasmata in T between *an* and the translocation breakpoint ($f=1$), a maximum of 0.601 ± 0.068 is found for z'. It is evident that the alternate orientation frequency among translocation multivalents with interstitial chiasmata (z') never reaches the level of that among multivalents without (z). However, f is smaller than 1 (see above) and a realistic estimate for z' of 0.530 ± 0.080 is obtained with $f=0.862$. Thus, it is very reasonable to conclude that $z'=0.5$, which corresponds with the view that there are no a priori mechanical reasons for a preference of alternate orientation among translocation multivalents having interstitial chiasmata. For $f=0.813$ and $r=0.244\pm0.038$, z' becomes 0.500 ± 0.084.

Degree of "masking"

The degree in which the actual chiasma frequency between gene and breakpoint is masked in the observed recombination fraction is determined by d: $r=d\cdot f\cdot t$ (formula A). In the present case, $d=\frac{0.244}{0.813\cdot0.723}=0.415$ whereas with random overall orientation ($z=z'=0.5$) d would be 0.5. When $z'=0.5$, the numerator of d (see formula A) becomes 1. Thus if the conclusion that $z'=0.5$ is correct, masking of the recombinational effect of interstitial chiasmata can only be attributed to an alternate orientation frequency among multivalents without interstitial chiasmata (z) larger than 0.5, resulting in an excessive contribution of

viable (non-recombinant) spores to the total gamete pool (Fig. 2).

When $z'=0.500+0.084$ (see above), d becomes 0.350: masking of interstitial recombination becomes stronger due to the preferential inclusion of recombinant chromatids in the non-reproductive gametes caused by values of z' larger than 0.5 (Fig. 3). The minimum of d in the present case would be 0.285, i.e. when $f=1$ and $r=0.244-0.038$, giving $z'=0.669$ (see above).

Lamm (1948) was the first to present a formula quantifying the effect of centromere orientation and interstitial chiasma formation on the observed recombination fraction for interstitial loci. Identity of z and z' was assumed, but Lamm pointed out explicitly that no proper allowance was made for the possibility that interstitial chiasmata act upon the orientation of the configuration. Lamm's formula was subsequently refined by Hanson and Kramer (1949), who concluded that barley interchanges, on the basis of an average sterility of 25%, should at least show an alternate frequency of 0.75. Implicitly they assumed identity of z and z' and, therefore, a value for z' of at least 0.75. Apart from overall disturbed crossing over around the translocation breakpoint, the preferential inclusion of recombinant chromatids in the abortive gametes which results from $z' > 0.75$ (Fig. 3) was used to explain reduced recombination, without paying attention to the effect of the excessive contribution of viable, non-recombinant spores caused by values for z of at least 0.75. Similar incorrect explanations for masking of interstitial recombination are found in the reports of Burnham (1950), Hanson (1952), Kramer (1954), Burnham and Hagberg (1956), Ramage (1964), Kasha and Burnham (1965), Persson (1969) and Künzel (1982). The only parameter used to determine the frequency of alternate disjunction, implicitly assuming that z and z' are the same, is semi-sterility, but in none of these reports are cytological or other arguments found to support identity of z and z'. Kramer and Blander (1961) distinguished between z and z' (a and a' respectively in their report) and demonstrated that 25% sterility and a low recombination fraction between an interstitial marker and a translocation may very well be compatible with $z'=0.5$ and considerably higher values of z at the same time. On the basis of combined recombination and cytological data, the present report provides evidence that z can indeed greatly exceed z', and that the assumption of random centromere orientation among translocation multivalents having interstitial chiasmata ($z'=0.5$) is realistic. The reduction of recombination should, therefore, be attributed to the effect of a high value of z, z' being of minor or no importance. Since the effect of z is, in turn, limited (d can only reduce from 0.5 to a minimum of 0.25 when $z'=0.5$, see above), disturbance of pairing and crossing over in the region of the interchange breakpoints will in most instances be the main explanation of low recombination between interstitial markers and translocation breakpoints.

Use of interchanges in gene localization and balanced chromosomal systems

Reciprocal translocations can be used to locate marker genes on specific chromosomes (de Vries and Sybenga 1983). To determine a marker's chromosomal position, the measurement of linkage values between the marker and a series of reciprocal interchanges involving the chromosome which carries the marker, with breakpoints at various and known positions (Hanson 1952; Ramage 1964; Künzel 1982, and others) has proven to be a fruitful approach, provided that a fair number of such interchanges is available. Chiasma formation among the chromosomes involved often follows patterns which are not predictable, giving recombination values which may be misleading. The extremely high recombination fraction between *an* and translocation 303 is an illustrative exception to the rule that recombination in interstitial segments is very low. Sybenga and Mastenbroek (1980) established the arm position of *an* on the basis of the recombination fraction between *an* and translocation 282, the absence of linkage between *an* and the gene *br* (brittle) in a translocation 282 homozygote and a quantitative analysis of first metaphase configurations of the translocation heterozygote. It is obvious that the methods described above are rather laborious and that, therefore, when available, the use of telocentric and several types of translocation-derived trisomics (de Vries and Sybenga 1983) is to be preferred in determining the chromosomal position of a marker.

Several balanced chromosomal systems have been worked out to make use of genetic male sterility in hybrid breeding (Ramage 1965, for barley; Patterson 1973, for maize). Very low recombination between the male sterility gene, a selection marker and the breakpoint is an essential condition to make these systems operational. It has been suggested that interstitial location of the genes would guarantee low recombination. The present report demonstrates, that this is not necessarily true.

Other regions may appear to be favourable as well. For instance Künzel (1982) in barley studied two balanced tertiary trisomics, both with an *ms* gene in the exchanged segment of the extra translocation chromosome. No recombinants were observed among the selfed offsprings (433 and 293 individuals respectively). Similar results for exchanged segments as well as unchanged arms are obtained in studies concerning balanced trisomic systems in rye (de Vries et al., in preparation).

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Erratum: p.39, read "The assumption is made that adjacent-2 orientation, giving only unbalanced gametes, may be excluded, as co-orientation of non-homologous centromeres is very unlikely here."

3. Sources of tertiary trisomics for balanced chromosomal systems in hybrid rye breeding.

by
J.N. de Vries
Department of Genetics, Agricultural University
53, Gen. Foulkesweg
6703 BM Wageningen
The Netherlands

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ABSTRACT

To increase the efficiency of isolating tertiary trisomics, five rye translocations of the Wageningen translocation tester set with exchanged segments of different size were crossed with either a telocentric or a primary trisomic, or with each other. Progenies of two telocentric translocation trisomics, four translocation trisomics and one double-translocation trisomic thus obtained, were karyotyped, and MI configurations of the two telocentric translocation trisomics quantitatively investigated. Tertiary trisomics were obtained with the short translocation chromosome extra of each of the five interchanges, in frequencies of 0.5-1.0% in case of the telocentric translocation trisomics. This implies hardly an increase compared to the corresponding interchange heterozygous disomics (maximally 1%, tertiary and translocation trisomics combined). The translocation and double-translocation trisomics yielded higher percentages of tertiary trisomics, the difference being due to the extra chromosome (telocentric or normal) present in the mother plants, and to different ranges of possibilities for MI association and A1 disjunction, respectively. No tertiary trisomics carrying a long translocation chromosome as the extra chromosome were detected. A reduced viability of eggs or zygotes with large amounts of extra chromosomal material, rather than a low frequency of the appropriate MI orientation, is suggested to be the cause.

INTRODUCTION

The most effective way to exploit the large heterotic potentials of cultivated rye (*Secale cereale* L.) is hybrid breeding. As for grain yield, similar gains appear to be realizable as have been obtained with maize, whereas other important characters like resistance to lodging and sprouting, shortness of straw and feeding value are most effectively improved by using this system (Geiger, 1982).

Hybrid breeding requires an operational method to maintain and increase an all male sterile seed parent, for which purpose gametocides might become valuable tools. Recently, a sufficiently reliable wheat pollen killer seems to have been developed, but it might not become available on a commercial basis. Whether rye pollen is also affected, is as yet not known. Another approach is the use of cytoplasmic male sterility (CMS). A few sources of CMS and corresponding restorer genes have been identified (Geiger, 1982), but at present only the 'Pampa' cytoplasm is being used in hybrid breeding programs (Morgenstern and Geiger, 1982). Therefore, although advantageous with regard to operationality, the genetic base of these programs is quite vulnerable, so that it is useful to have alternative systems available. Several have been developed in other crop plants, making use of genic (chromosomal, nuclear) male sterility (GMS) and extra chromosomal material. In these systems, a dominant male sterility (m.s.) allele is located on extra chromosomal material, while the recessives are carried by the normal complement. Due to certation, the extra material is only transmitted through the female haplophase. Thus, when recombination between the dominant and recessive m.s.-alleles is prevented, all pollen taking part in reproduction is karyotypically normal, and exclusively carries the recessive allele. To prevent recombination, the extra material should be unable to simply replace a normal chromosome, which excludes primary trisomics as the male restrictive agent. Moreover, structural barriers to recombination between normal and extra material are required. For barley hybrid breeding, Ramage (1965) proposed the use of balanced tertiary trisomics in which the dominant allele is located on the extra translocation chromosome, close to the breakpoint. Using this trisomic as a pollinator to a number of male sterile diploids, the production of a completely male sterile, diploid

seed parent would be accomplished. In maize, GMS has been made operational by Patterson (1973) who used diploid duplication-deficiency heterozygotes. When only the eggs tolerate the duplication and the deficiency, pollen transmission of the dominant m.s.-allele is prevented when it is located close enough to the translocation breakpoint. Phillips and Springer (1972) proposed a similar use of a disomic double duplication heterozygote, in which the absence of two normal non-homologous chromosomes is compensated by two translocation chromosomes, originating from two different interchanges, which both include parts of the missing chromosomes. There is no deficiency, and the presence in three-fold of the two "between breakpoints" regions (Burnham, 1966) accomplishes that only karyotypically normal pollen is functional. The use of compensating trisomics, in which one chromosome is replaced by two others of aberrant structure, has been proposed by Sybenga (1982). In the XYZ system proposed by Driscoll (1972) for wheat, the extra chromosomal material carrying the dominant m.s.-allele is obtained from related genera or species, like rye, barley, *Aegilops*, *Agropyron* and *Triticum monococcum* (Driscoll, 1981). While in the systems mentioned above recombination is prevented by the presence of translocation breakpoints, in the XYZ system this is accomplished by reduced pairing between homoeologous chromosomes.

The spontaneous frequency of tertiary trisomics in the progeny of normal diploids is extremely low. According to Avery et al. (1959), among about 2 million *Datura* plants investigated, six tertiary trisomics were found. Interchange heterozygotes are the most common source of tertiary trisomics. As a result of numerical non-disjunction at first anaphase, four types of n+1 gametes can be formed by an interchange heterozygote, two of which contain a translocation chromosome in addition to the normal complement, whereas the other two carry the interchange complement with a normal chromosome extra. Thus, when fused with karyotypically normal gametes, two types of tertiary, and two types of translocation trisomics respectively are expected (Ramage, 1960; Khush and Rick, 1967). In this paper, the term 'translocation trisomic' (Sybenga, 1975) is used instead of 'primary trisomic interchange heterozygote' (Ramage, 1960).

Many cases of tertiary trisomics originating from interchange heterozygotes have been described (for a review see Khush, 1973), but in most reports data concerning the frequency of the various trisomic types are scarce, and if present, not exclusively based on cytological observations. In rye, on the basis of eleven well described translocations

(de Vries and Sybenga, 1976) in principle twenty-two different tertiary trisomics can be obtained. However, their progenies never contain more than 1% trisomics (tertiary and translocation combined) indicating a strong predominance of 2:2-segregation of the chromosomes involved in the translocation complex at first anaphase. Univalents which result from absence of chiasmata in specific chromosome segments orientate only occasionally, whereas translocation bivalents will again segregate 2:2. Chances for tertiary trisomics to be produced might be increased, when at meiosis the translocation complex is involved in multivalents consisting of more than four chromosomes. Not only will these multivalents have a wider range of possibilities for co-orientation and segregation, also the lack of chiasmata in specific segments may result in independently orientating meiotic configurations from which tertiary trisomics can originate relatively simply. This report deals with the efficiency of several types of translocation trisomics, with respect to the occurrence of tertiary trisomics in their progenies. The effects of the choice of the extra chromosome, centromere orientation, preferential association, absence of chiasmata in specific chromosome segments and size of the extra chromosomal material on type and frequency of the tertiary trisomics are evaluated, using quantitative meiotic observations, when available.

MATERIALS AND METHODS

The translocations used in this study make part of the Wageningen translocation tester set (Sybenga, 1983), described by de Vries and Sybenga (1976) and Sybenga and Wolters (1972). Chromosome nomenclature corresponds with that of the *Triticinae*, and is based on preliminary data from crosses between the translocation tester set and the 'Imperial' to 'Chinese Spring' standard addition set (de Vries and Sybenga, 1983). Telocentric translocation trisomics 240 and 273 (fig. 1a, 2a) were obtained from crosses between telocentric trisomic 5RS and translocation homozygotes 240 (between 3RS and 5RL) and 273 (between 1RL and 5RS) respectively, whereas crosses between a primary trisomic for chromosome 5R and translocation homozygotes 273, 282 (between 5RL and 7RS) and 501 (between 4RL and 5RL) yielded the corresponding translocation trisomics (fig. 1a, 3a). Data concerning the translocation trisomic 273 with chromosome 1R extra (table 3a) are from Sybenga (1966b), who obtained this trisomic

from a cross between primary trisomic 1R and interchange homozygote 273. One trisomic, containing the complements of both translocations 282 and 305(between 2RS or 2RL and 5RS) and an additional chromosome 5R (fig. 4a), appeared with low frequency in the progeny of an F1 between the homozygotes of these interchanges, which was backcrossed with karyotypically normal plants (table 6a). This type of trisomic will be referred to as a "double-translocation trisomic". The genetic background of all trisomics and translocations used is divergent and cannot be further specified. MI configurations were scored in 2592 and 500 pollen mother cells (PMCs) of telocentric translocation trisomics 240 and 273 respectively after carmine staining, whereas another 151 Giemsa C-banded configurations in PMCs of the latter were investigated. Except telocentric translocation trisomic 273, all trisomics were crossed with karyotypically normal, male parents, and the F1 was karyotyped. From telocentric translocation trisomic 273 a selfed progeny was examined. Using Feulgen staining, the short translocation chromosomes are easily recognized at mitotic metaphase, whereas the long interchange chromosome cannot be recognized unequivocally. In order to discriminate between tertiary and translocation trisomic offspring, either MI configurations in PMCs were investigated qualitatively, or a selfed progeny of limited size (about 15-20 plants) of these trisomics was karyotyped, whenever anthers or seeds were available. Plants were grown in a greenhouse at a temperature of 18-20 ^{o}C throughout 1979-1982. Differences in day length and light intensity may therefore have somewhat affected the results quantitatively. Karyotypes were scored in root tip squashes after pre-treatment for 2 hr. in a saturated aquaeous alpha bromonaphthalene solution at 24 ^{o}C, fixation-maceration in 1 N HCl for 12 min. at 60 ^{o}C, and Feulgen staining. Anthers were fixed in 1:3 acetic alcohol and stored at -10 ^{o}C. First metaphase chromosomes were stained either with Giemsa as described by Giraldez et al. (1979) leaving out the xylene immersion step, or with 2% aceto carmine followed by mounting in Euparal.

RESULTS AND DISCUSSION

Telocentric translocation trisomic 240 (telo 5RS extra)

Tertiary trisomics originating from telocentric translocation trisomic 240 with 5RS extra (fig. 1a) may have either 5^{3} or 3^{5} as extra transloca-

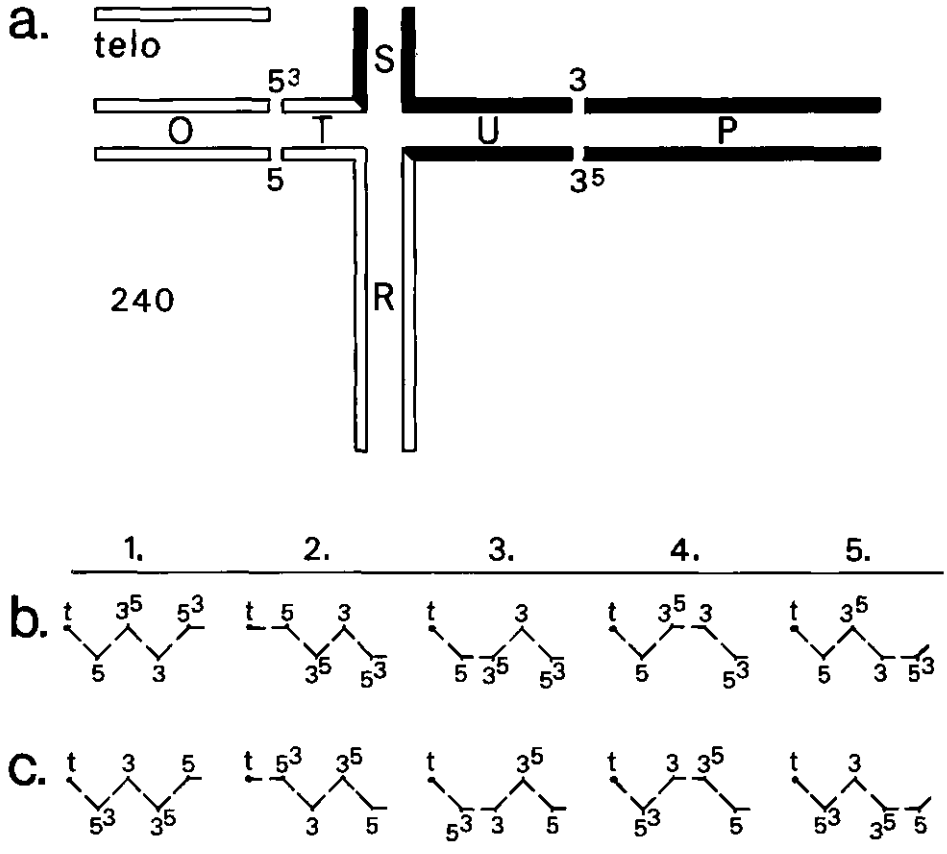


Fig. 1. a.: Pairing diagram of telocentric translocation trisomic 240 (3RS, 5RL) with 5RS as extra telocentric (not paired in diagram). Centromeres as gaps. O, P: unchanged arms, R, S: exchanged segments, T, U: interstitial segments. Translocations 282 (5RL, 7RS) and 501 (4RL, 5RL) have a breakpoint in 5RL somewhat more proximal and somewhat less proximal respectively as 240, while there is also only little difference in length of the exchanged segments of 3RS, 7RS and 4RL respectively.

b.: Orientations of chain-of-5 with telocentric (t) associated with 5R. Left to right (1-5): alternate orientation, adjacent orientation of telo and 5R, of 5R and 3^5, of 3^5 and 3R and of 3R and 5^3 respectively. Complete pairing: no partner exchange; chiasmata in all paired, except interstitial segments. Other orientations considered improbable (see text).

c: Orientations of chain-of-5 with telocentric associated with 5^3. Left to right (1-5): alternate orientation, adjacent orientation of telo and 5^3, of 5^3 and 3R, of 3R and 3^5 and of 3^5 and 5R respectively. For further details see 1b.

tion chromosome. However, the six trisomics in table 1a, which do not have a telocentric, all contain one short translocation chromosome 5^3.In first metaphase PMCs of four of these rings-of-4 were observed which is only possible when they are translocation trisomics, probably having 5R as extra chromosome (fig. 1b, c and below). No rings-of-4, and a relatively high frequency of chains-of-3 and -5 were detected in the other two, so that they were concluded to be tertiary trisomics with the small 5^3 chromosome as extra translocation chromosome ('tertiary trisomic 240'). The fact that no trisomics were found with 3^5 extra can only be partly explained by assuming that the amount of extra chromosomal material is too large to be tolerated, since this tertiary trisomic - although having a reduced viability - was observed in the progeny of a telocentric tertiary compensating trisomic 240, in which one chromosome 3R is replaced by telocentric 3RS and translocation chromosome 3^5 (de Vries and Sybenga, 1984; de Vries, in prep.). The chromosomal constitution of the gametes resulting from chains-of-5 (60.5%, table 1b) depends on the centromere orientation at first metaphase, which is illustrated in fig. 1b, c. Since alternate multivalent orientation predominates in rye, orientations b1 and c1 will have the highest frequency. With two adjacent centromeres moving to the same pole and the others alternately orientated, orientations b2-b5 and c2-c5 will result. The karyotypes of all diploids and trisomics in table 1a can be explained from the fusion of gametes resulting from fig. 1b, c with haploid normal spores. This does not take into account the linear orientation observed occasionally with one chromosome tied up between two neighbours of which the centromeres are directed to opposite poles. The middle chromosome then fails to orientate. The chromosomal constitution of gametes resulting from this situation, however, is not different from that of fig. 1b, c. When occasionally the middle chromosome is included in a daughter nucleus, progeny with a different karyotype can result. The plant with 16 chromosomes in table 1a might have had such origin, but other origins are possible. For instance, two pairs of centromeres instead of one can show adjacent orientation, and three neighbouring chromosomes moving to the same pole is also possible. However, due to the coorientation of the centromeres in the chain and as a result of continuous reorientation till the end of MI (Sybenga, 1968; 1975), these disjunctions are less probable. On the basis of figure 1b, c no gametes with translocation chromosome 3^5

additional to a normal, haploid genome are expected and this might explain the absence of the corresponding tertiary trisomic in table 1a. Also the suggestion made above that the four translocation trisomics in this table all contain one extra chromosome 5R (instead of 3R) is thus explained, viz. the absence of orientations resulting in spores containing the translocation genome with 3R extra. Thus, the choice of the telocentric determines which of the two possible tertiary (and translocation) trisomics will have the greatest chance to emerge from a telocentric translocation trisomic. For instance, an extra telocentric 3RL (segment P, fig. 1a) is expected to give tertiary trisomics with 3^5 extra if viable, whereas telocentrics 5RL and 3RS are expected to result in tertiary trisomics with 3^5 and 5^3 extra respectively, when associated with the interchanged segments R and S (fig. 1a) respectively. The effects of interstitial association of these telos via segments T and U respectively can be predicted with the same rules, when the translocated segment distal to the point of association of the telo has no chiasma. In addition, of course, the amount of extra genetical material will set a limit to the actual viability of the trisomics.

Ratios of 1:1 between translocation heterozygous (tn) and normal (nn) diploids, as well as between telocentric translocation and telocentric normal trisomics are expected in the offspring from the cross in table 1a. The simultaneous shortages of disomic translocation heterozygotes (χ^2 = 20.18) and telocentric normal trisomics (χ^2 = 3.92) are both significant at the 5% level, suggesting preferential association of telocentric 5RS with the normal chromosome 5R, which results in preferential combination of the telo with the translocation complement (fig. 1, b.1). Preferential attachment of the telocentric to the normal, instead of the translocation chromosome was also found in a telocentric interchange trisomic studied by Sybenga (1972), who pointed out that variations in preference within a population can be explained by genetical factors which determine the zygomeric activity of specific chromosome parts.

Absence of chiasmata in certain segments results in the breakdown of the chain-of-5 into smaller configurations which orientate independently. When no chiasma is formed in segment P between chromosomes 3R and 3^5, a trivalent and a bivalent will result, from both fig. 1b and 1c. Although the chromosomal constitution is different in the two instances, it is seen that from both the same gametes are formed. Lack of chiasmata in S between

Table 1a. Karyotype frequencies in the offspring of a cross between telocentric translocation trisomic 240 (fig. 1a) female parents and karyotypical normal plants. Progenies of 8 parents pooled. Normal karyotype: n; translocation: t. Number of chromosomes between brackets. Question mark is unidentified extra chromosome, which is not 1R. Distinction between tertiary ((15)nn+5^3) and translocation ((15)tn+?) trisomics based on MI configurations (see text)

karyotype	number of plants
(14)nn	93
(14)tn	41
(15)nn+5RS	18
(15)tn+5RS	32
(15)nn+5^3	2
(15)tn+?	4[a]
(16)1x5^3+5RS	1
Total	191

[a] : most probably (15)tn+5R (see text)

Table 1b. MI configuration frequencies in PMCs of three female parents of table 1a pooled

configuration	number of cells	frequency
telocentric 5RS not associated	969	0.374
telocentric 5RS associated in a		
- ring-of-4	3	0.001
- chain-of-5 (end position)	1568	0.605
- chain-of-4 (end position)	8	0.003
- chain-of-3 (end position)	39	0.015
- heteromorphic bivalent	5	0.002
	2592	1.000

3R and 5^3 in fig. 1b results in a univalent (5^3) which in most instances will not orientate and be lost during first anaphase. From fig. 1c, however, a heteromorphic bivalent consisting of telocentric 5RS and translocation chromosome 5^3 next to a chain-of-3 with 3R, 3^5 and 5R results. With alternate orientation of the chain, a cell containing 5^3 in addition to the normal haploid genome is expected in 50% of the dyads. Preferential association of 5RS with 5^3 (fig. 1c) instead of 5R would thus increase the fraction of tertiary trisomic 240 offspring, when simultaneously the frequency in which 5^3 and 3R are bound, is low. However, the telocentric preferentially associates with chromosome 5R (see above). Moreover, heteromorphic bivalents also result when in fig. 1b 5R and 3^5 are not associated, giving gametes from which translocation trisomic instead of tertiary trisomic progeny will originate with alternate chain-of-3 orientation. In fig. 1c, the result would be a univalent chromosome 5R. No morphological distinction can be made between the two types of heteromorphic bivalents,

but their total frequency (in 0.2% of the PMCs, table 1b) is much too low to account for the two tertiary and four translocation trisomics observed (3.1%, table 1a). Therefore, in the material studied, preferential pairing has no influence on the frequency of tertiary trisomic offspring, and non-alternate orientation of chains-of-5 is concluded to be the main source of tertiary trisomic 240.

Telocentric translocation trisomic 273 (telo 5RS extra)

In telocentric translocation trisomic 273 (fig. 2a), part of telocentric 5RS is homologous with the interstitial segment T. Since the probabilities of interstitial segments to have chiasmata in interchange 273 heterozygotes are low (Sybenga, 1966a, 1970), the frequency in which 5RS is associated via segment T of translocation chromosome 5^1 will be negligible. Hence, attachment of the telocentric is practically always realized by chiasmata with either chromosome 5R (fig. 2b) or 1^5 (fig. 2c), resulting in an end position for 5RS when a chain-of-5 is formed. Thus, the same considerations as for telocentric translocation trisomic 240 are valid in spite of the fact that 5RS is homologous with a non-translocation arm of interchange 240. The orientations b1-b5 and c1-c5 of fig. 2 are expected, on which the suggested chromosomal constitutions of the nine non-telocentric trisomics in table 2a (2.0%) among the progeny are based.

Table 2a. Karyotype frequencies among the selfed offspring of telocentric translocation trisomic 273 (fig. 2a). Pooled results of 15 parent plants. For legend, see table 1a.

karyotype	number of plants
(14)nn	90
(14)tn	158
(14)tt	23
(15)nn+5RS	21[a]
(15)tn+5RS	103[a]
(15)tt+5RS	31[a]
(16)tn+2x5RS	3[a]
(16)tt+2x5RS	5[a]
(15)tt+5R	3
(15)1x1^5,1x1R	4[b]
(15)1x1^5,2x1R	2[c]
Total	443

[a]: part of the telocentric trisomic and tetrasomic offspring might have resulted from spores in which the absence of 5R is compensated by 5RS together with 5^1 (fig. 2,b4 and c4). However, none of the plants investigated so far appeared to have the corresponding constitution.
[b]: most probably (15)tn+5R (fig. 2b, c)
[c]: most probably (15)nn+1 (fig. 2b, c)

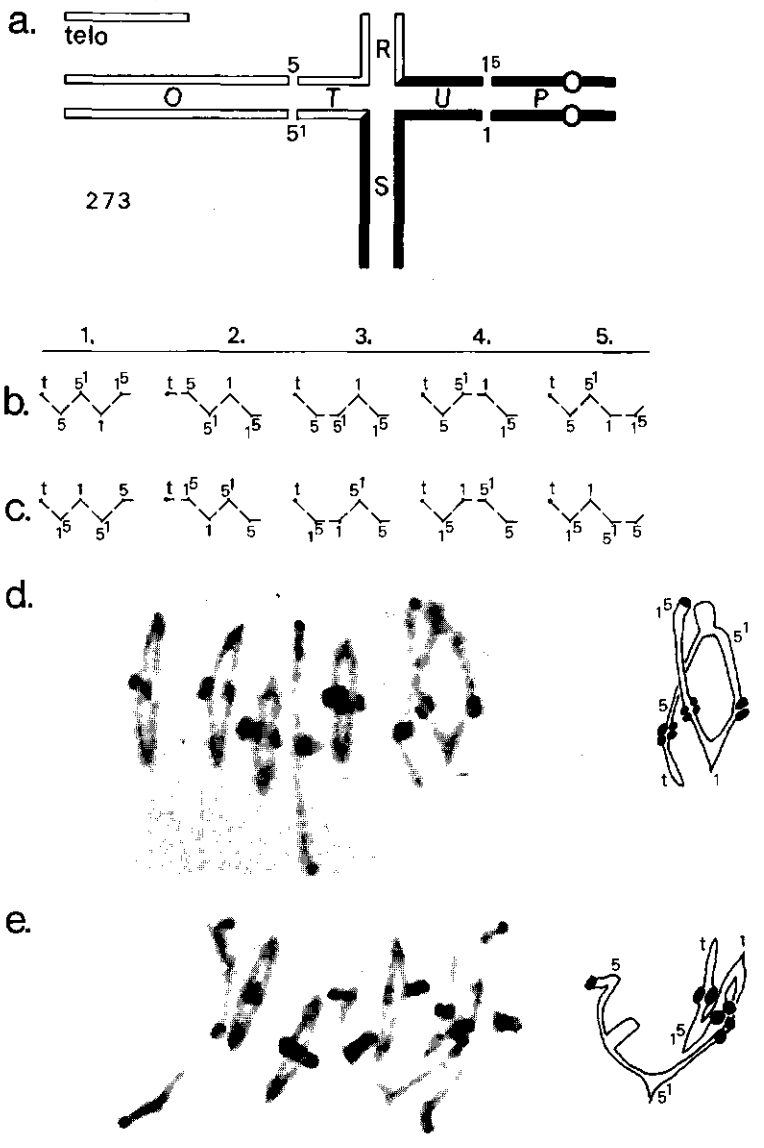


Fig. 2. a.: Pairing diagram of telocentric translocation trisomic 273 (1RL, 5RS) with 5RS as extra telocentric (not paired). Circles in segment P represent nucleolar organizer region. See Fig. 1.a. for further legend.

b., c.: Orientations of chain-of-5 with telocentric associated with 5R and 1^5 respectively. For further details see Fig. 1.b.

d.: Left: C-banded MI-chromosomes of PMC containing four ring bivalents, one open bivalent and a chain-of-5; right: interpretative drawing of chain-of-5, showing association of telo with 5R, which has no terminal band in its long arm (cf. Sybenga, 1983). Adjacent orientation b.3.

e.: Left: C-banded MI-chromosomes of PMC containing three ring and two open bivalents and a chain-of-5; right: interpretative drawing of chain-of-5, showing association of telo with 1^5 which has clear terminal band in its unchanged arm (1RS; Sybenga, 1983). Alternate orientation c.1.

MI configurations or progenies of these plants could not be further investigated. PMCs in telocentric translocation trisomic 273 with heteromorphic bivalents consisting of telocentric 5RS and either 5R (fig. 2b) or 1^5 (fig. 2c) are too scarce (0.8%, table 2b) to fully account for the 2.0% non-telocentric trisomics observed, so that again non-alternate multivalent orientation must be considered their major source. Without association preference of the telocentric, the situations of fig. 2b and 2c should occur in equal frequency, and among the diploid as well as the translocation trisomic progeny a tt:tn:nn-ratio of 1:2:1 is expected. The observed numbers (table 2a) do not fit this ratio. The significant ($P < 0.01$) excess of karyotypically normal diploids ($\chi^2 = 40.60$) and of telocentric translocation trisomics ($\chi^2 = 18.07$) suggests a strong preference for the situation in fig. 2b, i.e. - again - for association of the telocentric with a normal, instead of a translocation chromosome. This was also observed in a C-banded MI preparation of PMCs from one mother plant of table 2a (not the same as of table 2b), showing association of telocentric 5RS with 5R in 124 cells and with translocation chromosome 1^5 in 27 cells (fig. 2d, e). Tertiary trisomics with the long translocation chromosome 5^1 were not found for analogous reasons as in the case of telocentric translocation trisomic 240: intolerance for the amount of extra chromosomal material, or absence of $n+5^1$-spore formation (fig. 2b, c).

Translocation trisomic 273 (1R extra)

Primarily with the intention to test several hypotheses on the system of chromosome pairing in rye, Sybenga (1966b) extensively investigated a translocation trisomic 273, in which the satellite chromosome 1R was present as extra chromosome (fig. 3a). Based on detailed models, taking into account the type of multivalent orientation at MI as observed in 1000 PMCs and assuming terminal pairing initiation and absence of preferential pairing, a good fit was obtained between the calculated and the observed karyotype frequencies in the offspring of this translocation trisomic, which, among other arguments, confirmed the general validity of the assumptions for the material investigated. As Sybenga pointed out, the chains-of-5 in fig. 3b, c and d have an equal chance to arise (2/9) when,

Table 2b. MI configuration frequencies observed in 500 PMCs of one parent plant of table 2a

configuration	number of cells	frequency
telocentric 5RS not associated	309	0.618
telocentric 5RS associated in a		
- chain-of-5 (end position)	182	0.364
- chain-of-3 (end position)	5	0.010
- heteromorphic bivalent	4	0.008
	500	1.000

without preferential pairing all end segments have at least one chiasma. Since alternate orientation predominates (fig. 3, b1, c1 and d1) it is evident that the extra chromosome is expected to segregate together with the interchange complement more often than with the normal set, whereas the majority of the haploid gametes will be karyotypically normal. Haploid normal gametes and spores with the translocation complement and chromosome 1R extra also result from a ring bivalent consisting of 2 x 1R and an alternately orientated chain-of-3, with a frequency of occurrence of 1/9 (not drawn in fig. 3). Non-alternate orientation of maximally two adjacent centromeres per chain results in three new types of gametes, of which one is not viable due to a deficiency. The other two result in the two possible types of tertiary trisomics, when combined with karyotypical normal spores in a test cross ('trisome shift'). Although theoretically expected, no trisome shift was observed by Sybenga in the translocation trisomic offspring (table 3a). However, the trisomics among this progeny had not been investigated meiotically, while the fact that the long translocation chromosome 5^1 cannot be mitotically distinguished from the other non-satellite chromosomes without special techniques, was not considered (Sybenga, pers. comm.). Therefore, some of the 49 plants that were con-

Table 3. Karyotype frequencies in the progeny of a cross between a translocation trisomic 273 with 1R (a; from Sybenga, 1966b) or 5R (b) as extra chromosome and karyotypically normal individuals. For legend see table 1a

(a) Karyotype	number of plants
(14)nn	163
(14)tn	84
(15)3x1R,0x1^5	18
(15)2x1R,1x1^5	49
Total	314

(b) Karyotype	number of plants
(14)nn	36
(14)tn	10
(15)tn+5R	6
(15)2x1R,0x1^5	1^a
(15)2x1R,1x1^5	7^b
Total	60

^{a} : most probably (15)nn+5R (fig. 3d,1)
^{b} : most probably (15)nn+1^5 (fig. 3b, c and d)

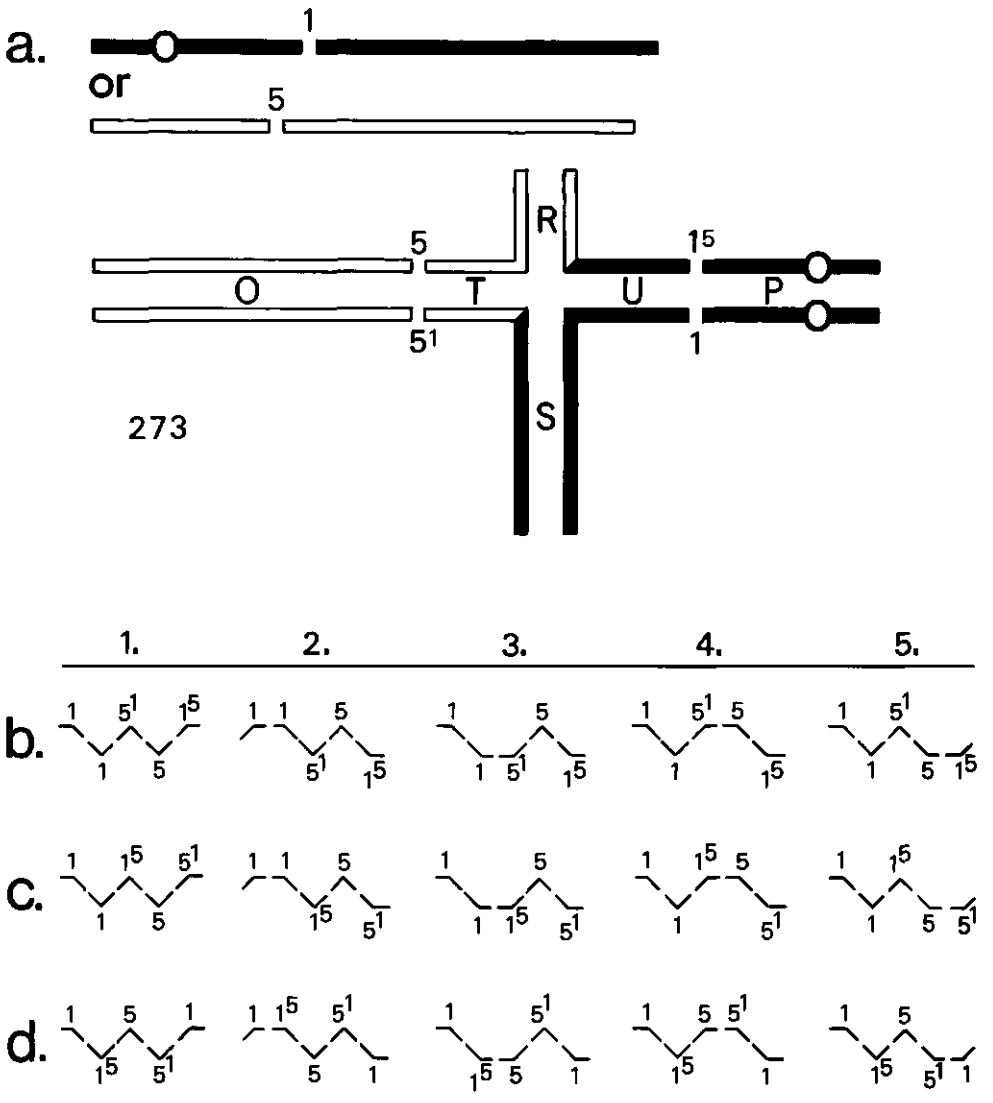


Fig. 3. a.: Pairing diagram of translocation trisomic 273 (1RL, 5RS) with 1R or 5R respectively as extra chromosome (not paired in diagram).

b., c. and d.: The three possible chains-of-5 in case 1R is extra, with five different MI-orientations (cf. Figs. 1.b., c. and 2.b., c.). Configurations in case 5R is extra are obtained by replacing "1" by "5", and vice versa; 1^5 and 5^1, however, are not replaced.

cluded to have originated from eggs containing 1R in addition to the translocation complement may in fact have resulted from eggs carrying the normal set with 1^5 extra, so that "shifts" might simply have been overlooked. On the other hand, trisome shift resulting in eggs with 5^1 extra to the normal complement was not observed, but should have been readily noted, since then two, instead of three, satellite chromosomes would be visable in mitotic metaphase. Moreover, "n+1^5" and "n+5^1" spores would be expected in equal frequency (fig. 3). An explanation can be that the viability of eggs or zygotes carrying the large extra chromosome 5^1, is reduced.

Translocation trisomic 273 (5R extra)

Table 3b contains the karyotype frequencies in the offspring of a cross between a translocation trisomic 273 with 5R as extra chromosome (fig. 3a) and karyotypically normal plants. Meiotic observations on neither the parent nor its trisomic offspring are available, which severely limits the validity of the conclusions based on table 3b. For instance, as expected under the assumption of non-preferential pairing, the progeny contains more normal diploids than interchange heterozygotes. However, a similar segregation can in an extreme case result when the situation of fig. 3c does not occur, while simultaneously the situation in fig. 3b is more frequent than in fig. 3d. Meiotic investigations could probably also have given an explanation for the high level and certainly the kind of trisome shift. The seven "shifted" trisomics in which one "short" (1^5) and two normal satellite chromosomes were observed, might have originated from eggs containing either the normal complement with 1^5 extra or the interchange complement with 1R extra. Origin of the latter is considered highly improbable, as this requires the inclusion of three neighbouring chromosomes in the same dyad cell. Breakdown of the chain-of-5 in fig. 3d by lack of chiasmata in segment P or S together with adjacent orientation of two neighbouring centromeres in the chain-of-3, is also considered very unlikely in view of the high frequencies in which P and S are bound in the interchange heterozygote (0.960 and 0.992 respectively; Sybenga, 1966a). Therefore, and since the short translocation chromosome 1^5 segregates together with the normal chromosome set already when only two neighbouring centromeres are orientated adjacently, the seven trisomics mentioned are concluded to be tertiary trisomics with 1^5 extra. On the basis of the absence of disomic interchange heterozygotes and homo-

zygotes in its selfed offspring, this was confirmed for at least one plant. An explanation for the surprisingly high level of trisome shift, however, is as yet not available. Genetic as well as environmental factors might have had an effect, for instance through a decrease of the average chiasma frequency. When chiasmata lack in segment P between chromosomes 1R and 1^5, in fig. 3c and d this results in a chain-of-3 and a bivalent which orientate independently, giving rise to gametes with 1^5 extra to the normal complement in 50% of the cases. The trisomic with two chromosomes 1R, in which no short translocation chromosome 1^5 was present (table 3b), probably is a primary trisomic for 5R, but since no meiotic observations were made, it can not be excluded that the long translocation chromosome 5^1 was present extra instead.

Translocation trisomics 282 and 501 (5R extra)

First metaphase configurations have been qualitatively investigated in PMCs of all nine trisomics in the offspring of a translocation trisomic 282 with 5R extra (compare fig. 1a, see table 4). In three plants, neither rings-of-4 nor chains-of-5 were observed, chains-of-3 being the main type of multivalent. These plants were concluded to be primary trisomics with 5R extra, since 7R as extra chromosome would require non-disjunction of three neighbouring chromosomes at meiosis. For the same reason, 5R is supposed to be the extra chromosome in three translocation trisomics, in which rings-of-4 were detected. Chains-of-3 and -5, but no rings of-4 were seen in the remaining three individuals, indicating their tertiary trisomic 282 constitution (5^7 extra). In the progeny of a cross between translocation trisomic 501 (compare fig. 1a) and karyotypically normal plants, 16 trisomics having one short translocation chromosome 5^4 were found (table 5). Of these, four were translocation trisomics, most probably having the maternal karyotype, and three were tertiary trisomics with 5^4 extra, while nine trisomics remained unidentified. The two trisomics in which no short translocated chromosome could be detected, are probably primary trisomics 5R, although the presence of an extra chromosome 4^5 instead can not be excluded, since MI data are lacking.

Double-translocation trisomic 282-305 (5R extra)

In F1 individuals of a cross between the translocation homozygotes 282 and 305, all segments of 5R are present two-fold, but are divided over four translocation chromosomes (fig. 4a). Chiasma formation in the diffe-

Table 4. Karyotype frequencies in the progeny of a cross between a translocation trisomic 282 (compare fig. 1a) (one female parent), and karyotypically normal individuals, For legend see table 1a.

Karyotype	number of plants
(14)nn	14
(14)tn	16
(15)tn+5R	3
(15)nn+5R	3
(15)nn+5^7	3
Total	39

Table 5. Karyotype frequencies in the progeny of a cross between a translocation trisomic 501 (compare fig. 1a) and karyotypically normal plants. Pooled results of 4 female parents. For legend see table 1a.

Karyotype	number of plants
(14)nn	38
(14)tn	11
(15)tn+5R	4
(15)nn+?	2[a]
(15)nn+5^4	3
(15)1x5^4	9
Total	67

[a]: most probably (15)nn+5R (fig. 3d,1)

rential segments T or T' is necessary to produce a normal chromosome 5R, required for the construction of a complete set of normal chromosomes. This again is a prerequisite for obtaining directly one of the four possible tertiary trisomics. The other prerequisite is numerical non-disjunction. The two events are independent and both occur in low frequency: only one out of 260 plants in the progeny of a backcross with karyotypically normal testers had the normal karyotype, whereas four trisomics were found (table 6a). Obviously, this double interchange disomic is no efficient direct source of tertiary trisomics.

The four trisomics is table 6a contained both short translocation chromosomes: 5^7 of 282, and 2^5 of 305.

Table 6.(a) Karyotype segregation in progeny of a cross between disomic heterozygotes for translocations 305 and 282 (compare fig. 4a) and normal plants. Result of two parents pooled. (b) Karyotype segregation in the pooled progeny of crosses between the three double-translocation trisomics of table 6a, (fig. 4a) and karyotypically normal plants

(a)

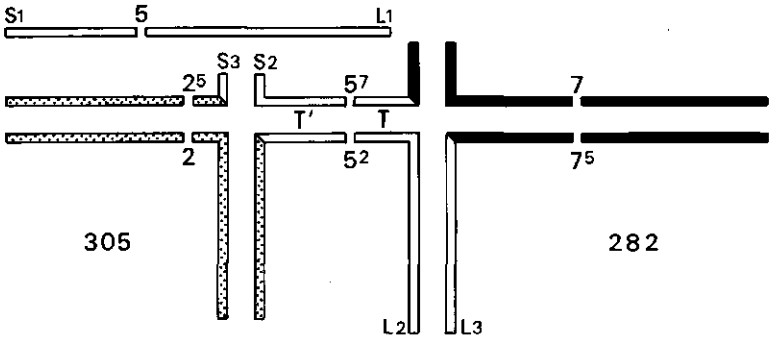
Karyotype	number of plants
(14)tn282	135
(14)tn305	120
(14)nn	1
(15)t282,t305+5R	3
(15)1x5^7,1x2^5	1
Total	260

(b)

Karyotype	number of plants
(14)nn	29
(14)tn282	33
(14)tn305	19
(15)1x5^7	11[a]
(15)1x2^5	15[b]
(15)1x5^7,1x2^5	13
(16)1x5^7	1
Total	121

[a]: 3x(15)tn282+5R, 4x(15)nn+5^7, remaining four not identified (see text)
[b]: 4x(15)tn305+5R, 1x(15)nn+2^5, remaining ten not identified (see text)

a.



b.

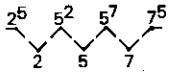
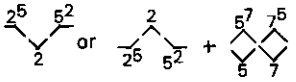
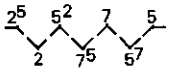
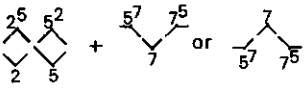
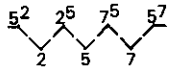
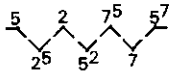
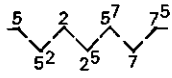
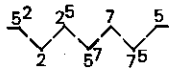
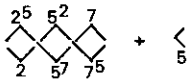
ASSOCIATION	CONFIGURATION
S1_S2 L1_L2	
S1_S2 L1_L3	
S1_S2 L2_L3	
S1_S3 L1_L2	
S1_S3 L1_L3	
S1_S3 L2_L3	
S2_S3 L1_L2	
S2_S3 L1_L3	
S2_S3 L2_L3	

Fig. 4. a.: Pairing diagram of double-translocation trisomic 282 (5RL, 7RS)/305 (2R, 5RS) with 5R extra (not paired). S1, S2 and S3: three homologous end segments of short arm of 5R; L1, L2 and L3: same of long arm. T and T': differential segments.

b.: The nine possible combinations of two-by-two associations of end segments S1, S2 and S3, and L1, L2 and L3, and resulting configurations (no chiasmata in interstitial and differential segments). Only alternate orientations drawn.

They could have been the result of numerical non-disjunction in the double interchange heterozygote in three different ways: either 5^7 is included in one egg together with the translocation 305 complement, or 2^5 with the interchange 282 chromosomes, or both sets of translocation chromosomes (5^7-7^5 and 2^5-5^2) are directed to one pole. In addition, the trisomics of course contain the normal chromosome complement derived from the tester parent. Backcrosses of three of the four trisomics with karyotypically normal testers yielded progenies segregating both (single) translocation 282 and 305 heterozygotes as well as normal disomics (table 6b), indicating that in addition to the normal complement, both interchange sets, and consequently all segments of chromosome 5R in three-fold (fig. 4a) were present in the mother plants. The pooled results of the three progenies are listed in table 6b; the fourth trisomic did not produce offspring.

On the basis of fig. 4b it is expected that gametes carrying the normal or one of the two interchange sets are formed in a ratio of 6:7:7. The fit with the observations ($\chi^2_2 = 4.755$; $0.05<P<0.10$) does not allow for any conclusion on association preferences. No quantitative examination of MI-configurations was made, as their interpretation would require an extremely detailed and complex model. In the same offspring (table 6b), 11 trisomics were detected in which one chromosome 5^7 of 282 was present. MI-configurations, or karyotype segregations among the selfed offspring showed that four of these were tertiary and three were translocation trisomics, while four remained unidentified. One of the 15 trisomics which contained one chromosome 2^5 of 305 appeared to be a tertiary trisomic, four were found to be translocation trisomics, whereas the remaining 10 could not further be examined. With seven chromosomes involved in the complex (fig. 4a), a high number of different non-alternate orientations as well as independently orientating configurations resulting from absence of chiasmata in specific end segments can be expected, and gametes containing one of the four possible translocation chromosomes additional to the normal set can be imagined to originate relatively easy. These four types of spores are expected to be formed in about equal frequency, since according to fig. 4b each translocation chromosome has a specific position in the multivalent - at the end, next to the end, next to the middle and in the middle - as often as any other translocation chromosome. However, none of the 39 trisomics in table 6b can be a tertiary trisomic with either 5^2 or 7^5 as extra chromosome, again suggesting that these amounts of extra genetic material are too large to be tolerated.

Comparison of sources

The various types of translocation trisomics studied yield higher percentages of tertiary trisomics (table 7) than the corresponding interchange heterozygous disomics, except perhaps telocentric translocation trisomic 273 with 5RS extra (see below). With respect to chains-of-4 in translocation heterozygotes, tertiary trisomics can only originate from non-alternate disjunction. Chains-of-5 (or more), however, not only have more possibilities for non-alternate orientation, they can also fall apart in smaller configurations which orientate independently, whereas breakdown of a chain-of-4 results in either a chain-of-3 and a univalent which usually gets lost, or two bivalents, so that here numerical non-disjunction is practically excluded. The maximum number of tertiary trisomics in table 7 is overestimated in the cases of translocation trisomics 273 with 1R extra and 501 with 5R extra, and the double-translocation trisomic (see notes a and b in table 7), the actual number being somewhere in between the maximum and the numbers of tertiaries identified unequivocally. Nevertheless, there are two points of interest. One is, that the frequency of tertiary trisomics in the offspring of both telocentric translocation trisomics is lower than that in the progenies of the other types. With respect to the translocation trisomics, an explanation might be, that with two members available of a normal chromosome which is involved in the translocation, the assembly of a complete normal haploid set is more easily accomplished than when there is only one member present. Variation in the frequency of chains-of-5, however (table 1b, 2b) presumably caused by genetic and environmental factors, may also have played a role. As for the double-translocation trisomic, the higher number of chromosomes involved in the multivalent probably allows for more variation in orientation and breakdown, resulting in a higher tertiary trisomic frequency. Another interesting fact is that tertiary trisomics with a long translocation chromosome are formed in a low frequency - if any. As explained above, this is not surprising in the case of the telocentric translocation trisomics, but they would have been expected to originate from the other types, theoretically in frequencies equal to those of the tertiary trisomics having a short extra translocation chromosome. Difficulties to obtain both types of tertiary trisomics

Table 7. Number and percentage of tertiary trisomics originating from various types of translocation trisomics, as based on tables 1a, 2a, 3a and b, 4, 5 and 6b. Corresponding interchange heterozygotes never yielded more than 1% trisomics (tertiary and translocation). In all tertiary trisomics identified unequivocally, the extra chromosome is the short translocation chromosome

Translocation trisomic	Size of progeny	Numbers (percentages) of tertiary trisomics identified unequivocally	Maximum possible numbers (percentages) of tertiary trisomics observed; extra translocation chromosome:	
			short	long
240+5RS	191	2(1.0)	2(1.0)	0(0.0)
273+5RS	443	0(0.0)	2(0.5)	4(0.9)[c]
273+1R	314	0(0.0)	49(15.6)[a]	0(0.0)
273+5R	60	1(1.7)	7(11.7)	1(1.7)[c]
282+5R	39	3(7.7)	3(7.7)	0(0.0)
501+5R	67	3(4.5)	12(17.9)[b]	2(3.0)[c]
282/305+5R	121	282:4(3.3)	282: 8(6.6)[b]	282:0(0.0)
		305:1(0.8)	305:11(9.1)[b]	305:0(0.0)

[a]: a substantial number of translocation trisomics with 1R extra will be present among these plants

[b]: a substantial number of translocation trisomics with 5R extra will be present among these plants

[c]: most probably primary trisomics 5R

from interchange heterozygotes have been reported for instance by Blakeslee et al. (1936), Sutton (1939), Khush and Rick (1967), Eicher (1973) and de Boer (1975). Ibrahim (1950) and Ramage and Humphrey (1964), however, could obtain the two types without much trouble. With increasing size of the extra material, viability may become more reduced, but also some types of multivalent orientation might occur less frequent than others, depending on factors like length of the chromosome and their translocated and interstitial segments, position of chiasma formation, chiasma terminalization, etc. (Khush, 1973). When the construction of a tertiary trisomic with a specific translocation chromosome extra is desired, for instance because an m.s.-gene is known to be located interstitially or close to the breakpoint, a cross between the appropriate telocentric trisomic and the translocation as outlined might be of use. Blakeslee et al. (1936) used a secondary trisomic for the same purpose. This method may be very efficient, since here tertiary trisomics already can originate from alternate chain-of-5 orientation. Secondary trisomics, however, are not as readily available as primary or translocation trisomics.

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4. Meiotic behaviour, stability, transmission and recombination in four balanced tertiary trisomics of rye (*Secale cereale* L.).

by
J.N. de Vries
Department of Genetics, Agricultural University,
Gen. Foulkesweg 53,
6703 BM Wageningen,
The Netherlands.

ABSTRACT

F3 and F4 progenies of four balanced tertiary trisomics (BTTs) of rye carrying the short translocation chromosomes of their corresponding interchanges were karyotyped and scored for the monogenic recessively inherited tigrina marker (*ti*) which is located terminally on the short arm of chromosome 5R. The segment carrying the dominant *Ti*-allele was included in the extra translocation chromosome of each BTT, while the normal 5R pair carried the recessive allele. Recombination fractions between *ti* and the interchange breakpoint varied between 0.005 $\pm$ 0.003 and 0.032 $\pm$ 0.005 for the different BTTs. Interstitial chiasma formation could be demonstrated in only one BTT, in 1% of the first metaphase configurations, without, however, resulting in recombination. Chiasma localization just proximal to the terminal C-band of 5RS was concluded to cause the low recombination observed. Less than 0.5% of the progenies consisted of plants with a deviant karyotype able to reach maturity, indicating a high level of meiotic stability of the trisomics. The results further suggest that selection towards low male and high female transmission rates of the extra chromosome is possible. The suitability of the BTTs investigated for use in hybrid breeding programs is evaluated.

Keywords: *Secale cereale* L. - balanced tertiary trisomics - hybrid breeding - recombination - transmission

In the diagram of fig. 1a, a hypothetical tertiary trisomic is shown with translocation chromosome a^b as the extra chromosome. Three copies are available of segments O (unaltered arm) and T (interstitial segment), two of which are present in the normal pair *a*. Of the three copies of segment R (exchanged segment) two are present in pair *b*. It is seen from fig. 1b, that with random, terminally initiated pairing and association of all paired end segments O, P, R and S the extra translocation chromosome appears as a univalent in 1/9 of the cases, whereas in 8/9 this chromosome is associated and is consequently able to orientate at MI. Thus, 0.5 x 8/9 or 44.4% of the gametes of the tertiary trisomic are expected to contain chromosome a^b in addition to the normal haploid complement. This is the maximum expected, since the end segments do not necessarily always form a chiasma when paired. Usually, male gametes carrying extra chromosomal material do not participate in reproduction due to certation. This does not apply to female gametes, and when their viability is not severely affected by the extra material, the progeny of a selfed tertiary trisomic is expected to consist of maximally 44.4% tertiary trisomics and minimally 55.6% normal disomics. Unlike primary trisomics in which three identical, interchangeable copies of the same chromosome are present, in tertiary trisomics only one copy of the extra translocation chromosome is available which cannot simply be replaced by another chromosome. Hence, with the dominant allele of a marker located on the extra chromosome a^b, very closely linked with the interchange breakpoint, and pair *a* or *b* carrying the recessive alleles, a balanced system arises in which transmission of the dominant allele through the male haplophase is prevented, but female transmission is permitted. Consequently, the selfed progeny of this balanced tertiary trisomic (BTT) is expected to consist of maximally 44.4% individuals having the dominant phenotype and containing the extra chromosome, whereas the remainder are disomic recessives.

Ramage (1965) proposed a system for barley hybrid breeding based on genetic (nuclear) male sterility (m.s.), with the dominant m.s.-allele located on the extra chromosome of a tertiary trisomic and the recessives on the normal complement. For hybrid rye breeding, this balanced

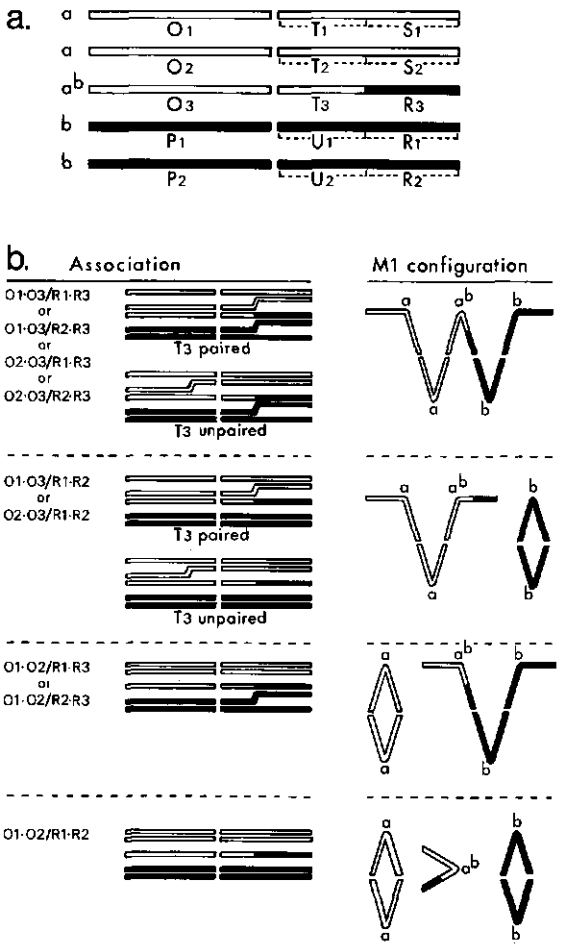

Fig. 1

a. Diagram of chromosomes involved in the complex of a tertiary trisomic with translocation chromosome a^b as extra chromosome. O1, O2 and O3: non-translocation arms of chromosome *a*, P1 and P2: same of chromosome *b*; R1, R2 and R3: exchange segments of chromosome *b*, S1 and S2: same of chromosome *a*; T1, T2 and T3: interstitial segments of chromosome *a*, U1 and U2: same of chromosome *b*.

b. Pairing diagrams (complete pairing) and MI configurations (alternate orientation) of the tertiary trisomic of fig. 1a, resulting from the nine possible combinations of two-by-two association of segments O1, O2 and O3, and R1, R2 and R3. With random terminal pairing initiation, the chance of occurrence for each combination is 1/9; each chromosome can change pairing partner only once. The point of partner change between chromosome a^b and one of the chromosomes *a* may be located at either side of the centromere, so that T may be paired completely, partly or not at all. Chiasmata are present in all segments paired, except the interstitial segments T and U. With chiasmata in T and U, "frying-pans" and other "closed" and "branched" configurations result (see text).

tertiary trisomic (BTT-) system may offer a useful alternative for systems based on cytoplasmic male sterility (CMS), which are relatively vulnerable due to the limited number of operational combinations of CMS-sources and corresponding restorer genes available (Geiger, 1982; Morgenstern and Geiger, 1982). In the BTT-system, the propagation of an all male sterile progeny to be used as seed parent in the hybrid seed production is accomplished by repeated cycles of pollination of male sterile normal disomics by fertile balanced tertiary trisomics. The BTT itself is maintained by selfing (Wiebe and Ramage, 1971). Especially for wind pollinated crops like rye, the seed parent should be completely free of male fertile individuals, as their occurrence affects both performance and homogeneity of the hybrid, whereas removal of fertile plants by hand is time consuming and expensive. Thus, male transmission of the dominant m.s.-allele should be completely absent and, therefore, pollen transmission of the extra chromosome carrying this allele as well as recombination with the recessives on the normal chromosome complement must be prevented. An obstacle to recombination is the presence of the interchange breakpoint, which should be located as close to the m.s.-allele as possible, whereas the extra translocation chromosome should be of sufficiently large size to prevent its male transmission. The vigour of the BTT, however, should not be affected too drastically, and for an efficient maintenance, the extra translocation chromosome should be transmitted through the eggs with as high a frequency as possible, which sets a limit to its size. This is in conflict with the requirement of reduced pollen transmission. In addition, transmission can only be accomplished when the extra chromosome orientates well at first meiotic metaphase and, consequently, chiasma formation between translocation and normal chromosomes is necessary. This conflicts with the requirement of the obstruction of recombination between the dominant and recessive m.s.-alleles, when linkage between m.s.-locus and translocation breakpoint is not very close.

To avoid extensive karyotyping or hand elimination of disomics in the maintenance of the BTT stock by selfing, Wiebe and Ramage (1971) have worked out a self-rogueing system making use of a monogenic recessive lethality gene with the dominant allele on the extra chromosome, again very closely linked with the translocation breakpoint. The normal chromosome complement again carries the recessives. Infrequent

disomic recombinants - which must be removed by hand before flowering - are distinguished morphologically from the trisomics in rye inbred lines.

This paper deals with the behaviour of four BTTs of rye in which the extra chromosome is composed of a segment of varying length including part or all of the short arm of chromosome 5R, and a translocated segment of other chromosomes. In the segment of 5R, the dominant allele of the monofactorial marker *ti* (tigrina; de Vries and Sybenga, 1983) is located on the extra chromosome, whereas the normal homologues carry the recessive alleles. Recombination between the marker and the translocation, male and female transmission and the occurrence of deviant karyotypes in selfed BTT progenies are studied in relation with a quantitative examination of meiotic first metaphase (MI) configurations. Conclusions are presented on chiasma localization and on the most favourable location of m.s.- and marker genes relative to the centromere and the interchange breakpoint of the extra chromosome.

MATERIALS AND METHODS

The four tertiary trisomics investigated (fig. 2) carry the short translocation chromosomes and the code numbers of the corresponding interchanges 240, 273, 282 and 305, which are part of the Wageningen translocation tester set of rye (Sybenga and Wolters, 1972; de Vries and Sybenga, 1976; Sybenga, 1983). The tertiary trisomics were isolated in F1-progenies of different translocation trisomics crossed with karyotypically normal male parents (de Vries, 1983), which were homozygous recessive for the tigrina gene (*ti*) causing transverse yellow striping in the leaves. As the tigrina gene is located on the short arm of chromosome 5R distally to the breakpoint of translocations 273 and 305 (fig. 2; cf. de Vries and Sybenga, 1983), tertiary trisomic F1 individuals carry the dominant allele on the extra translocation chromosome ($\frac{Ti}{T}$) and on one of the normal chromosome 5R ($\frac{Ti}{N}$), the other carrying the recessive allele ($\frac{ti}{N}$). In their F2s, 15-20% tigrina plants segregated, which agrees with the expectation of a 3:1 segregation ratio of wildtype: tigrina among the disomic F2 plants. With low recombination between *ti*-locus and interchange breakpoint (see below) most tertiary trisomic F2 plants are wildtype. In order to determine the genotype of

the wildtype trisomic F2 plants ($\frac{Ti}{T}\frac{Ti}{N}\frac{Ti}{N}$, $\frac{Ti}{T}\frac{Ti}{N}\frac{ti}{N}$ or $\frac{Ti}{T}\frac{ti}{N}\frac{ti}{N}$, the latter representing the BTT) they were selfed, F3s of about 15 plants were grown and the fraction of tigrina plants scored. F3s with 25% or less tigrina plants were concluded to have originated from an F2 parent in which one normal 5R chromosome carried the dominant *Ti*-allele, or at least one when no tigrina plants were observed. F3 samples with more than half of the plants showing the tigrina phenotype were karyotyped. The F2 parent was concluded to be a BTT, when almost all disomic F3 plants were tigrina and when wildtype F3 individuals contained the extra translocation chromosome.

Two balanced F2 plants of tertiary trisomics 240, 273 and 282, and one of 305 were used for further investigation. The two plants of BTT 240 were derived from two different F2s, those of 273 and 282 from one F2 each. F3 and F4 progenies were karyotyped and scored for the tigrina marker, providing data on stability of the tertiary trisomic, on male and female transmission of the extra chromosome and on recombination. Recombination fractions between the *ti*-locus and the breakpoint of interchanges 273 (1RL-5RS) and 282 (5RL-7RS) are 0.076 $\pm$ 0.020 and 0.009 $\pm$ 0.005 respectively (de Vries and Sybenga, 1983). Linkage between *ti* and interchange 240 (3RS-5RL) must be very close also, as there is practically absolute linkage between *ti* and *br* (brittle; de Vries and Sybenga, l.c.) while in large F2s recombinants between *br* and translocation 240 have so far not been observed. Absence of recombinants in the offspring of a testcross (190 plants) between translocation heterozygote 305 (2R-5RS) heterozygous for *ti*, and karyotypically normal tigrinas also indicates close linkage between locus and interchange breakpoints.

Meiotic configurations were scored in PMC preparations at mid-MI, containing maximally 10% cells at first anaphase. Two preparations of BTT 240 (240a, 240b) and two of BTT 305 (305a, 305b) from plants of different F4-lines derived from the same F3, were investigated. In case of BTT 273, two preparations (273a, 273b) from two different plants of the same F3 were studied. Two preparations of BTT 282 (282a, 282b) derived from anthers of different ears of one F2 parent plant, and one preparation of an F4-descendant of this plant (282c) were investigated as well. In all tertiary trisomics the extra chromosome is easily recognized at mitotic metaphase (fig. 2). MI-univalents of the extra chromo-

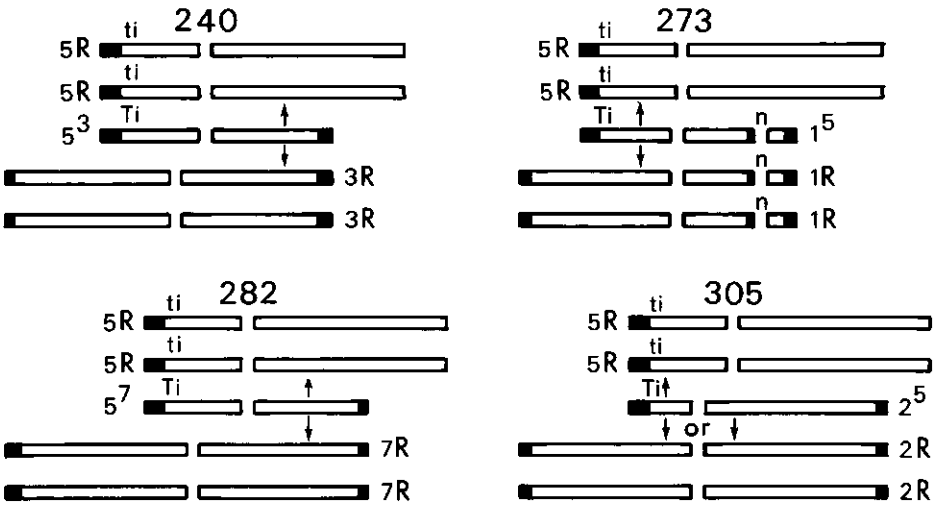

Fig. 2. C-banded diagrams of chromosomes involved in tertiary trisomics 240 (3RS-5RL), 282 (5RL-7RS), 273 (1RL-5RS) and 305 (2R-5RS). Chromosome nomenclature according to de Vries and Sybenga (1983). Approximate positions of translocation breakpoints at arrows. Centromeres as gaps, nucleolar organizer at 'n'. Tigrina locus (*Ti*, *ti*) in 5RS, distal to breakpoint of translocation 305. Only terminal C-bands drawn, except in 1RS where the band next to 'n' is drawn also. Arm length ratios based on Sybenga and Wolters (1972), C-banding pattern on de Vries and Sybenga (1976) (compare Sybenga, 1983).

some are smaller than univalents of the normal chromosome complement, and occasionally the position of the extra chromosome in an MI-multivalent can be determined. In carmine-stained PMC preparations, however, the pattern of association of the extra chromosome, especially in trivalents, cannot be recognized unequivocally. Therefore, a number of PMC preparations were C-banded. Per carmine-stained MI preparation, all configurations were scored in 500 PMCs, whereas in the C-banded preparations scoring was restricted mainly to trivalents.

Plants were grown under greenhouse conditions at 18-20 ^{o}C throughout 1980-1982. Variations in day length and light intensity could therefore have had some quantitative effect. Karyotypes were scored in root tips squashes after pre-treatment for 2 hr. in a saturated aquaeous solution of alpha bromonaphthalene at 24 ^{o}C, fixation-maceration in 1 N HCl for 12 min. at 60 ^{o}C, and Feulgen staining. Anthers were fixed in 1:3 acetic alcohol and stored at -10 ^{o}C. C-banding was carried out according to the procedure described by Giraldez et al. (1979) leaving out the xylene immersion step. For carmine staining, 2% aceto carmine was used followed by mounting in Euparal.

RESULTS AND DISCUSSION

Chiasmata in 03 and R3 (fig. 1a)

An association of five chromosomes at MI potentially results from the upper two pairing diagrams of fig. 1b, which are different with respect to the position of the point of partner change relative to the centromere of the translocation chromosome. In each of these two diagrams, six segments are distinguished which may or may not have chiasmata, so that there are maximally 128 possible combinations of segments being associated or not, i.e. 2^{6} or 64 for each type. Several combinations result in identical MI configurations, and it can be derived that 17 morphologically different quinquivalents, or quadrivalents with a univalent are possible. In addition, three types of trivalents (chain, frying-pan or Y-shaped) may be formed, accompanied by a ring- or an

open bivalent or a pair of univalents. However, the latter can not normally be distinguished from the bivalents formed by the chromosomes not involved in the tertiary trisomic complex. For the same reason, translocation chromosome univalents form one configuration category, so that 17 + 3 + 1 = 21 MI configurations can be recognized unequivocally. It is seen in table 1, that 11 different configurations have been observed in the four tertiary trisomics studied. Among the configurations 1-7, the frequency of chains-of-5 is considerably higher than that of any other configuration. The majority of trivalents are chains-of-3. PMCs with either a chain-of-5,a chain-of-3 or a translocation univalent are expected to be found in a ratio of 4:4:1 (fig. 1b). However, in all cases studied a significant shortage of chains-of-5 was observed ($P< 0.01$). Evidently at least some of the requirements on which the 4:4:1 ratio is based (fig. 1b) are not fulfilled. Pairing may not be random, or the chiasma frequency in specific segments may be reduced more than in others. Without quantitative data from meiotic stages before first metaphase, it is impossible to discriminate between these causes properly. Nevertheless, an attempt to extract some quantitative information from the observations remains worthwhile.

Assuming that chiasmata have not been formed interstitially between T3 and either T1 or T2 (fig. 1), and provided chiasmata in O3 and R3 are formed independently, the frequencies of configurations 1-7, 8-10 and 11 (table 1) can be expressed in terms of o_3 and r_3, the frequencies of MI association of segments O3 and R3. Under the conditions described in the caption of fig. 1b, o_3 and r_3 are expected to be maximally 2/3. The frequency of PMCs with configurations 1-7 equals $o_3 \times r_3$, that of configurations 8-10 equals $o_3(1-r_3)+(1-o_3)r_3$ and that of configuration 11 equals $(1-o_3)(1-r_3)$. From these equations estimates for o_3 and r_3 can be obtained (table 2, columns I). It should be noted, that trivalents (configurations 8-10) can also consist of the translocation chromosome and one member of each of the normal chromosomes involved, i.e. with both O3 and R3 associated. In the same PMC, the two other normal chromosomes then appear as univalents. The number of such PMCs, however, was small. Moreover, these univalents may have been another pair of normal chromosomes as well. Hence, the frequency of this type of trivalent can be neglected without consequences. For reasons of mathematical symmetry, the roots obtained with the equations

Table 1. Meiotic configuration frequencies in 500 PMCs per preparation of BTT 240 (240a: one F4 plant), 282 (282a and 282b: same F2 plant, different ears, 282c: one F4 plant), 273 (273a: one F3 parent plant of table 3) and 305 (305a and 305b: 2 plants of different F4 s). Compare table 3 for origin of preparations. Bound bivalent arms (%): average percentage of associated bivalent arms in cells with a translocation chromosome univalent (configuration 11), being a measure for the level of chiasma formation in each preparation.

type no.	configuration	240a	282a	282b	282c	273a	305a	305b
1	○○	0.0	0.0	0.0	0.0	0.0	0.002	0.0
2	○○	0.032	0.004	0.004	0.006	0.044	0.026	0.018
3	○○>	0.0	0.0	0.002	0.0	0.0	0.0	0.0
4	○○	0.004	0.002	0.0	0.002	0.002	0.004	0.010
5	○○>	0.0	0.0	0.0	0.0	0.0	0.002	0.0
6	○○	0.324	0.126	0.108	0.052	0.156	0.248	0.166
7	○○>	0.020	0.002	0.002	0.008	0.004	0.016	0.004
	subtotal	0.380	0.134	0.116	0.068	0.206	0.298	0.198
8	○	0.010	0.024	0.026	0.010	0.138	0.038	0.086
9	○	0.002	0.002	0.0	0.002	0.002	0.0	0.004
10	○	0.478	0.648	0.628	0.690	0.442	0.550	0.536
	subtotal	0.490	0.674	0.654	0.702	0.582	0.588	0.626
11	univalents	0.130	0.192	0.230	0.230	0.212	0.114	0.176
	Total	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Bound bivalent arms (%)	92.20	94.64	95.71	85.21	88.14	91.85	85.88

Table 2. Association behaviour of the extra translocation chromosome. o_3 and r_3: frequencies of segments 03 and R3 respectively (fig. 1a) being bound, estimated on the basis of table 1; columns I: estimates based on formulae applied to observations in carmine preparations, II: estimates corrected using C-banded preparations. Assignment of frequencies to 03 and R3 based on association of these segments in chains-of-3 as observed in C-banded MI preparations (right three columns). PMC preparations from anthers of same florets as in table 1 have identical code number. Compare table 3 for origin of preparations.

Prep. code	Extra chrom.	I		II		C-banded chains-of-3 Percentage of association of the extra chromosome to chromosome		Total no.of PMCs
		o_3	r_3	o_3	r_3			
240a	5_3^3	0.728	0.522	0.628	0.622	-	-	-
240b	5_7	-	-	-	-	5R-50.7	3R-49.3	73
282a	5_7	0.767	0.175	0.774	0.168	-	-	-
282b	5_7	0.726	0.160	0.737	0.149	-	-	-
282c	5_5	0.747	0.091	0.734	0.104	5R-94.9	7R- 5.1	59
273a	1_5	0.294	0.700	0.339	0.655	1R-22.9	5R-77.1	144
273b	1_5	-	-	-	-	1R-21.2	5R-78.8	55
305a	2_5	0.821	0.363	0.631	0.553	2R-56.7	5R-43.3	30
305b	2^5	0.762	0.260	0.565	0.457	2R-58.6	5R-41.4	29

presented above cannot be assigned to O3 or R3 without further information. For this purpose, observations on C-banded chains-of-3 are used, in which the association of the tertiary chromosome with each of the normal chromosomes can be determined (fig. 3b, c; compare fig. 2); the number of these observations (table 2) is considered too limited to provide reliable (direct) estimates of o_3 and r_3. On the basis of C-banding data (table 2), differences in association percentages of O3 and R3 are clear, except for tertiary trisomic 240. In tertiary trisomic 282 the largest roots are assigned to the unchanged arm of chromosome 5^7, which is the same as O3 of chromosome 5^3 of tertiary trisomic 240. Because the values of the largest roots in both instances correspond well (table 2, columns I), it was decided to assign the highest association frequency to O3 of chromosome 5^3 of 240 also. When the assumption of independent chiasma formation in O3 and R3 is correct, i.e. when the formulae presented above are valid, the association frequencies of the unchanged arms of the extra chromosomes 5^3, 5^7 and 2^5 (O3) and of the interchanged segment of 1^5 (R3) exceed the theoretically expected maximum value of 2/3. Preferental pairing of the normal chromosomes with specific, homologous parts of the extra translocation chromosome might be an explanation. Since a chiasma is not necessarily always formed whenever a segment is paired, preferential pairing may be stronger than the figures in table 2 suggest. On the other hand, the association frequencies of the exchanged segments of 5^3, 5^7 and 2^5 and the unchanged arm of 1^5 are substantially smaller than 2/3, but it can not be determined whether these segments are preferentially excluded from pairing, since pairing initiation may be random, but subsequent chiasma formation in specific segments may be reduced for various reasons (length of the segment, proximity of a translocation breakpoint, change of pairing partner). The association frequency of the exchanged segment of chromosome 1^5 of tertiary trisomic 273 apparently is not severely affected by the presence of the interchange breakpoint and approaches that of the unchanged arms of chromosomes 5^3 and 5^7 in 240 and 282. Even the very small terminal part of the short arm of chromosome 5R in chromosome 2^5 of tertiary trisomic 305 is able to form chiasmata rather frequently. Whether the contrasting low association frequency of the unchanged satellite arm of chromosome 1^5 is a matter of pairing preference or of chiasma reduction

attributable to the presence of the nucleolar organizer region, can again not be determined. Low association probabilities for this arm were reported also by Orellana and Giraldez (1983) in disomic rye, and change of pairing partner may result in a further decrease.

Positive chiasma interference between 03 and R3 may be another explanation for the observed reduced frequency of chains-of-5 relative to chains-of-3 (table 1). This explanation implicates that incorrect values for the association frequencies of 03 and R3 are presented in table 2, columns I, as then the formulae on which the estimates of o_3 and r_3 are based would no longer be valid.

Alternative estimates for o_3 and r_3 are obtained by multiplying the frequency of trivalents (configurations 8-10) observed in the aceto carmine preparations (table 1) by the fractions in which 03 or R3 respectively are associated in chains-of-3 in the C-banded material (table 2). To these products, the frequencies of configurations with *both* 03 and R3 associated (configurations 1-7, table 1), are added. The results are listed in table 2, columns II. Compared with columns I, differences between o_3 and r_3 are considerably smaller in columns II, except for tertiary trisomic 282. In the latter, 03 may be preferentially paired with 01 or 02, although the estimates of o_3 may also exceed the theoretically expected maximum of 2/3 here as a result of a fortuitous high percentage of association of chromosome 5^7 with 5R instead of 7R in the particular C-banded preparation investigated. In columns II, none of the association frequencies o_3 or r_3 estimated for the other tertiary trisomics exceeds the value of 2/3, which does not contradict the view that pairing initiation is random (Sybenga, 1966), without necessarily excluding the possibility of some preferential pairing.

Thus, the large difference between o_3 and r_3 as derived from the equations presented above (columns I, table 2) is due apparently to positive chiasma interference rather than a true difference (Sybenga, 1975). When this is a reality, a reduction of the frequency of chains-of-5 relative to chains-of-3, but also a shift of the 4:1 ratio between chains-of-3 and translocation univalents in favour of the chain-of-3 frequency would be expected. Except for preparation 305a, the latter

has not been observed (table 1). Probably, in these other preparations the effect of interference is overruled by the fact that segments 03 and R3, when paired, do not always form chiasmata, for instance owing to between cell variation in the level of chiasma formation, or to a change of pairing partner.

Chiasmata in T3

Based on the relative frequencies and the morphology of the MI configurations, chiasma formation in the interstitial segments T3 of the extra translocation chromosome (fig. 1a) is concluded to be very rare in the tertiary trisomics investigated. Under the realistic assumption that each chromosome segment present three times changes pairing partner at most once, it can be derived from fig. 1b that none of the configurations 1, 2, 4, 6, 7 and 10 (table 1) can possibly have resulted from chiasma formation in T3. Of course, translocation chromosome univalents (configuration 11) have resulted from absence of chiasmata in T3 as well as in 03 and R3 (fig. 1b). However, chiasmata may have been formed in T3 in Y-shaped and frying pan quadri- and trivalents (configurations 3, 5, 8 and 9). The quality of the Giemsa stained preparations 273a and 273b allowed a detailed examination of the frying pan trivalents (configuration 8), which are frequently observed in this tertiary trisomic. Among a total of 23 C-banded frying pan trivalents, three were found in which unequivocally translocation chromosome 1^5 and one member of the 1R pair had formed a small ring with a large projection, representing segment S to which the other chromosome 1R was associated (fig. 3e). Hence, in these frying pans chiasmata were formed between S1 and S2, T2 and T3 and 02 and 03 (fig. 1a, fig. 2). In the remaining 20 frying pans, no chiasmata were formed in T3, and translocation chromosome 1^5 was associated with a ring consisting of either the 1R pair via segment 03 (14 cells; fig. 3d) or the 5R pair via segment R3 (6 cells). In these configurations, the handle is short, whereas the ring has the size of an average normal ring bivalent. Of the 69 frying pans observed in the 500 cells of carmine stained preparation 273a (table 1), 64 showed the morphology of that in fig. 3d, whereas in five cells (1.0%) the frying pan had a markedly small ring and a large handle (fig. 3e) indicating chiasma formation in T3. In the prepara-

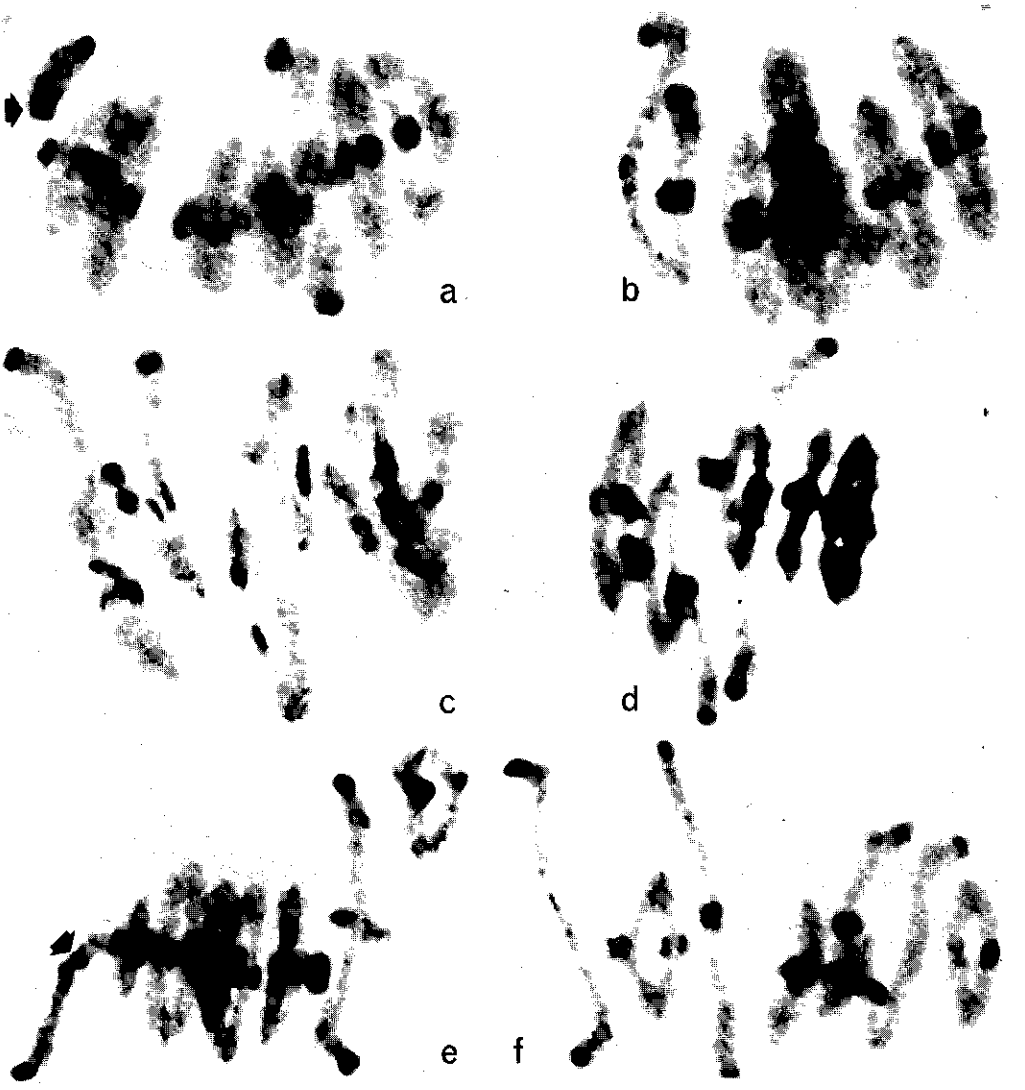

Fig. 3. C-banded MI configurations in PMCs of tertiary trisomic 273 (fig.2).
(a) 7 bivalents (6 ring, 1 open) and translocation univalent 1^5 (satellite arm at arrow).
(b) 6 ring bivalents and a chain-of-3 consisting of 1^5_5 and 2 x 5R
(c) 6 ring bivalents and a chain-of-3 consisting of 1^5_5 and 2 x 1R
(d) 6 bivalents (5 ring, 1 open) and a frying pan consisting of 1^5 and 2 x 1R. Chiasmata between O1 and O2, O1 (or O2) and O3 and T1 and T2 or S1 and S2 (fig. 1a, fig. 2).
(e) 6 bivalents (5 ring, 1 open) and a frying pan consisting of 1^5 and 2 x 1R. Chiasmata between S1 and S2, T1 (or T2) and T3 and O1 (or O2) and O3 respectively. Position of terminalized chiasma in 1RL at arrow. Note small size of ring and differences with frying pan of fig. 3d (size of ring and handle, position of bands).
(f) 5 bivalents (3 ring, 2 open) and a chain-of-5, with 2 x 1R at left and 2 x 5R at right.

tions of the other tertiary trisomics, no frying pans with morphological characteristics similar to those in fig. 3e were observed. In addition, in none of the carmine preparations investigated, frying pan quadrivalents (configuration 3) were found with a markedly small ring resulting from chiasma formation in T3. Since Y-shaped quadri- and trivalents (configurations 5 and 9) have essentially the same mode of origin as frying pans except for chiasma formation in one of the disomic segments P or S, their contribution to the class of configurations derived from chiasma formation in T3 may be safely considered nil, the more so since the frequency of configurations 5 and 9 is very low (table 1). Therefore, it is concluded that the frequency of chiasmata in the interstitial segments of the extra translocation chromosome in all four tertiary trisomics investigated, is extremely low, about 1.0% being the maximum in case of tertiary trisomic 273.

Deviant karyotypes

Interstitial chiasma formation can result in plants with a deviant karyotype. For instance, in case of BTT 273, two of the four gametes resulting from a PMC with a chiasma in T3 will contain seven chromosomes. In one, the normal haploid chromosome complement is present, whereas the other carries translocation chromosome 1^5, and, therefore, has a major deficiency for the distal part of 1RL. Eight chromosomes are found in the other two gametes, of which one carries chromosome 1^5 in addition to the normal haploid set, while in the other, two members of the satellite chromosome 1R are present. Thus, among the total of 544 plants of the BTT 273 progenies (table 3), 1/4 x 1% primary trisomics for 1R are expected, which is equal to one or two individuals. It is, therefore, not surprising that primaries for 1R have not been observed. Instead, however, eight other trisomics were found, seven of which presumably representing primaries for 5R, the other chromosome involved. These can only have originated from non-alternate multivalent orientation. Apparently, the frequency of 5R non-disjunction is higher than that of 1R. This contrasts with the suggestion of de Vries (1983) that the lack of certain types of tertiary trisomics in translocation trisomic progenies should be attributed to a reduced viability of gametes or zygotes carrying large amounts of extra chromosomal material

rather than to differences in the frequencies of specific non-alternate multivalent orientations.

Primary and telocentric trisomics have been found in the progenies of all BTTs (table 3). The latter might have originated from a centric split in a univalent chromosome, which does not necessarily have to be the translocation chromosome. In the offspring of BTT 240, one plant has been observed with 14 chromosomes including 5^3, apparently originating from the fusion of a normal and a deficient gamete. This plant survived for several weeks but did not reach maturity. The viability of the remainder of the individuals, with a deviant karyotype not specifically attributable to the tertiary trisomic constitution of their parents, is poor also. These plants did not produce any offspring, which also applied to tertiary tetrasomics, resulting from simultaneous male and female transmission of the extra chromosome. Tigrina individuals, when grown under field conditions in competition with wildtype plants, do not reach the flowering stage as well. Hence, not taking into account disomic recombinants (see below), only wildtype plants containing a telocentric are expected to destabilize the BTT system, for instance as a result of an increased male transmission of extra chromosomal material carrying a dominant allele. However, the frequency of these plants is very low, 0.42% being the maximum in case of BTT 282 (table 3), so that the disturbing effect will be limited.

Recombination

When the recombination fraction between the *ti*-locus and the translocation breakpoint is r, and when m and f are the transmission frequencies of the extra translocation chromosome through the male and the female haplophase respectively, the expected frequencies of recombinant and non-recombinant di-, tri- and tetrasomics in selfed BTT offsprings may be expressed as:

	Dominant	Recessive
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	$(1-m)(1-f)(2r-r^2)$	$(1-m)(1-f)(1-r)^2$

Table 3. Karyotype and *ti*-marker segregation, recombination fraction (r) and male (m) and female (f) transmission of the extra chromosome in F3s and F4s of four balanced tertiary trisomics. Code numbers of MI-preparations correspond with tables 1 and 2. s_r: standard deviation of the estimated recombination fraction. Deviant karyotypes: chromosome number between brackets, followed by indication of chromosome(s) recognized, phenotype (*ti*, if tigrina) and number of plants, respectively

BTT	MI-preparation	Generation (no. of lines pooled)	No. of plants scored (% of sown)	Wildtype			Tigrina			$r \pm s_r$	f (%)	m (%)	Deviant karyotypes
				2n+2	2n+1	2n	2n+2	2n+1	2n				
240	-	F3(1)[b]	67(73)	1	21	2	0	1	42	0.028±0.016	31.1	4.8	(15)5^3;5RS?-1
	-	F4(2)[b]	62(74)	2	25	6	0	3	25	0.108±0.036	46.5	7.0	(14)5^3-1
	-	F3(1)[b]	51(61)	1	19	1	0	0	28	0.013±0.013	37.4	5.5	(22)5^3-1
	240a,b	F4(8)[b]	282(70)	4	89	6	0	1	181	0.015±0.006	30.2	4.7	(30)5^3;5^3-1
Total, mean of BTT 240[b]			462	8	154	15	0	4	277	0.026±0.006	32.7	5.3	
282	282a,b	F3(1)[b]	116(63)	0	29	5	0	0	82	0.025±0.011	25.0	0.0	(15)5RS?-2
	282c	F4(9)[b]	400(74)	3	112	11	0	2	271	0.019±0.015	27.3	2.7	(23)5^7;5^7-1
	-	F3(1)[b]	83(69)	0	24	4	0	2	52	0.044±0.018	31.8	0.0	
	-	F4(4)[b]	165(62)	2	72	12	0	5	73	0.073±0.017	47.3	2.6	
Total, mean of BTT 282[b]			764	5	237	32	0	8	479	0.032±0.005	31.5	2.1	
273	-	F3(1)	14(93)	0	2	0	0	0	12	0.0	14.3	0.0	(15)≠1R,*ti*-7
	-	F4(2)	209(77)	0	61	5	0	3	137	0.023±0.008	31.1	0.0	(15)telo 1^5?-1
	273a,b	F3(1)	44(98)	1	11	2	0	2	28	0.055±0.028	25.3	9.0	(8)1^5-1
	-	F4(3)	277(66)	2	90	14	0	1	164	0.034±0.009	32.9	2.2	
Total, mean of BTT 273			544	3	164	21	0	6	341	0.031±0.006	31.2	1.8	
305	-	F3(1)	15(100)	0	5	0	0	0	10	0.0	33.3	0.0	(15)≠1R,*ti*-1
	305a	F4(1)	143(95)	0	71	2	0	1	67	0.014±0.008	51.1	0.0	(15)2^5;telo-1
	305b	F4(1)	209(95)	0	82	0	0	0	125	0.0	39.6	0.0	(15)2^5;?-2[c]
Total, mean of BTT 305			367	0	158	2	0	1	202	0.005±0.003	43.8	0.0	

[a]: MI-preparations of the F2-mother plant of this F3

[b]: plants with tigrina phenotype were not karyotyped; numbers presented give best fit with numbers expected, when using maximum likelihood estimates of r, m and f (see text)

[c]: in one plant, one chromosome 1R missed its satellite, in the other, one ring chromosome (≠ 1R) was present

Maximum likelihood estimates of r, m and f based on these formulae are presented in table 3. The assumption has been made that non-alternate disjunction leads to inclusion in the same cell of the extra translocation chromosome and each of its neighbours in the multivalent in equal frequency, so that there is no effect of orientation on the finally observed number of plants per class (table 3) and, therefore, on r. The error made when one of these neighbours does show preferential non-disjunction with the extra chromosome is limited due to the high frequency of alternate multivalent orientation in rye (Sybenga, 1968). Also, part of the non-alternate frequency is the result of adjacent orientation of homologous pairs within the multivalent. This does not affect recombination, since then either spores with a deficiency result, or spores which produce primary trisomic offspring when fused with karyotypical normal gametes. Due to the low frequency of primaries, neglecting them has no serious consequence for the estimation of r. Tigrina plants in the F4s of BBTs 240 and 282 have not been karyotyped (table 3). In these instances and in the case of the totals and means of these BTTs, estimates of r, f and m are based on the realistic assumption that the frequency of tertiary tetrasomic tigrina plants is nil (compare BTTs 273 and 305, table 3). The numbers of recessive F4 disomics and tertiary trisomics 240 and 282 presented in table 3 are obtained by iteration and give the best fit with the numbers calculated after introduction of the maximum likelihood estimates of r, m and f in the formulae given above. It is seen in table 3, that BTT 305 has the lowest mean recombination fraction, which is not surprising on the basis of what was already known of recombination between the *ti*-locus and the translocation breakpoints in the corresponding interchange heterozygous disomics (de Vries and Sybenga, 1983). The recombination fractions in the latter are expected to be about 1.5 x that of r estimated presently, assuming non-preferential pairing and a minor effect of change of pairing partner on chiasma formation. This agrees reasonably well with the estimates obtained for interchange 273, where 0.076 ± 0.020 is found in the interchange heterozygote (de Vries and Sybenga, 1983) and 0.031 ± 0.006 in the tertiary trisomic. The value obtained for BTT 282 (0.032 ± 0.005) exceeds that of the recombination fraction between the corresponding interchange heterozygote and *ti* reported by these authors (0.009 ± 0.005). The latter value, however, may be an underestimation,

since 0.056 ± 0.013 has been found for recombination between translocation 282 and the gene *br* (brittle) by Sybenga and Mastenbroek (1980) whereas *ti* and *br* are absolutely linked (de Vries and Sybenga, 1983).

Transmission

In the F3s and F4s examined, the rates of male and female transmission (*m* and *f* respectively, table 3) show considerable variation, which may be caused by differences in genetic background, environmental factors or both. In order to establish the significance of the transmission differences observed, tests of heterogeneity were carried out between F3 or F4 families. All tertiary trisomics - wildtype and tigrina - were pooled. A significant difference ($\chi^2 = 4.457$; $0.02 < P < 0.05$) between the two F4 lines of BTT 305 was found. Since their mothers originated from the same F3, all members of which were grown in one period, and because both F4s showed the same germination (table 3), this difference suggests a genetic base. No significant heterogeneity could be detected between F4 lines descending from members of the same F3 in case of the other BTTs. However, heterogeneity was significant between pooled F4s descending from different F3s in case of BTT 240 ($\chi^2 = 4.782$; $0.02<P<0.05$) and BTT 282 ($\chi^2 = 17.940$; $P<0.01$), but here environmental factors may have been important as the parental F3s were grown in different seasons. Heterogeneity could not be detected between F4s in case of BTT 273, and between F3s. Nevertheless, the results suggest that the genetic component determining variation in transmission may be large enough to enable selection of BTT lines with high female transmission. Tentatively it may also be concluded from table 3, that this does not necessarily result in a simultaneous increase of the male transmission as is illustrated by the F4s of BTT 282.

F4s are expected to show lower values for *m* and *f* than the F3s from which they descend as a result of a decreasing chiasma frequency in subsequent inbreeding generations (cf. Sybenga, 1958). However, a high average chiasma level does not necessarily result in a low univalent frequency (preparation 282b, table 1), whereas the univalent frequency itself does not entirely determine the transmission rate. For instance, on the basis of the univalent frequency in one of the BTT 273 F3 plants (preparation 273a, table 1), the expected value for *f* in their F4 pro-

geny is 0.5x (1-0.212), or 39.4%, whereas 32.9% has been observed (table 3). Similarly, f is 25.0% in an F3 of BTT 282 (table 3), whereas minimally 38.5% would be expected on the basis of the univalent frequencies in preparations 282a and 282b (table 1), which represent two ears of the parent plant of this F3. In several F4s a lower transmission relative to their F3s is indeed observed, but in others no differences or even an increase is found. No significant heterogeneity could be detected between F3s and their F4 progenies, except for one case of BTT 282 (χ^2 = 5.588; 0.01<P<0.02) in which f showed an increase from 31.8% in the F3 to 47.3% in the F4. The sources of the variation in male and female transmission have clearly not been completely identified.

Use of BTTs in hybrid breeding

In each of the four BTTs investigated presently, a low chiasma frequency in the interstitial segment is established meiotically, which therefore is a favourable location for an m.s.-allele. In spite of its relatively terminal location in either the unchanged arm (BTTs 240 and 282, fig. 2) or the exchanged segment (BTTs 273 and 305), the tigrina allele showed a very modest recombination with the breakpoints of each of the translocation chromosomes (table 3). This is in agreement with the finding of Jones (1978) that in rye chiasmata generally occur subterminally, in most cases at or near the proximal boundary of the terminal C-bands. As a consequence, the m.s.-allele may be located in other regions than the interstitial segment, for instance in the unchanged arm close to the centromere, or in the exchanged segment proximal to the interchange breakpoint. Künzel (1982) who investigated two BTTs in barley, both carrying a dominant m.s.-allele in the exchanged segment, did not observe any recombinants in their selfed offsprings (433 and 293 individuals respectively).

Of the four BTTs, 305 shows the lowest frequency of viable progeny with a deviant karyotype, the lowest recombination with *ti*, and the lowest male and simultaneously the highest female transmission of the extra chromosome (table 3). The few wildtype disomic recombinants are morphologically easily distinguished from the trisomics. As tigrina plants grown under field conditions in competition with wild type individuals do not reach maturity, it is obvious that a suitable basis for

5. Isolation of telotertiary compensating trisomics from telocentric translocation trisomics and telo-substituted translocation heterozygotes of rye (*Secale cereale* L.).

by

J.N. de Vries

Department of Genetics, Agricultural University,

Gen. Foulkesweg 53,

6703 BM Wageningen.

The Netherlands

ABSTRACT

Telotertiary compensating trisomics (CTs) of rye (*Secale cereale* L.), in which the absence of one normal chromosome is compensated by the presence of a telocentric and a translocation chromosome, were isolated in progenies of telocentric translocation trisomics, and telo-substituted translocation heterozygotes, respectively. These two sources were obtained from crosses between five interchanges of the Wageningen translocation tester set, and telocentric normal trisomics (for 1RS, 1RL and 5RS), or telocentric substitutions (for 1R and 3R), respectively. In test crosses with normal male plants, CTs were identified using either critical meiotic configurations, the segregation of karyotypes in selfed trisomic progenies, or the segregation of a marker located on the compensated chromosome. CT yields ranged from 0.0-6.3%. These frequencies were concluded to be determined mainly by the frequency of the exchanged segment of the translocation chromosome involved in the CT complex being associated at first meiotic metaphase (MI) in the source plants. The lower association frequencies result in the higher CT yields. The correlation between high association frequency of this segment and low CT yield suggests that infrequent adjacent orientation of one critical segment is also responsible for the origin of CTs. This agrees with cytogenetic theory.

Keywords: *Secale cereale* L. - compensating trisomics - translocations - telocentrics - hybrid breeding

INTRODUCTION

The large heterotic potential of cultivated rye (*Secale cereale* L.) is most effectively exploited in hybrid varieties. For grain yield, similar gains appear to be feasable as have been obtained with maize. Improvement of other important characters such as shortness of straw, feeding value and resistance to sprouting, is also effectively realized by hybrid breeding (Geiger, 1982).

Hybrid breeding based on the use of male sterility requires an operational method to maintain and increase an all male sterile seed parent. Gametocides might become valuable tools and recently, a sufficiently reliable wheat pollen killer appears to have been developed, which, however, might not become available on a commercial basis. Whether rye pollen is also affected, is as yet not known. Another approach is the use of cytoplasmic male sterility (CMS). A few sources of CMS and corresponding restorer genes have been identified (Geiger, 1982), but at present only the 'Pampa' cytoplasm is being used in hybrid breeding programs (Morgenstern and Geiger, 1982). Therefore, although advantageous with regard to operationality, the genetic base of these programs is quite vulnerable, so that it is useful to have alternative systems available. Several have been developed in other crop plants, making use of genic (chromosomal, nuclear) male sterility (GMS) and extra chromosomal material. In these systems, the dominant male sterility (m.s.) allele is located on extra chromosomal material, while the recessives are carried by the normal complement. Due to certation, the extra material is only transmitted through the female haplophase. Thus, when recombination between the dominant and recessive m.s.-alleles is prevented, all pollen taking part in reproduction is karyotypically normal, and exclusively carries the recessive allele. To prevent recombination, the extra material should be unable to simply replace a normal chromosome, which excludes primary trisomics as the male restrictive agent. Structural barriers to recombination between normal and extra material are usually required. For barley hybrid breeding, Ramage (1965) proposed the use of balanced tertiary trisomics in which the dominant allele is located on the extra translocation chromosome, close to the breakpoint. Using this trisomic as a pollinator of male sterile diploids, the production of a completely male sterile, diploid seed parent is accomplished. In maize, GMS has

been made operational by Patterson (1973) who used diploid duplication-deficiency heterozygotes. When only the eggs tolerate the duplication and the deficiency, pollen transmission of the dominant m.s.-allele is prevented when it is located close enough to the translocation breakpoint. Phillips and Springer (1972) proposed a similar use of a disomic double duplication heterozygote, in which the absence of two normal non-homologous chromosomes is compensated by two translocation chromosomes, originating from two different interchanges, which both include parts of the missing chromosomes. In this system, plants have no deficiencies, and owing to certation caused by a three-fold presence of the two "between breakpoint" regions (Burnham, 1966), only karyotypically normal pollen takes part in reproduction. Sybenga (1982) proposed the use of compensating trisomics in which the absence of one normal chromosome is compensated by the presence of two modified chromosomes (see below). In the XYZ system proposed by Driscoll (1972) for wheat, the extra chromosomal material carrying the dominant m.s.-allele is obtained from related species or genera like rye, barley, *Aegilops*, *Agropyron* and *Triticum monococcum* (Driscoll, 1981). While in the systems mentioned above recombination is prevented by the presence of translocation breakpoints, in the XYZ system this is accomplished by reduced pairing between homoeologous chromosomes.

In compensating trisomics, the absence of one member of a pair of normal homologues is compensated by the presence of two others of aberrant structure. Together - but not separately - the latter contain at least the complete genetic material of the missing chromosome. Hence, the presence of *both* compensating chromosomes is indispensable for gametes of a strictly diploid species like rye to be viable, when this chromosome is missing.

The simplest kind of a compensating trisomic is the Robertsonian split in which a normal chromosome is substituted by its two telocentrics. This results in an increase in chromosome number by one, without a net increase of the genetic material, so that transmission of the aberrant karyotype is not prevented by certation. Consequently, the Robertsonian split is not suited for use in a hybrid breeding system as outlined above. This does not apply to five other types of compensating trisomics described by Khush (1973), which all contain more chromosomal material than strictly necessary for compensation, thus, owing to cer-

tation, allowing only karyotypically normal pollen to take part in reproduction. In these five types, the missing chromosome is compensated by a combination of either two iso chromosomes (diiso-), two translocation chromosomes (ditertiary-), one iso and one translocation chromosome (isotertiary-), one iso and one telocentric chromosome (isotelo-), or one telocentric and one translocation chromosome (telotertiary compensating trisomic). Of course, more types are possible. For instance, in a compensating trisomic of barley described by Lehmann et al. (1976) - to which the authors did not refer as a compensating trisomic - one copy of chromosome 6 was replaced by translocation chromosome 6^3 and a translocated telocentric containing the missing segment of chromosome 6.

Very few reports dealing with compensating trisomics or their origin have been published. Several compensating trisomics have originated from radiation experiments. In the selfed progeny of a karyotypically normal *Datura* plant which had been exposed to radium emanation, Blakeslee in 1921 found the first 'compensating type' (Avery et al., 1959) called 'Nubbin' which appeared to be a ditertiary compensating trisomic. Smith (1947) reported about an isotelo compensating trisomic, isolated in the X_2 of X-rayed seeds of *Triticum monococcum* . Compensating trisomics can also be purposely synthesized by intercrossing the appropriate carriers of translocations, telocentrics and iso chromosomes, followed by an analysis of the progenies of the F1s. Among the 76 plants from the progeny of an F1 between a secondary trisomic and an appropriate translocation homozygote of *Datura* , Blakeslee and his co-workers isolated two isotertiary compensating trisomic individuals (Avery et al., 1959). The same authors state that the *Datura* workers isolated about 50 di-, telo- and isotertiary compensating trisomics, a considerable number of which having been intentionally synthesized. The compensating trisomics reported by Lehmann et al. (1976) were found in percentages of 0.8 and 2.5% in two selfed progenies of barley translocation heterozygotes in which one of the translocation chromosomes was replaced by its two telocentrics. Recently, Saini and Minocha (1981) reported about a ditertiary compensating trisomic of pearl millet, isolated in the offspring of a double translocation trisomic. They did not, however, provide data concerning the size of the progeny examined relative to the number of compensating trisomics isolated from it. Unusual meiotic events, resulting in aberrant chromosomes which were not present originally in the parent plant, form

another category from which compensating trisomic offspring can arise. For instance, one isotelo compensating trisomic plant of tomato was found in the selfed progeny (2798 plants) of a tertiary monosomic parent (Khush, 1973), which originally did not contain the telocentric nor the isochromosome, having only the absence of the compensated chromosome in common (Khush and Rick, 1966; 1967). One of the 183 plants in a test-cross progeny of this trisomic with a karyotypically normal male parent, appeared to be the related diiso type. No logical explanation could be given for the origin of a second diiso compensating trisomic, observed in a different progeny (Khush and Rick, 1967).

The number of compensating trisomics which can purposely be synthesized is only limited by the availability of modified chromosomes, and, of course, by the tolerated quantity of extra genetic material. When compared with the use of balanced tertiary trisomics in hybrid breeding programs (Wiebe and Ramage, 1971; de Vries, 1984), compensating trisomics potentially cover larger segments of the genome, in which m.s.-alleles may be located to give an operational system. An additional advantage of compensating over tertiary trisomics is, that in the first the suppression of recombination between the dominant and the recessive m.s.-alleles is expected to be stronger, owing to the presence of more than one translocation breakpoint, or of abnormal centromere conditions, or both. Moreover, for an efficient maintenance of the balanced trisomic (Wiebe and Ramage, 1971), the extra material should be transmitted in as high a frequency as possible. For several types of compensating trisomics, levels of female transmission up to 50% are expected (Khush, 1973; fig. 1), whereas maximally 44.4% is expected for tertiaries, which is practically never realized (de Vries, 1984). However, in compensating trisomics, the meiotic complications may be greater, and may lead to reduced fertility and more aberrant progeny than when tertiary trisomics are used.

A hypothetical telotertiary compensating trisomic (CT) is shown in the diagram of fig. 1a. Only one copy is available of normal chromosome a. The absence of the other member of the a pair is compensated by the presence of telocentric a and translocation chromosome a^b. As a consequence, three copies are available of the interstitial segment T: one in translocation chromosome a , one in the telocentric and one in a^b. Two of the three copies of interchanged segment S are present in normal pair b, the third is found in a^b . It is seen from fig. 1b, that MI association

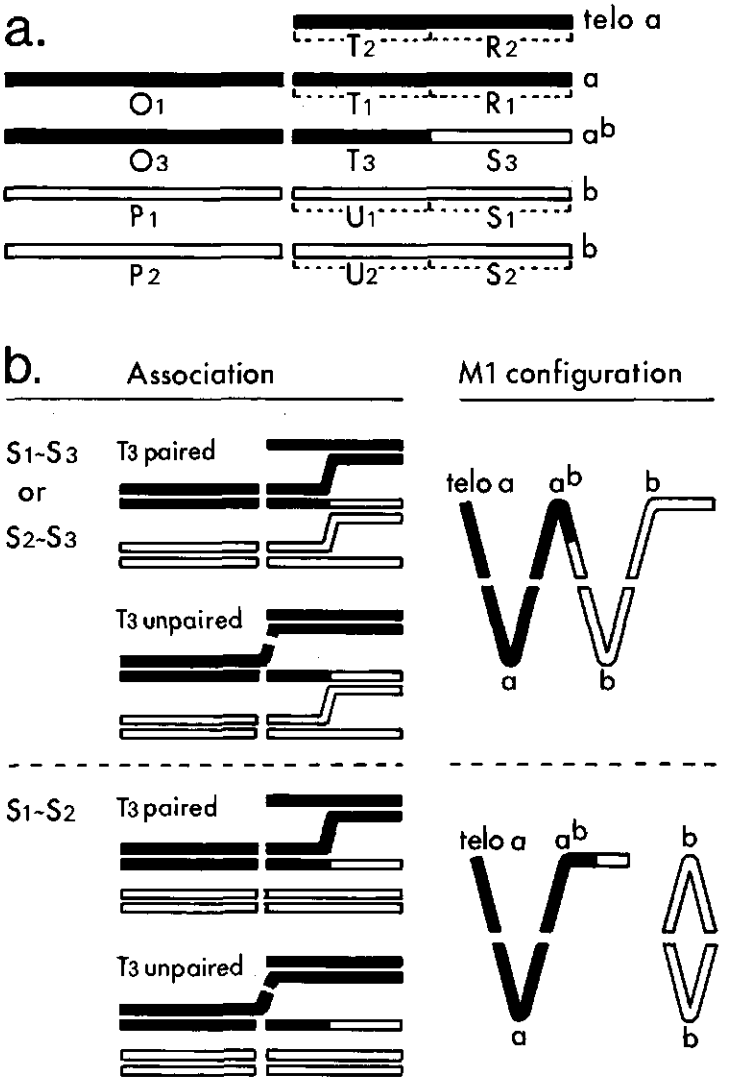

Fig. 1

(a) Diagram of chromosomes involved in the complex of a telotertiary compensating trisomic, in which the absence of one copy of normal chromosome *a* is compensated by the presence of telocentric *a* and translocation chromosome a^b. O1 and O3: non-translocation arms of chromosome *a*, P1 and P2 : same of *b*; R1 and R2 : exchange segments of chromosome *a*, S1, S2 and S3 : same of *b*; T1, T2 and T3 : interstitial segments of chromosome *a*, U1 and U2 : same of *b*.
(b) Pairing diagrams (complete pairing) and MI configurations (alternate orientation) of the compensating trisomic of fig. 1a, resulting from the three two-by-two associations possible for segment S. With random terminal pairing initiation, the chance for each possibility to occur is 1/3. Each chromosome can change pairing partner only once. The point of partner exchange is variable, so that T3 may be paired completely, partly or not at all. Chiasmata are assumed to be present in all segments paired, except the interstitial segments T and U.

of all paired end segments (O, P, R and S) and alternate multivalent orientation, result in gametes of which 50% contain the compensating chromosome complement (telo a + a^b), the other 50% containing the normal haploid set. However, probably less than 50% of the testcross progeny from a CT and a karyotypically normal father will consist of CTs again, since these segments are not necessarily always associated. This also applies to selfed CT progenies as, due to certation, only karyotypically normal pollen is able to take part in reproduction. Hence, with the recessive allele of an m.s.- or a marker gene located on a, and the dominant allele located on either a^b (segment O3), telo a (segment R2), or both (segments T2 and T3), and provided that recombination between the dominant and the recessive alleles is absent, a balanced system arises in which transmission of the dominant allele through the male haplophase is prevented, but female transmission permitted. This is also accomplished when *both* members of pair b carry the recessives, while a^b carries the dominant allele (segment S, fig. 1a). Consequently, the selfed progeny of each of these balanced telotertiary compensating trisomics (BCTs) is expected to consist of maximally 50% BCTs having the dominant phenotype, whereas the remainder are disomic recessives.

CTs are expected to arise in a substantial frequency only in the progenies of parent plants in which the compensating chromosomes (telo a and translocation chromosome a^b, fig. 1a) are already available. This applies to translocation heterozygotes which contain either one extra telocentric (fig. 2a), or two telocentrics instead of a normal chromosome (fig. 2b), respectively. These parental types are referred to as telocentric translocation trisomics (de Vries, 1983), and telo-substituted translocation heterozygotes (Sybenga et al., 1973), respectively. The specific segregational behaviour at meiosis required to yield CTs, appears to be about the same for both, as their meiotic configurations are related. Moreover, these specific requirements are limited in number, which becomes evident from the following:

1. There is only one type of non-alternate orientation per chain of five (fig. 2a, b) which results in the inclusion in one gamete of the desired combination of chromosomes (telo a, a^b and b; cf. fig. 1a).

2. Only the absence of chiasmata in the exchanged segment of the translocation chromosome to be involved in the CT complex (a^b, fig. 2a, b, at arrows) leads to breakdown of the multivalent in a chain of three

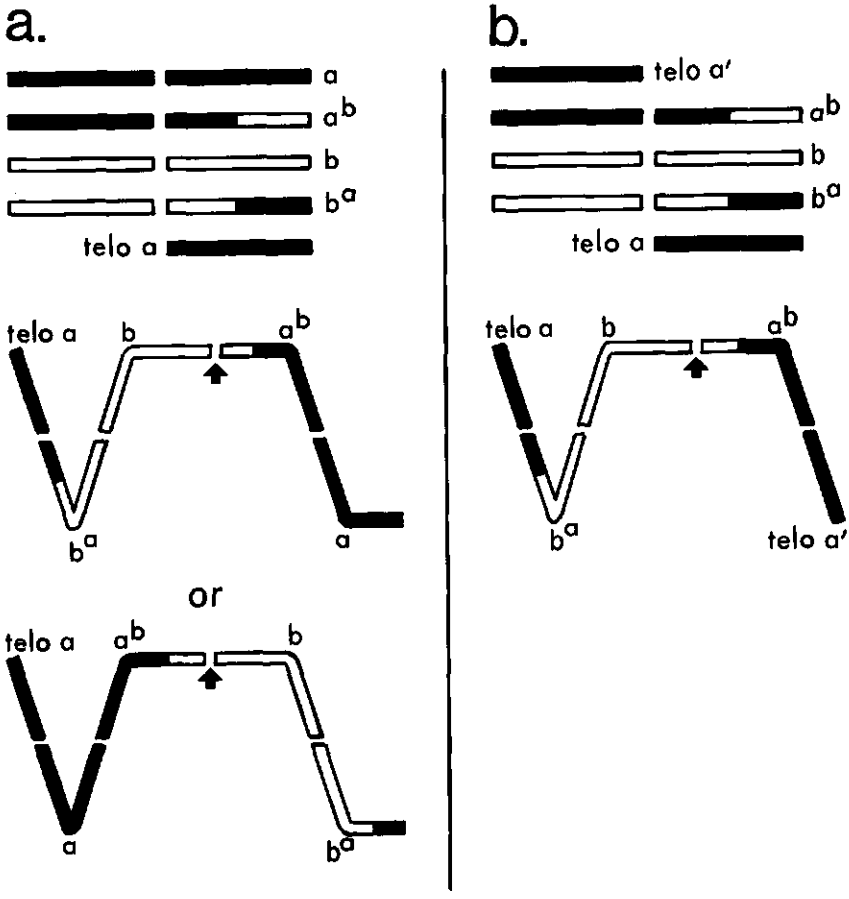

Fig. 2

(a) Diagram of a telocentric translocation trisomic, involving a translocation between chromosomes a and b. The extra telocentric (telo a) is homologous with the translocation arm of a.
(b) Diagram of a telo-substituted translocation heterozygote involving the same translocation as in fig. 2a. Chromosome a is replaced by its two telocentrics: telo a, homologous with the translocation arm of a, and telo a', homologous with the unaltered arm.
From both karyotypes the CT of fig. 1a can arise, when chromosome b is included in one gamete together with telo a and translocation chromosome a^b, the latter two compensating the absence of chromosome a. This chromosome make up is expected in all n+1-gametes resulting from the non-alternate chain-of-five orientation drawn, and in 50% of the n+1-gametes resulting from absence of chiasmata in the exchanged segment of the translocation chromosome to be involved in the CT complex (a^b) (arrows). Of the n-spores, 50% will be abortive in the latter instance.

and a bivalent which undergo independent orientation, resulting in 50% of the n+1-spores having the desired chromosomal make up. The other 50% of the n+1-spores contain telocentric a in addition to either the haploid normal complement, or the translocation complement. Half of the n-spores are abortive due to a deficiency. There is no other chromosome segment than the exchanged segment of a^b in which absence of chiasma formation has the same effect (cf. fig. 2a, b).

3. A chiasma in the interstitial segment of the translocation chromosome to be included in the CT complex (a^b) can also result in CT progeny. In case of a telo-substituted translocation heterozygote (fig. 2b) this chiasma can only be formed between a^b and telo a. The chance for a gamete with a compensating chromosome combination to result equals 1/16, whereas 9/16 of the combinations are abortive owing to a deficiency. The same applies to a telocentric translocation trisomic (fig. 2a), in which case a compensating combination can also result from interstitial chiasma formation between a^b and normal chromosome a, when simultaneously telo a is (non-interstitially) associated to enable its MI orientation. Spores with eight different chromosome combinations will result in equal frequency, one of which containing the compensating set, whereas deficiencies cause lethality of three others.

In the present article, the relation is discussed between the meiotic behaviour of specific chromosome segments in telocentric translocation trisomics or telo-substituted translocation heterozygotes of rye, and the frequencies in which CTs are found in their progenies. Three methods for CT isolation are presented, involving (a) the determination of critical first meiotic metaphase (MI) configurations, (b) the scoring of karyotype segregations in selfed trisomic progenies, or (c) of the segregation of a marker gene located on the compensated chromosome.

MATERIALS AND METHODS

Four telocentric translocation trisomics (cf. fig. 2a) and two telo-substituted translocation heterozygotes (cf. fig. 2b) were used as CT source. The source plants were obtained by crossing telocentric 'normal' trisomics for 1RS, 1RL or 5RS, and telocentric substitutions for 1R or 3R, with the appropriate translocations. The latter are part of the Wageningen

translocation tester set of rye (Sybenga, 1983), described by de Vries and Sybenga (1976) and Sybenga and Wolters (1972), and carry the code numbers 240 (3RS, 5RL), 248 (1RS, 6RS), 273 (1RL, 5RS), 305 (2R, 5RS) and 306 (1RS, 6RL). Chromosome nomenclature corresponds with that of the *Triticinae* and is based on preliminary data from crosses between the tester set and the standard addition set of 'Imperial' rye to 'Chinese Spring' wheat (Sybenga, in prep.).

Meiotic configurations were quantitatively studied in pollen mother cells (PMCs) at first meiotic metaphase (MI) from six anthers (250 PMCs each) of a telocentric translocation 248 trisomic with 1RS as the extra telocentric. Meiotic data on 5RS-telocentric translocation trisomic 273 are from de Vries (1983). In case of the other CT sources, data concerning the meiotic behaviour of specific chromosome segments were derived from related karyotypes.

All telocentric translocation trisomics and telo-substituted translocation heterozygotes were testcrossed with karyotypically normal male parents, and the testcross progenies karyotyped. In case of 5RS-telocentric translocation trisomic 273, data concerning an F2 progeny were available as well (de Vries, 1983). A discrimination between CTs and other kinds of telocentric trisomics found in these progenies could not be made directly on the basis of the morphology of their mitotic metaphase chromosomes, as with the techniques used only one of the two translocation chromosomes could be unequivocally recognized. To isolate CTs despite this, a choice was made between three alternative methods:

(a) A qualitative study of meiotic configurations in pollen mother cells (PMCs) at first meiotic metaphase (MI) from trisomics having a telocentric in the testcross progenies. As CTs contain only one translocation chromosome, they are not able to form rings of four at MI; chains of five, on the other hand, are expected to be found regularily (cf. fig. 1b). These configurations enable a discrimination between CTs and telocentric translocation trisomics (rings of four present), or telocentric 'normal' trisomics (chains of five absent).

(b) Karyotyping of small samples (about 20 seedlings) of the selfed progeny of the trisomics mentioned at (a). Translocation homozygous, heterozygous and karyotypically normal disomics appear in the selfed progeny of a telocentric translocation trisomic, whereas disomics in the selfed progeny of a CT are always karyotypically normal (see above, cf. fig. 1b).

Of course, using this method no distinction can be made between CTs and telocentric 'normal' trisomics, as the disomic progeny of the latter is karyotypically normal also.

(c) Scoring of the frequency of recessives in the selfed progeny of the trisomics mentioned at (a), when the male testcross parent is homozygous recessive for a monogenically inherited marker located on the chromosome to be compensated. All plants in the testcross progenies mentioned contain one copy of the normal chromosome to be compensated (*a*), derived from the male parent. When in the latter both members of the *a* pair carry the recessive allele of a monogenically inherited marker - the mother plants (figs. 2a, b) carrying the dominant alleles - a CT arising from this testcross (fig. 1a) will throw 50% or more recessives in its selfed offspring. This is caused by the fact that at least half of the selfed CT progeny consists of karyotypically normal disomics (see above), having the recessive phenotype obtained from the only copy of chromosome *a* available in the CT parent. The other half of this progeny - or less - are dominant CTs. (Recombinants are not taken into account). Less than 25% recessives are expected in selfed offsprings of telocentric translocation trisomics and telocentric 'normal' trisomics. This is because all trisomics are dominant again, whereas dominant: recessive ratios of 3:1 are expected among the disomics. This method of CT isolation is not feasable for markers located on chromosome *b* (fig. 1a), as then 3:1 ratios are found among the disomic CT progeny also.

Plants were grown in a greenhouse at 18-20 °C. Karyotypes were classified in root tip mitoses after pre-treatment in a saturated aquaeous solution of alpha bromonaphthalene for 2 h at 24 °C, fixation-maceration in 1 *n* HCl at 60 °C for 12 min, and Feulgen staining. Anthers were fixed in 1:3 acetic alcohol and stored at -10 °C. MI chromosomes were stained with 2% aceto carmine, and the preparations were mounted in Eurapal in case of a quantitative cytological analysis.

RESULTS AND DISCUSSION

Isolation of CT248,1RS and CT273,5RS (fig. 3)

The karyotype segregation in the offspring of the testcross between telocentric translocation trisomic 248 (1RS, 6RS) with 1RS as the extra telocentric, and karyotypically normal fathers, is presented in table 1.

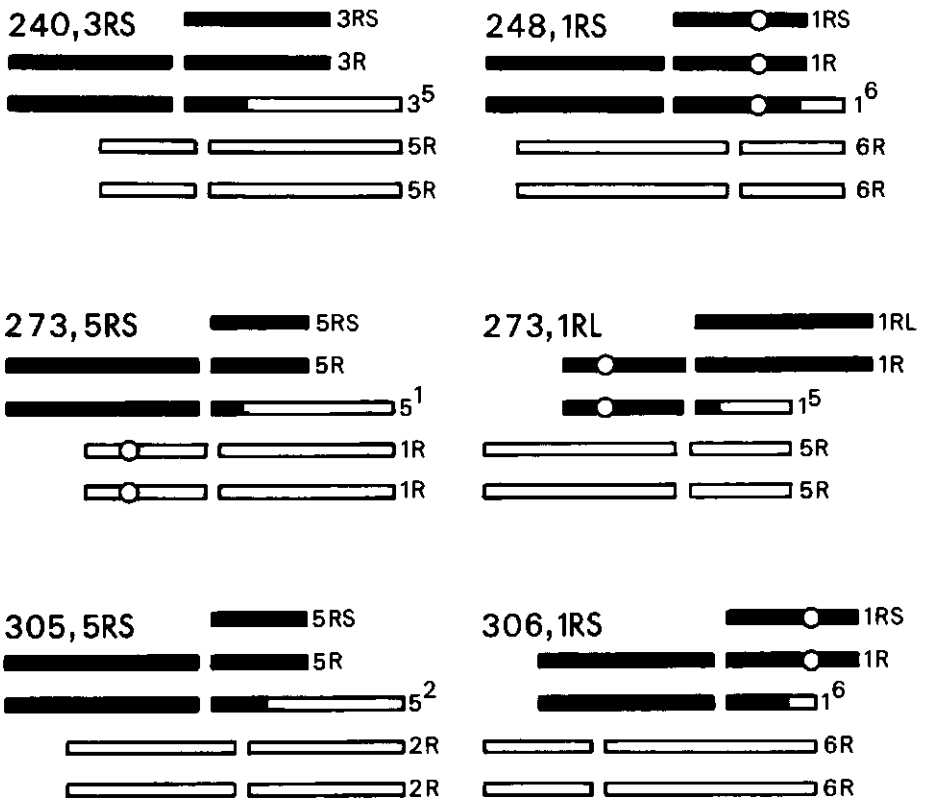

Fig. 3

Diagrams of chromosomes involved the six telotertiary compensating trisomics, the isolation of which is described in the present study. Chromosome nomenclature according to de Vries and Sybenga (1984), arm length ratios based on Sybenga and Wolters (1972) and de Vries and Sybenga (1976). Circle in 1RS represents nucleolar organizer region. 240,3RS : absence of 3R compensated by 3RS and 3^5; 248,1RS : absence of 1R compensated by 1RS and 1^6; 273,5RS : absence of 5R compensated by 5RS and 5^1; 273,1RL : absence of 1R compensated by 1RL and 1^5; 305,5RS : absence of 5R compensated by 5RS and 5^2; 306,1RS : absence of 1R compensated by 1RS and 1^6. All combinations could be obtained, except with 273,5RS and 273,1RL (see text)

Table 1. Karyotype segregation in the offspring of female parents of telocentric translocation trisomic 248 (breakpoints in 1RS and 6RS) with 1RS as extra telocentric, and karyotypically normal plants. Results of 9 crosses pooled. Number of chromosomes between brackets. Haploid normal karyotype: n, translocation: t. Distinction between CTs ((15)nn-1R+1^6+1RS) and telocentric translocation trisomics ((15)tn+1RS) based on karyotype segregations in selfed progenies of trisomics with (15)1^6;1RS;1R (see text)

Karyotype	No. of plants
(14)nn	41
(14)tn	48
(15)nn+1RS	11
(15)1^6;1RS;1R	9[a]
(15)telo 1^6S;1R;1R	1
(21)1R;1R;1R	1
Total	111

[a] : 7xCT(15)nn-1R+1^6+1RS; 2x(15)tn+1RS

In CTs derived from the maternal karyotype, the absence of one member of the 1R pair is compensated by the presence of telocentric 1RS and translocation chromosome 1^6 (248, 1RS; fig. 3). Nine of the trisomics found in the testcross progeny contained both 1RS and 1^6. Since at mitotic metaphase an unequivocal distinction between normal chromosome 6R and translocation chromosome 6^1 of 248 is not always possible, it could not be determined directly which of the nine trisomics mentioned were CTs and which had the maternal karyotype. Therefore, selfed progenies of about 20 plants of each of the nine trisomics were karyotyped. Two progenies contained disomic translocation homozygotes, heterozygotes and karyotypically normal plants, which is only possible when the parents were telocentric translocation trisomics. The remaining seven trisomics were concluded to

Table 2. Karyotype segregation in the offspring of a cross between wild-type female parents of telocentric translocation trisomic 273 (breakpoints in 1RL and 5RS) with 5RS as extra telocentric, and karyotypically normal tigrina plants (*titi*). Results of 5 crosses pooled. For legend, see table 1. Distinction between CTs ((15)nn-5R+5^1+5RS) and telocentric normal trisomics ((15)nn+5RS) based on segregation of tigrina marker in selfed progenies of trisomics with (15)5RS;1R;1R (see text)

Karyotype	No. of plants
(14)nn	110
(14)tn	71
(15)5RS;1R;1R	23[a]
(15)tn+5RS	43
(15)1^5;1R;1R	3
Total	250

[a] : all were (15)nn+5RS

be CTs, as in their progenies all disomics had the normal karyotype.

Efforts to obtain a CT from a telocentric translocation trisomic containing translocation 273 (1RL, 5RS) and 5RS as the extra telocentric, were not successful. In this CT, the absence of one copy of 5R is compensated by translocation chromosome 5^1 and telocentric 5RS (273, 5RS; fig. 3). No distinction at mitotic metaphase can be made between this CT and a telocentric 'normal' trisomic for 5RS, since translocation chromosome 5^1 cannot be identified unequivocally. A distinction between the two types of trisomics based on karyotype segregations in their selfed progenies is not possible here either, because the disomic progeny is karyotypically normal in both instances. To isolate the CT despite this, MI configurations were qualitatively investigated in PMCs of trisomics which contained telocentric 5RS without showing any other apparent chromosome aberration at mitosis. The selfed progeny (443 plants) of a 5RS-telocentric translocation trisomic 273 contained 21 of these trisomics (de Vries, 1983), and of nine plants, anthers were available. No chains of five were detected in PMCs of all nine, while chains of three were frequently observed, so that the nine plants were concluded to be telocentric 'normal' trisomics for 5RS. In a second effort the 5RS-telocentric translocation trisomic 273 was testcrossed with a karyotypically normal male parent, which was homozygous recessive for the "tigrina" marker (*ti*) causing transverse yellow striping in the leafs. The tigrina gene is located on the short arm of 5R, distally to the breakpoint of translocation 273 (segment R, cf. figs. 1a and 3), and is closely linked with the centromere of 5R (de Vries and Sybenga, 1984; de Vries, 1984). Table 2 lists the karyotype segregation in the testcross progeny. Each of the 23 telocentric, seemingly normal trisomics of table 2 contain one copy of 5R carrying the recessive *ti*-allele. In a CT, this is the only copy available (fig. 3), whereas in a telocentric 'normal' trisomic a second copy of 5R is present, carrying the dominant allele *Ti*. Disomics in the selfed progenies of both are karyotypically normal, but among the disomics in the selfed progeny of a telocentric 'normal' trisomic a wildtype: tigrina segregation ratio of 3:1 is expected, whereas most disomics in a selfed CT progeny are expected to have the tigrina phenotype, owing to the close linkage between *ti* and the centromere of 5R. Most telocentric trisomics in both progenies will be wildtype because of the presence of the dominant allele on telocentric 5RS. Hence, since the progeny of a selfed CT consists of maximally 50% CTs (see above, cf. fig. 1b), a wildtype: tigrina ratio of 1:1 or less is expected in case of a CT. The progenies of the 23 selfed telocentric trisomics of table 2, however, all contained less than 25% tigrina plants, which is expected in case

their parents were telocentric 'normal' trisomics. It was concluded, therefore, that no CTs were present among the 250 individuals of table 2.

Thus, from telocentric translocation trisomic 248, CTs could be obtained with relative ease (6.3% of the testcross progeny, table 1), whereas in large offsprings of telocentric translocation trisomic 273 none were found (table 2; de Vries, 1983). This difference is principally caused by a difference in MI-association frequency in the source plants of the exchanged segment of the critical translocation chromosome having a centromere homologous with that of the chromosome to be compensated (cf. fig. 1a). Association frequencies of the exchanged segments of chromosome 1^6 of translocation 248, and 5^1 of 273 (cf. fig. 3) have been assessed by Sybenga et al. (1973). Based on observations in 600 PMCs of each of two preparations, values of 0.428 and 0.475 were found in case of 1^6, whereas the association frequency of the translocated segment of 5^1 varied between 0.990 and 0.997 (eight preparations, 1000 PMCs each). Chains of three including the telocentric, which result from absence of chiasmata in these segments (cf. fig. 2a), were observed in five out of 500 PMCs (1.0%) of a 5RS-telocentric translocation trisomic 273 plant (de Vries, 1983), whereas in six anthers (250 PMCs each) of a 1RS-telocentric translocation trisomic 248 plant the chain-of-three percentage averaged 39.7%, varying between 35.6% and 45.2%. As explained above (cf. caption of fig. 2), 1/4 of the gametes arising from a cell with a chain of three including the telocentric contain a compensating chromosome combination, whereas 1/4 are abortive. Cells with other meiotic configurations may be expected to produce abortive gametes in negligible frequencies. Hence, in case of the 1RS-telocentric translocation 248 trisomic, the percentage of gametes with a compensating combination is expected to be 1/4 x 39.7%, or 9.9% of all gametes produced, abortive and viable combined. Relative to viable gametes this percentage becomes $(1 - 1/4 \times 0.397)^{-1} \times 9.9\%$, or 11.0%. Only 6.3% CTs have been observed (table 1). The difference may be due for instance to a fortuitously high chain-of-three frequency in the plant investigated meiotically, which was not one of the testcross mother plants. In the same preparations, the frequency of PMCs with MI configurations having interstitial chiasmata in either 1^6, 6^1, or both in addition to association of telocentric 1RS, varied between 2.4% and 4.0%, with an average of 3.3%. A distinction between MI configurations resulting from chiasma formation in either of the two interstitial segments in aceto carmine preparations cannot always be made, but even when it is assumed that no chiasmata have been formed interstitially in 6^1, the percentage of PMCs with a chiasma in

the interstitial segment of 1^6 is still low relative to that of cells with a chain of three (35.6%-45.2%, average 39.7%; see above). Moreover, only 1/8 or 1/16 respectively of the gametes resulting from interstitial chiasma formation between 1^6 and 1R or 1RS respectively give rise to a CT when fused with a karyotypically normal gamete (1/4 in case of a chain of three, see above). It is evident, therefore, that in 1RS-telocentric translocation trisomic 248 the contribution to the CT offspring resulting from presence of chiasmata in the interstitial segment of chromosome 1^6, is small compared to that of absence in the exchanged segment.

In case of 5RS-telocentric translocation trisomic 273, only 0.25% CTs are expected on the basis of 1.0% PMCs with a chain of three observed in a preparation of one of the parents of the selfed progeny of 443 plants (de Vries, 1983). In the same preparation, no interstitial chiasma formation was detected. It is, therefore, not surprising that no CTs were isolated from this telocentric translocation trisomic. At the same time, the non-occurrence of CTs indicates, that the types of non-alternate chain-of-five orientation required for CT combinations (fig. 2a), are very rare. This, in turn, agrees with the observation that in rye non-alternate multivalent frequencies are low (Sybenga, 1968; 1975; de Vries, 1983).

Isolation of CT305,5RS and CT273,1RL

Table 3 presents the segregation in the progeny of a cross between telocentric trisomic mothers, heterozygous for translocation 305 (2R, 5RS) with telocentric 5RS as the extra chromosome, and normal male testers, homozygous recessive for the "tigrina" marker (*ti*). CTs derived from this trisomic contain the combination of translocation chromosome 5^2 and telocentric 5RS, compensating the absence of 5R (305, 5RS; fig. 3). No distinction can be made in standard mitotic preparations between these CTs and telocentric 'normal' trisomics, since 5^2 is not markedly different from the other normal, non-satellited chromosomes. However, just like in translocation 273, the tigrina gene is located distally to the breakpoint of translocation 305 (de Vries and Sybenga, 1984), i.e. in segment R (cf. fig. 1a), so that it is possible to discriminate between CTs and telocentric normal trisomics in the same way as in case of the 5RS-telocentric translocation 273 trisomic (table 2). Selfed progenies of about 20 plants each, obtained from the 10 telocentric, apparently normal trisomics ((15)5RS, table 4), were grown and scored for the *ti*-segregation. In nine progenies, less than 25% tigrina plants were observed, so that their mothers were concluded to be telocentric 'normal' trisomics with an extra

Table 3. Karyotype segregation in the offspring of a cross between wild-type female parents of telocentric translocation trisomic 305 (breakpoints in 2R and 5RS) with 5RS as extra telocentric, and karyotypically normal tigrina testers (*ti ti*). Results of 3 crosses pooled. For legend, see table 1. Distinction between CTs ((15)nn-5R+5^2+5RS) and telocentric normal trisomics ((15)nn+5RS) based on segregation of the tigrina marker in selfed progenies of trisomics with (15)5RS (see text). In all plants, two copies of 1R were present

Karyotype	No. of plants
(14)nn	28
(14)tn	32
(15)5RS	10[a]
(15)tn+5RS	8
(15)	4
Total	82

[a] : 9x(15)nn+5RS; 1xCT(15)nn-5R+5^2+5RS

5RS, carrying a recessive *ti*-allele on one of the 5R chromosomes, while dominant alleles were carried by the other copy of 5R and the telocentric. More than 50% tigrinas were observed in one progeny. Of this progeny, all available seeds were sown, and the seedlings karyotyped and scored for the *ti*-marker. The results are shown in table 4 and will be discussed in detail later (de Vries, in prep.). The segregation in table 4 can only be explained, when the mother plant is a CT in which the absence of one member of the 5R pair is compensated by translocation chromosome 5^2 and telocentric 5RS.In this CT, the only available copy of 5R, carrying the recessive *ti*-allele, is derived from a karyotypically normal tester parent of the progeny in table 3, whereas only the telocentric 5RS carries the do-

Table 4. Combined segregation of karyotypes and tigrina marker in the selfed progeny of the CT in table 3. For legend, see table 1. Distinction between CTs ((15)nn-5R+5^2+5RS) and telocentric normal trisomics ((15)nn+5RS) based on MI configurations observed in PMCs of trisomics with (15)5RS (see text). In all plants, two copies of 1R were present

Karyotype	Phenotype		No. of plants
	Wildtype	Tigrina	
(14)nn	3	78	81
(15)5RS	38	0	38[a]
(15)	0	7	7
(16)5RS;5RS	1	0	1
(13)+telo?	0	1	1
Total	42	86	128

[a] : 17xCT(15)nn-5R+5^2+5RS, 2x(15)nn+5RS; no anthers of remaining 19 plants studied

Table 5. Karyotype segregation in the offspring of a cross between wildtype female parents of telocentric translocation trisomic 273 with an extra telocentric 1RL, and karyotypically normal plants. Results of 6 crosses pooled. For legend, see table 1. Distinction between CTs ((15)nn-1R+1^5+1RL) and telocentric translocation trisomics ((15)tn+1RL) based on MI configurations observed in PMCs of trisomics with (15)1^5;1RL;1R (see text)

Karyotype	No. of plants
(14)nn	76
(14)tn	49
(15)nn+1RL	2
(15)1^5;1RL;1R	18[a]
(16)1^5;1RL;1R;1R	1
(15)1^5;1R	1
(15)1R;1R	1
(21)1R;1R;1R	1
(30)1RL;1RL;1R;1R;1R;1R	1
Total	150

[a] : 7x(15)tn+1RL; remaining 11 plants not further identified

minant allele (cf. fig. 3). Consequently, most karyotypically normal disomics in the selfed offspring of this "balanced telotertiary compensating trisomic" (BCT) show the tigrina phenotype, whereas plants carrying 5RS are wildtype (table 4).

No quantitative study of meiotic configurations in the 5RS-telocentric translocation 305 trisomic mother plants of table 3 could be carried out. Data on related karyotypes are not available. Nevertheless, considering its relative length, it is reasonable to assume that the frequency of the exchanged segment of 5^2 (fig. 3) being associated at MI in the source plants, approaches unity. As a result, a low frequency of cells with a chain of three (cf. fig. 2a) and, therefore, very few gametes with a compensating combination are expected. The contribution to this class of gametes resulting from chiasma formation in the interstitial segment of 5^2 is expected to be very small also, in view of its relatively small size (fig. 3). Hence, chances for a CT to arise from a 5RS-telocentric translocation 305 trisomic are about as small as that for a CT to arise from a 5RS-telocentric translocation 273 trisomic. That no CTs were isolated from the testcross progeny of the latter (table 2), presumably is a matter of - not unexpected - misfortune.

The karyotype segregation in the progeny of a cross between a telocentric trisomic including translocation 273 (1RL, 5RS) with the non-satellited 1RL as the extra telocentric, and normal male tester parents, is listed in table 5. Eighteen trisomics were found in which the chromosomes 1R, 1^5 and 1RL were present once each. These may include CTs in which one

of the satellite chromosomes 1R is replaced by the short translocation chromosome 1^5 and telo 1RL (273, 1RL; fig. 3). In standard mitotic preparations these CTs cannot be distinguished from 1RL-telocentric translocation 273 trisomics. However, from seven of the 18 trisomics mentioned, preparations of PMCs at MI could be obtained, in all of which rings of four chromosomes were observed. This is only possible when all chromosomes involved in translocation 273 (1R, 5R, 1^5 and 5^1) are present. As CTs 273, 1RL (fig. 3) contain two copies of 5R but lack the 5^1 chromosome, they are not able to form rings of four at meiosis, so that the seven plants investigated meiotically were concluded to have the maternal karyotype.

In 1RL-telocentric translocation 273 trisomics, a low chain-of-three frequency is expected on the basis of the high association frequency of the exchanged segment of the translocation chromosome to be involved in the CT complex (1^5), which varies from 0.779-0.982 (average 0.894, 8 preparations of 1000 PMCs each), whereas in none of these 8000 PMCs chiasma formation in the interstitial segment of 1^5 could be detected (Sybenga et al., 1973). Chances for a CT to arise from a telocentric translocation trisomic 273 with an extra 1RL are, therefore, expected to be small.

Isolation of CT 240,3RS and CT 306,1RS

CTs 240,3RS and 306,1RS were isolated from the progenies of their related telo-substituted translocation heterozygotes (cf. fig. 2b). Four CTs were obtained from a cross between mother plants, heterozygous for translocation 240 (3RS, 5RL) in which the normal chromosome 3R was replaced by two telocentrics, and karyotypically normal testers (table 6). In these CTs, the absence of one member of the 3R pair is compensated by the pre-

Table 6. Karyotype segregation in the offspring of a cross between female parents of translocation heterozygote 240 (breakpoints in 3RS and 5RL) in which the normal chromosome 3R is replaced by its two telocentrics (3RS, 3RL), and karyotypically normal plants. In the CTs ((15)nn-3R+3^5+3RS), the long translocation chromosome 3^5 could be distinguished mitotically (see text)

Karyotype	No. of plants
(15)nn-3R+3RS+3RL	101
(14)tn	84
(15)nn-3R+3^5+3RS	4
(15)tn+3R (S or L)	2
(16)5^3;3RS;3RL	1
(22)3RS;3RL	1
Total	193

sence of both telocentric 3RS and the long translocation chromosome 3^5 (240, 3RS; fig. 3). Owing to the exceptionally high quality of the root tip squashes, the latter could be identified directly. The occurrence of chains of five at MI in PMCs of trisomic progeny of the four CTs confirmed the correctness of the mitotic observations.

A low association frequency of the exchanged segment of translocation chromosome 3^5 of 240 would increase the chances for a CT to originate from telo-substituted translocation heterozygote 240 (cf. fig. 2b). Meiotic data on this karyotype are not available. However, in a 'normal' translocation 240 heterozygote, the association frequency of this segment was estimated to be 0.930 (Sybenga, 1970). Owing to negative chiasma interference between the two exchanged segments, and considering the size of the exchanged segment of 3^5 (fig. 3), most probably this is an underestimation and the actual association frequency is expected to be close to unity. Therefore, absence of chiasmata in the exchanged segment of translocation chromosome 3^5 is not expected to contribute substantially to the origin of CTs in case of 3R-telo-substituted translocation heterozygote 240. This also applies to presence of chiasmata in the interstitial segment, as among the 1000 PMCs of translocation heterozygote 240 investigated, Sybenga (1970) observed only 11 cells in which the MI configuration could have resulted from interstitial chiasma formation, in either 3^5, 5^3 or both. The only remaining alternative for a CT to arise, is non-alternate chain-of-five orientation (cf. fig. 2b). As 2.1% of the testcross offspring of table 6 consists of CTs, this type of non-alternate multivalent orientation has apparently occurred in about 4% of the cells, which, for rye, is not unusual (Sybenga, 1968).

CT 306, in which one of the satellite chromosomes 1R is replaced by translocation chromosome 1^6 and telocentric 1RS (306, 1RS; fig. 3) has resulted from a telo-substituted translocation heterozygote 306 (1RS, 6RL) containing the two telocentrics 1RS and 1RL instead of the normal copy of 1R. No data are available from which a meaningful estimate can be derived of the frequency in which CT 306 arises from this source. It is plausible, however, to assume that absence of chiasma formation in the exchanged segment of 1^6 has played an important role, considering its small size (fig. 3).

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General discussion and conclusions

Genes for male sterility and (conditional) lethality

In the systems for breeding and propagation of male sterile seed parents for hybrid varieties of rye described in the present thesis, genes causing male sterility (m.s.) are indispensable, whereas (conditionally) lethal markers may be of great value.

As yet, m.s.-genes are very scarce in rye. One or two are probably available in Poland and the U.S.S.R. In chapter 1, the gene *ms* has been described (table 1), the chromosomal location of which was hampered by the occurrence of male sterility among translocation homozygous F2-plants which obviously was caused by the translocation rather than by *ms*. An effort was made to obtain new m.s.-genes. Dry seeds of spring type inbred lines were soaked in 0.5%-2.0% ethylmethanesulphonate for 4 h at 20 °C. The attempt was not successful. In a few M2s (40 plants each), plants with strongly reduced pollen production segregated, but no consistent male steriles could be isolated. In several M2s, chlorophyll mutants were observed which segregated 3:1, suggesting a monofactorial recessive inheritance. Most of these did not survive in the field, but some reached maturity in the greenhouse, where crosses with a selection of the Wageningen translocation tester set (chapter 1) were made. As yet, these markers have not been localized.

Introduction of lethality genes into balanced chromosomal systems may be achieved by crossing wildtype F2 plants (of which 2/3 are expected to be heterozygotes) as pollinators to tertiary or other kinds of suitable trisomics, and selection of segregating trisomic lines in later generations. M.s.-genes can be introduced similarily. Apparently, (conditional) lethality genes can be induced with relative ease (cf. Müntzing and Bose, 1969).

Most of the markers described in chapter 1 (table 1) were available at the Department of Genetics. Others, for instance the lethals *wh* ('white') and *gr* ('grass type') segregated spontaneously in the material under study. Even with non-competitive greenhouse conditions, *wh* and *gr* are not able to produce offspring, and can in principle be used in the construction of self-reproducing balanced trisomic systems (chapters 3, 4 and 5). This also seems to apply to some of the other markers described in chapter 1, which are conditionally lethal. For instance, homozygous recessives for *lg1* and *lg2* ('light green'), and for *ti* ('tigrina') are capable of producing offspring in the greenhouse, but are practically completely eliminated under field

conditions in competition with normal wildtype neighbours. That complete elimination of these conditionally lethal recessives also occurs in progenies of balanced tertiary trisomics (BTTs) or balanced telotertiary compensating trisomics (BCTs) may be assumed, but has not been tested. However, pure stands of homozygous recessives for these genes produce seeds under field conditions, whereas in progenies of BTTs or BCTs, these plants are expected to be surrounded on an average by 64% (chapter 4, table 3) or 67% (chapter 1, tables 12b and 13; chapter 5, table 4) identical disomics, respectively. Moreover, the competitive ability of inbred wildtype BTTs and BCTs is reduced, since, compared with wildtype disomic recombinants which occasionally appeared in selfed BTT and BCT progenies (cf. same tables), the trisomics are clearly shorter and generally showed a somewhat reduced vigour. Hence, recessives for *lg1*, *lg2* and *ti* may not be eliminated completely, and hand-elimination may be necessary. Therefore, only *true* (or more perfectly conditional) lethals should be used to set up self-reproducing balanced chromosomal systems for hybrid seed production. Another potentially useful category of markers are genes affecting seed colour, enabling a preselection of di- and trisomics, for instance by making use of a photo-sensitive device. Of course, the phenotype of the seed should represent that of the seedlings (xenia). Of the markers described in chapter 1, only *an* ('anthocyaninless') meets this requirement, as the other seed colour gene, *Ps* ('purple seed'), is expressed in the seed coat, representing maternal tissue. *an*, however, is expressed to a variable extent in the seed, probably insufficient for mechanical selection.

Ramage (1976) reports, that in commercial hybrid barley seed production via the BTT-system, no use is made of monogenic recessively inherited markers. To produce nearly pure stands of male sterile diploids, BTT offspring is sown at a rate of 25–30 kg/ha, resulting in an almost complete elimination of trisomics by competition from the male sterile diploids, which are longer and more vigorous. To perpetuate and increase the BTT-stock, selfed seed from trisomics are sown sparsely (5–7 kg/ha), and the disomics are pulled up as seedlings. Trisomics are easily recognized at this stage, having longer and narrower leaves. Ramage does not point out, however, to which extent the cost of the hybrid seed is enhanced by this laborious operation.

In inbred lines of rye, di- and trisomics can be morphologically distinguished before maturity also. As yet, however, preference should be given to the construction of balanced chromosomal systems for hybrid breeding

which require the lowest possible input of labour. Lethality markers for this purpose are available or can be induced with relative ease.

Set-up of trisomic systems

With the limited number of genes causing male sterility or lethality available at present in rye, it is important to be able to construct as wide a variety of balanced chromosomal systems as possible, to increase chances of finding one or more suited for use in hybrid breeding. The construction of two systems is descibed in chapters 3 and 5.

Chapter 3 deals with the isolation of tertiary trisomics from several types of translocation trisomics. Translocation heterozygotes carrying an extra telocentric did not yield substantially more tertiary trisomics than the corresponding translocation heterozygous disomics. Other types of translocation trisomics, containing a complete normal chromosome additional to one or two translocations, showed an increased frequency of tertiary trisomic offspring (chapter 3, table 7). The difference was concluded to be caused by the availability of an extra complete, instead of a telocentric chromosome, offering an easier opportunity for the assembly of a complete set of chromosomes to which a translocation chromosome is added. Moreover, from the presence of a normal chromosome in addition to two translocations having this particular chromosome in common, multivalents of up to seven chromosomes arise, resulting in more variation in non-alternate orientation at first meiotic metaphase, or in breakdown into smaller configurations. These undergo independent orientation, potentially resulting in tertiary trisomic progeny. However, from all sources tested, only tertiaries with a *short* translocation chromosome extra could be isolated, suggesting that the size of the extra material of a long translocation chromosome is too large to be frequently transmitted even by the eggs. Consequently, the number of tertiary trisomics which may be obtained will hardly exceed that of the translocations available. Hence, to cover large parts of the genome with tertiary trisomics, a wide variety of translocations is required. As yet, the Wageningen translocation tester set consists of 11 translocations involving all chromosomes at least once, and the isolation of several new interchanges from X- and neutron rayed seed is underway.

A method to cover a larger part of the genome than possible with tertiary trisomics without isolating new translocations, is the construction of compensating trisomics (chapter 5), in which one normal chromosome is re-

placed by two others of modified structure. The latter may be two translocation chromosomes, but there is a large potential of other possibilities, including combinations of translocation-, telocentric- and iso-chromosomes. Chapter 5 describes the isolation of four telotertiary compensating trisomics, in which a telocentric and a translocation chromosome replace one normal chromosome. These trisomics were isolated in progenies of testcrosses between translocation heterozygotes carrying either an extra telocentric, or two telocentrics instead of a normal chromosome, and karyotypically normal male parents. The four telotertiary compensating trisomics could be obtained with relative ease, especially when in the source plants the frequency of first metaphase association of the exchanged segment of the compensating translocation chromosome was low. A more important finding was that of the four trisomics isolated, three contained a *long* translocation chromosome (chapter 5, fig. 3). Hence, starting from a given set of translocations, about twice as many telotertiary compensating trisomics as tertiaries may be isolated.

One isotelotertiary and three ditertiary compensating trisomics have been synthesized as well, the isolation of which will be reported later. The conclusion seems justified, that the stock of structurally aberrant chromosomes presently available, offers sufficient opportunity to construct balanced chromosomal systems suited for hybrid breeding purposes, even in spite of low numbers of m.s.- and lethality genes.

Desturbing factors: male transmission and recombination

The most important requirement to be met in hybrid seed production, especially in case of wind pollinated crops like rye, is, that the disomic seed parent is *completely* male sterile. Unlike barley, a single male fertile rye plant in the male sterile rows is capable of pollinating many seed parents, thus affecting the performance and homogeneity of the hybrid. Removal of these plants is not only laborious, but has also a limited effect, as by the time that the male fertile plants can be pulled up,pollen has already been shed. Therefore, male transmission of the dominant m.s.-allele should be absolutely prevented. Hence, because the dominant allele is located on the extra genetic material, absence of pollen transmission of this material must be complete, and recombination with the recessive m.s.-alleles on the normal chromosome complement must be obstructed.

In most tertiary and telotertiary compensating trisomics tested, some male transmission of the extra chromosomal material could be demonstrated

(chapter 1, tables 10 and 13; chapter 4, table 3; chapter 5, table 4). However, the results presented in chapter 4 led to the tentative conclusion, that a response to selection for low rates of male transmission might be feasable. Moreover, the competitive ability of inbred trisomics is reduced, so that these may be eliminated by competition from disomic male sterile neighbour plants, when the seed parent is sufficiently thickly sown. On the basis of the findings in chapter 4, this is also expected to apply to deviant karyotypes, including trisomics with fragments of the extra material which carry the dominant m.s.-allele.

The absolute prevention of recombination between the dominant m.s.-allele on the extra material and the recessives on the normal complement may represent a more serious obstacle for a successful use of balanced chromosomal systems in hybrid rye breeding. Of all chromosome segments involved in the balanced chromosomal systems studied, the lowest chiasma frequencies were assessed in the interstitial segment of the extra translocation chromosome in the four tertiary trisomics studied (chapter 4), 1.0% being the maximum in case of tertiary trisomic 273 (fig. 2). Meiotic investigations of the four telotertiary compensating trisomics of chapter 5, showed 0.0%-7.2% interstitial chiasmata (de Vries, in prep.), the highest value belonging to the large interstitial segment of chromosome 1^6 of translocation 248 (chapter 5, fig. 3). An exceptionally high interstitial chiasma frequency (72.3%) was observed in chromosome 7^5 in translocation heterozygote 303 (chapter 2, table 8). Hence, although usually chiasmata in the interstitial segment of a translocation chromosome are formed less frequently than in other parts, it seems unlikely that this segment will be absolutely free of chiasmata, unless it has an extremely small size. In that case, however, chances for an m.s.-gene to be located interstitially, will be very small.

Low recombination fractions were established between the 'tigrina' marker, located on the unchanged arms of the chromosomes 5^3 and 5^7 of translocations 240 and 282, respectively, and the translocation breakpoints of these chromosomes (chapter 4, table 3). Terminal chiasma localization in 5RS was concluded to be the cause. Probably, this phenomenon acts in other chromosome arms as well. Evidence for this has been obtained with regard to the short arm of chromosome 6R (de Vries, in prep.; cf. chapter 1, table 13 and chapter 5, fig. 3). Although, consequently, larger parts of the genome may offer favourable locations for m.s.-genes, a sufficiently close linkage with the extra material may only be accomplished when these genes are located

as closely as possible to the translocation breakpoints of this material. When this is difficult, for instance owing to a limited number of m.s.-genes available, it should be assessed whether a slight reduction in the performance and homogeneity of the hybrid, caused by a very small fraction of male fertiles in the seed parent, is tolerated.

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Samenvatting

Van rogge is bekend, dat belangrijke opbrengst- en kwaliteitsverbeteringen zijn te bereiken, wanneer op een effectievere manier dan tot nu toe gebruik zou kunnen worden gemaakt van de aanwezige 'bastaard groeikracht' of 'heterosis'. Om de mogelijkheden van heterosis volledig uit te buiten, moet men hybride rassen kweken. Een bijkomend voordeel van hybride rassen is hun uniformiteit.

Het zaaizaad van een hybride ras ontwikkelt zich op planten die niet in staat zijn functioneel stuifmeel te vormen. De bestuiving van deze 'mannelijk steriele' (m.s.)-lijn, of 'moederlijn', gebeurt door een vaderlijn waarvan de combinatiegeschiktheid met de m.s.-lijn na uitvoerige proefnemingen is komen vast te staan.

Vooral bij windbestuivers als rogge is volstrekte mannelijke steriliteit van de moederlijn een vereiste: één enkele mannelijk fertiele - overigens niet tot de vaderlijn behorende - plant tussen de planten van de moederlijn kan tientallen m.s.-buurplanten bestuiven. Het zaad dat uit zo'n bestuiving voortkomt, is geen hybride zaaizaad, maar wordt wel meegeoogst en heeft uiteindelijk een negatieve invloed op prestatie en uniformiteit van het hybride ras.

Gedurende de afgelopen tien jaar is in rogge een systeem ontwikkeld, dat het mogelijk maakt, m.s.-lijnen op een betrouwbare en betrekkelijk eenvoudige - en dus commercieel aantrekkelijke - manier in stand te houden en te vermeerderen. In dit systeem wordt gebruik gemaakt van cytoplasmatische mannelijke steriliteit. Omdat momenteel slechts één steriliserend cytoplasma operationeel is, is de genetische basis van hybride rassen, die zijn veredeld met dit systeem, bijzonder smal en dus kwetsbaar.

Voor de hybride veredeling van een aantal andere gewassen zijn alternatieve methoden ontwikkeld of voorgesteld, die in principe ook in rogge toepasbaar zijn. In dit proefschrift zijn een aantal aspecten van de problemen, die zich bij de toepassing van deze alternatieven kunnen voordoen, nader belicht.

Alle voorgestelde alternatieven hebben met elkaar gemeen, dat gebruik wordt gemaakt van een monofactorieel recessief overervend, chromosomaal gelocaliseerd m.s.-gen. Het dominante allel van dit gen ligt op extra chromosoommateriaal met een van normaal afwijkende structuur, terwijl het normale chromosoomcomplement de drager is van het recessieve allel. Het doel van deze constructie is, te voorkomen dat het dominante allel via het

stuifmeel wordt overgedragen op de nakomelingschap. Daarvoor is nodig, dat stuifmeel met het extra materiaal niet aan de bevruchting kan deelnemen ('certatie'). Bovendien moeten de structurele afwijkingen in het extra materiaal verhinderen, dat door recombinatie het dominante allel op de normale chromosomen terecht komt. Wordt aan beide voorwaarden voldaan, dan is de vermeerdering van een volledig mannelijk steriele ouderlijn mogelijk, door normale, homozygoot recessieve m.s.-planten te bestuiven met pollen van fertiele planten met een chromosomale en genotypische constitutie als hierboven beschreven. Planten met dergelijke 'gebalanceerde chromosoomsystemen' kunnen door zelfbestuiving worden vermeerderd, omdat eicellen met extra chromosoommateriaal wèl reproductief zijn. Om bij de vermeerdering zoveel mogelijk arbeid te besparen, zou gebruik gemaakt kunnen worden van een recessief overervend gen dat letaliteit veroorzaakt. Net als bij een m.s.-gen, moet het dominante allel op het extra chromosoommateriaal en het recessieve allel op de normale chromosomen liggen. Deze constructie leidt ertoe, dat na zelfbestuiving uitsluitend planten met extra chromosoommateriaal in leven blijven.

Recombinatie in gebalanceerde chromosoomsystemen kan worden bestudeerd door splitsende nakomelingschappen van kruisingen tussen planten met deze systemen en dragers van monofactorieel overervende morfologische kenmerken te onderzoeken. Daartoe is allereerst geprobeerd 17 van dergelijke genen - waaronder een m.s.-gen - te localiseren op één van de zeven roggechromosomen (hoofdstuk 1). In 13 gevallen lukte dat, en bij negen genen kon tevens worden vastgesteld op welke chromosoomàrm ze liggen. Vier genen konden niet worden gelocaliseerd. Dit onderzoek resulteerde in de eerste genenkaarten voor de chromosomen 2R en 5R van rogge.

Bij de in hoofdstuk 1 beschreven genlocalisatie werd gebruik gemaakt van reciproke translocaties: permanente uitwisselingen tussen niet-homologe chromosomen. Reeds lang is bekend, dat in het chromosoomsegment tussen het punt van uitwisseling ('translocatiebreukpunt') en het centromeer recombinatie doorgaans gereduceerd is. Dergelijke 'interstitiële' segmenten zouden derhalve geschikt zijn als plaats voor een m.s.-gen. Een duidelijke uitzondering op de regel van lage interstitiële recombinatie wordt behandeld in hoofdstuk 2, waar het splitsingsgedrag in de nakomelinschap van een kruising tussen een translocatie en een interstitieel gelocaliseerd gen wordt beschreven. Zowel op grond van de waargenomen splitsing, als op basis van de fre-

quenties van karakteristieke chromosoomconfiguraties gevormd tijdens de metafase van de reductiedeling ('MI-configuraties') in de translocatieheterozygoot kon worden geconcludeerd, dat bij deze translocatie een extreem hoge chiasmafrequentie in het interstitiële segment optreedt. Een vanuit theoretisch oogpunt belangwekkende gevolgtrekking uit dit onderzoek was, dat de centromeeroriëntatie van translocatiemultivalenten in planten met (sub-)metacentrische chromosomen door interstitiële chiasmavorming kan veranderen van 'bij voorkeur zig-zag' in 'geen voorkeur'.

Een aantal voorwaarden waaraan voldaan moet worden om twee typen gebalanceerde chromosoomsystemen te construeren, is beschreven in de hoofdstukken 3 en 5. Hoofdstuk 3 behandelt de isolatie van vier verschillende tertiaire trisomen: planten die naast het normale chromosoomcomplement een translocatiechromosoom bevatten. Tertiaire trisomen komen in een lage frequentie voor in de nakomelingschap van translocatieheterozygoten. Uit de experimenten beschreven in hoofdstuk 3 bleek, dat de aanwezigheid van een extra chromosoom in een translocatieheterozygoot deze frequentie aanzienlijk kan verhogen. Voorwaarde is,dat dit extra chromosoom compleet is: de aanwezigheid van een extra 'telocentrisch' chromosoom (bestaande uit slechts één van de twee armen van een normaal chromosoom) bleek de ontstaansfrequentie van tertiaire trisomen niet wezenlijk te beïnvloeden. Tevens kwam naar voren, dat de kans op tertiaire trisomen in de nakomelingschap groter is naarmate bij een MI-configuratie in de ouderplanten meer chromosomen betrokken zijn (tot zeven stuks in dit onderzoek). De vier geïsoleerde tertiaire trisomen bevatten alle een extra translocatiechromosoom dat korter is dan de gemiddelde lengte van de normale chromosomen; tertiaire trisomen met een 'lang' translocatiechromosoom extra konden niet worden verkregen.

De isolatie van vier verschillende telotertiaire compenserende trisomen is beschreven in hoofdstuk 5. Het ontbreken van een normaal chromosoom wordt in dit type trisoom gecompenseerd door de aanwezigheid van een translocatie- en een telocentrisch chromosoom. De vier telotertiaire compenserende trisomen kwamen voor in de nakomelingschap van een aantal translocatieheterozygoten, waarin een telocentrisch chromosoom extra aanwezig was, of waarin twee telocentrische chromosomen de plaats in hadden genomen van een normaal chromosoom. Geheel in overeenstemming met wat op grond van theoretische overwegingen verwacht mocht worden, werd gevonden dat de frequentie van telotertiaire compenserende trisomen hoger is, naarmate het uitgewisseld segment van het translocatiechromosoom met de compenserende rol korter is. Een

belangrijke constatering was, dat telotertiaire compenserende trisomen niet alleen korte, maar ook lange translocatiechromosomen kunnen bevatten. In vergelijking met tertiaire trisomen (hoofdstuk 3) betekent dit, dat uitgaande van een gegeven aantal translocaties, tweemaal zoveel verschillende telotertiaire compenserende trisomen geconstrueerd kunnen worden, waardoor de mogelijkheid om een geschikte plaats voor een m.s.-gen te vinden, wordt vergroot.

Het gedrag van de vier tertiaire trisomen uit hoofdstuk 3 wordt beschreven in hoofdstuk 4. Het extra translocatiechromosoom van elk van deze trisomen bevat een stuk van chromosoom 5R met daarop het dominante allel van het 'tigrina' gen. De normale chromosomen dragen de recessieve allelen, zodat er sprake is van vier 'gebalanceerde' tertiaire trisomen. Het tigrina gen veroorzaakt een gele dwarsstreping van het blad ('tijgering'). In zelfbevruchtingsnakomelingschappen van de vier gebalanceerde tertiaire trisomen werd geconstateerd dat recombinatie tussen dit gen en het translocatiebreukpunt erg laag is, zelfs in de twee trisomen waarbij gen en breukpunt aan weerszijden van het centromeer liggen, en ondanks het feit dat het tigrina gen betrekkelijk ver van het centromeer afligt. Aangezien onderzoek aan MI-configuraties uitwees dat overkruising in de chromosoomarm met het tigrina gen zeer frequent optreedt, moest worden geconstateerd dat een groot deel van de overkruisingen plaats heeft bij het uiteinde van deze chromosoomarm. Dit is in overeenstemming met conclusies uit ander onderzoek aan rogge, waarbij werd gevonden dat deze 'terminale chiasmalocalisatie' ook in andere chromosoomarmen voorkomt. Wordt hierdoor het gebied waarin een m.s.-gen zou kunnen liggen op zich al vergroot, ook de interstitiele segmenten van de vier bestudeerde extra translocatiechromosomen komen voor de locatie van het m.s.-gen in aanmerking: slechts in één tertiair trisoom kon worden aangetoond, dat interstitiele chiasmavorming optrad, in een frequentie van 1%. Overigens vond enige recombinatie ook plaats in een tertiair trisoom waarbij gen en breukpunt vlak bij elkaar lagen.

Bij drie van de vier tertiaire trisomen kon worden aangetoond, dat het extra chromosoom via het stuifmeel op de nakomelingschap wordt overgebracht, echter in lage frequenties. Mannelijke transmissie kon niet worden vastgesteld bij het vierde tertiaire trisoom, maar door de proefopzet kon een geringe mannelijke transmissie onopgemerkt blijven. Aanwijzingen werden verkregen dat het transmissieniveau door selectie is te beïnvloeden. Bovendien bleek dat in inteeltlijnen trisomen minder groeikrachtig zijn dan normale planten zonder extra chromosoommateriaal, waardoor bij voldoende dicht

zaaien van de moederlijn een gering aantal trisomen zou kunnen worden weggeconcurreerd door normaal groeikrachtige m.s.-planten. Hiervan wordt in de hybride zaaizaadproductie van gerst gebruik gemaakt. Sporadisch traden tijdens de reductiedeling van tertiaire trisomen onregelmatigheden op, resulterend in nakomelingen met een gewijzigde chromosoomsamenstelling. Meestal waren deze planten niet in staat nakomelingen voort te brengen, en gezien hun geringe groeikracht zouden ook zij waarschijnlijk geëlimineerd worden in concurrentie met normale buurplanten.

Onderzoek aan de vier telotertiaire compenserende trisomen uit hoofdstuk 5 leidde in hoofdzaak tot dezelfde conclusies ten aanzien van recombinatie, chiasmalocalisatie, transmissie en meiotische stabiliteit als voor de onderzochte tertiaire trisomen.

Hybride rogge is alleen economisch aantrekkelijk, wanneer de extra zaaizaadkosten van een hybride ras worden gecompenseerd door de extra baten verkregen door het verbouwen ervan. De zaaizaadproductie moet daarom tegen zo laag mogelijke kosten worden gerealiseerd. Hiervoor zijn arbeidsbesparende letaliteitsgenen beschikbaar of kunnen met betrekkelijk gemak worden geïnduceerd door mutagene behandelingen. Ook het aantal m.s.-genen zou langs deze weg kunnen worden verhoogd. Vooralsnog lijkt het aantal beschikbare translocaties en andere chromosoomafwijkingen voldoende groot om enkele voor hybrideveredeling geschikte gebalanceerde chromosoomsystemen op te zetten. Wel dient het dominante m.s.-allel absoluut gekoppeld te zijn met de translocatiebreukpunten van het extra chromosoommateriaal om mannelijke fertiliteit in de moederlijn te voorkomen. Indien, om wat voor reden dan ook, een dergelijke koppeling niet te verwezenlijken is, maar een iets zwakkere wel, dient nagegaan te worden of een beperkte afname van de prestatie en de uniformiteit van de hybride te toleren is.

Curriculum Vitae

Jaap de Vries werd geboren op 15 oktober 1954 te de Bilt. In 1972 behaalde hij het HBS-B diploma aan het College Blaucapel te Utrecht. In hetzelfde jaar werd een begin gemaakt met de studie aan de Landbouwhogeschool te Wageningen, waar hij in januari 1980 met lof afstudeerde in de richting plantenveredeling. Keuzevakken waren erfelijkheidsleer en plantenziektekunde. Vanaf oktober 1979 tot april 1983 was hij als promotie-assistent bij de vakgroep Erfelijkheidsleer in dienst van de Landbouwhogeschool. In januari 1984 hoopt hij te gaan werken bij de vakgroep Genetica van de Rijks Universiteit Groningen, binnen het project 'genetica van zetmeelvorming bij de aardappel'.