GENERATIVE TRANSMISSION OF THE TRANSLOCATION CHROMOSOME IN TERTIARY TRISOMICS OF RYE: GENETIC AND PHYSIOLOGICAL ASPECTS

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GENERATIVE TRANSMISSION OF THE TRANSLOCATION CHROMOSOME IN TERTIARY TRISOMICS OF RYE : GENETIC AND PHYSIOLOGICAL ASPECTS

Proefschrift
ter verkrijging van de graad van
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van de Landbouwuniversiteit te Wageningen.

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Aan Peter

Aan mijn moeder en vader

STELLINGEN

1) De door De Vries opgestelde formules voor de frekwenties van di-, tri- en tetrasomen in de zelfbevruchtingsnakomelingsschappen van tertiaire trisomen kunnen leiden tot onderschatting van de mannelijke transmissie van het extra chromosoom.
Vries J.N.de (1984) Chromosoma 89: 24-32
Dit proefschrift

2) Het selekteren van tertiair trisome genotypen met een zeer lage mannelijke transmissie van het extra chromosoom met behulp van toetskruisingen is in de praktijk bij rogge niet uitvoerbaar, zolang geen gebruik kan worden gemaakt van mannelijke steriliteit.
Dit proefschrift

3) De bewering van Ramage, dat de vertraagde ontwikkeling van aneuploide microsporen in tertiaire trisomen van gerst de mannelijke transmissie van het extra chromosoom verhindert, wordt niet onderbouwd door waarnemingen.
Ramage R.T. (1965) Crop Sci. 4: 177-178

4) De experimenten van Titarenko en Torop tonen duidelijk aan dat de invloed van de aneuploide sporofyt op de fysiologische eigenschappen van het pollen groter is dan vaak wordt verondersteld.
Titarenko A.V. en Torop A.A. (1985) Genetika 21: 1012-1020

5) McDaniel en Ramage houden in hun voorstel om primaire trisomen van gerst te identificeren aan de hand van de eiwitpatronen van het zaad onvoldoende rekening met het mogelijk optreden van genetische variatie.
McDaniel R.G. en Ramage R.T. (1970) Can.J.Genet.Cytol. 12: 490-495

6) De mogelijke toepassing van selektie op pollenniveau in de plantenveredeling verdient meer aandacht.
Mulcahy D.L., Bergamini Mulcahy G. en Ottaviano E. (1986) Proceedings of the Conference on Biotechnology and Ecology of Pollen.

7) Het is wenselijk dat in de wetenschappelijke literatuur de referentielijsten gestandaardiseerd worden.

8) Positieve discriminatie van vrouwen in sollicitatieprocedures heeft ook negatieve effecten.

9) Alle leerkrachten van basisscholen zouden verplicht moeten worden in het bezit te zijn van een geldig EHBO-diploma.

10) De visser doet er momenteel verstandig aan een kabeljauw uit te gooien om tenminste nog een spiering te vangen.

Stellingen bij het proefschrift van J. Janse:
"Generative transmission of the translocation chromosome in tertiary trisomics of rye: genetic and physiological aspects."
Wageningen, 15 januari 1988.

VOORWOORD

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CONTENTS

GENERAL INTRODUCTION

Use of tertiary trisomics in rye hybrid breeding

In rye, *Secale cereale L.*, hybrid varieties are expected to have three main advantages over open pollinated or synthetic varieties. First, they make optimal use of heterosis; second, they are uniform and third, specific characters can easier be introduced.

For hybrid breeding, inbred lines with good qualitative characters and combining ability must be developed. However, for the production of hybrid seed cross-fertilization is a prerequisite and self-fertilization of the female parent must be prevented. Four approaches can be used for this purpose:

(a) Application of gametocides
(b) Self-incompatibility
(c) Cytoplasmic male sterility with nuclear restorer genes
(d) Balanced chromosomal male sterility system.

The use of gametocides is promising for some crops but it is not expected to be practically available for rye in the near future.

Experiments carried out by Wricke (1986) showed that self-incompatibility may be useful for rye hybrid breeding. However, at least 17% of the "hybrid" seed was the result of fertilization within parents, which is expected to reduce the performance and uniformity of the hybrid.

Cytoplasmic male sterility (*cms*) has thus far been the only successfully applied mechanism. A few hybrid rye varieties have been released in Germany (Geiger 1986), that were based on the "Pampa" (P) plasma discovered by Geiger and Schnell (1970). A promising new type, the "G" plasma, has been reported but it seems to be more difficult to be maintained (Adolf and Winkel 1986). The genetic basis for the hybrid varieties on the basis of *cms* is still narrow and vulnerable, and good restorer genes are sometimes difficult to find in the male parents.

The use of balanced chromosomal systems with genetic male sterility (*gms*) may be an alternative. It was first proposed for barley hybrid breeding by Ramage (1965), who used balanced tertiary trisomics (BTTs). These trisomics carry an extra translocation chromosome, on which the dominant allele *Ms* of the male sterility gene is located. The two normal chromosomes carry the recessive allele *ms*. If the extra translocated chromosome is not transmitted through the pollen, all pollen grains that are able to contribute to fertilization will carry the *ms* allele. Thus, when the tertiary trisomic is used to pollinate male sterile disomic plants, the progeny will only consist of male sterile disomics. In this way the male sterile stocks that serve as the seed parents in

hybrid seed production can be propagated (Fig. 1). No special requirements have to be met in the choice of the male parent for the hybrid cross because all normal, not related, genotypes will carry the dominant *Ms* alleles.

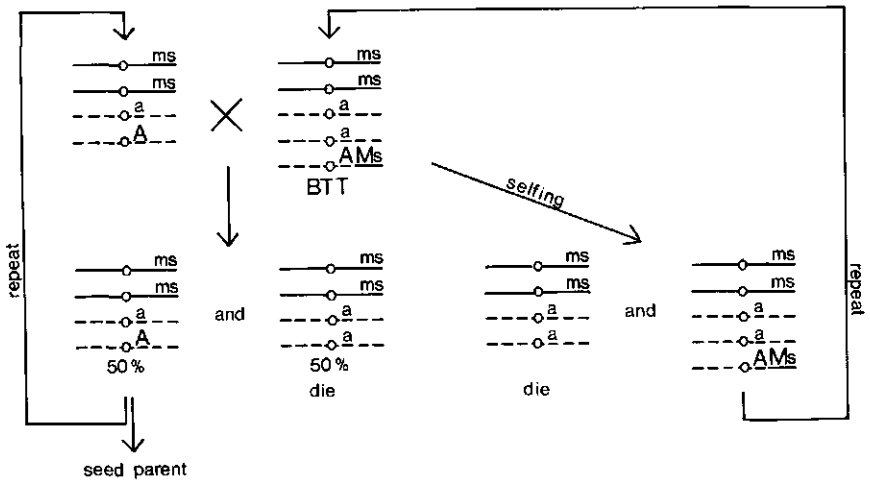

Fig. 1. Breeding procedure for propagation of male sterile seed parents for hybrid seed production with the use of balanced tertiary trisomics (BTTs). Maintenance of BTTs by selfing (After Wiebe and Ramage 1971).

The BTT itself is maintained by selfing. The progeny will contain male sterile disomics and male fertile trisomics, the latter having a constitution equal to that of the parent. For early selection of the trisomics, a recessive selective marker, usually conditional lethal, can be used (Wiebe and Ramage 1971). The dominant allele *A* of this marker gene should be located on the extra chromosome, whereas the recessive alleles *a* are carried by the normal chromosomes. After selfing, only viable and fertile ("balanced") trisomics will be produced. As a consequence, this lethal marker will then be introduced into the male sterile seed parent as well. In every propagation step 50% of the male sterile plants will die (Fig. 1).

In this system special requirements have to be met with respect to male and female transmission of the translocated chromosome (see below), fertility of trisomics, recombination between the genes *Ms* and *A* and the translocation breakpoint and meiotic behaviour of the trisomics.

Although some commercial barley hybrid varieties have been released in the 1970s by Ramage and coworkers, their success was rather limited due to practical difficulties (cf. Scholz and Künzel 1986). Apparently, insufficient attention had been paid to the aspects mentioned above.

Sybenga (1982) proposed the use of the BTT system for rye hybrid breeding. De Vries (1983) isolated several tertiary trisomics and studied their meiotic behaviour in inbred lines (De Vries 1984). Several selective markers (most of which are conditionally lethal) have been located on their respective chromosomes with the use of the Wageningen translocation tester set (De Vries and Sybenga 1984). In contrast with barley, male sterile mutants are scarce in rye and they are difficult to localize. Some of the problems arising in the construction and application of the BTT system in rye were reviewed by Sybenga (1986).

Male and female transmission in tertiary and other trisomics

One of the most important requirements for the practical application of the BTT system in hybrid rye breeding is that male transmission of the translocated chromosome is absent. If not, male fertile trisomic plants will occur within the male sterile disomic seed parent, resulting in undesirable fertilization. This can severely affect the vigour and uniformity of the hybrid. In addition, female transmission of the extra chromosome should be as high as possible for efficient maintenance of the BTT itself.

De Vries (1984) found some variation in male and female transmission rates in the progenies of different rye tertiary trisomics upon selfing. In some cases, additional variation was present between different lines of a tertiary trisomic. Male transmission was estimated to range from 0% to 7% and female transmission from 14% to 51%. However, separate estimates for male and female transmission after selfing were largely based on the frequency of tetrasomics that usually have a strongly reduced viability, particularly in inbred lines. Thus, male transmission may have been underestimated.

This was indeed found for tertiary trisomics of barley (Lehmann 1972). Tetrasomics were absent in progenies upon selfing but in crosses with disomics as female parents, transmission of the translocation chromosome through the pollen could even reach 13%.

Thus, male transmission rates in crosses with disomics can not be predicted from segregation data upon selfing. This is very important when developing BTTs for hybrid breeding.

Other studies on male and female transmission rates of extra chromosomes are mainly concerned with primary trisomics. In rye, male transmission of the extra chromosome in the primary trisomic "semistout" was 2.6% and in the other types it was even lower or zero (Kamanoi and Jenkins 1975). For barley, no transmission through the pollen could be found in any of the primary trisomics (Tsuchiya 1960) but in a *Nicotiana sylvestris* primary trisomic it reached 34% (Goodspeed and Avery 1939).

Khush (1973) listed the factors that determine the generative transmission of an extra chromosome through the male as well as through the female:

(a) meiotic behaviour of the supernumerary chromosome
(b) development of aneuploid gametophytes
(c) fertility of the mature aneuploid gametophytes
(d) competition between euploid and aneuploid gametophytes during fertilization processes
(e) development of aneuploid zygotes, embryo and endosperm
(f) germination of aneuploid seeds
(g) vigour of aneuploid seedlings.

Angiosperm pollen is known to be subjected to important selective forces, especially during germination and pollen tube growth (Mulcahy 1975). This may be the reason for the usually much lower male transmission when compared to the transmission through the eggs. On the female side, competition is not supposed to occur.

Germination of trisomic seeds and vigour of trisomic seedlings may be related to the degree of inbreeding as trisomic seeds are usually smaller and lighter than disomics, even in a heterotic background (Tsuchiya 1960; Khush 1973).

Aims of the present study

In this thesis experiments on male and female transmission of the supernumerary chromosome in rye tertiary trisomics are described. Special attention is given to the transmission through the male.

Studies were undertaken to determine:
(a) transmission of the extra chromosome in different inbred lines upon selfing and a possible correlation to the degree of inbreeding.
(b) male transmission of the extra chromosome in crosses with disomics.
(c) any possible genetic factors involved in (a) and (b).
(d) the "bottle-neck" for male transmission (i.e. the stage in which the largest part of aneuploid spores are eliminated) and the expression of any genetic factors herein.
(e) the biochemical basis for the aneuploid behaviour at the sporophytic and the (male) gametophytic level.

In chapter 1 a study is reported on the differential developmental rates of euploid and aneuploid microspores in a tertiary trisomic by means of comparative timing of the first pollen mitosis; from meiotic analysis the frequencies of both types are predicted.

Chapter 2 presents estimates for male and female transmission rates upon selfing and male transmission rates upon testcrossing, for several F_5 and F_6 lines of one tertiary trisomic. Genetic factors are assessed and male transmission rates are compared with the relative rate of aneuploid pollen development and to meiotic behaviour.

In chapter 3 the possible certation between euploid and aneuploid pollen grains is

investigated using abundant and strongly limited pollinations.

Chapter 4 describes an attempt to study the biochemical basis for the differential morphology and physiology of the trisomics and for the differential behaviour of aneuploid pollen grains.

In chapter 5 male and female transmission rates upon selfing and male transmission rates upon testcrossing in a tertiary trisomic are related to the degree of inbreeding and to pollen quality.

Finally, the combined results are discussed in chapter 6. Here, some additional, partly unsuccessful, smaller experiments are described briefly.

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CHAPTER 1
Relative rate of development of aneuploid and euploid microspores in a tertiary trisomic of rye, *Secale cereale L.*

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Key words: rye - tertiary trisomic - euploid microspores - aneuploid microspores - rate of development.

Abstract

Meiotic configurations were studied in pollen mother cells of a tertiary trisomic of rye. Chains of five and chains of three, in alternate orientation, were the most frequent configurations. Assuming loss of univalents in anaphase I or single chromatids in anaphase II, a total of 58.1% of the viable gametes resulting after meiosis were expected to contain the normal haploid complement, whereas 41.9% were expected to have the translocated chromosome in addition.

The percentages of uninucleate and binucleate microspores in anthers containing dividing microspores provided a time scale for the development of euploid and aneuploid spores during first pollen mitosis. Microspores containing the extra translocated chromosome tended to divide at a later stage than euploid microspores. The slower development was also illustrated by the course of the mitotic index of both types. It was found that 58.1% of all microspores passing through pollen mitosis contained seven and 41.9% contained eight chromosomes, which means that up to the end of first pollen mitosis aneuploid spores were not lost significantly more than euploid spores. It is likely that the delay in development already starts immediately after meiosis.

Introduction

A tertiary trisomic carries a translocation chromosome in addition to a normal karyotype. The most important source of this type of trisomy is the translocation heterozygote (Khush 1973; De Vries 1983).

Tertiary trisomics can be of special use in cytogenetics and plant breeding, both for gene localization (De Vries and Sybenga 1984) and for the construction of "balanced tertiary trisomics" (BTTs) for hybrid breeding, as proposed for barley by Ramage (1965). In a BTT the dominant allele of a selective marker gene is carried on the extra chromosome, whereas the normal chromosomes both carry the recessive allele. If the extra chromosome is not transmitted through the pollen, functional pollen will be homogeneous for the recessive gene. When a recessive gene for male sterility is used as the marker gene, this offers the possibility for producing all male sterile stocks (Ramage and Tuleen 1964). For this purpose the BTTs are used to pollinate male sterile disomics. The offspring will again be completely male sterile and disomic and in this way the seed parent for the production of hybrids is produced. The BTT stock itself is maintained by selfing. The progeny will contain male sterile disomics and male fertile trisomics, the latter having a constitution equal to that of the BTT parent (Ramage 1965; Wiebe and Ramage 1971).

Sybenga (1982) proposed the use of this BTT system for hybrid rye breeding. De

Vries (1984a) studied a number of aspects related with the construction and application of a BTT system with genetic male sterility in rye. Factors that could disturb the balanced system were found to be the possibility of recombination between marker genes and translocation breakpoint and the occurrence of male transmission of the translocated chromosome. According to De Vries (1984b) the male transmission rate in four BTTs was low (0-7%) and a response to selection against transmission through pollen may be expected. However, these values might be underestimates, as they were deduced from observations on selfed progenies. In these progenies trisomics can result from male as well as from female transmission. Separate estimates for male and female transmission were largely based on the frequency of tetrasomics. However, as tetrasomic individuals can have a strongly reduced viability, it is possible that trisomic male transmission estimates are much higher when crosses are made with disomics as female parents.

It seemed useful to study the sources of variation in the transmission of extra chromosomes in BTTs of rye. Special attention should be given to the causes of reduced male transmission.

Khush (1973) made an extensive review of studies on transmission of extra chromosomes. He summarized the factors that could contribute to the reduction of both male and female transmission:

1. Elimination of the extra chromosome in meiosis
2. Subnormal development of n+1 gametophytes
3. Reduced fertility of these gametophytes
4. Subnormal development of zygotes, embryos or endosperm containing extra chromosomes
5. Reduced and delayed germination of 2n+1 seeds
6. Reduced vigour of 2n+1 seedlings.

An additional factor was described that could contribute to the extremely low male transmission:

7. Competition between n and n+1 pollen during germination, pollen tube growth and fertilization.

It was suggested that the most important factors are those concerning development and functioning of the gametophytes (factors 1, 2, 3 and 7) and that the tolerance to genetic and chromosomal imbalance is usually lower in male than in female spores.

Ramage (1965) stated that in barley BTTs the n+1 microspores showed a slower development. The division of the generative nucleus (second pollen mitosis) occurred much later in these spores, often as late as anthesis. Experimental results were not given.

De Vries (1984a) suggested that in rye BTTs the reduced male transmission is due to certation of pollen, but this was not based on any observations. However, it was found that the transmission of the translocated chromosome through male meiosis is relatively high, as a result of high meiotic multivalent frequencies and alternate orientation in pollen mother cells (De Vries 1984b).

This paper deals with the behaviour of aneuploid and euploid spores during microsporogenesis in a rye BTT. Meiotic configurations were also studied. The chromosomal constitution of microspores in first pollen mitosis was determined in several preparations. Following Gülcan and Sybenga (1967) a physiological time scale was constructed by determining the percentages of binucleate and uninucleate microspores in these preparations. In addition, mitotic indices were determined. Conclusions will be drawn on the relative rate of development of euploid and aneuploid microspores and on the frequencies of both types of spores passing first pollen mitosis.

Material and methods

Material

The tertiary trisomic investigated in this study carries the short translocation chromosome of translocation 240, that is described by Sybenga and Wolters (1972) as a part of the Wageningen translocation tester set.

In this interchange the chromosome arms 5RL and 3RS are involved (nomenclature according to Sybenga 1983; Sybenga et al. 1985). The short translocated chromosome contains the unchanged segment, including the short arm, of 5R and the translocated segment from the short arm of 3R (Fig. 1).

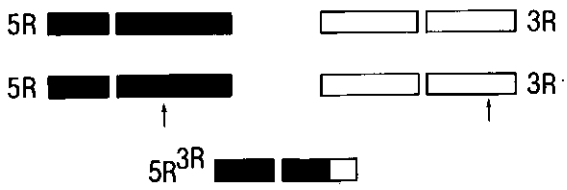

Fig. 1. Diagram of chromosomes involved in tertiary trisomic 240. Nomenclature according to Sybenga (1983) and Sybenga et al. (1985). Arm length ratios derived from Sybenga and Wolters (1972). Arrows indicate the translocation breakpoints.

The isolation of the tertiary trisomic, from translocation trisomics crossed with karyotypically normal plants, was carried out in our laboratory and described by De Vries (1984b). The observations are made on an F_5 plant derived from one of the F_4 lines De Vries (1984b) used for his studies.

The plant was grown in a cooled greenhouse (approximately 20 °C) during the summer of 1984.

Meiotic analysis

Anthers containing pollen mother cells (PMCs) in first meiotic metaphase (MI) were fixed in acetic alcohol (1:3) and stored at -10 °C. Anthers were stained in 2% aceto-carmine for 1 day, squash preparations were made in 45% acetic acid and mounted in Euparal. In the best preparation, meiotic configurations were studied in 350 PMCs.

Analysis of pollen mitosis

Ears to be used for analysis of pollen mitosis were kept in the refrigerator over-night. Next day, the content of one anther per flower was squeezed in 2% acetocar-mine, followed by squashing and heating. If this anther contained microspores in pollen mitosis, the other two anthers of this flower were fixed in acetic alcohol (1:3). For observations temporary preparations were made as described for the first anther (modi-fication from Gülcan and Sybenga 1967).

In a preparation three random samples of 100 microspores were taken. In each sample the numbers of binucleate and uninucleate spores were determined. Cells in mitotic prophase and telophase were considered to be uninucleate and binucleate, respectively. Cells in mitotic metaphase were not taken into account. The mean per-centage of binucleate cells corresponds with the percentage of cells that have already undergone division.

Subsequently, chromosomes were counted in all microspores in metaphase. The microspores can contain 7, 8 or (occasionally) an aberrant number of chromosomes.

Finally the total number of cells in the preparation was counted. These observa-tions were made on several preparations.

Results and discussion

Meiotic analysis

The exchanged segment of the translocated chromosome is homologous with the terminal segment of one of the arms of both chromosomes 3R. The remainder, including

the centromere and the unchanged arm, is homologous with a part of both chromosomes 5R (see Fig. 1). With three copies of each set of homologous segments, each segment has three ways of pairing at zygotene. For the two sets together, with terminally initiating pairing, there are nine (3x3) different possibilities of association.

Table 1. Frequencies of MI configurations involving the translocated chromosome in 350 PMCs in a tertiary trisomic of rye, and expected frequencies of gametes resulting from these configurations after meiosis, when univalents are lost.

	MI configurations		Expected gametes		
Type	Configuration	Frequency	7	7+5R^{3R}	not viable
1		0.300	0.150	0.150	0.0
2		0.006	0.003	0.003	0.0
3		0.077	0.039	0.0	0.039
4		0.491	0.246	0.246	0.0
5		0.006	0.003	0.003	0.0
6		0.003	0.002	0.002	0.0
7	Univalents	0.117	0.117	0.0	0.0
	Total	1.000	0.560	0.404	0.039
	Percentage of viable gametes		58.1	41.9	

The number and location of chiasmata in the paired segments of these associations determine the type of configuration at MI. Table 1 shows the frequencies of MI configurations involving the translocated chromosome that were observed in the meiotic analysis.

As usual for rye, most configurations show an alternate (zigzag) orientation. Types 1 and 4, chains of five and chains of three, are the most frequent configurations. These types, as well as type 2, will result in viable gametes after meiosis, of which 50% is expected to contain the translocated chromosome in addition to the normal haploid complement. Probably, this is also true for the types 5 and 6 (Table 1).

The constitution of gametes resulting from configuration types 3 and 7 will depend on the meiotic behaviour of the univalent chromosome. Univalents tend to stay behind, but later in anaphase I they may orient as mitotic chromosomes, followed by segregation of chromatids. At anaphase II, however, the single chromatids lag behind and are lost. This is quite common in rye. Another possibility is the occurrence of centro-

mere misdivision, resulting in two half chromosomes. If these chromosomes separate at anaphase II, functional telocentric chromosomes may arise. It is also possible that the two chromatids unite by sister reunion resulting in an isochromosome (Sybenga 1972). Occasionally a whole univalent can end up in one anaphase I group and show a normal segregation in anaphase II.

Configuration 3 can only arise when both parts of the translocated chromosome pair with one of the homologous parts of the normal chromosomes and when chiasmata have been formed in these paired segments. In addition, pairing and formation of at least one chiasma should occur between the homologues of either 5R or 3R (Fig. 2).

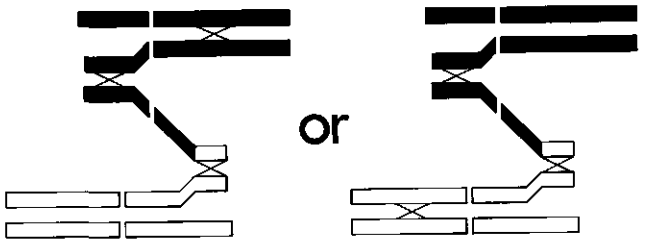

Fig. 2. Two associations in a tertiary trisomic that can lead to a MI configuration consisting of a chain of four and a univalent. The segments in which at least one chiasma should occur are marked with X.

Thus, in configuration type 3, the univalent chromosome is not the translocated chromosome, but one of the normal chromosomes 5R or 3R. If the univalent lags behind or its chromatids are lost in anaphase II, in 50% of the gametes one of the normal chromosomes is replaced by the translocated chromosome. These gametes will have a deficiency and will not be viable. The other 50% will contain the normal complement (Table 1).

Configuration type 7 will, if the univalent is lost, result in viable gametes with normal constitution.

Thus, if the univalent chromosomes are not transmitted to the gametes, a total of 58.1% of the viable gametes will contain the normal haploid complement whereas 41.9% will have the translocated chromosome in addition (Table 1).

When, in some PMCs, the univalent chromosome does not lag and is not lost in anaphase II, the percentage of aneuploid gametes will be somewhat larger than 41.9%. The surplus of aneuploid gametes can contain an isochromosome, a telocentric, or a translocated chromosome.

The frequency of euploid and aneuploid gametes resulting after meiosis is an important datum for the analysis of transmission of the extra chromosome, e.g. for comparison of the development of both types of gametes to mature pollen grains.

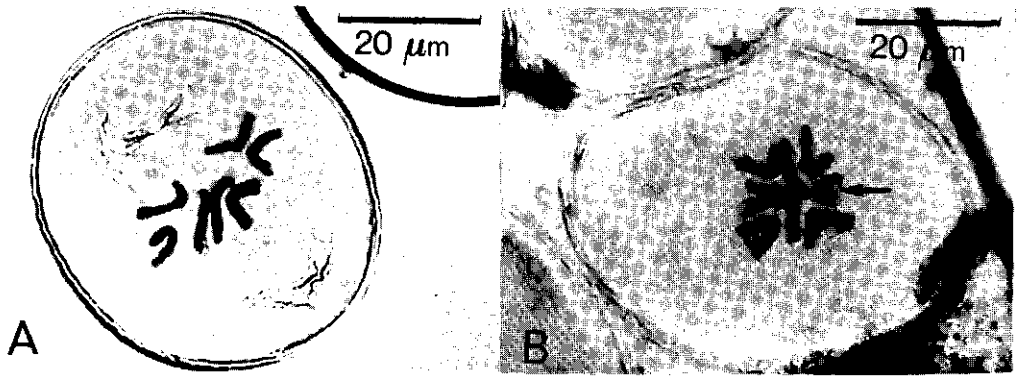

Fig. 3. Two types of microspores produced by a tertiary trisomic plant, in the metaphase of first pollen mitosis. A – Euploid microspore. B – Microspore containing the translocated chromosome (arrow) in addition to the normal haploid complement.

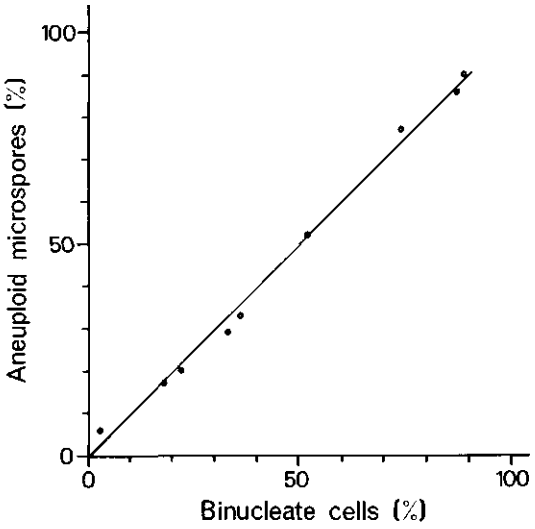

Fig. 4. Percentages of dividing microspores containing eight chromosomes during the process of pollen mitosis. Stages of anther development are represented by percentages of binucleate cells.

Analysis of pollen mitosis

In Fig. 3 a euploid and an aneuploid microspore in first pollen mitosis are shown. In Fig. 4 the percentages of dividing cells with eight chromosomes are plotted against the stages of anther development in which the observations were made. Each stage of development is characterized by the percentage of binucleate cells in the given preparation. All other dividing microspores had seven chromosomes. In most microspores containing eight chromosomes the translocated chromosome could be recognized (Fig. 3). Aberrant extra chromosomes such as isochromosomes or telocentric chromosomes have not been observed.

It can be seen from Fig. 4 that in an early stage of development only few cells at pollen mitosis contain eight and most contain seven chromosomes. As development proceeds the percentage of dividing aneuploid cells increases. This means that the aneuploid microspores tend to divide at a later stage than the euploid microspores, which implies that they show a slower development.

In every preparation the total number of microspores has been counted. This makes it possible to determine the mitotic index, being defined as the ratio between the number of dividing cells in metaphase and the total number of cells. The mitotic index is shown for both types of microspores separately and pooled, for different stages of anther development (Fig. 5).

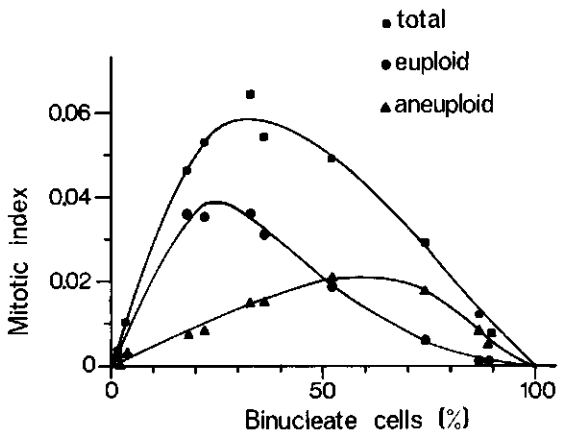

Fig. 5. Mitotic index during the process of pollen mitosis for the total of dividing microspores and for the euploid and aneuploid dividing microspores separately. Stages of anther development represented by percentages of binucleate cells.

The overall mitotic index reaches its maximum at the stage of 30–35% binucleate cells. However, the maximum mitotic index of euploid microspores is reached at a much earlier stage (20–25% binucleate cells) than the maximum index of the aneuploid microspores (55–65% binucleate cells), which again illustrates the difference in rate of development between these types of microspores.

The surfaces covered by the curves in Fig. 5 equal the numbers of cells passing through pollen mitosis and can be measured for both the aneuploid and the euploid microspores. The ratio between the two surfaces gives the ratio between the numbers of cells of both types that have come through pollen mitosis and equals 581:419. Thus, of all microspores passing pollen mitosis 58.1% contained seven and 41.9% contained eight chromosomes.

These percentages correspond precisely with the percentages expected from the observed meiotic configurations assuming univalent loss. (It should be noted that the exact similarity of these values must be a coincidence.) This leads to the conclusion that between meiosis and pollen mitosis, aneuploid microspores are not lost significantly more than euploid microspores. The aneuploid microspores apparently show a normal, but delayed development.

However, as synchronization within an anther stops after meiosis and as the development of the spores to mature pollen grains is a continuous process, it is likely that the delay in development observed at pollen mitosis starts immediately after the meiotic tetrad stage.

Considering this delay and the reduced male transmission in tertiary trisomics, it is interesting to know which proportion of the aneuploid microspores can reach the mature pollen stage before anthesis. If this is large, the cause of reduced male transmission must be found in the processes that occur after pollen maturation.

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CHAPTER 2
Male transmission of the translocated chromosome in a tertiary trisomic of rye: genetic variation and relation to the rate of development of aneuploid pollen grains.

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Summary

Variation in male and female transmission of the translocated extra chromosome ($5R^{3R}$) was studied in a tertiary trisomic of rye (*Secale cereale L.*). In two F_5 lines derived from a single F_4 line, female transmission was lower than in five others derived from another F_4 line. This could be caused by genetic factors or by the strong inbreeding depression in these lines, leading to low viability of trisomic progeny. After selfing, male transmission was estimated as very low, but this was primarily based on the occurrence of tetrasomics, that probably have a very poor viability. In testcrosses with disomic female parents, male transmission was much higher (up to 27%), without variation within F_5 lines. One F_5 line showed significantly higher male transmission than any other of the seven tested, including a sister line from the same F_4. This was consistent in the F_6. Apparently high male transmission is genetically determined. There was a positive correlation with recombination of the marker *ti* (tigrina) on the extra chromosome and the normal 5R chromosomes.

At the first meiotic metaphase, trivalents and quinquivalents were frequent in the trisomics. Assuming loss of univalents, 40% of the microspores should carry the translocated extra chromosome. In most lines, even more than 40% were found at pollen mitosis. Observations on timing of pollen mitosis showed a delayed development of aneuploid spores, with clear differences between plants, but no correlation with male transmission. The cause of reduced male transmission and the expression of genetic variation therein can, therefore, not be found in meiotic behaviour or delayed microspore development. Pollen germination and tube growth may be more important.

Key words: *Secale cereale L.* – tertiary trisomic – male transmission – genetic variation – aneuploid microspores.

Introduction

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

Self-fertile inbred lines can be developed but to produce hybrid seed, selfing of the seed parent must be prevented. One method for this uses genetic male sterility (*ms*). Then, however, the propagation of the male sterile seed parent is a problem. A possible solution is the use of the "Balanced Tertiary Trisomic" (BTT) system, as proposed by Ramage (1965) for barley. In this system the extra, translocated chromosome in a tertiary trisomic carries the dominant *Ms* allele and the dominant allele *A* of a

selective marker gene. The two normal chromosomes carry the recessive alleles *ms* and *a*. If the extra chromosome is not transmitted through the male gametes, all functional pollen will be homogeneous for the recessive alleles *m* and *a*. If the extra chromosome can be transmitted through the eggs, the progeny after selfing will consist of parental male fertile tertiary trisomics and male sterile disomics. As the latter will express the recessive marker, selection is possible. When the tertiary trisomic is used to pollinate male sterile plants, all progeny will be male sterile disomics again. Thus, the tertiary trisomic can be used as a maintainer line for male sterile stocks that serve as the seed parent in the production of hybrids. The BTT itself is maintained by selfing (Ramage 1965; Wiebe and Ramage 1971).

As the system works for barley, although not yet in practice, Sybenga (1982) proposed it for rye. A number of aspects of its construction and application for rye hybrid breeding was studied by De Vries (1984a). Several meiotically stable tertiary trisomics could be isolated (De Vries 1984b). A number of morphological selective markers has been located on their respective chromosomes. Male sterile mutants were more difficult to isolate and localize with the translocation tester set (De Vries and Sybenga 1984).

As mentioned, for the maintenance of male sterile lines using pollination with BTTs it is necessary that male transmission of the translocated chromosome is completely absent. For efficient maintenance of the BTT, female transmission should be as high as possible.

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

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

In primary trisomics of *Nicotiana sylvestris* Goodspeed and Avery (1939) found a male transmission rate of 0%–34% and a female transmission of 16%–29%, both in test-crosses. In some trisomics the total transmission after selfing, that ranged from 20% to 39%, was smaller than male and female transmission separately. In other cases, it was

even greater than male and female transmission together. Even when male transmission was high, tetrasomic individuals were very rare in selfed progenies of trisomics. The same phenomenon was observed in primary trisomics of *Lotus pedunculatus* (Chen and Grant 1968). This is also the only species studied whose trisomics showed an equal transmission through male and female (9% on an average). For primary trisomics of *Hordeum vulgare* it was shown that male transmission was zero in all testcrosses 2n x (2n+1). Still, for some trisomics the selfed progeny showed a transmission rate that was either significantly higher or lower than the female transmission in testcrosses (Tsuchiya 1960).

In most studies, data have been given for only one or a few plants per trisomic type. No attention has been paid to the possibility of (genetic) variation between plants carrying the same extra chromosome.

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

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

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

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

a) elimination of the extra chromosome in meiosis.
b) subnormal or delayed development of aneuploid microspores.
c) reduced fertility of aneuploid pollen grains.
d) competition between euploid and aneuploid pollen grains during pollen germination and tube growth.

e) subnormal development of aneuploid zygotes, embryos or endosperm.

f) reduced or delayed germination of 2n+1 seeds.

g) reduced vigour of 2n+1 seedlings (cf. Khush 1973).

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

In meiosis, the resulting percentages of n, n+1 or aberrant gametes depend on: the extent of pairing of the translocated chromosome with its homologous parts of the normal chromosomes, the number and location of chiasmata, the orientation of the resulting multivalents and the behaviour of any univalents (Sybenga 1972).

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

Few reports are available on the development of aneuploid microspores to mature pollen grains. According to Gülcan and Sybenga (1967), aneuploid microspores produced in autotetraploids reached their first mitotic division later than euploid microspores. In a previous paper (Janse 1985) it was shown that the same applies to the aneuploid microspores in a rye tertiary trisomic. A linear correlation was found between the percentage of dividing microspores containing the extra chromosome and the percentage of binucleate microspores, the latter expressing the stage of development. The total percentage of aneuploid microspores passing through pollen mitosis was high (41.9%) and equalled the number expected as a result of regular segregation of alternate multivalents in meiosis, assuming loss of univalents. It could not be concluded whether the delayed development of aneuploid microspores directly causes the reduction in male transmission.

Therefore, a possible correlation between the delay in development of aneuploid microspores and the male transmission rate of the extra chromosome was investigated here. If this correlation is strong, the delay may be one of the causes of reduced male transmission, for instance because the aneuploid microspores will frequently not be able to reach maturity in time for anthesis. For several tertiary trisomic plants from

different lines, the percentage of aneuploid microspores expected to result after meiosis was estimated from meiotic configuration frequencies. Then, the extent of delay in development was determined as described before (Janse 1985) and finally male transmission rates were obtained from testcrosses.

Material and methods

Plant material

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

The marker gene *Ti/ti* is used, located on the short arm of 5R (De Vries and Sybenga 1984). The normal chromosomes have the recessive allele whereas the extra translocated chromosome carries the dominant allele (Fig. 1). Homozygous recessive

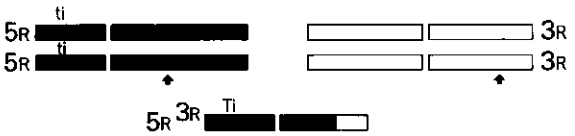

Fig. 1. Tertiary trisomic 240: the chromosomes involved. Nomenclature according to Sybenga et al. (1985). Arm length ratios derived from Sybenga and Wolters (1972). Ti and ti alleles of the tigrina locus (De Vries and Sybenga 1984). Arrows show the translocation breakpoints.

plants show the *"tigrina"* feature: coiling of leaves with yellow transverse striping. When grown under field conditions and in normal stands, these plants cannot compete with the wild types. Therefore, in the progeny of this BTT after selfing the parental tertiary trisomics are automatically selected. Here, all plants were grown in a greenhouse at 20 °C during the spring of 1984 (F_5) and 1986 (F_6). Then, *titi* genotypes are usually viable.

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

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

Segregation in progenies after selfing and testcross

Approximately 12 days after sowing the F_5 plants were scored for the *ti* marker. In the first two lines all plants were also karyotyped. The other lines contained many more plants and so in these cases only the plants carrying the *Ti* allele were scored for the presence of the extra, translocated chromosome. Root tips were pretreated in a saturated solution of α-bromonaphtalene for 1.5–2.5 h at 25 °C, macerated in 1N HCl for 12 min at 59 °–60 °C and stained with Feulgen reagent. Squash preparations were made in 45% acetic acid.

For testcrosses tertiary trisomics with the *Ti* allele were used as a father. Unless recombination had occurred, the *Ti* allele was located on the translocated chromosome, while the normal chromosomes carried the *ti* alleles, as in the parental trisomics. The female parents in the testcrosses had a normal karyotype (2n = 14) and were homozygous recessive for *ti*. They were emasculated and pollinated when the stigmas were receptive (approx. 8 days after emasculation). Plants in the progenies of the testcrosses were scored for *ti*. Wild types were karyotyped as they were expected to be tertiary trisomics (due to male transmission) unless recombination had occurred. From the frequencies of trisomics and disomics in this group, the actual male transmission rate and the recombination could be calculated.

Estimates of transmission and recombination

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

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

In this model it is assumed that multivalent orientation in meiotic metaphase is alternate, which is common in rye (Sybenga 1972, De Vries 1984b). Further, the *"tigrina"* phenotype is not supposed to affect viability. For the first two F_3 lines, in which all plants had been karyotyped, maximum likelihood methods had to be used to estimate m, f and r on the basis of these formulae. For the other lines, they were directly calculated from the frequencies of di-, tri- and tetrasomics among the wild types. With the same assumptions, it can be deduced that the frequencies of the possible types in the progeny of testcrosses can be expressed as:

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

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

Meiosis

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

Pollen mitosis

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

Table 1. *Segregation for ti and the extra chromosome in seven F_5 lines of balanced tertiary trisomic 240 and in progenies of testcrosses 2n x 2n+1 with trisomic F5 plants used as a pollinator. Estimates of male (m) and female (f) transmission rates and recombination fraction (r). Chromosome numbers in parentheses.*

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

[a]) *15 normal and 1 translocation chromosome*

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

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

Results and discussion

Transmission and recombination

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

Although total numbers were small in lines 1 and 2, there were indications that female transmission was lower here than in the other lines. This could have been caused by reduced viability and vigour of trisomic seeds and seedlings. Apart from the low germination percentage and the high seedling mortality, all plants from these two lines looked less vigorous than the others. They obviously suffered from severe inbreeding depression, which could have reduced the appearance of trisomics in the progeny. Another possible explanation for the observed differences in female transmission is the involvement of genetic factors, which influence female transmission directly.

The foregoing will also apply to the male transmission, estimated as very low in all progenies studied, due to the low frequencies of tetrasomics. Reduced viability and vigour of tetrasomic seeds and seedlings was probably even more important here.

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

Segregation for *ti* and the extra chromosome in the progenies of testcrosses

2n x (2n+1) and estimates for m and r are shown in Table 1 (columns at right). The male transmission rate in almost all progenies was much higher than estimated on the basis of the F_5 progeny to which the trisomic pollinator belonged. As mentioned above, this phenomenon had also been shown for primary trisomics of several species and for barley tertiary trisomics. It also emphasizes that the reduced viability of tetrasomics causes an underestimation of male transmission in progenies after selfing when compared to progenies after testcross. This will be stronger after subsequent inbreeding, as appears from the transmission rates in F_4 and F_5 lines.

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

Table 2. *Segregation for ti and the extra chromosome, male transmission rate (m) and recombination fraction (r) in progenies of testcrosses 2nx2n+1, with tertiary trisomics from different F_5 lines used as pollinators (pooled from Table 1). Significance of differences between lines.*

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

a) *Including one plant with an extra telocentric instead of a translocated chromosome*

The data for each line were pooled and m and r calculated again (Table 2); tests of heterogeneity were carried out for every pair of F_5 lines. The most striking

differences were between line 2 and all others. In this line male transmission rate as well as recombination fraction were exceptionally high.

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

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

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

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

Pooled testcross segregation data for every F_6 line and estimates for m and r based on them are given in Table 3 (right column). The harmonic means of m and r are given for line 2A. Again, tests of heterogeneity were carried out for every combination of two lines. For line 2A, calculation was modified as described above. It appeared from the tests that line 2A differed considerably from all other F_6 lines, due to its high male transmission rate and high recombination fraction. Two more differences were found: Line 1C differed from 6A and 7A, probably due to the higher recombination fraction in 6A and the lower transmission rate in 7A.

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

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

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

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

Finally, no significant differences were found between the segregation in testcrosses of F_6 lines and of their parental F_5 lines.

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

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

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

Meiosis

Frequencies of first metaphase configurations in tertiary trisomics of the different F_5 lines are given in Table 4. All configurations showed an alternate orientation, as is common for rye. Chain trivalents were most frequent, occurring in about 50% of all pollen mother cells. Also, chain quinquivalents were regularly found; in most remaining cases the translocated chromosome was univalent and the normal chromosomes 5R and 3R formed bivalents.

The number of aneuploid microspores after meiosis could be calculated from these frequencies, as described before (Janse 1985). Univalents are assumed to stay behind or be lost in meiosis, as is known to occur in rye (Sybenga 1972). Quinqui- and trivalents are expected to produce 50% euploid and 50% aneuploid microspores. Configurations

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

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

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

consisting of a quadrivalent plus a univalent will give rise to 50% unbalanced (not viable) and 50% normal gametes, but are rare.

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

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

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

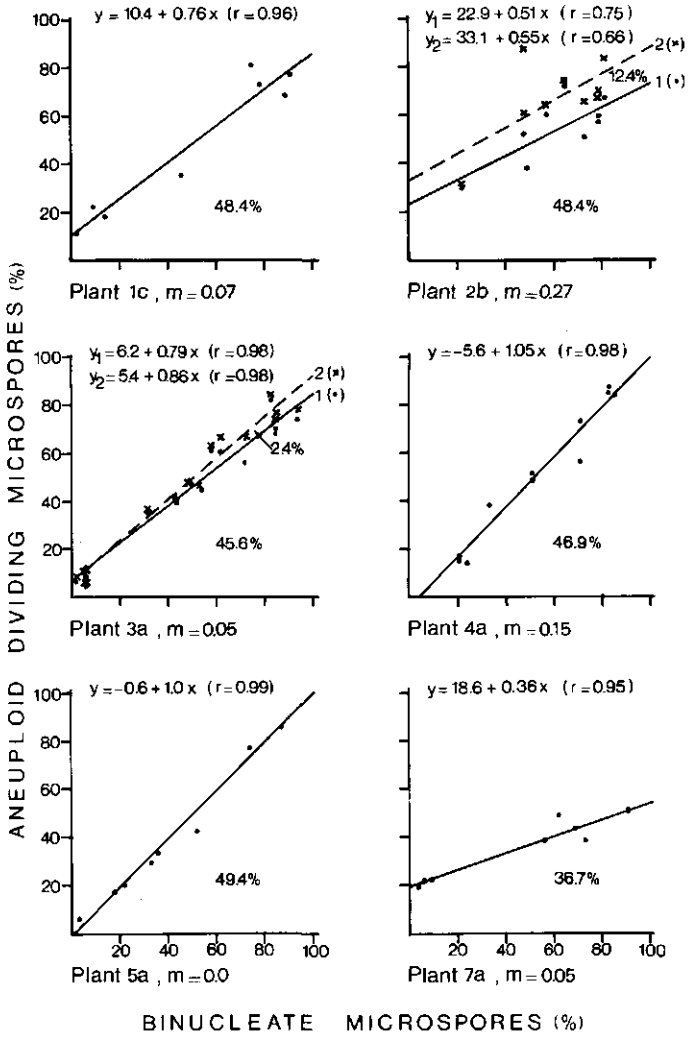

Fig. 2. *Percentage of dividing microspores with aneuploid chromosome number during pollen mitosis in six tertiary trisomic plants of different F_5 lines. Ordinate: percentage of binucleate cells, representing the stage of development. Abscissa: percentage of microspores carrying the extra, translocated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

Pollen mitosis

The relation between the percentage of binucleate microspores (i.e. the stage of development) and the percentage of aneuploid microspores in first pollen mitosis is shown in Fig. 2 for tertiary trisomics of six F_5 lines.

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

In anthers of all plants investigated, the percentage of aneuploid microspores increased with the percentage of binucleate cells, i.e. with the stage of development (Fig. 2). This means that the aneuploid microspores always showed a slower rate of development than the normal ones, as was described before (Janse 1985).

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

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

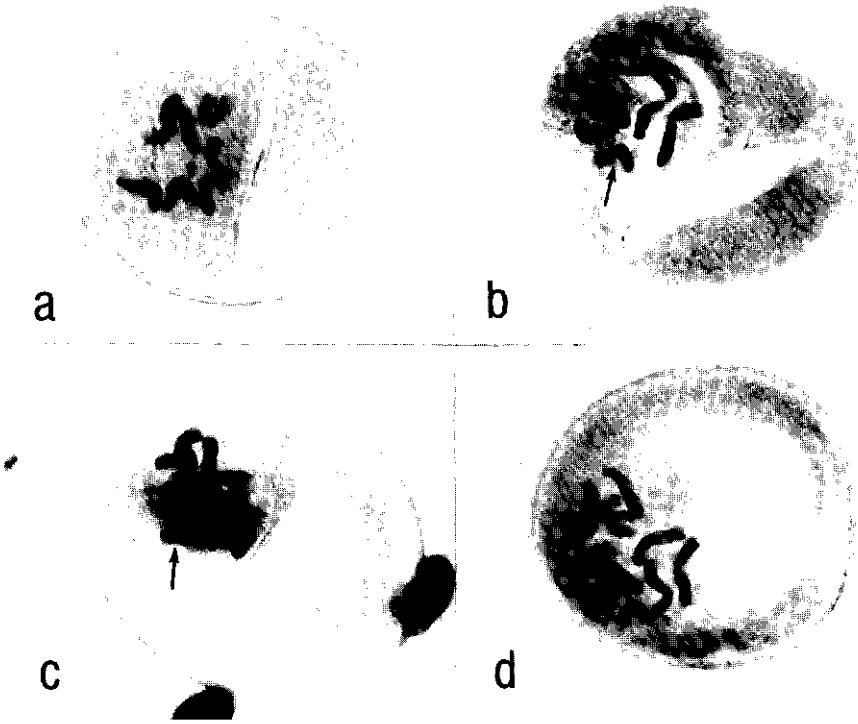

Fig. 3a–d. *Microspores formed in anthers of tertiary trisomics 240, in first pollen mitosis: a Normal microspore with seven chromosomes (including one satellite chromosome); b Aneuploid microspore with the translocated chromosome (arrow) in addition to the normal haploid complement; c Aberrant aneuploid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

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

From the analysis of meiosis and pollen mitosis two important conclusions can be drawn. Firstly, from the high percentages of aneuploid microspores resulting after meiosis and going through pollen mitosis, it is obvious that the causes of reduced male transmission and the genetic variation can not be found in the meiotic behaviour of the translocated chromosome. Also, the aneuploid microspores apparently all show a normal development, at least up to the young binucleate stage.

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

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CHAPTER 3
Certation between euploid and aneuploid pollen grains from a tertiary trisomic of rye, *Secale cereale L.*

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Key words: rye - tertiary trisomic - euploid pollen grains - aneuploid pollen grains - certation

Abstract

Male transmission of the translocated extra chromosome $5R^{3R}$ was studied in a tertiary trisomic of rye (*Secale cereale L.*), using two pollination densities. With abundant pollen, male transmission reached 4%. When a mean of four pollen grains were brought on every stigma (restricted pollination), a transmission rate of 20% was obtained. Seed set, mean seed weight, germination percentage, and the percentage of plants finally surviving were lower in the case of restricted pollination. It was concluded that certation between euploid and aneuploid pollen grains plays a decisive role in male transmission of the translocated chromosome. Although it was previously shown that aneuploid microspores have a delayed development, a large proportion must have reached maturity before anthesis. Therefore, genetic factors determining male transmission rate will primarily be expressed during pollen germination and tube growth rather than before anthesis.

Introduction

In angiosperm pollen, with its haploid genotype and large population size, important selective processes can take place, both during development and functioning of the gametophyte. The competitive ability of pollen is determined by the genetic content of the individual gametophyte as well as by quantitative genetic factors expressed through the sporophyte (Mulcahy 1974; Ottaviano et al. 1975). The biological significance of gametophytic competition can be considerable if the genes determining quality are expressed in the gametophyte as well as in the sporophyte (Mulcahy 1975).

Gametophytic competition can occur during microsporogenesis and during pollen germination and tube growth. In the latter case the term "certation" is used after Heribert-Nilsson (1920), who was the first to describe the phenomenon in *Oenothera lamarckiana*. Pollen grains carrying the dominant allele *R* for rednervedness were found to contribute to fertilization more than those having the recessive allele.

Not only single genes but also the presence of extra chromosomal material can strongly affect the competitive ability of pollen grains. Extensive studies were carried out with primary and secondary trisomics of *Datura stramonium* by Buchholz and coworkers in the 1930s. They studied germination and tube growth of pollen from these trisomics under favorable conditions. For most trisomics the frequency distribution of pollen tube length after 12 h showed a bimodal curve. In six primary and three secondary trisomics both groups of tubes had a normal appearance. The growth rate of the slow growing tubes, which were assumed to be aneuploid, ranged between 1/3 and 3/4 of the faster growing tubes. In these cases male transmission of the extra chromo-

some was possible, although the frequency was low. In six other primary and 11 other secondary trisomics either the slow growing tubes had swelled and burst, or the slow growing group was absent and germination was poor. In agreement with this, the extra chromosome could not be transmitted through the pollen (Buchholz and Blakeslee 1932). In the case of a trisomic carrying an extra translocated segment, a bimodal distribution for pollen tube growth was also found, but the difference between the modes was smaller and male transmission was higher (Buchholz et al. 1935).

Evidence for the aneuploid nature of the slow growing tubes was given for one of the primary trisomics with potential male transmission (Buchholz and Blakeslee 1930). When the styles were cut off after the rapidly growing pollen tubes had entered the ovary, transmission of the extra chromosome was absent. When restricted pollinations were carried out, transmission rate increased up to 26.7%, with most of the trisomic seeds found in the lower part of the seed capsule. As the ovules in the upper part were known to be fertilized first, it was concluded that the aneuploid gametes from the slower growing tubes had fertilized the ovules in the lower part, which were left unfertilized by the euploid gametes of the faster growing tubes.

Certation between aneuploid and euploid pollen grains was also found after crosses with maize plants carrying a supernumerary B^A translocated chromosome (Ghidoni 1975). Most seeds having the extra chromosome were found in the top section of the ear because the length of the silks decreases from the base flowers upwards. Competition is expected to be less severe when the silk is shorter.

The subject of the present report is a tertiary trisomic of rye, *Secale cereale L.* This trisomic carries a translocation chromosome in addition to the normal complement. It was previously shown (Janse 1987) that the trisomic produces a high percentage (about 46%) of microspores carrying the extra translocated chromosome in addition to normal, euploid microspores. This was due to frequent pairing of the translocated chromosome with homologous segments of the normal chromosomes during meiosis, a high level of chiasma formation, and a predominantly alternate orientation of the resulting multivalents in meiotic metaphase. Even when present as a univalent, the translocated chromosome was often transmitted to the daughter nuclei.

From analysis of the timing of first pollen mitosis, it appeared that aneuploid microspores showed a slower development than euploid microspores (Janse 1985). Although both the extent of this delay and the male transmission of the extra chromosome in testcrosses differed between lines, a correlation between these characters could not be found (Janse 1987). It was suggested that certation during pollen germination and tube growth was the cause of reduced male transmission compared with the expected rate of 46% and not the delayed development of aneuploid

microspores.

Pollen germination and tube growth *in vitro* is difficult to achieve for rye, as for most trinucleate pollen (Mulcahy and Mulcahy 1983). In particular, in *Graminae* pollen the hydrodynamics of early germination processes are very complex and critical (Heslop-Harrison 1979). The germination of rye pollen on the stigma (*in vivo*) was reported to be better (Titarenko and Torop 1985) but still appeared to be variable in preliminary experiments (Janse, unpublished). Also, when stained with aniline blue pollen tubes were brightly fluorescent in UV light when growing into the stigmatic papillae but not further in the style. Therefore, tube measurements were difficult to make.

The possible certation between euploid and aneuploid pollen from the tertiary trisomic was investigated by making restricted pollinations, as was done for a *Datura* primary trisomic (Buchholz and Blakeslee 1930). In contrast with *Datura*, in the ovary of which 400-600 ovules can be present, the rye floret contains only one ovule per ovary. The best way to examine certation would be to compare transmission rates after single-grain pollinations with abundant pollinations. However, seed set from single-grain pollinations is expected to be very low because not all pollen grains are sufficiently viable and germination can be variable, even on the stigma. Also, it is more laborious to carry out pollination with one pollen grain than with few pollen grains. Therefore, for restricted pollination, a mean of four pollen grains were applied to every stigma.

Material and methods

Plant material

One tertiary trisomic plant was used, which carried the short translocated chromosome of translocation 240 in addition to 14 normal chromosomes. In this inter-

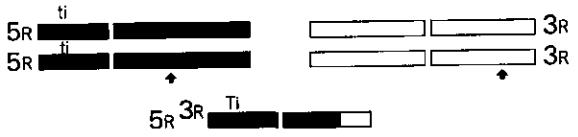


Fig. 1. *The chromosomes involved in a tertiary trisomic 240. Arm length ratios from Sybenga and Wolters (1972), nomenclature according to Sybenga et al. (1985). Ti and ti alleles of the tigrina locus (De Vries and Sybenga 1984). Arrows indicate the translocation breakpoints.*

change, which was first described by Sybenga and Wolters (1972), chromosomes 5R and 3R are involved (Sybenga et al. 1985). The marker gene *Ti/ti* was used, which was located on the short arm of chromosome 5R (De Vries and Sybenga 1984). In the tertiary trisomic the normal 5R chromosomes both carry the recessive allele, while the translocated chromosome has the dominant allele on its unchanged arm of 5R (Fig. 1).

This trisomic is a wild type. Homozygous recessive *titi* plants show the *tigrina* phenotype: coiling of leaves with yellow transverse striping. Usually the plants are smaller and weaker than the wild types but they can survive well in the greenhouse.

The tertiary trisomic was part of the progeny of a testcross 2n x(2n+1) with an F_5 tertiary trisomic 240 used as a pollinator. The F_5 line involved showed a moderate male transmission rate of the translocated chromosome in testcrosses, 7% on an average (Janse 1987). The plant was grown in the greenhouse at approximately 20 °C.

Male transmission and recombination in testcrosses

Male transmission rate was determined by testcrossing the tertiary trisomic described above as the pollen parent with homozygous recessive *titi* plants (2n=14) as female parents.

If no recombination between *ti* and the translocation breakpoint has occurred in the pollen parent, two types will be found in the progeny: disomic *titi* plants and trisomic *Tititi* (wild type) plants. The marker gene enables a simple selection of trisomics. However, if recombination has occurred, trisomic *tititi* plants and disomic *Titi* plants will also arise in the progeny.

Therefore, after scoring all plants in the progeny for the *ti* marker (approximately 12 days after sowing), the wild types were karyotyped to distinguish the trisomics from the disomics. Root tips were pretreated in α-bromonaphtalene (2 h at 25 °C), macerated in 1 N HCl (12 min at 60 °C), and stained with Schiffs reagent. Squash preparations were made in 45% acetic acid.

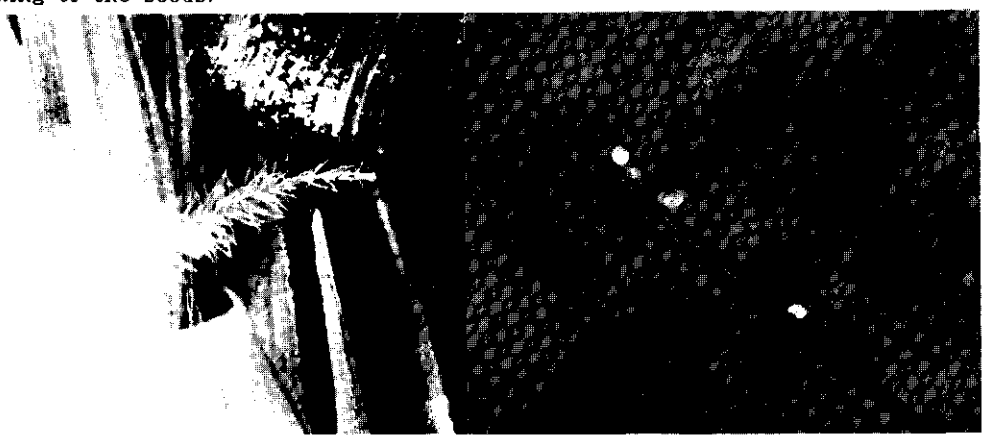
If m is the male transmission rate of the extra chromosome and r is the recombination fraction, the frequencies of trisomic and disomic wild types in the progeny can be expressed as m(1-r) and r(1-m) respectively (Janse 1987). From these, m and r can be estimated.

Methods of pollination

Ten disomic *tigrina* plants were used as female parents. Ears were emasculated. Pollinations were carried out when the stigmas were receptive and at the same time ears of the pollen parent (the tertiary trisomic) shed pollen. Pollen was collected in a watch glass from fresh, deep yellow anthers that had just dehisced. Older anthers had

been removed before.

For abundant pollinations a small brush was deeply dipped into the pollen sample until it was crowded with pollen (Fig. 2a). This was repeated for every floret of the ear that had to be pollinated. A total of 10 emasculated ears, with approximately 35 florets each, were pollinated in this way. One ear was accidentally lost during the ripening of the seeds.



a

Fig. 2. *Pollination methods for investigating certation. (a) Abundant pollination (approximately x 7), (b) Pollen sample of four grains for restricted pollination (approximately x 40).*

For restricted pollinations all hairs but one were removed from another brush. The single hair was lightly dipped into the pollen sample. Then, a very little white dot could just be seen at the end of the hair. Countings on 100 dips showed that 4 ± 2 pollen grains were attached to the hair (Fig. 2b). The dip was repeated for each of the approximately 35 florets in one ear. In total 16 emasculated ears were pollinated in this way.

As far as possible, abundant and restricted pollination were carried out simultaneously with the same pollen sample and on ears with equal quality of stigmas.

Results and discussion

Segregation for *ti* and the extra chromosome in the progenies of testcrosses with abundant and restricted pollination is given in Table 1, together with estimates for male transmission rate and recombination fraction. In addition, some characteristics of the seeds obtained are presented.

Male transmission of the translocated chromosome appeared to be significantly

higher ($P<0.01$) when restricted pollinations had been carried out. This demonstrates clearly that certation takes place between the euploid and the aneuploid pollen grains.

There can be two ways in which male transmission rate of the extra chromosome increases as a result of restricted pollination. First, if some pollen grains are defective, not fertile or simply do not germinate, there will be a number of stigmas on which only one pollen grain is germinating. Then, certation is absent. Second, even with two or more pollen grains germinating, competition is less severe than in the case of abundant pollination. If the tube growth distributions of euploid and aneuploid pollen are overlapping, some aneuploid gametes have a greater chance of reaching the single ovule. The way in which male transmission increased here is basically different from that in *Datura stramonium* (Buchholz and Blakeslee 1930). There, the aneuploid gametes were only able to fertilize the ovules that were left unfertilized by the euploid gametes.

Table 1. *Results of testcrosses 2n x(2n+1) for determining male transmission of the translocated chromosome in a tertiary trisomic 240, using abundant and restricted pollinations. Segregation for ti and the extra chromosome (in parentheses) and derived estimates for male transmission and recombination are given.*

Progeny characteristics	Abundant pollination	Restricted pollination
No. of seeds	79	77
Seed set (seeds/ear)	8.8	4.8
Mean seed weight (mg)	18	14
Germination (%)	92	84
Finally surviving (%)[a]	92	79
Segregation: (15) *Ti*	3	12
(14) *Ti*	1	1
ti	69	48
Total	73	61
Male transmission	0.04	0.20
Recombination	0.01	0.02

[a] *Equals germination percentage minus mortality in the seedling stage.*

The mean seed set after abundant pollination was not high (Table 1). This was probably due to the relatively poor quality of ears produced by the *tigrina* plants. Still, seed set was almost twice as high as in the case of restricted pollination. Probably, it often happened that none of the few pollen grains applied to a stigma was able to fertilize the ovule. Another possibility is that trisomic embryos aborted more often than normal euploid embryos, resulting in low seed set. In that case, the proportion of trisomic embryos resulting from fertilization after restricted pollination was even higher than 20%.

The mean seed weight, the germination percentage, and the percentage of plants eventually scored were also lower after restricted pollination (Table 1), possibly caused by the higher proportion of trisomics among these seeds. Trisomic seeds are generally smaller, lighter and have a poorer germination capacity than disomic seeds (cf. Khush 1973). Thus, it is likely that the frequency of trisomics in the seeds resulting from restricted pollination was even higher than eventually found in the progeny.

Considering the possible loss of trisomic embryos, seeds and seedlings, the proportion of aneuploid pollen grains that were able to contribute to fertilization after restricted pollination was probable even more than 20%.

The results presented here demonstrate that certation plays an important role in the male transmission of the translocated chromosome from this tertiary trisomic. Despite the delay in development of aneuploid microspores to mature pollen grains (Janse 1985), at least 20% (but probably more) of the mature pollen grains were aneuploid.

Genetic factors were found to be involved in determining male transmission rate but a correlation with the extent of the delay in development of aneuploid spores was absent (Janse 1987). The results from the experiment described here indicate that these factors are probably expressed quantitatively during pollen germination and tube growth, causing different degrees of certation.

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CHAPTER 4
Protein and glucose—6—phosphate dehydrogenase patterns in leaves and pollen of tertiary trisomics and disomics of rye.

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Abstract

Protein patterns have been studied in trisomic and disomic plants of different inbred lines of two tertiary trisomics of rye, by means of electrophoresis of leaf and pollen extracts. In leaves, there were minor differences between and within lines of both tertiary trisomics. A correlation with the presence of the extra translocation chromosome could not be found, although its presence was known to cause morphological and physiological deviations. Pollen protein patterns differed from those of leaves, but again no effect of the extra chromosome was observed. Tertiary trisomics are expected to produce at least 20% aneuploid mature pollen grains, that are known to have a selective disadvantage during pollen germination and tube growth.

G6PDH patterns were also studied. The supernumerary chromosome in both trisomics carries the structural gene for one G6PDH isozyme. In leaves, its activity was slightly decreased, indicating that regulatory genes added with the extra chromosome probably exert a stronger influence than the extra structural gene dose. Similar effects on activities of other enzymes may, in addition, be responsible for the aneuploid syndrome. G6PDH patterns of pollen showed that the isozyme in question was not expressed at the gametophytic level. No additional differences between pollen from trisomics and disomics were found. Thus, a biochemical basis for the differential behaviour of aneuploid and euploid pollen grains and for the differences in male transmission rate of the extra chromosome could not be established.

Key words: rye – tertiary trisomics – protein – glucose-6-phosphate dehydrogenase – sporophyte – gametophyte.

Introduction

Tertiary trisomics carry a translocation chromosome in addition to the normal complement. They occur with variable frequency in the progeny of translocation heterozygotes. In rye, the extra chromosome is usually the shorter translocation chromosome (De Vries 1984). Tertiary trisomics can be applied in hybrid rye breeding in combination with genetic male sterility (Ramage 1965). The trisomics are then used for the maintenance and propagation of male sterile lines (Sybenga 1982; De Vries 1984).

Therefore, differential male and female transmission rates of the extra chromosome are essential. In general, transmission through the eggs is relatively frequent in various types of trisomics, whereas transmission through pollen is low (Khush 1973; literature reviewed by Janse 1987b). For use in hybrid rye varieties male transmission should approach zero.

In several F_5 lines of a rye tertiary trisomic, female transmission rates after selfing varied between 19% and 42%. Genetic factors may be involved and the degree of inbreeding depression may also play a role (Janse 1987b), because trisomic seeds tend to be lighter and show a poorer germination than disomic seeds and trisomic seedlings are less vigorous (Khush 1973). These effects may be stronger after inbreeding. Estimates of male transmission of the extra chromosome were very low in these selfed progenies. This was probably partly caused by the very low viability of tetrasomics, which are essential in these estimates. Transmission through pollen was indeed estimated to be higher when testcrosses were made with disomic plants as female parents. Genetic factors appeared to be involved here, one line showing a consistent high rate of about 20% (Janse 1987b).

Most of the difference between male and female transmission rates probably has its basis in the fact that the male gametophyte in Angiosperms is subjected to important selective forces, during microsporogenesis and to a greater extent during pollen germination and tube growth (Khush 1973, Mulcahy 1975).

In the tertiary trisomic of rye mentioned, the analysis of meiotic behaviour and pollen mitosis showed that a high percentage (about 46%) of the microspores contained the extra translocated chromosome (Janse 1985, 1987b). These aneuploid microspores showed a slower development than the normal spores (Janse 1985). Although differences between plants were found, these were not correlated with male transmission rates, indicating that probably other processes were more important in determining transmission (Janse 1987b). In crosses with very small numbers of pollen grains, male transmission rate could be raised from 5% up to 20%. This demonstrated that certation between euploid and aneuploid pollen grains plays an important role in determining male transmission and that, despite their delayed development, a large proportion of the aneuploid spores reaches the mature pollen stage (Janse 1987a).

Apparently, the presence of the extra chromosome has a distinct effect on cellular processes at the sporophytic as well as at the gametophytic level. Trisomic plants of most diploid species have been known for a long time to show physiological disturbances and morphological and developmental deviations. In many species the different primary trisomics can be morphologically distinguished from each other and from the disomics (See review by Khush 1973).

The biochemical basis of the aneuploid syndrome has not yet been completely clarified. The classic view was that addition of an extra chromosome disturbs the "gene balance" of proteins involving structural genes (Patterson et al 1940). However, Yielding (1967) suggested that, together with structural genes, a number of specific regulatory genes is added with the supernumerary chromosomes. These genes direct the synthesis

of repressor molecules and thus suppress the expression of structural genes. This theory was affirmed by Birchler and Newton (1981), who found the dosis of several chromosome arms in maize to be negatively correlated with specific protein levels.

In the experiments described here, an attempt is made to define the effect of the translocated chromosome on protein patterns in rye tertiary trisomics. Possible differences between two different tertiary trisomics are investigated. Furthermore, trisomic and disomic plants of different inbred lines, known to show differential behaviour with respect to transmission of the extra chromosome, are compared to establish any biochemical basis for these differences.

Another approach is to study the expression of specific (iso)enzymes in trisomics and disomics. Dosage effects with respect to a specific isozyme or, when different allelic forms are present, different allelic banding ratios in segregating populations can occur then (Birchler 1983).

In rye, two different isozyme markers have been localized on the short arm of chromosome 5R, which is present in the extra translocation chromosome of both tertiary trisomics used in this study. Koebner and Shepherd (1982) found the structural gene for a shikimate dehydrogenase isozyme to be located on 5RS. However, the clearest bands were obtained from embryos, which are then lost for further analysis. By means of wheat-rye addition lines, Salinas and Benito (1983) located a gene controlling one of the six isozymes of glucose-6-phosphate dehydrogenase (G6PDH) on this arm. Extracts were obtained from leaves. Then, individuals can be preserved e.g. for obtaining transmission data. G6PDH has therefore been chosen to study dosage effects in the two tertiary trisomics. Like with the proteins, G6PDH enzyme patterns are studied in trisomics and disomics of inbred lines.

The occurrence of certation between euploid and aneuploid pollen grains indicates that cellular processes may be disturbed in aneuploid pollen grains. The effects at the gametophytic level seem to be comparable to those at the sporophytic level.

It has become clear that the gametophytic phase in higher plants is metabolically very active (Brewbaker 1971; Mascarenhas 1975). Mature pollen grains contain many proteins. Many enzymes are involved in the metabolism of internal or external substrates and are essential for pollen germination and tube growth (Mascarenhas et al 1986).

Most proteins present in pollen seem to be of gametophytic origin. Direct evidence for gametophytic transcription of acid phosphatases and other proteins was presented by Mulcahy and coworkers who succeeded in carrying out micro electrophoresis with single *Cucurbita* pollen grains. Proteins showing segregation in pollen grain populations from heterozygous plants are under the control of the haploid genome (Mulcahy et al

1979; Mulcahy et al 1981). By comparing the gene expression of multimeric enzymes in the sporophyte with that in the gametophyte it was shown that 60% of the sporophytic genes investigated in tomato (Tanksley et al 1981) and 72% of those studied in maize (Sari Gorla et al 1986) were also expressed in pollen.

The detrimental effect of the extra chromosome on germination and tube growth of aneuploid pollen grains may be caused by the presence of a number of additional genes that are transcribed and translated in the gametophyte. The micro electrophoresis technique for isoelectric focusing of individual pollen grains (Mulcahy et al 1981) is in principle very promising to study this effect. However, rye pollen grains are much smaller (about 60 μ longitudinal) than the Cucurbita grains (over 200 μ), i.e. about 30 times smaller in volume. Several modifications of Mulcahy's method have been tested on rye pollen grains. Different extraction buffers were used and applied to the pollen grains before or after crushing them on the gel. In some experiments the gel, including the pollen grains, was frozen and defrozen before use. It was also tried to extract the proteins previously. The grains were placed in small vials or in micro capillaries or on small paper wicks, with very small amounts of extraction buffer. The extracts were applied to the gel freshly, or after freezing and thawing. Also, the dimensions of the gel and its composition were modified. However, in all experiments the amount of protein extracted from individual pollen grains appeared to be too small to give consistent bands in the micro gel (Janse, unpublished).

In the experiments described here, a large amount of pollen was taken from rye tertiary trisomics and their overall protein profiles were compared to those from disomics. It should then be taken into account that the pollen from the trisomic contains a mixture of euploid and aneuploid grains. Furthermore, G6PDH patterns from pollen produced by trisomics and disomics were studied. Considering the extent of sporophytic-gametophytic overlap in many species, the structural gene for the isozyme on chromosome 5RS may also be expressed in the rye gametophyte. G6PDH has been shown to be present in pollen from *Lilium longiflorum* (Desborough and Peloquin 1968; Dickinson and Davies 1971), but not yet in other species.

Material and methods

Plant material

The tertiary trisomics 240 and 282 that are used in this study carry the short translocated chromosome of translocation 240 and 282 respectively, that were first described by Sybenga and Wolters (1972). In both translocations, the long arm of

chromosome 5R has exchanged a segment with 3R and 7R respectively (Nomenclature according to Sybenga et al 1985). Tertiary trisomic 240 can thus be designated as $2x + 5R^{3R}$ and tertiary trisomic 282 as $2x + 5R^{7R}$. They both have 15 chromosomes.

These trisomics proved to be meiotically stable (De Vries 1984; Janse 1987b) and were used to produce several inbred lines. Two F_5 lines and two F_6 lines of tertiary trisomic 282 (282A, B, C and D) and two F_7 lines of tertiary trisomic 240 (240E and F) were used here. In these lines segregation for the extra chromsome and male transmission of the supernumerary chromosome in testcrosses has been determined (Janse, submitted).

Leaf extracts

For protein analysis about 100 mg of fresh green leaves of medium age were taken from every plant, folded into small packets in aluminum foil and stored at −80 °C. After thawing, the softened leaves in the packets could be pressed in a lemon-squeezer, resulting in about 70 µl of clean extract. One drop of electrode buffer (see below) and one drop of marker dye solution (0.5% bromophenol blue in 20% glycerol) were added. The extracts were stored at −10 °C until use.

For G6PDH analysis about 100 mg of fresh leaves were cut into small pieces and crushed in 100 µl of cold 0.1 M sodium acetate, pH 7.2 (Salinas and Benito 1983). After centrifugation at 14,000 rpm for 30 minutes, the supernatant was removed and mixed with one drop of electrode buffer and one drop of marker dye. These extracts were also stored at −10 °C.

Pollen extracts

Fresh pollen was collected from young, newly dehisced anthers and used either directly or after storage at −80 °C. The weight of the pollen samples was not determined because rye pollen looses its viability rapidly, and the water content prior to and after dehiscence varies considerably within a short period of time (Heslop-Harrison 1979), which strongly influences the weight of the pollen samples. For both protein and G6PDH analysis, roughly the same volume of distilled water was added to each pollen volume. After violent stirring the samples were kept at 4 °C for 1−1½ h in order to allow the proteins to diffuse out of the pollen grains (Mäkinen and Brewbaker 1967). Then, one drop of electrode buffer and one drop of marker dye were added. The samples were used directly.

Electrophoresis

Electrophoresis was performed in vertical polyacrylamide slab gels (250x150x3 mm).

The gel mixture contained 10% acrylamide, 0.4% methylenebisacrylamide, 0.02% ammonium persulphate and 0.06% TEMED in 0.375 M Tris-HCl, pH 8.9. A stacking gel (5% acrylamide, 0.2% methylenebisacrylamide, 0.02% ammonium persulphate and 0.06% TEMED in electrode buffer) was used for the upper layer of the gel. Wells were made in this layer by means of a "comb". Samples of 50 µl were loaded into the wells.

The electrode buffer consisted of 0.04 M Tris and 0.1 M glycine (pH 8.9). The gels were run at 4 °C with a constant voltage of 350 V, until the marker dye had reached the end of the gel (about three hours). By that time, the current had fallen from about 140 to 70 mA.

Staining

The most sensitive general protein staining methods are those using silver nitrate. The one developed by Morrissey (1980), which is good and relatively simple, was used here. The gels were prefixed in 50% methanol/10% acetic acid (30 min) and 5% methanol/7% acetic acid (20 min), followed by fixation in 10% glutaraldehyde for at least 30 min. After extensive rinsing in distilled water, the gels were treated with 5 µg/ml dithiotreitol and then soaked in 1% silver nitrate, both for 30 min. They were rinsed rapidly with a small amount of water (once) and developer (twice) and then placed into an excess of developer (200 ml 3% sodium carbonate with 100 µl 37% formaldehyde). When the desired level of staining was reached, the reaction was stopped by adding 10 ml of 2.3 M citric acid. After 10 min the gel was washed with distilled water several times.

Staining for G6PDH activity was performed according to Shaw and Prasad (1970): 400 mg glucose-6-phosphate (disodium salt), 60 mg $NADP^+$, 40 mg nitro blue tetrazoliumchloride, and 4 mg phenazinemethosulphate were dissolved in 25 ml 0.5 M Tris-HCl (pH 7.1) and 90 ml H_2O per gel. The gels were incubated in the dark at room temperature overnight, with gentle shaking. Then, they were washed and fixed in 20% glycerine for several hours.

Results and discussion

Leaf protein patterns

In Figure 1A samples of protein patterns from the leaf extracts of tertiary trisomics and disomics are shown. In some plants the total amount of proteins extracted appeared to be smaller than in others. This variation was probably introduced through the extraction procedure, particularly in the steps of folding and squeezing of the leaf

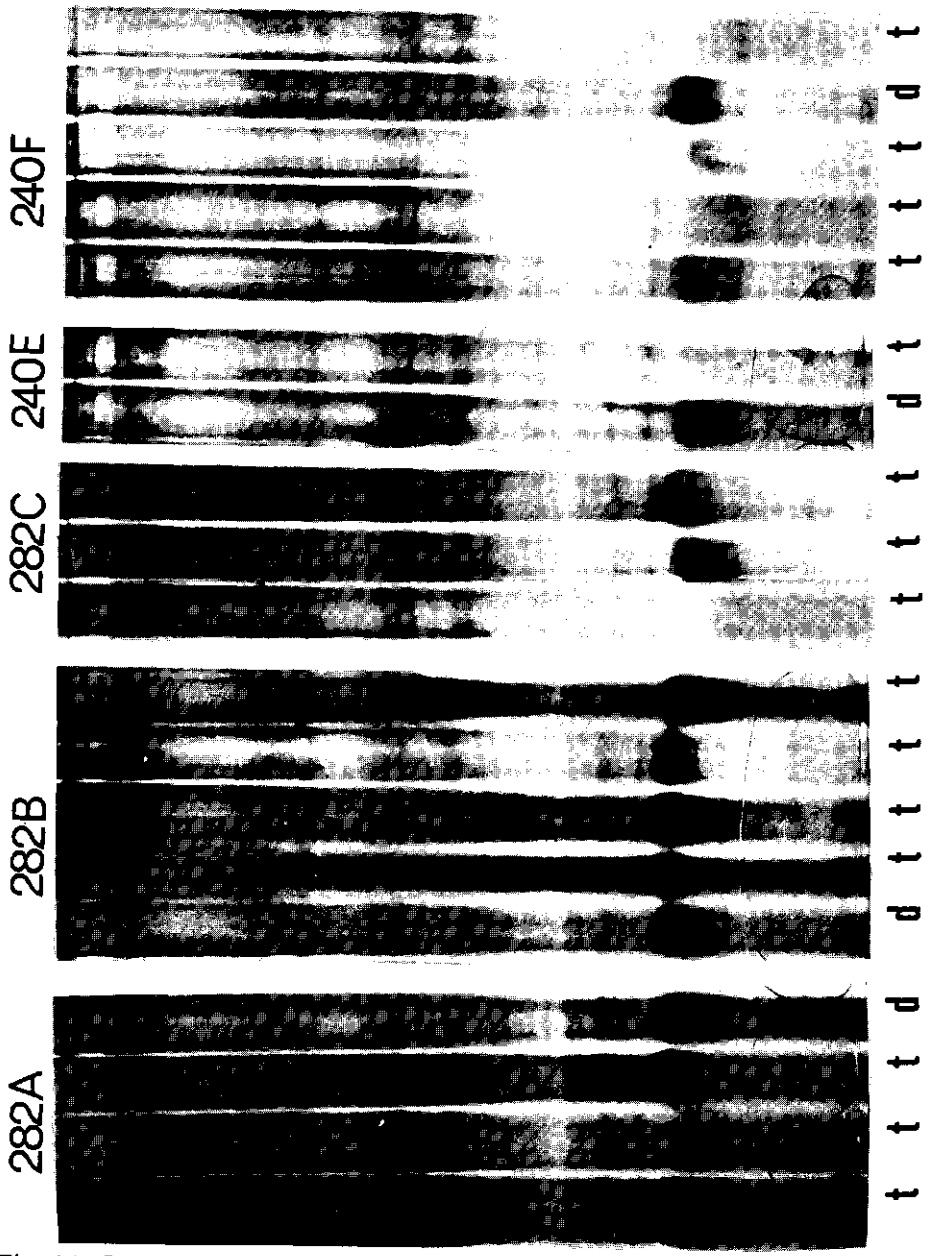

Fig. 1A. Protein patterns of leaf extracts from trisomic (t) and disomic (d) plants of inbred lines from tertiary trisomic 282 (A, B, C) and 240 (E, F).

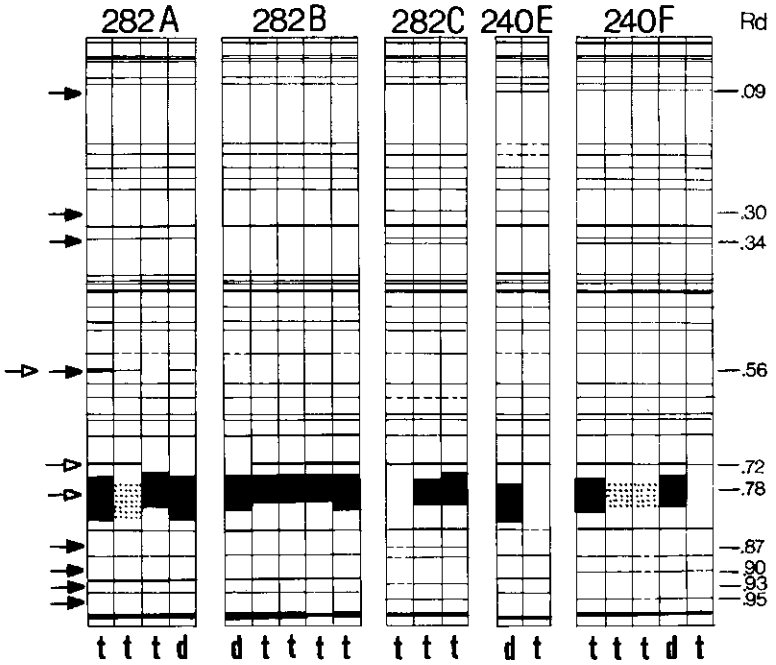

Fig. 1B. Diagram of Fig. 1A and relative distances (Rd) of some protein bands from disomic (d) and trisomic (t) plants. Dotted bands (....) are bands clearly visible in some plants, but their presence in the plant in question is dubious. Open arrows indicate clear segregation within at least one line. Filled arrows indicate differences between lines.

packets. Also, there may be some variation caused by differences in leaf age. In Figure 1B a representative diagram is presented for the protein profiles of Figure 1A, including relative distances of important bands. Bands present in some plants, but not clearly present or absent in others have been indicated in dots for the latter. It should be noted that this may be the case when for instance the total amount of protein extracted is small for a particular plant, while the band in question is only weak in plants yielding larger quantities of pollen. Then, the specific protein is not necessarily absent when not clearly visible.

The protein patterns of plants from different lines show a great resemblance. Some minor differences can be observed between lines (Fig. 1B, filled arrows). No specific differences were found between the 282 lines on the one hand and the 240 lines on the other, except perhaps for a minor band at Rd 0.09. This is somewhat surprising because the two groups of lines are not related, while within each group the lines have

common ancestors.

Some differences within lines are observed (Fig 1B, open arrows). A broad and intensely staining protein band at Rd 0.78 shows a clear segregation in all lines except for 282B. The disomic plants express this band in the samples shown, but this does not necessarily apply to all possible disomics. Some of the trisomics do and some do not contain this protein band. Thus, the differences are not related to the presence of the extra translocation chromosome. Neither a positive nor a negative gene dosage effect is demonstrated here. The same conclusion can be drawn for the band at Rd 0.72 that segregates in 282 lines A and B (Fig. 1B) and D (not shown). It probably also applies to the band at Rd 0.56, that seems to be present and segregate only in 282A, but the segregation is not very clear due to the intense background staining.

No bands showing a consistent higher or lower staining intensity in trisomics compared to disomics can be observed in these gels. Also, no "novel" proteins due to the presence of the extra chromosome could be found.

McDaniel and Ramage (1970) compared the seed protein patterns of all seven, morphologically distinguishable, primary trisomics of barley with each other and with disomics. Alterations of protein bands in the trisomics illustrated three types of gene action: a positive dosage effect, production of a "novel" protein band and a negative dosage effect (up to complete suppression of bands). They were able to identify each primary trisomic on the basis of its protein profile and stated this was also possible for tertiary trisomics. Birchler and Newton (1981) studied protein profiles of scutellar tissue of maize carrying different extra chromosome arms. They found that the dosis of most particular regions was negatively correlated with specific protein levels, while in some cases the amount of certain proteins was increased by the addition of a specific chromosome arm.

In the light of these facts, it is surprising that no evidence for a specific effect of the extra translocation chromosome on the leaf protein patterns of tertiary trisomics could be demonstrated here, particularly since the silver staining method used here has proven to be far more sensitive than the amido black method used by McDaniel and Ramage (1970) and the Coommassie blue staining in the experiments of Birchler and Newton (1981). It is possible that the effects of the extra chromosome remained undetected here. In some parts of the gel the considerable background staining may mask (minor) bands.

On the other hand, it should be kept in mind that during the development of the inbred lines, selection for tolerance of aneuploidy – and thus against strong effects of the extra chromosome – as well as selection for heterozygosity will have taken place automatically. Still, in all lines investigated the trisomics were less vigorous than the

disomics: they grew slower, flowered later, and their leaves and ears were smaller, especially in the 282 lines. Thus, an effect of the extra chromosome is still present and this effect was expected to be demonstrable in the present experiments.

Pollen protein patterns

In Figure 2A samples of protein patterns of pollen extracts from disomic and trisomic plants are shown; a diagram is given in Figure 2B. Not all plants, from which leaf extracts had been analysed, produced a sufficient amount of fresh pollen in the period the experiment was undertaken. Thus, the number of plants analysed for pollen was smaller than that analysed for leaf proteins.

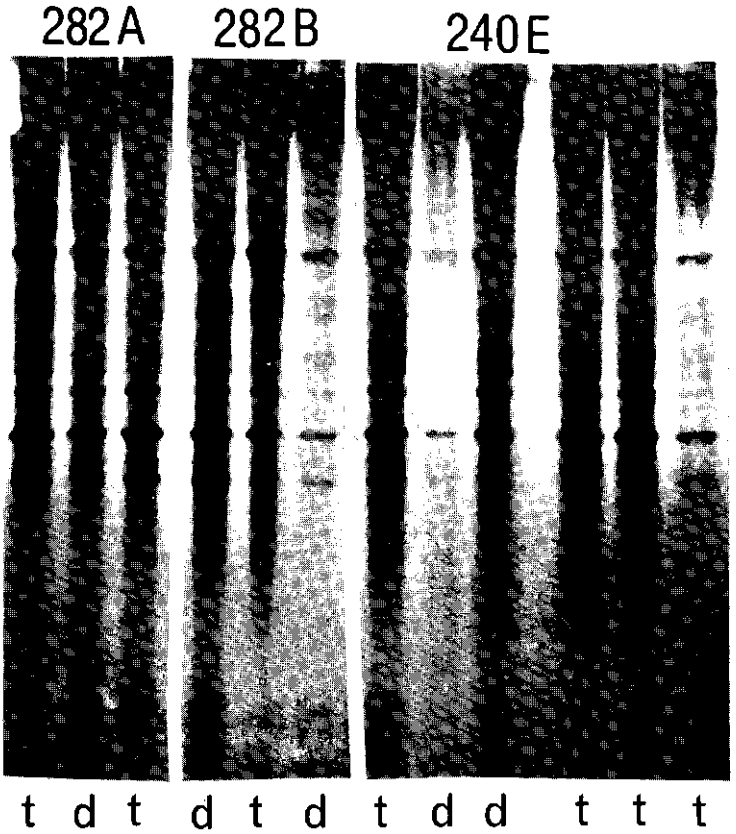

Fig. 2A. Protein patterns of pollen extracts from trisomic (t) and disomic (d) plants of inbred lines from tertiary trisomic 282 (A, B) and 240 (E).

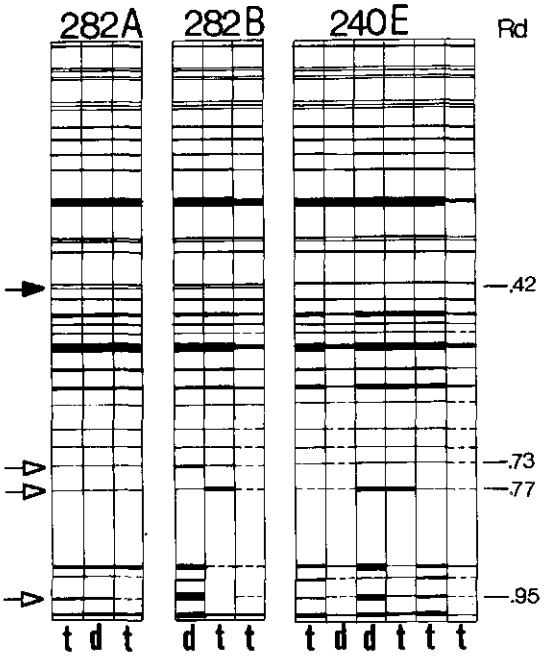

Fig. 2B. Diagram of Fig. 2A. See for further explanation Fig. 1B.

The number of protein bands exhibited by the pollen is about as high as the number present in leaves (30–35). The amount of protein extracted again shows some variation (Fig. 2A). Some protein bands in leaf and pollen appear at the same relative distances, but this does not necessarily mean that the same proteins are involved. Most studies concerned with the "sporophytic-gametophytic" overlap make use of specific enzyme activities (Tanksley et al. 1981; Sari Gorla et al. 1986). However, it is apparent from Figs. 1A and 2A that the major proteins present in rye pollen differ from those in leaves.

For the pollen proteins, only one minor difference between lines was found at Rd 0.42 (Fig. 2B). Differences within lines can also be observed, but no segregation occurs as for the leaf proteins. The bands at Rd 0.73, 0.77 and 0.95 are probably present in the pollen of all plants, but they show differences in staining intensity that are not proportional to the total amount of proteins extracted from the pollen. This is most clearly visible for the band at Rd 0.95 that shows a high intensity in the disomic of

282A, in one disomic of 282B, but also in a trisomic and disomic of 240E. Other pollen samples, including some with a high level of extracted protein, express this band only weakly.

A relation with the presence of the extra chromosome in the pollen of the trisomics does not seem to exist. If the protein in question is of sporophytic origin, the reasoning is similar to that for the leaf proteins, but if it is controlled by the haploid genome the situation is more complex. The trisomic plant produces a mixture of euploid and aneuploid pollen grains. The latter account for at least 20% (Janse 1987a) and at most 45% (Janse 1987b) of the total. Again, the extra chromosome can exert a negative or a positive dosage effect on proteins produced in the aneuploid. To what extent the level of a specific protein can be modified depends on the strength of this effect, but also on the percentage of aneuploids in the pollen mixture. The protein band at Rd 0.95 in the trisomic can be expressed stronger or weaker than in a disomic (Fig. 2) and its expression is therefore not related to the presence of the extra chromosome.

The same is true for protein bands at Rd 0.73 and 0.77, that show some variation in all lines except 282A. It is interesting to note that proteins at approximately the same relative distances (0.72 and 0.78) showed segregation in the leaf protein patterns (Fig. 1). However, these segregations do not correspond with the staining variation observed in the pollen of the same plants. Therefore, it is not likely that the same proteins are involved that are controlled by genes expressed in both sporophyte and gametophyte.

From the experiments with pollen protein extracts from trisomic and disomic plants, no evidence could be obtained for a specific effect of the extra translocated chromosome on the aneuploid pollen grain. Considering the differential behaviour of euploid and aneuploid pollen grains during pollen development and tube growth, processes accompanied by a high enzyme activity, such an effect would have been expected. Again, it is possible that the effects remained undetected in the present experiments. This is especially true for quantitative effects, because the pollen of the trisomic contains a mixture of euploid and aneuploid pollen grains, the exact composition of which is unknown. Qualitative effects, such as the appearance of new protein bands, will usually be detected easier.

Leaf G6PDH patterns

In Figure 3, the G6PDH patterns for some trisomic and disomic plants of tertiary trisomic lines 240 and 282 are shown. Because entire leaves had been crushed in the extraction medium for this analysis, the enzyme activities show less variation due to extraction procedures than the protein samples in the first experiments.

Of the six G6PDH isozymes that have been described by Salinas and Benito (1983), four could be recognized in this gel: R_1, R_2, R_3 and R_4 (Fig. 3). Isozymes R_5 and R_6, that were reported to be very faint in the three varieties studied by Salinas and Benito (1983), could not be detected here. According to Salinas and Benito (1983) the structural gene r_4, involved in the production of isozyme R_4, is located on chromosome arm 5RS. This isozyme does not show an equal activity in the plants investigated here (Fig. 3, arrow). More specifically, it seems to be present in higher intensity in disomics than in trisomics when plants within lines are compared. Apparently, the presence of the extra chromosome $5R^{3R}$ or $5R^{7R}$ causes a reduction in enzyme activity (negative dosage effect) in all lines studied.

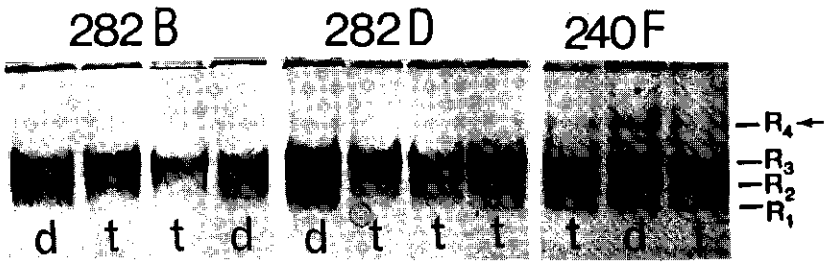

Fig. 3. G6PDH leaf patterns of trisomic (t) and disomic (d) plants of inbred lines of tertiary trisomic 282 (B, D) and 240 (F). Isozymes (R_1...R_4) numbered according to Salinas and Benito (1983). The arrow indicates the isozyme that is controlled by a gene on chromosome arm 5RS.

Positive and negative dosage effects in trisomics with respect to isozyme activity have been reported for many species (See review by Birchler 1983). However, it was often found that an extra dose of the chromosome carrying the structural gene for a specific isozyme resulted in an increase in the activity up to 150% of the disomic level. Decreases in activity in different trisomics occurred for isozymes encoded by structural genes on other chromosomes than the one present in triplicate. These negative effects are apparently caused by the addition of regulatory genes that suppress enzyme activity (Fobes 1983, Birchler 1983).

The results obtained here do not correspond with those findings. It is possible that

extra regulatory genes have been introduced together with the structural gene in the added chromosome, exerting a stronger influence than the extra structural gene dose.

Isozyme R_3 also shows some variation in staining intensity but this is not related to the presence of the extra chromosome (Fig. 3).

There is no evidence for genetic polymorphism in the material investigated with respect to G6PDH regulation.

Pollen G6PDH patterns

In Figure 4 samples of the G6PDH patterns of pollen from tertiary trisomics and disomics are shown. In the period this experiment was conducted, pollen production in the inbred lines was limited, whereas a large amount of pollen was needed to demonstrate G6PDH activity. Therefore, a few trisomics and a disomic from some F_1 populations have also been studied.

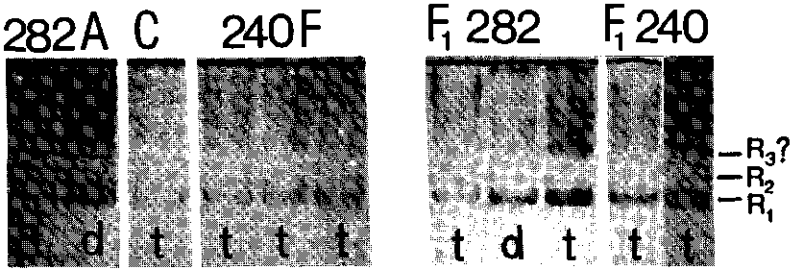

Fig. 4. G6PDH pollen patterns of trisomic (t) and disomic (d) plants of inbred lines of tertiary trisomic 282 (A, C) and 240 (F) and from some F_1 progenies. R_1 and R_2 are different isozymes, nomenclature according to Salinas and Benito (1983)

One isozyme, occurring at the same relative distance as isozyme R_1 in the leaves, was clearly present in all pollen samples. In most samples a very faint band could be observed at the location of isozyme R_2. The presence of isozyme R_3 in pollen is dubious. Isozyme R_4 that showed a negative dosage effect in the leaves, was not present in pollen zymograms. A dosage effect for this isozyme due to the presence of an extra chromosome arm 5RS in the aneuploid pollen grains could thus not be demonstrated. Such an effect would have been expected if sporophytic–gametophytic genetic overlap would have been involved, that is if the gene r_4 was also expressed at the gametophytic level.

In the experiments described here, no evidence for a specific effect of the extra translocated chromosome in tertiary trisomics on the general protein patterns of leaves

or pollen has been found. Still, the trisomic plants differ morphologically and physiologically from the disomics and the aneuploid pollen grains show a slower germination and/or tube growth than euploid pollen grains. A negative gene dosage effect has been found when studying the activity of a glucose-6-phosphate dehydrogenase isozyme in leaves but not in pollen. This effect was the same in different inbred lines of both tertiary trisomics.

Although in barley general protein patterns could be used to identify all primary trisomics (McDaniel and Ramage 1970), specific enzyme activities seem to be more suitable to study gene doses effects in trisomics (See Birchler 1983).

A biochemical basis for differences in male and female transmission rates found in several inbred lines of two tertiary trisomics could not be shown here.

Acknowledgements

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CHAPTER 5
Generative transmission of the extra chromosome in a rye tertiary trisomic and its relation with inbreeding depression and pollen quality.

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Abstract

Transmission of the translocated chromosome was studied in several inbred lines of a tertiary trisomic of rye (*Secale cereale L.*). Upon selfing, total transmission rate ranged from 0% to 36%. The trisomics in these progenies had resulted from either male or female transmission, but the contribution of the latter was greater. Tetrasomics, arising from simultaneous male and female transmission, were not viable. Seed germination percentage was positively correlated with seed weight and with transmission rate. Also, in lines with a poor seed germination and a low transmission rate, the tertiary trisomics were less vigorous than in other lines. It was concluded that the extent of inbreeding depression influences transmission rate through its effect on relative viability of trisomic seeds or seedlings.

Male transmission, in crosses with disomics, was found in all lines and averaged 7%. One F_5 line showed a higher rate and in another, segregation for higher and lower rate seemed to occur. The differences were less distinct in the corresponding F_6 and F_1 progenies and thus it was not clear whether genetic factors were involved. Pollen quality of trisomics was, on the average, a little lower than that of disomics from the same line and was in some cases higher in F_1 tertiary trisomics. It was not correlated with male transmission rate, which seems to be determined mainly by relative pollen tube growth of euploid and aneuploid gametophytes.

The results are discussed in relation with the use of tertiary trisomics in chromosomal balanced systems for hybrid breeding.

Key words: rye – tertiary trisomic – transmission – inbreeding – pollen quality.

Introduction

Tertiary trisomics carry an extra, translocated chromosome. In rye, *Secale cereale L.*, the most important source of this type of trisomy is the translocation heterozygote. Then, the extra chromosome is usually the shortest of both translocation chromosomes (De Vries 1983).

An interesting application of tertiary trisomics is their use in hybrid breeding with genetic male sterility. This system was first described by Ramage (1965) for barley. When the dominant allele *Ms* of the male sterility gene is located on the translocated chromosome and the recessive allele *ms* on both corresponding normal chromosomes, this trisomic is fertile. If the extra chromosome is not transmitted through the pollen and no recombination between the *ms* gene and the translocation breakpoint occurs, all pollen grains participating in fertilization will carry the *ms* allele. Thus, when the

tertiary trisomic is used to pollinate male sterile disomics, the progeny will completely consist of male sterile disomics. In this way, the male sterile seed parent for a hybrid cross can be propagated.

The tertiary trisomic can be maintained by selfing, resulting in fertile tertiary trisomics (due to transmission of the translocated chromosome through the eggs) and male sterile disomics. For early selection of trisomics, a recessive selective marker gene (for instance for a conditional lethal character) can be used. When the dominant allele is again located on the translocated chromosome and the recessive alleles on both normal chromosomes, the trisomics in the progeny will be automatically selected. Then, it is termed a "balanced tertiary trisomic" or BTT (Wiebe and Ramage 1971).

Some aspects of the construction and application of this system in rye breeding were studied by De Vries (1984), who also isolated several different tertiary trisomics.

For successful application of the BTT system with genetic male sterility in hybrid breeding, male transmission of the translocated chromosome in crosses with disomics should be absent but pollen production and quality should be high. Also, in inbred lines transmission of the extra chromosome through the female should be high for efficient maintenance of the BTT after selfing.

For one tertiary trisomic of rye, estimates of transmission upon selfing were found to range from 19% to 42% in different inbred lines. Tetrasomics, resulting from simultaneous male and female transmission, were rare in these progenies. In crosses with disomics as female parents, transmission through the male occurred in plants from all lines and it could even reach 27%. Probably, the rareness of tetrasomics in the progenies upon selfing was due to reduced viability of these seeds or plants, rather than to absence of male transmission of the translocated chromosome (Janse 1987a).

There were indications that the degree of inbreeding depression may play a role in determining transmission rates upon selfing. Also, genetic factors were shown to be involved in determining male transmission rates in crosses with disomics. Lines with male transmission rates approaching zero were not found (Janse 1987a).

In an attempt to establish the "bottle-neck" for male transmission in this tertiary trisomic it was found that transmission of the extra chromosome through meiosis was high. Aneuploid microspores showed a slower development than euploids but there was no correlation with male transmission rate (Janse 1985, 1987a). Restricted pollination showed that certation between euploid and aneuploid pollen grains plays a predominant role in determining male transmission. Also, a large part of the aneuploid microspores, although retarded, must have reached maturity before anthesis (Janse 1987b).

The studies mentioned were all carried out on different genotypes of one tertiary trisomic of rye. It is not yet known whether the results obtained were typical for this

tertiary trisomic or are representative for the general behaviour of tertiary trisomics in rye. More specifically, the genetic factors involved in determining male transmission rate are of interest. Differentiation in high and moderate rates was found. The question arises whether low rates can be found in other tertiary trisomics.

Also, the effect of inbreeding depression on transmission rates upon selfing needs further investigation. Finally, the quality of pollen produced by a tertiary trisomic is an important character because, firstly, a high quality is a prerequisite for good seed set and, secondly, pollen quality may influence the competition between euploid and aneuploid pollen grains.

In this paper, generative transmission of the translocated chromosome in inbred lines of a new tertiary trisomic of rye is studied. A large number of F_5 lines was used to make the genetic basis as broad as possible. Seed weight, germination percentage and seedling mortality have been recorded and studied for their correlation with transmission rates. Male transmission rates in testcrosses with disomics have been studied for tertiary trisomics of some lines and, in some cases, also in the next generation (F_6). Finally, the quality of pollen produced by trisomics and disomics has been determined.

Material and methods

The tertiary trisomic

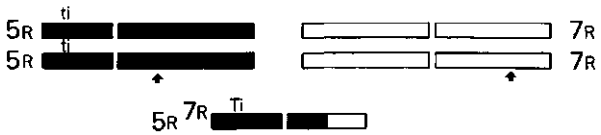

Fig. 1. Chromosomes involved in tertiary trisomic 282. Translocation breakpoints (arrows) and arm length ratios by Sybenga and Wolters (1972). Alleles of the tigrina locus (De Vries and Sybenga 1984).

The extra chromosome in this tertiary trisomic (that contains 15 chromosomes) is the short translocation chromosome of the reciprocal interchange 282. It is part of the translocation tester set developed by Sybenga and Wolters (1972). The extra chromosome consists of a part, including the short arm and the centromere, of chromosome

5R and the translocated segment of the short arm of 7R (Fig. 1) (Nomenclature according to Sybenga et al. 1985).

A recessive, conditionally lethal, selective marker gene *Ti/ti* located on the short arm of 5R (De Vries and Sybenga, 1984) is used. In the tertiary trisomic studied, the dominant allele *Ti* is carried by the translocation chromosome and the recessive alleles by the unchanged 5R chromosomes (Fig. 1). Homozygous recessive *titi* plants will show coiling of leaves with yellow transverse striping (*"tigrina"*). They are usually viable when grown in the greenhouse at about 20 °C. In the tertiary trisomic studied in previous papers (Janse 1985, 1987a and b) the same gene had been used. There, the translocation chromosome also had the short arm and a part of the long arm of 5R, but the exchanged segment came from 3R.

Segregation, recombination and transmission in inbred lines

A total of 18 F_5 lines, resulting from self-fertilization of F_4 tertiary trisomics, were used in this study. Some of them were grown in the spring of 1985 and 1986 but most of them in 1987. Their origin and relation to each other is shown in Fig. 2.

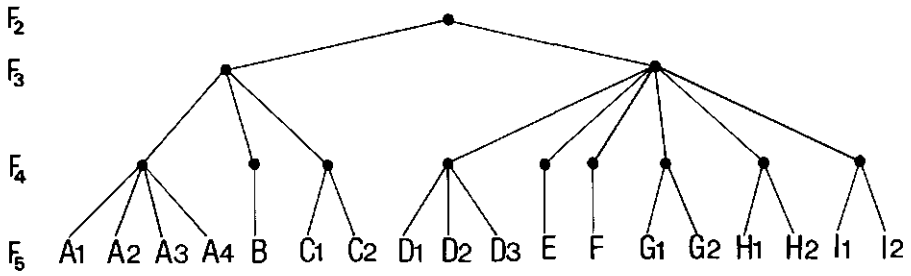

Fig. 2. Origin of the F_5 lines with tertiary trisomic 282.

For each line mean seed weight, germination percentage and seedling mortality were recorded. About 12 days after sowing, the plants were scored for the *ti* gene. The dominant allele of this gene could be used as a marker for the presence of the translocation chromosome. However, all plants were karyotyped in order to study recombination between *ti* and the breakpoint. Root tips pretreated with a saturated solution of α-bromonaphtalene (2 h, 26 °C), were macerated in 1 N HCl for 12 min at 59 °-60 °C and stained with Schiffs reagent. Squash preparations were made in 45% acetic acid. In trisomics, the translocated chromosome can be recognized because it is smaller than the unchanged chromosomes.

If m and f represent male and female transmission rates, respectively, and r is

used for the recombination fraction, the frequencies of disomic and trisomic wild types and *tigrinas* can be expressed as:

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

These formulae, as well as those for the tetrasomics ($mf(1-r^2)$ for the wild types and mfr^2 for *tigrinas*) were deduced by De Vries (1984). As the very low viability of tetrasomics can lead to a considerable underestimation of male transmission rates (Janse 1987a), their frequencies are not taken into account. Then, good estimates of male and female transmission separately can not be obtained from the formulae presented above. However, the total fraction of trisomics indicates the transmission either through the male or through the female, but not both.

The recombination fraction can then be estimated from the segregation of wild types and *tigrinas* within the trisomics and the disomics with the use of the formulae mentioned above. When different values for r are obtained, maximum likelihood estimates will be presented.

Segregation, transmission and recombination in testcrosses

In testcrosses for determining male transmission rates, female parents with a normal karyotype (2n=14) and homozygous recessive for *ti* were used. Ears were emasculated, and pollinated after the stigmas had become receptive.

As male parents, two or three tertiary trisomics were selected from F_3 lines with relatively good transmission upon selfing. They were also selected for their general appearance as they should be vigorous enough to produce several ears and enough pollen to make several testcrosses.

The plants in the testcross progenies were scored for *ti*. Wild types were expected to be trisomic, unless recombination had occurred. These plants were karyotyped. The frequency of wild type trisomics in the testcross progeny can be expressed as $m(1-r)$ and that of wild type disomics as $r(1-m)$ (Janse 1987a). Then, m and r can be estimated. In addition, testcrosses with tertiary trisomics from some testcross progenies were made and analysed in the same way. These trisomics have a heterotic background and this may affect male transmission rates. Also, as all *tigrina* plants used in testcrosses have the same genetic background, genetic factors may be expressed in the F_1 tertiary trisomics as well. Some F_3 plants were selfed to obtain F_6 lines and testcrosses were made with a few of these F_6 tertiary trisomics.

Pollen quality

Pollen quality was assessed using the fluorochromatic reaction (FCR), which tests (a) the presence of an active esterase and (b) the integrity of the plasmalemma of the vegetative cell (Heslop-Harrison and Heslop-Harrison 1970). A high correlation with germinability is usually obtained (Heslop-Harrison et al. 1984). In partly dehydrated pollen, however, the membranes are largely dissociated and need rehydration that normally takes place on the stigma (Shivanna and Heslop-Harrison 1981).

Freshly dehisced pollen was collected from the first ears of trisomic and disomic plants. It was put in a watchglass in a petri dish with moistened filterpaper to allow rehydration for 1 h. Fluorescein diacetate (FDA) was made up as a stock solution in acetone at 2 mg/ml. Immediately before use, drops were added to 1 ml of a solution of 20% sucrose with 10^{-2} M $CaCl_2$ until persistent clouds indicated saturation. This medium was added to the pollen and incubated at room temperature in the dark for about 45 min. Then, fluorescein, resulting from hydrolysis of FDA by esterase, had accumulated in the viable pollen grains. A preparation was made on a slide and 500 pollen grains were studied under a Zeiss UV microscope with a mercury light source and a BP365 excitation and LP397 barrier filter. The test was repeated twice for each plant during its flowering period.

Results and discussion

Transmission and recombination in inbred lines

In Table 1 segregation for chromosome number and marker gene and the estimates for the total transmission rate and recombination fraction are given for the F_5 lines.

Tetrasomics did not appear in any of the progenies, while the percentage of trisomics ranged from 0% to 36%. If the absence of tetrasomics is caused by the failure of transmission through the male, these trisomics have resulted from transmission through the eggs. Another explanation is that tetrasomic zygotes or embryos, resulting from simultaneous male and female transmission, were not viable. Then, the trisomics could be the result of either male or female transmission. In general, total transmission rates seem to be smaller in this tertiary trisomic than in the one studied previously, were transmission varied between 19% and 42% in F_5 (Janse 1987a).

For all lines mean seed weight, germination percentage and seedling mortality are given in Table 1. A significant positive correlation ($r = 0.78$; $P<0.01$) was present between seed germination and transmission rate, whereas germination and seed weight seemed to be correlated too ($r = 0.45$; $0.05<P<0.10$). Seedling mortality showed a signifi-

Table 1. Segregation for ti and chromosome number in 18 F_5 lines. Mean seed weight, germination and seedling mortality in each line and estimates for total transmission (t) and recombination fraction (r)

F_5 line	No. of seeds	Mean weight mg	Germination (%)	Seedling[a] mort. (%)	*Ti* (15)	*Ti* (14)	*titi* (14)	*titi* (15)	Total	t	r
A1	81	12.3	19(23)	1(5)	0	2	15	0	17[b]	0.0	0.06
A2	66	16.8	45(68)	5(11)	1	4	35	0	40	0.03	0.05
A3	29	10.0	1(3)	1(100)	-	-	-	-	-	-	-
A4	40	14.0	11(28)	1(9)	0	4	6	0	10	0.0	-[c]
B	122	13.8	39(32)	8(21)	1	3	27	0	31	0.03	0.05
C1	70	19.3	47(67)	14(30)	3	1	29	0	33	0.09	0.02
C2	49	18.4	11(22)	2(18)	0	0	9	0	9	0.0	0.0[d]
D1	95	19.3	86(91)	8(9)	28	2	48	0	78	0.36	0.02
D2	87	18.7	67(77)	20(30)	6	4	37	0	47	0.13	0.05
D3	77	17.4	58(75)	10(17)	10	2	36	0	48	0.21	0.02
E	64	11.3	18(28)	4(22)	0	3	11	0	14	0.0	0.11
F	39	17.2	16(41)	4(25)	3	2	7	0	12	0.25	0.12
G1	64	18.3	46(72)	9(20)	8	3	24	2	37	0.27	0.11
G2	41	17.8	30(73)	5(17)	5	2	18	0	25	0.20	0.05
H1	76	16.3	51(67)	9(18)	11	2	29	0	42	0.26	0.03
H2	33	17.6	7(21)	3(43)	0	0	4	0	4	0.0	0.0[d]
I1	98	19.7	47(48)	12(26)	2	2	30	0	34	0.06	0.03
I2	46	19.8	10(22)	5(50)	0	0	5	0	5	0.0	0.0[d]

[a] *Seedling mortality as percentage of plants germinated*
[b] *Plus one tetraploid (2n = 28) tigrina plant*
[c] *This parent was probably a recombinant itself*
[d] *Recombination not reliable due to small numbers of plants*

cant negative correlation ($r = -0.50$; $P<0.05$) with germination, but it should be considered that seedling mortality percentages are sometimes based on very few plants (Table 1).

In many species, germinating ability and seedling viability of trisomics is known to be poor compared to disomics (Khush 1973). However, this can not explain the strong positive correlation between germination and transmission rate. Correlation would then rather be negative, as seed populations with a high percentage of trisomics would exhibit a poorer germination, but would nevertheless express a higher transmission rate.

Apparently, relative germinability and seedling viability of trisomics differ between lines. In rye, strong inbreeding effects can occur in F_3 (Sybenga 1958). It may be expected that lines suffering from severe inbreeding depression will produce seeds with poor germinating ability and in these lines germination of trisomics will be affected more severely than in lines more tolerant to inbreeding. The latter can therefore exhibit a higher transmission rate, even if the percentages of trisomics in the seeds were the same. It seems likely, however, that the inbreeding depression has also affected earlier processes, i.e. fertilization, and development of embryos.

Observations on the general appearance of plants in the inbred lines support the hypothesis that inbreeding depression decreases transmission rate. In lines with a poor germination of seeds and a low transmission rate, the (few) trisomic plants were very weak: they remained small, flowered late and had narrow leaves and short ears that produced little pollen. Trisomics from the lines D1, D2, D3, F, G1 and G2, with higher germination percentages and transmission rates, were more vigorous although they were always weaker than wild type disomics appearing in the same lines.

Between lines, the mean seed weight was correlated with germination and both will be related to the degree of inbreeding depression. However, with respect to transmission rate, there is also a tendency towards the opposite direction: in seed populations with a higher trisomic frequency the mean seed weight will be lower because trisomic seeds are usually lighter than disomic seeds (Khush 1973). This is probably the reason why a significant correlation between seed weight and transmission rate could not be found ($r = 0.37$; $P>0.10$).

The results obtained here indicate that the degree of inbreeding depression plays a very important role in the transmission rate of the extra chromosome upon selfing. On an average, only 48% of the seeds germinated, whereas 26% of the seedlings died at an early stage.

The lines A1, A2, A3, A4, B, C1 and C2 that descended from the same F_3 line (Fig. 2) all showed a very low transmission rate and a corresponding strong inbreeding depression (the germination percentage averaged 29%). The other lines, descending from

another F_3 line (Fig. 2) were generally more vigorous and showed higher transmission rates. However, within this group large differences occurred. Most F_5 lines descending from the same F_4 line showed a similar behaviour, except for H1 and H2. It is clear that the genetic background influences transmission rate at least in part through its effect on inbreeding depression.

The recombination fraction in the F_5 lines varied between 0% and 12% (Table 1). The progenies with the smallest recombination fractions contained few plants, which makes these figures not very reliable. Some F_5 lines belonging to the same F_4 group seemed to show a slightly higher or lower recombination than others, but these differences are not very clear.

Transmission and recombination upon testcrossing

Table 2 shows segregation for *ti* and for chromosome number in the wild type class in the progenies of the testcrosses with tertiary trisomics from some F_5 lines. Male transmission rates (m) and recombination fractions (r) have been estimated.

In all testcross progenies, germination was good. Male transmission of the extra chromosome had occurred in all lines. In one case a primary trisomic was found in the progeny. It can arise from adjacent orientation of multivalents in meiotic metaphase. Chi-square tests were carried out to analyse differences in segregation in the progenies of tertiary trisomics from the same F_5 line. (In some cases, the euploid *Ti* class was so small that it had to be added to the *tigrina* group). No significant differences (α = 0.05) were found except for line G1, were one plant (G1/1) showed a lower and the other (G1/2) showed a higher male transmission rate (Table 2).

For the other lines, the numbers in each category were pooled and m and r were estimated again (Table 2). Then, comparisons between lines could be made. Line D1 differed significantly ($P<0.05$) from D2 and G2 because it had a relatively high male transmission rate and a relatively low recombination frequency. Segregation in testcross progenies from D3 trisomics differed from that of trisomics from line G2 but this was probably largely due to differences in recombination. When both trisomics from line G1 (G1/1 and G1/2) were compared with the other lines separately, it was found that G1/1 differed significantly ($P<0.05$) from D1 and G1/2 from G2. Other differences were not found to be significant at the 0.05 level, but G1/1 showed the lowest and G1/2 showed the highest transmission rate of all progenies.

As found in the tertiary trisomic studied previously, where one line showed a consistently high male transmission rate (Janse 1987a), genetic factors may be present here. One or more factors may have segregated in F_5 line G1 and may also have caused the higher male transmission rate in line D1 when compared to some other

Table 2. Segregation for ti and for chromosome number among the wild types in progenies of testcrosses 2n x 2n+1, with tertiary trisomics from six different F_5 lines used as a male parent. Estimates of male transmission (m) and recombination fraction (r)

F_5 line	Plant	No. of seeds	Germination (%)	*Ti* (15)	*Ti* (14)	*titi*	Total	m	r
D1	1	83	72(87)	10	0	59	69	0.15	0.0
	2	38	27(71)	1	0	23	24	0.04	0.0
	Total	121	99(82)	11	0	82	93	0.12	0.0
D2	1	91	72(79)	2	2	61	65	0.03	0.03
	2	138	134(97)	10	8	115	133	0.09	0.07
	3	31	31(100)	2	1	28	31	0.07	0.03
	Total	260	237(91)	14	11	204	229	0.06	0.05
D3	1	79	78(99)	7	1	66	74	0.10	0.02
	2	20	18(90)	1	0	17	18	0.06	0.0
	3	18	18(100)	1	0	15	16	0.07	0.0
	Total	117	114(97)	9	1	98	108	0.08	0.01
F	1	21	21(100)	1	1	19	21	0.05	0.05
	2	67	61(91)	4	2	53	59	0.07	0.04
	Total	88	82(93)	5	3	72	80	0.07	0.04
G1	1	76	66(87)	1	2	63	66	0.02	0.03
	2	103	93(90)	12	3	78	93	0.13	0.04
G2	1	114	107(94)	4	8	94	106	0.04	0.08
	2	89	89(100)	3[a]	5	80	88	0.04	0.06
	Total	203	196(97)	7	13	174	194	0.04	0.07

[a] *including one unidentified primary trisomic*

lines.

To further study these possible genetic factors, both trisomics from line G1 were selfed and new testcrosses were made with some F_6 tertiary trisomics, the results of which are shown in Table 3. Unfortunately, not enough F_6 seeds were obtained from line D1 because it had been grown in an unfavorable season. Additional testcrosses have been made with three F_1 tertiary trisomics that were members of the testcross progenies of G1/1, G1/2 and a D1 trisomic, respectively. Results of these crosses are also shown in Table 3.

In the progeny of one of the two F_6 tertiary trisomics originating from F_5 tri-

somic G1/1, an abnormal segregation was observed (Table 3). Firstly, the number of disomic *Ti* plants was too high to solve m and r. Probably, the parental F_6 trisomic was a recombinant itself and carried a *Ti* allele on one of the normal chromosomes. New formulae could be deduced for this situation. However, secondly, both trisomic *Ti* plants appearing in this progeny were primary trisomics. Estimates for m would then refer to male transmission rate of an extra normal instead of a translocated chromosome. This progeny will therefore not be taken into account for comparisons of male transmission rates. However, it should be noted that it is very surprising that two primary trisomics occurred in one testcross progeny. Apparently, adjacent orientation is more frequent here than in other tertiary trisomics. As one of the tertiary trisomics of F_5 line G2 originating from the same F_4 line (Fig. 2) produced a primary trisomic as well, the genetic background may play a role. At the time the testcross progenies were analysed, these parental plants were no longer available for meiotic analysis.

It can be seen in Table 3 that the other F_6 tertiary trisomic descending from F_5

Table 3. Segregation for ti and chromosome number in progenies of testcrosses 2n x 2n+1. As a pollinator, tertiary trisomics from F_6 lines and from earlier testcross progenies with F_5 plants (see Table 2) are used. Estimates of male transmission (m) and recombination fraction (r)

Origin	Plant	No. of seeds	Germination (%)	*Ti* (15)	*Ti* (14)	*titi*	Total	m	r
F_6 (G1/1 ⊗)	1	71	64(90)	1	3	60	64	0.02	0.05
	2	77	59(77)	2[a]	25	29	56	-[b]	-[b]
F_6 (G1/2 ⊗)	1	69	53(77)	5	0	49	54	0.09	0.0
	2	122	87(71)	4	5	76	85	0.05	0.07
	Total	191	140(73)	9	5	125	139	0.07	0.04
F_1 (*titix*G1/1)	1	81	69(85)	4	2	62	66	0.06	0.03
F_1 (*titix*G1/2)	1	112	105(94)	6	2	93	101	0.06	0.02
F_1 (*titix*D1)	1	70	58(83)	0	0	56	56	0.0	0.0

[a] *Both trisomics were probably primaries, as the translocated chromosome could not be detected*

[b] *The number of (14) Ti plants was too high to solve m and r. The parental trisomic was probably a recombinant itself, carrying a Ti allele on one of the normal chromosomes (see text)*

trisomic G1/1 again showed a relatively low male transmission rate (0.02). However, in two F_6 tertiary trisomics from F_5 trisomic G1/2, m was not high as would be expected from the high rate in F_5, but was only moderate. The difference between the F_6 trisomic from G1/1 on the one hand and both F_6 trisomics from G1/2 on the other was not significant ($\alpha = 0.05$). This may mean that either the difference observed within the F_5 line G1 did not have a genetic basis or the genetic effect was modified in F_6 tertiary trisomics by unknown factors.

The results of testcrosses with F_1 tertiary trisomics are also presented in Table 3. Any differences in male transmission rates between F_1 trisomics must have their origin in the corresponding F_5 trisomics, as all *tigrina* female parents had the same genetic background. Both F_1 tertiary trisomics from F_5 trisomics G1/1 and G1/2 showed a moderate male transmission rate, and thus did not express the difference found between their parental trisomics. Of course, genetic factors are not expressed in the heterotic trisomic when they are recessive.

The F_1 tertiary trisomic derived from F_5 line D1 did not show any male transmission of the extra chromosome (Table 3), while the trisomics from the parental line showed a relatively high rate (Table 2). Although the total number of plants was not high, the difference was large enough to be highly significant ($P<0.02$). This difference is very surprising and makes it more likely that the higher rate of transmission in the F_5 line was not genetically determined. An interesting point is that recombinants were absent in testcrosses with both this F_1 tertiary trisomic and the corresponding F_5 trisomics.

Thus, from the results of the testcrosses, no evidence could be obtained for the presence of genetic factors determining male transmission rate and recombination in tertiary trisomics 282. There were some differences within and between F_5 lines, but these were not clearly present in the corresponding F_6 and F_1 tertiary trisomics.

Pollen quality

Results of pollen quality tests are given for tertiary trisomics and disomics from two F_5 and two F_6 lines and for F_1 tertiary trisomics (Table 4). The plant numbers correspond with those used in Table 3. All plants had been grown in the same season. Pollen from trisomics belonging to the F_5 lines G1 and D1 was not tested because these plants had of course been grown in an earlier season than their derived F_6 and F_1 tertiary trisomics. Although these plants had been maintained, they were in such a poor physiological condition that the results of pollen tests were not expected to be representative for the pollen used in the testcrosses.

Generally, the quality of pollen grains was quite high, ranging from 69%–95%. In

Table 4. Quality of pollen from tertiary trisomics 282 and disomics in F_5 and F_6 lines and in a heterotic background (FCR method). The percentage of fluorescent pollen grains (n = 500) is given at three sampling dates (I, II, III) during the flowering season

Line	Plant	Type	Fluorescent			
			I	II	III	Mean
F (F_5)	1	(15) *Ti*	73	69	65	69
	2	(15) *Ti*	84	87	72	81
	3	(15) *Ti*	73	72	-	73
	4	(14) *Ti*	87	83	73	81
G2 (F_5)	1	(15) *Ti*	90	93	87	90
	2	(15) *Ti*	86	94	86	89
	3	(15) *Ti*	89	90	-	90
	4	(14) *Ti*	88	85	90	88
G1/1 ⊗ (F_6)	1	(15) *Ti*	91	90	-	91
	2	(15) *Ti*	85	78	79	81
	3	(15) *Ti*	70	72	69	70
G1/2 ⊗ (F_6)	2	(15) *Ti*	76	85	87	83
	3	(15) *Ti*	68	72	-	70
	4	(14) *Ti*	85	86	77	83
titi x G1/1 (F_1)	1	(15) *Ti*	73	75	-	74
titi x G1/2 (F_1)	1	(15) *Ti*	94	95	97	95
titi x D1 (F_1)	1	(15) *Ti*	92	94	96	94

the inbred lines, the quality of pollen from trisomics varied between individual plants and between sampling dates. As a mean, it was a little lower than in a comparable disomic. An exception was line G2, where all plants investigated produced pollen of high quality (Table 4).

Sybenga (1958) showed that pollen fertility in rye is usually strongly affected by inbreeding. After five generations of subsequent inbreeding it averaged 55%. Here, pollen quality was much higher, even in trisomics. However, some selection against strong inbreeding effects had been carried out when lines with good transmission rate upon selfing (Table 1) were selected and vigorous trisomics with acceptable pollen production were used for testcrosses and quality tests.

Pollen from two of the three F_1 trisomics tested showed a constant and very high

quality. However, the third produced pollen of relatively low quality.

A relation between pollen quality and male transmission rate does not seem to be present. This is most clearly seen in the F_1 trisomics. The trisomic derived from G1/2 showed a very high quality and a moderate transmission rate, while that from G1/1 had the same transmission but a poorer quality. The F_1 trisomic from D1 produced pollen of high quality that was not capable of transmitting the extra chromosome.

There are two ways in which pollen quality could be related to male transmission of the extra chromosome. Firstly, reduced viability of aneuploid pollen grains could lower the quality of the total sample. Secondly, the quality is affected by the physiological condition of the plant. This condition will be influenced by the tolerance to aneuploidy and, in this case, to inbreeding.

In primary trisomics of many species, pollen fertility has been shown to be high. In *Nicotiana sylvestris*, it ranged from 92% to 98%, whereas male transmission varied from 0% to 34% (Goodspeed and Avery 1939). In different barley primaries, pollen fertility was 72%–97% (Tsuchiya 1960) and in rye it could vary from 63% for the primary trisomic *stout* to 93% for *pseudonormal* (Kamanoi and Jenkins 1975).

Tertiary trisomics were studied of pearl millet, where pollen fertility ranged from 3% to 95%. However, it appeared from meiotic analysis that the tertiary trisomics exhibiting a low pollen fertility carried a long translocation chromosome (Singh et al. 1982).

It should be noted that in all studies mentioned above, pollen fertility was assessed using "stainability" tests, that determine the content of the vegetative cells. Heslop-Harrison et al. (1984) showed that this does not always provide good estimates for potential germination. The FCR test, used in this study, would provide a better guide to germinating ability. Still, a high FCR score is a prerequisite but not a guarantee for germination. When conditions for germination are suboptimal or the stigma quality is insufficient, germination will be poor.

The experiments described here indicate that, in inbred lines of tertiary trisomic 282, the degree of inbreeding depression plays a very important role in the transmission of the translocated chromosome upon selfing. The trisomics arising in the progenies resulted partly from male and partly from female transmission, but the contribution of the latter was probably greater. Tetrasomics, resulting from simultaneous male and female transmission, were not viable. In the rye tertiary trisomic 240 studied previously (Janse 1987a), the average transmission rate upon selfing was higher, but the tolerance to inbreeding was also greater. This was confirmed by the appearance of tetrasomics, although in low frequency. It seems likely that the

differences in mean transmission rate between both tertiary trisomics were mainly caused by differences in tolerance to inbreeding, rather than to a differential effect of the extra translocation chromosome.

Male transmission rate of the extra chromosome, as estimated by means of testcrossing with disomics, showed some variation in and between F_5 lines. No clear evidence for genetic factors could be obtained, but one F_5 trisomic and a derived F_6 trisomic showed a lower rate (0.02) than the others. In tertiary trisomic 240 male transmission rate reached about the same level, but genetic factors were shown to be present that caused a consistent high male transmission rate in one line (Janse 1987a). No lines of either 240 or 282 were without male transmission.

Pollen quality was relatively high in some of the inbred lines selected for testcrosses. On the average, it was a little lower in trisomics than in disomics. A relation with male transmission rate was not present. Probably, both the euploid and the aneuploid pollen grains are well capable of germinating in these trisomics.

In one tertiary trisomic 240 male transmission rate could be raised from 0.05 to 0.20 when pollinations with very few pollen grains were carried out (Janse 1987b). The same experiment was carried out with one tertiary trisomic 282, F_5 plant G1/2. Here, m could be raised from 0.13 (Table 2) to 0.27. Thus, certation between euploid and aneuploid pollen grains occurred in tertiary trisomic 282 as well. Also, it can be concluded that in this plant at least 27% of the germinating pollen grains were aneuploid and that the pollen produced by this tertiary trisomic possibly contained even more.

When BTT lines with a fair tolerance to inbreeding and to aneuploidy are selected, female transmission rate of the translocated chromosome upon selfing will probably be high enough for efficient maintenance of BTTs. Simple selection for high transmission rate upon selfing, however, includes the risk of selecting genotypes with higher male transmission rate.

Pollen quality is expected to be high in tertiary trisomics selected for good viability and this is necessary for good seed set. It is not related to male transmission rate, which is probably mainly determined by the relative pollen tube growth of aneuploid pollen grains. In this process selection for the necessary low male transmission may be possible.

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CHAPTER 6
General discussion

In this thesis, studies on the genetic and physiological aspects of the generative transmission of the extra chromosome in rye tertiary trisomics are described. Two different tertiary trisomics have been used that differ with respect to the exchanged segment in the translocated chromosome and that both contain the marker gene *Ti/ti* on the short arm of this chromosome. The results will be discussed here and related with the use of balanced tertiary trisomics in hybrid rye breeding. Some additional experiments will be described.

Transmission upon selfing

Observations on different inbred lines of the two tertiary trisomics have shown that transmission after selfing could vary from 0% to 42% (Chapters 2 and 5). Variation was greater for tertiary trisomic 282, but more lines were tested. The trisomics in the progenies did not result as predominantly from transmission through the eggs as often assumed, but also from transmission through the pollen. The contribution of the latter, however, was apparently smaller. Tetrasomics were expected to result from simultaneous male and female transmission, but they were infrequently observed. This was apparently a consequence of their strongly reduced viability, particularly in inbred lines. The low frequency of tetrasomics has in the past led to the assumption that male transmission of the translocated chromosome is usually absent or very low. It was shown here that this assumption was not correct.

In male meiosis, the extent of pairing and chiasma formation and the alternate orientation of multivalents guarantee a high percentage of aneuploid microspores (Chapters 1 and 2). Transmission of the extra chromosome through female meiosis is expected to be high too. Meiotic behaviour of tertiary trisomic 282 was similar to that of tertiary trisomic 240 (results not shown).

Female aneuploid gametophytes may have a reduced viability during development and functioning, but they are generally known to tolerate chromosomal imbalance better than the male gametophytes (Khush 1973). As even the latter can still function quite normally (Chapters 3 and 5) and as competition between euploid and aneuploid egg cells does not occur, a high percentage of aneuploid zygotes may be formed after selfing of a tertiary trisomic. Development of these zygotes to embryos and/or viability of aneuploid seeds and seedlings are then considered to be the most important factors in determining transmission rate.

For tertiary trisomic 282, the inbreeding depression was shown to play an important role in determining transmission upon selfing, probably through its effect on vigour of aneuploid seeds and seedlings and perhaps also on that of zygotes and embryos (Chapter 5). This probably also applied to tertiary trisomic 240 (Chapter 2) and to a third tertiary trisomic (501), the translocation chromosome of which also contained the 5RS arm and the *ti* gene but again another exchanged segment. This trisomic was not used for further analysis, because it showed an unpracticably high recombination frequency.

According to Khush (1973) the size of the extra chromosome in trisomics largely determines its detrimental effect on viability of aneuploid sporophytes and gametophytes. Here, both translocation chromosomes 240 and 282 (and also 501) had about the same size. The observed differences in transmission rates were mainly dependent on the degree of inbreeding depression. No evidence could be found for a specific differential effect of the different translocated segments.

The effect of the extra chromosome in the tertiary trisomics was also studied biochemically (Chapter 4). No systematic differences in general protein pattern could be found between trisomics and disomics, between the two tertiary trisomics or between different genotypes of each trisomic. For one isozyme of glucose-6-phosphate dehydrogenase (G6PDH), the activity seemed to decrease slightly in the tertiary trisomics 240 and 282, that contained an extra dose of the structural gene involved. This may be exemplary for the effects of the extra chromosome on cellular processes in the trisomics.

The results obtained indicate that, with selection for tolerance to aneuploidy and to inbreeding, a high transmission of the translocated chromosome may be obtained, even after several generations of inbreeding.

Male transmission in crosses with disomics

When disomic female parents were crossed with tertiary trisomics from different inbred lines (using an excess of pollen), male transmission of the translocated chromosome was shown to occur in all progenies and averaged 7-8% for both tertiary trisomic 240 and 282 (Chapters 2 and 5). In a limited number of testcrosses with tertiary trisomic 501, the same level was reached.

For tertiary trisomic 240 one line with a consistent, apparently genetically determined, high male transmission rate and a high recombination frequency was found (Chapter 2). For tertiary trisomic 282 two plants with a higher and a lower rate, respectively, seemed to segregate in an F_5 line, but it was not entirely clear whether genetic factors were involved (Chapter 5). No correlation between male transmission

rate and recombination frequency was present here.

When tertiary trisomics would be used for propagation of male sterile stocks in hybrid rye breeding, male transmission of the translocated chromosome should be low, preferably zero. Although the rates obtained here were relatively high, the presence of genetic variation indicates that selection for low transmission may be possible.

Experiments have been carried out to find the "bottle-neck" for male transmission and the stage in which genetic factors are expressed. This would provide a way for efficient selection, avoiding extensive and time-consuming testcrossing.

Development and functioning of aneuploid male gametophytes

Observations on meiotic configurations and microspores in first pollen mitosis showed that transmission of the extra chromosome through meiosis was high for tertiary trisomic 240. Aneuploid microspores tended to pass first pollen mitosis later and thus showed a slower development than euploids. Although differences between plants were present, they were not correlated with male transmission rate. Therefore, the delayed development of aneuploid pollen grains was not expected to be the main factor in determining male transmission rate (Chapters 1 and 2). Some observations on tertiary trisomic 282 gave comparable results (not shown).

The quality of dehisced pollen, according to the fluorochromatic reaction and indicating the potential germinability, was on the average a little higher in disomics than in tertiary trisomics 282 of the same inbred lines. In some cases, it was higher in heterotic tertiary trisomics than in disomics or trisomics from inbred lines. It was not correlated with male transmission rate (Chapter 5). A limited number of observations on tertiary trisomics 240 showed that pollen viability of trisomics was relatively high too (results not shown).

In both tertiary trisomics, pollination with a limited number of pollen grains (average four) gave a much higher transmission rate than normal (abundant) pollination, indicating that certation during pollen germination and tube growth was probably the most important factor determining male transmission of the translocated chromosome (Chapters 3 and 5). It also showed that at least 20% (for one line of tertiary trisomic 240) and 27% (for one of tertiary trisomic 282) of the mature pollen grains must have contained the extra chromosome and probably more. If all aneuploid microspores observed in first pollen mitosis would be able to reach maturity, a percentage of about 48% could be obtained for tertiary trisomic 240 (Chapter 2) and probably also for 282.

It would be useful to know more exactly, how many aneuploid microspores have reached the mature pollen stage, despite their delayed development. Possible differences

between the different lines of a tertiary trisomic could then be studied.

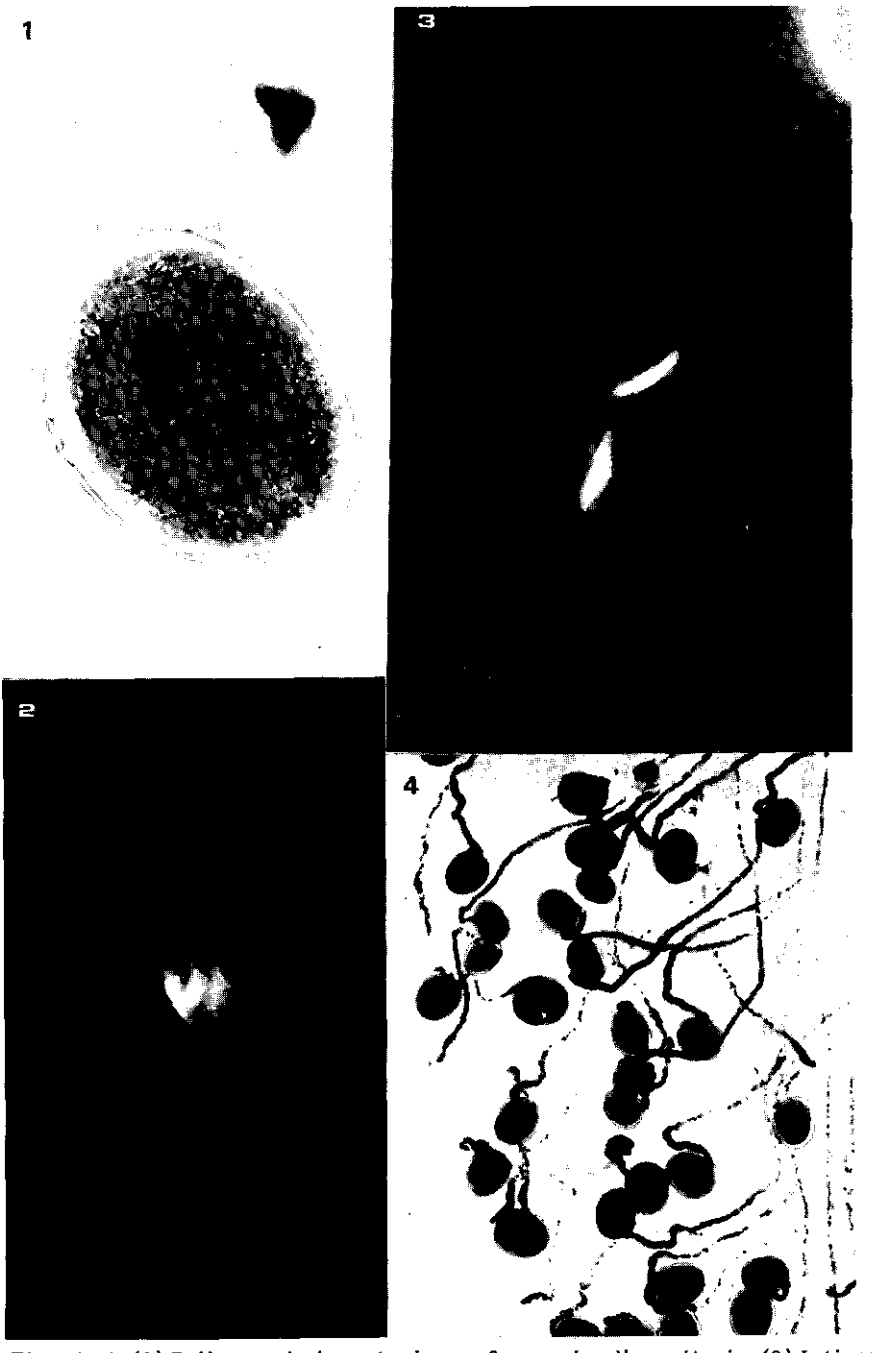
After first pollen mitosis, a second division occurs in the generative cell of the pollen grain. The timing of this second pollen mitosis in euploid and aneuploid pollen grains, as carried out for the first pollen mitosis, would provide further information on the rate of development and the number of aneuploid pollen grains.However, this cell is very small, as it contains only the generative nucleus and hardly any cytoplasm. In this division it is therefore very difficult to count the chromosome number in metaphase (Fig. 1). With some squashing techniques this could be slightly improved, but not enough for quantitative analysis, as done for first pollen mitosis.

Attempts have been made to distinguish euploid and aneuploid pollen grains by means of quantitative DNA measurements of their nuclei. This would offer the possibility of determining the percentages of aneuploid pollen grains at anthesis or at any other stage of development. Possibly, even germinated pollen grains could be identified by means of their DNA content.

Because of the small difference in DNA content (approximately 10%), the traditional method of microspectrophotometry after Feulgen staining is not accurate enough (Bennet and Smith 1976; Allison 1985). Also, satisfactory Feulgen staining of nuclei in mature pollen grains of rye was difficult to obtain.

DNA-specific fluorochromes in combination with flow cytometry have proved to be very suitable for quantitative DNA studies, mainly in animal cells (Crissman et al. 1982; Otto and Tsou 1985). Some fluorochromes have also been used to study the vegetative and sperm nuclei during pollen development (Coleman and Goff 1985; Hough et al. 1985). In a number of experiments, we have tested the fluorochromes ethidium bromide, mithramycin, Hoechst 33258, Hoechst 33342 and 4',6-diamidino-2-phenylindole (DAPI) in several concentrations and buffer solutions on rye pollen grains of disomics and trisomics. The best staining of pollen nuclei was obtained in 5 µg/ml DAPI in McIlvaine's buffer at pH 4.0 (after Coleman et al. 1981). However, we observed that the pollen wall showed some fluorescence too, due to autofluorescence or perhaps to aspecific staining. The fluorescence intensity of a large number of pollen grains was measured using a flow cytometer. Due to the staining of the wall, the variance of the measurements was much too high to detect any difference between euploid and aneuploid pollen grains.

Elimination of the pollen wall (especially the exine) could solve this problem. The exine of most species dissolves in hot 2-ethanolamine (Southworth 1974), but this method is so rigid that the rye pollen nuclei visibly degenerated in my experiments. Baldi et al. (1986) described a method with 4-methylmorpholine N-oxide (MMNO) and cyclohexylamine at room temperature, that gave rise to intine-enclosed "sporoplasts". For rye pollen grains, we have tested several treatments using the components



***Figs. 1–4**: (1) Pollen grain in metaphase of second pollen mitosis. (2) Intinoplast (pollen grain without the exine) stained with DAPI, under UV. (3) Sperm cells in a pollen tube in vitro, stained with DAPI, under UV. (4) Pollen tubes in the stigma, stained with Lugol's reagent.*

mentioned at different temperatures. A mixture of ethanolamine and cyclohexylamine at 60 °C was most suitable to produce intine-enclosed cells, that I prefer to call "intinoplasts".

These intinoplasts were again stained with DAPI (Fig. 2) and measured with a flow cytometer. The coefficient of variation was still high (15%-20%), both in disomics and trisomics. In the latter, euploid and aneuploid pollen grains could therefore not be distinguished. Possibly, the treatment had still affected the nuclei. Another reason for the great variation may be that the sperm cells have an irregular shape and are very compact, while the vegetative nucleus is round and more diffuse (Fig. 2). Measurements of fluorescence with a microspectrofluorometer did not give better results.

Probably the only way to quantitatively measure the differences in DNA content between euploid and aneuploid male gametes is to use isolated sperm cells, that consist mainly of their nucleus and do not have a cell wall. Isolation of sperm cells is very complicated but recently considerable progress has been made, especially in Graminae (Dupuis 1987; Matthys-Rochon 1987). Thus, an efficient method to isolate rye sperm cells may be available in the near future.

The experiments described above did not succeed in determining the percentages of aneuploids in mature pollen or between first pollen mitosis and dehiscence. If the micro-electrophoresis technique of single pollen grains would have succeeded in visualizing differential biochemical behaviour of euploid and aneuploid grains (Chapter 4), it would also have provided information on the frequency of both types.

Experiments on pollen germination and tube growth of trisomics and disomics have been carried out. Methods for *in vitro* germination of rye pollen have been described (Pfahler 1965; Heslop-Harrison 1979a), but as for most trinucleate pollen the results were poor and variable. This was affirmed in my experiments. In a few of the pollen tubes that were formed, the sperm cells could be stained with DAPI (Fig. 3), but in others they did not emerge into the tube. *In vitro* experiments with rye pollen are therefore not expected to be representative for its behaviour *in vivo*.

In the next experiment, carried out in the greenhouse, pollen from tertiary trisomics 240 and disomics was dusted over the receptive stigmas of emasculated ears. The pistils were fixed in acetic alcohol after 10 min, cleared in 10% potassium hydroxide and treated with Lugol's reagent (Adamchuk et al. 1979), which stains starch in the pollen tubes (Fig. 4). Pollen germination and tube growth was still very variable, probably due to its dependence on relative humidity and condition of the stigma. Due to the dehydration of pollen prior to dehiscence and its rehydration during early germination, the hydrodynamics of Graminae pollen germination and tube growth is very complex (Heslop-Harrison 1979b). In one experiment, were germination was high, a

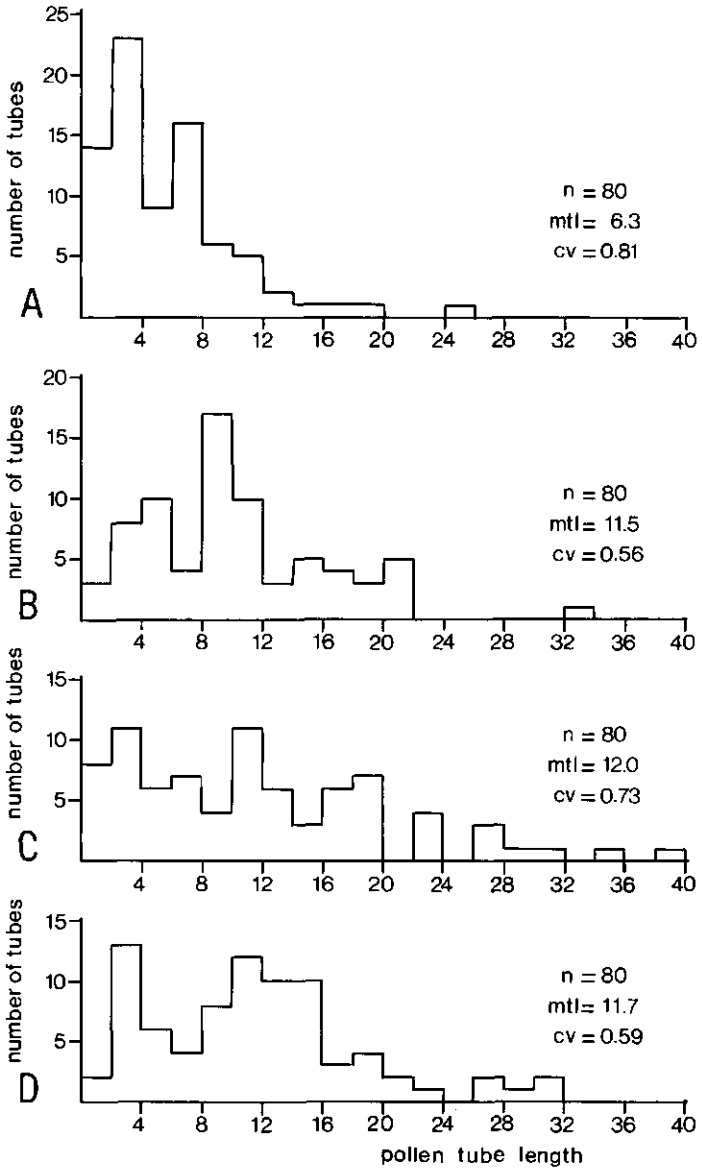


Fig. 5. *Pollen tube length frequency distribution after 10 min of germination on the stigma. (A) Tertiary trisomic with moderate male transmission rate. (B) Disomic comparable to A. (C) Tertiary trisomic with high male transmission rate. (D) Disomic comparable to C. Pollen tube length in units of an ocular micrometer. Mean tube length (mtl) and coefficient of variation (cv) are given.*

bimodal frequency distribution of pollen tubes was found in a tertiary trisomic 240 with moderate male transmission rate (Fig. 5). Simultaneously, pollination with a disomic of comparable genotype was carried out on an ear of the same plant. Pollen tube growth showed a considerable variance too, but less than that of the trisomic and no obvious bimodal distribution was found. Also, the mean tube growth was higher than in the trisomic (Fig. 5). On another day with good pollen germination, tube growth of pollen from a trisomic with high transmission rate did not show a bimodal distribution but mean tube growth was as high as in a comparable disomic (Fig. 5). Although one should be very careful in interpreting these incomplete and variable results, they seem to confirm the results found in Chapters 3 and 5: Certation between euploid and aneuploid gametes during pollen tube growth is an important factor in determining male transmission rate. It seems likely, that in genotypes with a lower male transmission rate, tube growth of aneuploid pollen is reduced more severely than in genotypes with a higher rate. Viability and germinating ability seem to be less affected. This may be the reason why no biochemical differences could be found between euploid and aneuploid mature pollen grains. Differences may perhaps be more evident when proteins would be extracted from germinated pollen grains.

Selection for low (or high) male transmission rate should thus be sought in the process of pollen tube growth and perhaps also in the penetration of the embryo sac. In the future, methods for identifying euploid and aneuploid gametes may become available. If, also, a better knowledge of germination and tube growth of rye pollen would lead to the development of standardized methods to study tube growth, selection at this stage may become possible.

Implications for use of BTTs in hybrid breeding

Tertiary trisomics are of potential use in chromosomal balanced systems for hybrid breeding. A high female and a low male transmission of the translocation chromosome are important prerequisites for the successful application of these systems.

It has been shown for two different tertiary trisomics that selection for tolerance to inbreeding and to aneuploidy may provide the best basis for high female transmission and thus for efficient maintenance of BTTs. It should be avoided to select simply for high transmission rates, as this includes the danger of selecting for high male transmission too. The trisomics in the selfed progenies partly result from male transmission, and its rate is probably determined by genetic factors.

It should also be kept in mind that, in rye, the strongest inbreeding effects come to light only after several generations of selfing. Thus, a broad genetic basis is required.

In testcrosses with disomics, male transmission of the translocated chromosome appeared to be higher than previously assumed. When pollinating male sterile disomics with male fertile tertiary trisomics for propagation of the former, this would lead to the appearance of male fertile trisomics in the male sterile seed parent. This will disturb the further propagation of this parent and will decrease uniformity and vigour of the "hybrids". De Vries (1984) suggested that these trisomics may be eliminated when the progeny is sufficiently thickly sown, because inbreeding decreases the competitive ability of the trisomics. However, as mentioned above, selection against strong inbreeding and aneuploidy effects is necessary for effective maintenance of BTT lines. Also, when a conditional lethal, selective marker gene (like *Ti/ti*) is used, half of the disomic progeny will die and reduce competition. In fact, the male transmission rate is probably too high for competition to be sufficient.

Thus, efforts have been made to reduce male transmission of the extra chromosome. Genetic factors are probably involved, but genotypes with a low rate seem to be very rare. The experiments described here show that selection of these genotypes by means of testcrossing is very laborious and probably unworkable in practice. The growth of pollen tubes in the pistil is now assumed to be the "bottle-neck" for transmission of the extra chromosome. It is probably also the stage in which the genetic factors are expressed, although it is still possible that some aneuploid male gametophytes were lost earlier. Selection of suitable genotypes by means of studying their pollen tube growth distribution requires more knowledge on conditions that influence this tube growth.

No differential effects of the different translocation chromosomes 240 or 282 (or 501) on the aneuploid sporophytic or gametophytic behaviour have been found. It is therefore suggested that, as for some other species, the size of the extra chromosome mainly determines these effects, rather than its genetic composition. For successful use of a tertiary trisomic in a BTT system, its translocation chromosome should be large enough to prevent male transmission and small enough to guarantee good viability of trisomic plants and aneuploid female gametophytes. It is not excluded, however, that specific chromosome segments occur that do have a special effect on male or female transmission. These have not been found here.

Finally, it should be noted that the recombination frequency in the tertiary trisomics studied was higher than expected from earlier experiments (De Vries 1984). In the translocation heterozygote 240, recombination between *ti* and the breakpoint had not been found, and in 282 it was very low. The higher recombination frequencies found in the corresponding tertiary trisomics studied in this thesis, indicate that this gene may not be very useful as a marker for selecting the tertiary trisomics from the

disomics. Although several other markers are available in rye, they should better not be selected solely on their behaviour in the translocation heterozygote.

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SAMENVATTING

In dit proefschrift is de generatieve transmissie (overdracht) van het extra translocatiechromosoom in tertiaire trisomen van rogge (*Secale cereale L.*) bestudeerd.

Tertiaire trisomen hebben een extra translocatiechromosoom en bevatten dus, in het geval van diploide rogge, 15 chromosomen. Zij ontstaan in lage frequenties in de zelfbevruchtingsnakomelingschappen van translocatieheterozygoten en worden gebruikt in (cyto)genetisch onderzoek. Een mogelijke toepassing in de plantenveredeling is het gebruik van tertiaire trisomen als "gebalanceerd chromosoomsysteem" voor de hybrideveredeling met behulp van genetische mannelijke steriliteit.

Bij het gebruik van deze steriliteit (die meestal recessief overerft) voor de productie van hybridezaad is de instandhouding en vermeerdering van de mannelijk steriele (*msms*) moederlijn een probleem. Dit kan opgelost worden met behulp van "gebalanceerde tertiaire trisomen" (BTT's) volgens een methode die voor gerst ontworpen werd. Als op het translocatiechromosoom van een tertiair trisoom het dominante allel *Ms* ligt, en op beide overeenkomstige normale chromosomen het recessieve *ms* dan is de trisoom mannelijk fertiel (*Msmsms*). Als het extra chromosoom niet via het pollen overgedragen wordt en men gebruikt deze trisoom om mannelijk steriele disomen te bestuiven, dan ontstaan in de nakomelingschap weer uitsluitend mannelijk steriele disome (*msms*) planten. Deze kunnen wederom bestoven worden, etc.

De tertiaire trisomen zelf worden gehandhaafd en vermeerderd door zelfbevruchting. Aangezien een extra chromosoom normaliter wel via de eicel overgedragen kan worden, ontstaan in de nakomelingschap weer tertiaire trisomen (*Msmsms*) en disomen (*msms*). Om deze tijdig van elkaar te scheiden, kan gebruik worden gemaakt van een recessief (conditioneel) letaal marker gen dat eveneens op het translocatiechromosoom ligt. Wanneer het extra chromosoom weer het dominante allel (*A*) bevat en de normale chromosomen het recessieve (*a*), dan zijn de na zelfbevruchting ontstane disomen (conditioneel) letaal (*aa*). Uiteindelijk zullen alleen de tertiaire trisomen overleven: zij zijn "gebalanceerd".

Voor het succesvol toepassen van een dergelijk systeem moet aan diverse voorwaarden voldaan worden. De recombinatie tussen de genen *Ms* en *A* en het translocatiebreukpunt moet laag zijn. Het extra translocatiechromosoom moet een dusdanige nadelige invloed op de aneuploide mannelijke gametofyt uitoefenen, dat transmissie via het pollen voorkomen wordt. Tegelijkertijd mag het effect op de vrouwelijke gametofyt niet te groot zijn, aangezien een hoge transmissie via de eicel vereist is voor een efficiënte vermeerdering van de BTT. Ook de fertiliteit van de trisome sporofyt mag niet te sterk aangetast worden, met name wat betreft productie en kwaliteit van het

pollen.

Deze transmissie-problematiek is in dit proefschrift nader bestudeerd waarbij naast de genoemde fysiologische ook de genetische aspecten de aandacht hadden. Indien genetische variatie aanwezig is, kan selectie resultaat geven.

In de experimenten is gebruik gemaakt van de tertiaire trisomen 240 en 282 (en soms van 501). Hierin bestaat het translocatiechromosoom telkens uit de korte arm en een stukje van de lange arm van chromosoom 5R, terwijl het uitgewisselde stuk verschilt. Op de korte arm 5RS ligt het marker gen *Ti* dat, in homozygoot recessieve vorm, bladafwijkingen veroorzaakt. Deze planten zijn levensvatbaar onder non-competitieve condities.

In diverse F_5 lijnen van 240 en 282 en enkele van 501 werd de uitsplitsing bestudeerd en de transmissie en de recombinatie berekend. De transmissie varieerde van 0% tot 42%. Dit werd tot nog toe geacht uitsluitend het gevolg te zijn van transmissie via de eicel, aangezien tetrasomen meestal zeer zeldzaam zijn. Het laatste was ook in deze experimenten het geval. Echter, m.b.v. toetskruisingen werd hier aangetoond dat de transmissie via het pollen meestal 7% à 8% bedraagt. Het vrijwel ontbreken van tetrasomen kan dan verklaard worden door een verminderde levensvatbaarheid, met name in inteeltlijnen (Hoofdstukken 2 en 5).

Er was, voor tertiair trisoom 282, een significante positieve correlatie tussen zaadkieming en transmissie in de inteeltlijnen. Ook waren bij hogere transmissie de trisomen groeikrachtiger. Dit impliceert dat er een relatie is tussen inteeltdepressie en transmissie: in inteeltlijnen met een sterke inteeltdepressie ondervinden trisome embryo's en zaailingen een extra selectief nadeel en gaan vaker verloren dan die in meer vitale lijnen (Hoofdstuk 5).

Electroforese van bladextracten, gevolgd door eiwitkleuring, gaf kleine verschillen tussen en binnen lijnen te zien. Er werden geen systematische verschillen gevonden tussen trisomen en disomen of tussen tertiair trisoom 240 en 282. De activiteit van een glucose-6-fosfaat-dehydrogenase isoenzym, waarvan het structurele gen op 5RS gelocaliseerd was, was iets lager in trisomen dan in disomen. Soortgelijke effecten t.a.v. andere essentiële enzymen kunnen, gecumuleerd, de oorzaak zijn van het "aneuploidie syndroom" (morfologische en fysiologische afwijkingen) (Hoofdstuk 4).

De mannelijke transmissie werd voor een groot aantal trisomen bepaald m.b.v. toetskruisingen met disomen. Trisomen van één F_5 lijn van tertiair trisoom 240 gaven een hogere transmissie (± 19%) dan andere. Dit verschil was ook aanwezig in de F_6 generatie en klaarblijkelijk genetisch bepaald (Hoofdstuk 2). In een F_5 lijn van 282 was

de transmissie eveneens hoog, terwijl in een andere lijn een uitsplitsing in hogere en lagere transmissie leek op te treden. Deze verschillen waren minder duidelijk in de F_6 (Hoofdstuk 5).

De aanwezigheid van genetische variatie biedt mogelijkheden voor selectie. Echter, genotypen met lage mannelijke transmissie waren vrij zeldzaam en het uitvoeren van toetskruisingen is zeer bewerkelijk. Daarom is getracht na te gaan, waar in de ontwikkeling en het functioneren van de aneuploide mannelijke gametofyten deze een selectief nadeel ondervinden. Het lijkt logisch te veronderstellen dat in hetzelfde proces de genetische verschillen zich zullen manifesteren.

Uit analyse van de meiose bleek dat, dankzij goede paring en chiasmavorming en het overwegend zig-zag oriënteren van multivalenten, minstens 40% aneuploide microsporen verwacht mogen worden (Hoofdstukken 1 en 2).

Tellingen in de eerste pollenmitose gaven zelfs een hoger percentage (46%) aan. Tevens bleek dat deze deling in aneuploide microsporen later optreedt dan in euploide, m.a.w. de aneuploide ontwikkelen zich trager. Hoewel er verschillen waren in de mate van deze vertraging, was deze niet gecorreleerd met de hoogte van de mannelijke transmissie. De vertraging is dus niet de factor die deze transmissie bepaalt en het lijkt zelfs waarschijnlijk dat de aneuploide microsporen zich normaal, zij het vertraagd, tot rijpe pollenkorrels kunnen ontwikkelen (Hoofdstukken 1 en 2).

Wanneer toetskruisingen met een zéér klein aantal (gemiddeld vier) pollenkorrels uitgevoerd werden, bleek de mannelijke transmissie een stuk hoger te zijn dan bij normale (overvloedige) bestuivingen. Dit betekent dat certatie tussen euploide en aneuploide pollenkorrels optreedt en dat dit een zeer belangrijke factor in de hoogte van de mannelijke transmissie is. Ook werd duidelijk dat tenminste 20% (voor 240) resp. 27% (voor 282) van de rijpe pollenkorrels aneuploid is geweest en waarschijnlijk zelfs meer (Hoofdstukken 3 en 5).

Er is getracht na te gaan hoe hoog dit percentage was door het uitvoeren van DNA-metingen aan de pollenkernen met behulp van fluorochromen en flowcytometrie. De fluorescentie van de dikke exinewand van het pollen verstoorde de metingen. Er werden succesvolle pogingen ondernomen de exine te verwijderen. Hoewel in de ontstane "intinoplasten" nu uitsluitend de kernen fluoresceerden, bleek de variantie in de metingen nog steeds te groot te zijn om de euploide en aneuploide pollenkorrels te onderscheiden (Hoofdstuk 6).

De kwaliteit van het door tertiaire trisomen geproduceerd pollen bleek vrij hoog te zijn, zelfs in inteeltlijnen. Heterotische trisomen gaven soms een nog iets hogere kwaliteit. Er was geen duidelijke correlatie tussen de hoogte van de mannelijke transmissie en de pollenkwaliteit (i.e. de potentiële kieming). (Hoofdstuk 5).

Biochemisch was geen verschil aantoonbaar tussen pollen van trisomen en dat van disomen. Het gen voor het isoenzym van glucose-6-fosfaat-dehydrogenase, dat in de trisome sporofyt een verlaagde activiteit te zien gaf, kwam in de gametofyt niet tot expressie (Hoofdstuk 4).

Tenslotte werden de kieming en buisgroei van pollen van trisomen en dat van disomen bestudeerd na bestuivingen onder kascondities. De resultaten waren zeer variabel, vaak was de kieming laag en de buisgroei gering. Het pollen van Gramineeën is i.h.a. zeer gevoelig voor condities die de rehydratatie van pollenkorrels op de stempel beïnvloeden. Echter, in één experiment met hoge kieming gaf de frequentieverdeling van de pollenbuisgroei van een trisoom twee toppen te zien, wat in een vergelijkbare disoom niet duidelijk het geval was. Ook was in de trisoom de gemiddelde buisgroei lager en de variantie relatief groter. Deze trisoom vertoonde een gematigde mannelijke transmissie.

In een trisoom met hoge mannelijke transmissie week de gemiddelde buisgroei niet af van die van een vergelijkbare disoom. Ook waren hier geen duidelijke pieken te onderscheiden (Hoofdstuk 6). Hoewel deze resultaten niet erg volledig waren, lijken ze de conclusie te bevestigen dat de mannelijke transmissie voornamelijk bepaald wordt door de relatieve buisgroei van euploide en aneuploide pollenkorrels. Het lijkt waarschijnlijk dat in genotypen met lagere transmissie de buisgroei van de aneuploide gametofyten sterker geremd is dan in andere genotypen. Vooralsnog lijkt het moeilijk op grond hiervan deze genotypen te selecteren. Het is aan te bevelen eerst de condities die de pollenbuisgroei beïnvloeden nader te bestuderen en gestandaardiseerde en accurate methoden te ontwikkelen om deze te meten.

Met betrekking tot de toepassing van gebalanceerde tertiaire trisomen in de hybrideveredeling, zoals in het begin van dit hoofdstuk beschreven, kan een aantal conclusies getrokken worden.

Voor een efficiënte vermeerdering van BTT's is een hoge vrouwelijke transmissie bij zelfbevruchting noodzakelijk. Gebleken is dat selectie tegen een sterke inteeltdepressie en tegen gevoeligheid voor aneuploidie de beste basis is om deze te bereiken. Het dient vermeden te worden uitsluitend op hoge transmissie bij zelfbevruchting te selecteren. Een deel van de trisomen is immers via mannelijke transmissie ontstaan en de kans is groot dat men tevens genotypen met een hoge mannelijke transmissie selecteert.

In tegenstelling tot wat in het verleden aangenomen werd, kan de mannelijke transmissie van het translocatiechromosoom wel een belangrijk struikelblok vormen voor toepassing van het BTT-systeem. De transmissie is i.h.a. zo hoog dat ook niet verwacht

mag worden dat de trisome nakomelingen in een overigens disome nakomelingschap weggeconcurreerd kunnen worden. De mannelijk fertiele trisomen, die dan in de mannelijk steriele moederlijn voorkomen, kunnen een aanzienlijk deel van deze lijn bevruchten en zo de groeikracht en de uniformiteit van de "hybriden"-populatie reduceren.

Duidelijke verschillen tussen de tertiaire trisomen 240 en 282 (en 501) zijn in geen enkel experiment gevonden. Waarschijnlijk is het vooral de grootte van het translocatiechromosoom die de effecten op de gametofyten en de sporofyt bepaalt. Het kan echter niet worden uitgesloten dat er bepaalde chromosoomsegmenten bestaan, die wel een specifieke invloed op de mannelijke of vrouwelijke transmissie uitoefenen.

Tenslotte is het opmerkelijk dat de recombinatiefrequentie tussen *ti* en het translocatiebreukpunt in de tertiaire trisomen vrij hoog was. In de translocatieheterozygoten, waaruit de tertiaire trisomen ontstaan zijn, was deze zeer laag. Het lijkt dus weinig zinvol om de voor het BTT-systeem benodigde selectieve markers te selecteren op grond van de recombinatie in de heterozygoot. Het aantal recessieve semi-letale markers, dat voor rogge beschikbaar is, is echter vrij groot.

CURRICULUM VITAE

Jakoba Janse werd op 15 november 1958 geboren te Westkapelle. In juni 1977 behaalde zij het diploma Atheneum-B aan de Stedelijke Scholen Gemeenschap Middelburg en startte in datzelfde jaar met de studie plantenveredeling aan de Landbouwhogeschool te Wageningen. Haar praktijktijd bracht zij door op een freesia veredelingsbedrijf in het Westland en een veredelingsinstituut in Stuttgart. In maart 1984 studeerde zij af, met naast het hoofdvak plantenveredeling de keuzevakken erfelijkheidsleer, plantenfysiologie en planteziektenkunde. Tevens volgde zij de cursus pedagogiek en didactiek, resulterend in een 1e graads onderwijsbevoegdheid biologie. Van februari 1984 tot juni 1987 was zij werkzaam bij de vakgroep Erfelijkheidsleer van de Landbouwhogeschool/universiteit, waarvan 3 jaar als promotie-assistent ten behoeve van het in dit proefschrift beschreven onderzoek.