DEVELOPMENT OF COMPLEX INTERCHANGE STOCKS IN BARLEY (*HORDEUM VULGARE* L.) AND THEIR POSSIBLE DIPLOIDIZING EFFECT AT THE TETRAPLOID LEVEL

by

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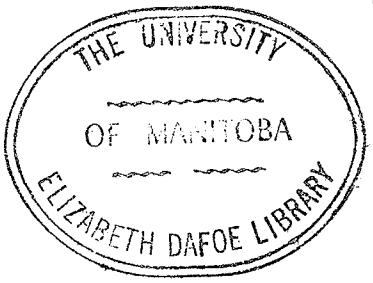

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ABSTRACT

The primary object of this investigation was to synthesize a homozygous translocation stock involving all the chromosomes in barley and to obtain preliminary information on the diploidizing effect of translocation complexes at the tetraploid level.

Two methods for the synthesis of complex interchange stocks were used and compared viz., irradiation of existing translocation stocks and intercrossing different complex interchanges in order to develop more complex stocks. Using X-rays, a total of 17 translocations were induced, of which 14 were established as homozygotes. Five different lines, homozygous for translocations involving 12 chromosomes were isolated from X-rayed populations of the stock 5903, homozygous for translocations involving ten chromosomes. The chromosomes involved in translocations of four stocks were completely identified. Using the intercross method, a stock homozygous for $0_6+0_4+2^{II}$ was isolated from progenies of the hybrid T3-5-7a x T1-2a. This stock has been crossed with 5903 to obtain a stock homozygous for a $0_{10}+0_4$. The relative merits of both the methods were discussed and it was concluded that the irradiation method is more desirable for the synthesis of complex interchanges in barley particularly at a higher level of chromosome participation.

The fertility of translocation heterozygotes of varying complexity was recorded. Translocation heterozygotes with a $0_{10}+0_4$, $0_{12}+1^{II}$ and 0_{14} were almost sterile. The practicability of the "Oenothera" method was discussed. The present procedure of synthesis of translocations without any consideration of fertility appears to be responsible for the high sterility of complex interchanges in barley.

Colchicine induced tetraploids were obtained from five complex translocation hybrids, in which the expected MI configurations were a $010+2^{II}$, $012+1^{II}$, 06+04+04, 010+04 and 014. The diploidizing effect was measured by comparing the fertility of hybrid tetraploids with the tetraploids of O.A.C.21, used as a control.

Fertility of hybrid tetraploids was lower and the frequency of aneuploid plants was much higher than that of the control. In view of the limited data of the present study, it was considered that further investigations are necessary before final conclusions about diploidization could be made.

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INTRODUCTION

The existence of true breeding, multiple translocation heterozygotes with alternate disjunction in Oenothera, and the finding that translocations can be produced by means of irradiation (131) led to a proposal by Burnham (14) known as the "Oenothera" or "multiple translocation" method of establishing homozygous lines from promising hybrids. In order to test the practicality of the method, a homozygous multiple translocation stock*, which in the heterozygous condition will produce a complete ring of all chromosomes at metaphase I is a prerequisite. As pointed out by Burnham, this method will be applicable only to crops with low chromosome numbers, in which such a translocation complex is sufficiently fertile in the heterozygous condition. He suggested that barley would be such a crop, because of its low chromosome number (n=7) and high fertility (approximately 75%) of a stock heterozygous for one translocation.

A second possible use of such a stock suggested by Prof. Shebeski (123) would be to produce tetraploids, which display diploid pairing or 'diploidization'. Theoretically, if a translocation heterozygote involving all the chromosomes is doubled, according to Darlington's hypothesis of 'preferential pairing', autosyndetic pairing would occur in the resulting tetraploid. Fertility of the tetraploid barley should therefore be improved.

Synthesis of a complete translocation stock is the first requisite to test these theoretical possibilities. Two methods have been suggested for the synthesis of complex translocation stocks, viz., intercrossing two homozygous translocation stocks involving a common

* Hereafter referred to as complete translocation stock.

chromosome and cyclic irradiation of existing homozygous translocation stocks. Evidence indicates that the second method is more promising although Wang (144), found that instead of inducing a new translocation, the original translocation was lost in a number of lines. This could indicate differential sensitivity of the chromosomes to irradiation. If this is true, with the increase of the translocation complex in a stock it would be more and more difficult to make further progress using the irradiation technique.

The present study was undertaken primarily to synthesize a complete translocation stock in barley and to obtain preliminary information on the diploidizing effect of translocation complexes at the tetraploid level. Since both these aspects are independent of each other, the results are presented in two parts. Part A deals with the synthesis of complex interchange stocks and Part B, deals with the diploidizing effect of translocation complexes in autotetraploid barley.

PART A

SYNTHESIS OF COMPLEX INTERCHANGE STOCKS IN BARLEY

LITERATURE REVIEW

I. Studies on Reciprocal Interchanges

That non-homologous chromosomes can exchange segments, was first suggested by Bridges (12) to explain the linkage of a gene in *Drosophila* with genes situated on two different chromosomes. Earlier Gates (44) observed the association of more than two chromosomes in a ring in *Oenothera*. However, Muller (80) considers Stern (135) to be the first to provide both genetic and cytological evidences proving that chromosome breakage had been followed by the attachment of one of the pieces to a different chromosome.

In plants, Belling (5) was the first to report a probable case of interchange, based on his results of interspecific crosses in *Stizolobium*. The hybrid was found to be "semisterile". He proposed a "two factor" hypothesis to explain the results, but pointed out that it could also result from the abnormal behaviour of two chromosomes. No cytological evidence was obtained in this case, but was obtained later from work on *Datura* (8,9). Belling (6) suggested that the *Stizolobium* case also could be the result of "segmental interchange between non-homologues". Since then this phenomenon has been studied in a number of plants (18,19).

The first report of chromosomal interchanges in barley is credited to Smith (125). This was of a spontaneous origin. Subsequently a number of reports on interchanges produced artificially by X-rays and other mutagenic agents have appeared (20,21,22,23,26,50,51,54,57, 58,65,73,90,91,92,93,101,102,124,140,143,144,146). X-rays have been

used most extensively for the induction of mutations including chromosomal translocations. In X-rayed barley, Caldecott and Smith (23) observed reciprocal translocations as the most common type of chromosomal aberration. The optimum dosage of X-rays varies with different species. For common cereals including barley, an X-ray dose of 10,000 to 20,000r units is the most suitable (48). A $\overset{*}{\odot}4$ (i.e. an interchange between two non-homologous chromosomes) has been reported to be the most common type of translocation induced, but multiple translocations involving more than two chromosomes giving a ⊙6, 2⊙4, ⊙8 and ⊙6+⊙4 also have been observed (22,23,91,92,146). Most of these stocks have been established in the homozygous condition without any difficulty. A number of cytogenetic and linkage studies on barley interchanges have been carried out (22,53,54,58,59). For most of the translocations, the participating chromosomes have been identified. Based on root tip analysis, Hagberg and Tjio (53,54) and Burnham and Hagberg (20) were able to determine the chromosome arms in which the exchange had occurred. For a few translocations involving the satellite of chromosome 6 and 7, the localization of breaks was somewhat more precise than in the rest of the translocations. Burnham and Hagberg (20) found a high break frequency in the "b" chromosome in the 27 interchanges of the variety Mars,but this was not the case in the 13 interchanges obtained from two-rowed varieties. Hagberg and Tjio (53) proposed a standard system of designating barley chromosomes using

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* The symbol ⊙ refers to a ring of chromosomes observed at metaphase I, and shall be used hereafter.

Arabic numerals. According to this system chromosomes 1 to 5 are designated in order of decreasing length; chromosome 6 has the longer satellite and chromosome 7 has the smaller satellite.

(a) Methods of identifying chromosomes involved in interchanges

Among the methods of identifying chromosomes involved in interchanges summarized by Burnham (19), the most common method is by crossing the unknown interchanges with a set of testers covering all the chromosomes and studying the F_1's cytologically at meiosis, For example in barley, the tester set is built up of five stocks (22). The chromosome configurations at meiosis of the F_1's will give information as to which of the chromosomes are involved in the unknown interchange (T_1) as follows:

Test-cross	Metaphase I Configuration in the Hybrid	Interpretation
T(1-5) x T_1	$20_4 + 3^{II}$	1 and 5 not involved
T(1-7) x T_1	$20_4 + 3^{II}$	1 and 7 not involved
T(1-6) x T_1	$06 + 4^{II}$	1 or 6 involved
T(3-4) x T_1	$06 + 4^{II}$	3 or 4 involved
T(2-4) x T_1	$20_4 + 3^{II}$	2 and 4 not involved

On this basis the chromosomes involved are 3 and 6. The tester sets are established initially by crossing different interchanges and analysing the F_1 meiosis in a similar manner. Such tester sets have also been established in maize (18) and Pisum (68). Other methods include pachytene analysis in corn (72), salivary gland analysis in

Drosophila (63) root tip analysis as reported in barley (20,53,54), use of trisomics as reported by Burnham (15) in corn and linkage tests between partial sterility and marker genes (59).

(b) Sterility, segregation and factors affecting segregation in a translocation heterozygote

Considerable differences between species have been reported in sterility of individuals heterozygous for a translocation involving two chromosomes. Certain species show predominantly random segregation with about 50 per cent sterility such as corn (13), peas (67,68,115,116), sorghum (41), radish (74), soybean (147) and rice (91). Other species such as Datura (9), Triticum monococcum (139, 149), Hordeum vulgare (22,125), Lycopersicum esculentum (4) and Oenothera (28) show predominantly directed segregation, the sterility being as low as 5 to 10 percent in T. monococcum and on the average 25 per cent in barley. In these species, there is a high proportion of alternate disjunction of the chromosomes in a ring (58).

Differences in degree of sterility also exist within a species for different interchanges. Thus in barley the range in average per cent sterility of an individual heterozygous for a single translocation has been reported to be 20.6 to 92.2 per cent (22), 20 to 69 per cent (91) and 26.4 to 61.5 per cent (124). Length of the interstitial segment (segment between centromere and break point) has been considered responsible for these differences (18). A cross-over in this region will produce spore abortion even if segregation is all alternate, a maximum of 50 per cent, if every meiocyte has one or more such cross-

overs. Hanson and Kramer (58) suggested that in barley, interchanges ranging from almost complete fertility to 50 per cent sterility should be expected. They also found that low sterility was associated with a short interstitial segment.

An important question still unsolved is why segregation is directed in some species and not in others. Burnham (18) has analysed the evidence for and against different views proposed to answer this question. In general, species showing a high frequency of alternate segregation have been found to possess certain cytological features in common; the chromosomes are relatively uniform in length, the centromeres are median or nearly so, and the chiasmata are mostly terminal (18). Evidence in favor of the view that terminal chiasmata tend to favor alternate segregation was obtained by Gairdner and Darlington (39) in Campanula, Sax and Anderson (119) in Tradescantia, and Ganesan (40) in Nicotiana. Levan (69), however, reported random segregation in Allium cernuum, in spite of chiasmata being terminal. In 1937, Darlington and Gairdner (30) stated that the absence of terminalization prevents regular zigzag orientation of the chromosomes in a multiple ring.

Burnham (16,17) studied crossing-over and the kind of segregation, using interchanges involving chromosome 6 in maize, which has the nucleolus organizer as a marker. He found that the length of the interstitial segment is one factor affecting segregation. Relative length of the two axes does not appear to have any effect on segregation. In Oenothera, the translocations that have survived in nature

are those with a short interstitial segment (16). Catcheside (25) reported alternate segregation in Oenothera with very unequal interchange segments produced by X-rays. Hence equality in length of the chromosomes in the interchange complex is not a necessary factor.

Frolik (38) suggested the possibility of a genetic control of segregation as did Burnham (17) and Garber (42). Search for such a factor in maize has not been successful (18). Evidence against the genetic control hypothesis comes from the work of Lewis (71) in Clarkia elegans. Further in Collinsia heterophylla, the rings produced by X-rays show an excess of alternate segregation (43), whereas those produced by colchicine show random segregation (136). Garber and Dhillon (43) considered that perhaps colchicine induced breaks in the chiasma forming segments and radiation in the internal segments of the chromosomes. The interchanges thus produced, differ in the length of translocated segments, which may be responsible for the observed differences in the type of segregation.

Burnham (19) considers that orientation may be a matter of timing. In some species, all the chromosomes may be synchronized to pass to the plate together. In others, it may be a progressive process and the first one then determines the manner in which the successive ones will be oriented. He also considers that differences in centromere activity may be responsible for the different types of segregation.

(c) Experimental production of bigger rings

Burnham in 1946 (14) proposed the "Oenothera" or "multiple

translocation method" of gamete selection. As pointed out in the introduction, a complete translocation stock is a prerequisite to test the applicability of this method. Two different methods of producing large chromosome rings have been described by Burnham (14). One is the 'intercross method' and other is by cyclic irradiation of a homozygous translocation stock. In the 'intercross method', two separate interchanges involving a common chromosome are crossed. A cross-over in the differential segment (the region between the break points of two translocations involving a common chromosome) will combine the two rings into one big ring. The success of this method is dependent, therefore, on the recovery of the cross-over in the differential segment. Burnham (14) suggested that the longer the differential segment the greater the chance of obtaining a cross-over in this segment. The intercross method has been used successfully in Campanula persicifolia (30), einkorn wheat (150) and maize (62). In einkorn wheat, Yamashita (150) reported synthesis of a ring involving all the chromosomes ($2n = 14$) but both parents of the cross contributed interchanges.

Sterility in interchange heterozygotes increases parallel to the size of the ring (124). This apparently raises a problem in using the intercross method at a higher level of translocation. Inman (62), however, suggested a method to overcome this difficulty. In this method interchanges are chosen such that they have translocations in common and differ by only two independent translocations. This would give sterility comparable to 20% in crosses of interchanges. By crossing

two stocks homozygous for a ⊙6, with one translocation in common, he obtained a stock homozygous for a ⊙8. He diagrammed different methods of crossing and selection for building larger rings in corn. But these methods involve considerable crossing work and may not be applicable to a self pollinated plant such as barley. MacDonald (73) in barley using the method suggested by Inman (62) did not succeed in identifying a single cross-over that combined the two interchanges, in more than 10,000 plants representing progenies of eight hybrids. He attributed this failure to the inadequacy of the method of selection used or to the low frequency of crossing-over in the proper differential and interstitial segments followed by the appropriate types of segregation. According to him the stability of a large ring built by selecting cross-overs in the differential segment may not be as great as a ring produced by radiation with no selection for cross-overs in this region.

The second method of cyclic irradiation has been successfully applied in corn (18) and barley (26,91,143,144). Nishimura and Kurakami (92) have outlined the procedure of producing larger rings by cyclic irradiation.

Burnham (18) reported synthesis of a ⊙10 in maize. In barley, Nishimura (91) synthesized lines homozygous for ⊙8+3^{II}, ⊙6+⊙4+2^{II}, 3⊙4+1^{II}, ⊙10+2^{II}, and ⊙8+⊙4+1^{II}. Intercrosses between some of these lines produced plants heterozygous for ⊙10+⊙4, ⊙12+1^{II} and ⊙14. Also Tuleen (143) reported synthesis of a stock homozygous for ⊙10+2^{II}. Recently he* has obtained two stocks homozygous for ⊙10+⊙4. One

* Tuleen 1963, personal communication.

of these was produced by crossing two stocks, one being homozygous for $\odot 10+2^{II}$ and the other for $\odot 4+5^{II}$. This stock has been identified as T(3-5-1-6-7)(2-4). The other stock was produced by X-raying a stock homozygous for $3\odot 4+1^{II}$.

Chang (26) obtained several lines homozygous for $\odot 8+3^{II}$ by irradiation. His results support the efficiency of the X-ray method over that of intercrossing but according to him the probability of inducing additional desired interchanges by X-rays is highest in an interchange stock involving a median number of chromosomes in the interchange complex. The interchange pattern and also the position of break points is not known in large rings produced by X-rays. Sometimes instead of inducing a new translocation by X-rays, a retranslocation may occur in the original interchange homozygote, restoring to normal one of the translocated chromosomes (26,144), but at present evidence on this phenomenon is not sufficient to establish that this is a regular occurrence.

Once a complete translocation stock is synthesized, the application of the "Oenothera" method could be tested, but it's success will depend on the fertility of this stock in the heterozygous condition. In a crop such as barley, with predominantly alternate segregation (58) such a stock involving all the chromosomes in one or two rings was found to be almost completely sterile (91,94,124). Shih and Shebeski (124) reported that when an equal number of interchange chromosomes participated, sterility was dependent on the size of the chromosome ring or rings. For this reason Nishimura and

Kurakami (92) suggested that a stock combining all the chromosomes in two rings such as 08+06 or 010+04 would be more useful. They estimated that the fertility of a stock with 08+06 should be 12.6 per cent. Nishimura (91) found fertility of stocks with 08+06, $012+1^{II}$, 010+04 and 014 to vary between 0 to 10 per cent and therefore, suggests that a stock with $206+1^{II}$ might be of more practical value, if it could be synthesized. The fertility of plants heterozygous for a 014 was reported to be 13.72 per cent in einkorn wheat (150).

MATERIALS AND METHODS

The work reported in this study was done during the years 1961-1963. Two methods, 'irradiation' and 'intercross', were used for the synthesis of translocation stocks in barley.

I. Irradiation Method.

A number of homozygous translocation stocks from various sources were selected for irradiation to induce further translocations as listed in Table I. The stock 5903 homozygous for a 010*, was synthesized in the Plant Science Department in two steps by X-radiation. From irradiated seeds of the stock T3-5-7a (old designation 4256-1) homozygous for a 06, a line was obtained, that was homozygous for 06+04. Seed of this line, designated as 161 was X-rayed, and in subsequent progenies 5903 was isolated.

Throughout the study, Montcalm was used as the standard normal variety in crosses for identification purposes.

In May 1959, 200 seeds of each of the translocation stocks listed in Table I (excluding 5903) were irradiated by 10,000r units of X-rays, and planted in the field. Partially sterile spikes were selected from the X_1 generation, and were available for further analysis at the time this study was begun. Seeds from 76 of these spikes representing 76 plants, were planted in a green house in February 1961. Subsequently, in the summer of 1961, approximately 500 seeds of the

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* The expression "homozygous for a 010" is used and will be used hereafter to refer to a homozygous translocation stock involving ten chromosomes which when crossed with a normal stock will produce a ring or chain at metaphase I in the F_1.

TABLE I. Description of the barley translocation stocks used for the induction of further translocations.

Designation		Parental Source + (new designation)	Originating station
New System	Old System		
*	5903	T3-5-7a	
T(1-7)(2-3)a	XXXIX-14 (b-d)(c-f)	T 1-7 a x T2-3a	Univ. of Man.
T(3-4)(6-7)b	III-15 (d-g)(c-e)	T 3-4 a x T6-7a	"
T(2-5)(3-4)a	VII-9 (a-f)(c-e)	T 2-5 a x T3-4a	"
T(1-5)(2-4)a	XVII-2 (a-b)(e-f)	T1-5a x T2-4a	"
T1-5 c	C1343 (a-b)	Mars	Univ. of Minn.
T2-4 a	C1420 (e-f)	Mars	"

* New designation could not be assigned to this stock, because all the chromosomes involved in the translocation complex as well as their interchange pattern were not known at the commencement of the project.

\+ T2-3a, T1-5a and T6-7a, T2-5a were obtained from the varieties Gull and Bonus respectively at Svalöf.
T1-7a, T3-4a, T2-4a and T3-5-7a were derived from the variety Mars at the University of Minnesota.

stock 5903 were treated by the same dose of X-rays and planted in the field. A total of 76 partially sterile spikes, representing 49 different plants was selected from this population for further analysis. The necessary steps of the method for the induction, detection and identification of new translocations, are outlined below:

(1) Irradiate approximately 200 to 500 seeds of homozygous translocation stocks by 10,000r units of X-ray.

(2) Grow the X_1 generation. Include control after every tenth row.

(3) Select partially sterile spikes at maturity; harvest them separately and record their fertility. Partial sterility was used as a marker for possible translocations.

(4) Plant six to ten seeds from each partially sterile X_1 spike.

(5) Mark the X_2 families segregating for fertility.

Theoretically, half of the plants in such a family would be fertile and half partially sterile. In judging for partial sterility, emphasis was placed on the pattern of sterility in a spike in addition to the per cent sterility. This was considered important, since two spikes may have the same degree of sterility, but in one case, the sterility may be restricted to one area alone, and in the other, distributed all along the head as illustrated in Figure 1. For the purpose of this study, the latter would be more important.

Harvest one or two heads from each plant of the segregating families and record their fertility, plants with 80 per cent or more fertility were considered fertile under greenhouse conditions.

	1	2
Percent fertility	56.2	44.2

FIGURE 1. Two different patterns of ovule sterility in barley. (In the above figure, the empty florets have been removed to increase contrast). Although, the percent fertility in the two spikes is almost equal, the pattern of sterility is different. In type 1, sterility is restricted to one area, whereas in type 2, it is randomly distributed. Type 1 is more likely to be environmental.

(6) X_3 families segregating for fertility:- Plant six to ten seeds from a partially sterile plant of each of the X_2 segregating families. Examine PMC's of these plants for ring formation. If a translocation has occurred, half of these plants would show ring formation.

Harvest fertile plants of only those families in which ring formation has been observed.

(7) X_3 fertile families: - Plant six to ten seeds from each fertile plant of the X_2 families segregating for fertility (step 5). Make crosses with Montcalm and the appropriate parental stock.

Harvest the crossed seeds and one or two heads from the plant which has been used in crossing.

(8) Grow three to four plants from each cross and examine PMC's at metaphase I*. This would identify the lines homozygous for new translocations, in cases where a ring formation has been observed in the X_3 families segregating for fertility. Theoretically, half of the X_3 fertile families would be homozygous for a new translocation and half homozygous for the original translocation.

(9) Also grow eight to ten plants from each of the fertile plants harvested in step 6, in case of failure to identify the line homozygous for a new translocation in steps 7 and 8. Make crosses

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* Hereafter referred to as MI.

with Montcalm and the appropriate parental stock.

(10) Plant 20 to 30 seeds from each of the lines homozygous for the new translocations. Make crosses with Montcalm and other tester stocks. The tester stocks used are listed in Table II.

(11) Grow three to four plants from each test-cross. Examine PMC's at MI and identify the chromosomes involved in the translocation complex.

TABLE II. Description of the tester stocks used for the identification of the chromosomes involved in the new translocations.

Designation		Parental Source	Originating Station	Remarks
New System	Old System			
T1-5b	C1385 (a-b)	Mars	Univ. of Minn.	Burnham's tester
T1-5d	C1384 (a-b)	"	"	
T1-5f	XT12 (a-b)	Bonus	Svalöf	
T1-7a	C1358(b-d)	Mars	Univ. of Minn.	Burnham's tester
T1-6a	C1483(b-g)	"	"	" "
T1-2a	C1310 (b-f)	"	"	
T2-3d	C1336 (c-f)	"	"	
T2-3c	Ert 47 (c-f)	Bonus	Svalöf	
T2-4a	C1420 (e-f)	Mars	Univ. of Minn.	Burnham's tester
T3-4a	C1432 (c-e)	"	"	" "
T5-6a	XT9 (a-g)	Bonus	Svalöf	

In the fall of 1961, a total of 76 X_2 families of the stock 5903 were grown in greenhouse (step 4). These families were damaged from sulphur burning and therefore had to be replanted. Fourteen of these families could not be grown, since no extra seed was available. For further analysis, therefore, only the remaining 62 families representing 44 plants were available. During the summer of 1962, the material suffered badly from hail damage. Most of the crosses (step 7) were lost. Because of space limitation in the greenhouse as well as time limitation, all the X_3 fertile families of the stock 5903 could not be grown and only those which had been identified as carrying a translocation (step 6) were grown and crossed with Montcalm and the parent stock.

II. Intercross Method

This method was used in an attempt to obtain a stock homozygous for 0l0+04. If such a stock is produced, it could be further X-rayed to unite the two translocations.

The homozygous translocation stocks 5903, T3-5-7a (4256-1)* and T1-2a(C1310)* were selected for this study. The inter-relationship between the stocks 5903, and T3-5-7a has already been mentioned earlier (page 14). The stock T1-2a was obtained from the variety Mars from the University of Minnesota. This stock was selected because these two chromosomes are not involved in the translocation complex of either of the other two stocks.

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* Represents old designation.

Montcalm was used as the standard normal variety in crosses for identification purposes.

Two approaches to produce a stock homozygous for ⊙10+⊙4 were used.

(a) Direct approach by crossing the stocks 5903 and T1-2a

Two methods of completing the synthesis were used.

1. Back crossing.

   (i) Cross 5903 (5TT.2NN)* x T1-2a (5NN.2TT)

   (ii) Backcross the F_1 hybrid (5TN.2TN) to 5903. Use F_1 hybrid as the male parent to eliminate the unbalanced gametes from functioning. Functional F_1 gametes should be 5T.2T, 5T.2N, 5N.2T and 5N.2N. In pollination, use as much pollen as possible.

   (iii) Plant the back cross seeds and study PMC's at MI. Theoretically, the chromosome constitution, and MI configuration of the back cross population would be as follows:

MI Configuration	Chromosome Constitution	Frequency	Remarks
$⊙4 + 5^{II}$	5TT.2TN	1	desired
7^{II}	5TT.2NN	1	not desired
$⊙10 + ⊙4$	5TN.2TN	1	" "
$⊙10 + 2^{II}$	5TN.2NN	1	" "

* Represents chromosome constitution of the corresponding translocation stock. 'T' refers to a translocated chromosome, and 'N' refers to a normal chromosome.

(iv) Select and self the desired plants ($\odot 4+5^{II}$). One quarter of the progeny should be homozygous for the desired genotype.

(v) Grow 15 to 20 plants from step iv; examine meiosis and cross the plants which show 7^{II} with Montcalm and 5903.

(vi) Grow three to four plants from each cross, and examine in meiosis. The desired plant will show $\odot 10+\odot 4$ and $\odot 4+5^{II}$, MI configurations in crosses with Montcalm and 5903 respectively.

2. F_2 analysis.

This method is essentially similar to the one outlined above except that the F_1 hybrid is selfed. The F_2 progenies are analysed in the same way.

Theoretically the chromosome constitution and MI configuration of the F_2 population would be as follows:

Chromosome constitution of the F_1/5TN.2TN

Functional balanced F_1 gametes 5T.2T, 5T.2N, 5N.2T, 5N.2N.

MI Configuration	Chromosome Constitution	Frequency	Remarks
7^{II}	5TT.2TT	1	most desirable
	5NN.2TT	1	not desirable
	5TT.2NN	1	" "
	5NN.2NN	1	" "
$\odot 4 + 5^{II}$	5TT.2TN	2	desirable
	5NN.2TN	2	not desirable

MI Configuration	Chromosome Constitution	Frequency	Remarks
⊙IO + 2^{II}	5TN.2TT	2	Not desirable
	5TN.2NN	2	" "
⊙IO + ⊙4	5TN.2TN	4	" "

Size of the F_2 population required:

	Frequency	Chromosome Constitution	Size of Population 1% level	5% level
Most desirable plant	1	5TT.2TT	72	47
Desirable plants	3	5TT.2TT, 5TT.2TN	23	15

The above expectation is based on the assumption that only the balanced gametes function on both male and female side. It is known, however, that unbalanced gametes will function particularly on the female side and give rise to aneuploids. These aneuploids, could be discarded on the basis of their appearance or by checking mitotic chromosome number in root tips.

(b) Bridging approach

This approach is based on the idea suggested by Inman (62) that the problem of sterility in building large chromosome rings by the intercross method may be overcome by choosing interchange parents having translocations in common and differing by only two independent interchanges. This approach is termed 'bridging' because the synthesis of the desired stock is to be carried out in two steps.

The chromosomes involved in translocation of the stock T3-5-7a,

are also involved in the translocation complex of the stock 5903. Therefore, it was planned, first to synthesize a stock homozygous for $0_6+0_4+2^{II}$ by crossing the stocks T3-5-7a and T1-2a, and then to cross this stock with 5903. The resulting hybrid would show $0_6+0_4+2^{II}$ configuration at MI and its fertility would be higher than the 5903 x T1-2a hybrid. A stock homozygous for $0_{10}+0_4$ could be isolated from the selfed progenies of this hybrid.

The initial crosses between the stocks 5903, T3-5-7a and T1-2a were made in the summer of 1961, and subsequently handled in the manner outlined above.

III. Fertility of Translocation Heterozygotes

Data on fertility of various test cross hybrids were recorded. For this purpose the first two spikelets from the top and from the bottom of a spike were removed and then the number of seeds and total number of florets were counted. Fertility percentage was determined by:

$$\frac{\text{Number of seeds}}{\text{Total number of florets}} \times 100$$

IV. Cytological Techniques

Meiosis was studied in PMC's using the acetocarmine smear technique described by Smith (126). The spikes were fixed and stored in Carnoy's solution (6:3:1) of ethyl alcohol, chloroform and glacial acetic acid respectively. The MI configurations were recorded as rings and/or bivalents, irrespective of whether they were open or closed types. The variation in configurations observed in the same

slide was scored. Where variation existed some configurations appeared more often than others but the configuration involving the maximum number of chromosomes in a ring was accepted as the MI configuration in that particular material.

RESULTS AND DISCUSSION

I. Irradiation Method

(a) Induction and detection of new translocations

Germination of the X-rayed populations of 5903 grown in the summer of 1961 was very low. Out of a total of 500 seeds planted, only about 80 seeds germinated. This poor germination could be attributed to the after-effects of radiation as discussed by Nilan (89), since there was a lapse of four to five days between irradiation and planting.

The results from the partially sterile X_1 spikes studied are presented in Tables III,IV and V. Table III presents a complete list of the X_2 families segregating for fertility as well as the fertility of the parent X_1 spikes and X_2 plants, the segregation for fertility in X_2, and the frequency of ring formation in X_3. The information with respect to those lines, in which new translocations were detected is presented in Table IV. The relationship between partial sterility in X_1 and X_2,and the frequency of detectable translocations in X_3 is given in Table V.

Stadler (130) proposed that a mutation in one primordium may not be duplicated in other primordia, and therefore plants from X-rayed seeds may be chimeras. The present data (available only for 5903, Table III) are in agreement with this view. For example, four spikes viz. 5903-39a, 5903-39b, 5903-39c and 5903-39d, were selected from the same plant, but only two of them 5903-39c and 5903-39d gave both partially sterile and fertile progenies, whereas the other two gave only fertile

TABLE III. The percent fertility of parent X_1 spikes and selected X_2 plants, the segregation for fertility in X_2, and the frequency of ring formation in X_3

X_2 line designation Column 1	Percent fertility of parent X_1 spikes (available only for 5903) Column 2	Number of partially sterile X_2 plants Column 3	Number of fertile X_2 plants Column 4	Percent fertility of the X_2 plant selected for growing X_3 Column 5	X_3 line designation Column 6	Number of X_3 plants cytologically examined Column 7	Number of X_3 plants with a 04 Column 8
T(1-7)(2-3)a - 14		1	5	69	T(1-7)(2-3)a..6	6	0
- 16		1	4	35	- 5	6	0
- 18		3	3	41	- 4	6	0
- 21		1	1	69	- 1	5	0
- 24		4	2	52	- 3	3	1
- 25		1	5	65	- 6	4	0
- 26		2	4	47	- 6	6	0
T(3-4)(6-7)b - 1		1	5	63	T(3-4)(6-7)b.- 2	6	0
- 6		1	5	59	- 1	6	5
T(2-5)(3-4)a - 4		2	4	53	T(2-5)(3-4)a.. - 2	2	0
- 6		3	1	33	- 3	6	2
- 8		6	0	42	- 2	6	3
- 10		3	2	49	- 5	3	0
- 11		3	3	55	- 1	6	0
T(1-5)(2-4)a - 5		5	1	55	T(1-5)(2-4)a..- 3	3	*
- 6		3	1	49	- 2	7	0
T(1-5)c - 3		2	2	49	T(1-5)c.. - 3	6	3
- 4		2	4	60	- 2	6	0
- 6		3	3	42	- 5	3	0
- 7		2	3	51	- 2	6	0
- 10		4	2	53	- 5	6	0
- 12		4	2	45	- 6	6	0
- 14		2	3	51	- 4	6	0
- 15		5	1	50	- 4	3	0

TABLE III CONTINUED

Column 1		Column 2	Column 3	Column 4	Column 5	Column 6		Column 7	Column 8
	- 16		4	0	51		- 4	6	0
	- 18		2	3	55		- 2	6	0
T2-4a	- 1		4	2	51	T2-4a..	- 1	6	0
	- 2		3	3	25		- 5	7	1
5903	- 2b	32	2	4	51	5903..	- 1	8	4
	- 3a	31	1	4	58		- 4	13	9
	- 5a	33	4	1	40		- 5	8	0
	- 7a.	41	3	2	53		- 2	7	3
	- 17a	29	1	4	40		- 3	6	0
	- 19a	39	1	5	49		- 6	7	0
	- 21a	43	1	5	70		- 1	9	0
	- 24a	60	2	4	47		- 5	7	0
	- 27a	28	1	4	67		- 3	6	0
	- 27b	74	3	3	67		- 3	8	0
	- 27c	65	3	3	58		- 6	8	0
	- 30a	19	1	3	54		- 2	8	5
	- 31a	60	3	3	66		- 4	9	6
	- 34a	21	2	2	31		- 5	9	8
	- 35a	40	1	5	73		- 5	8	0
	- 38a	30	5	1	33		- 1	9	6
	- 38b	35	3	3	16		- 2	7	*
	- 39c	33	2	4	56		- 3	8	7
	- 39d	16	2	2	27		- 1	7	0
	- 40a	66	3	2	34		- 4	5	0
	- 42a	3	1	0	18		- 1	5	0
	- 43a	33	4	1	31		- 1	8	0
	- 45a	67	2	4	54		- 2	8	0
	- 46b	44	2	4	57		- 5	8	0

TABLE III CONTINUED

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8
- 48a	22	2	2	50	- 2	7	4
- 49a	53	4	2	50	- 5	7	0
- 49b	18	2	1	52	- 3	8	0
Total		138	154			356	67

* Although ring formation was not observed in these families, a new translocation was detected from the test-cross results (Table VI).

TABLE IV. The percent fertility of parent X_1 spikes and selected X_2 plants, the segregation for fertility in X_2 and the frequency of ring formation in X_3, of those lines in which new translocations were detected.

X_3 line designation		Percent fertility of parent X_1 spikes (available only for 5903)	Number of partially sterile X_2 plants	Number of fertile X_2 plants	Percent fertility of the X_2 plant selected for growing X_3	Number of X_3 plants cytologically examined	Number of X_3 plants with α 04
T(1-7)(2-3)a	- 24-3		4	2	52	3	1
T(3-4)(6-7)b	- 6-1		1	5	59	6	5
T(2-5)(3-4)a	- 6-3		3	1	33	6	2
	- 8-2		6	0	42	6	3
T(1-5)(2-4)a	- 5-3		5	1	55	3	*
T1-5c	- 3-3		2	2	49	6	3
T2-4a	- 2-5		3	3	25	7	1
5903	- 2b-1	32	2	4	51	8	4
	- 3a-4	31	1	4	58	13	9
	- 7a-2	41	3	2	53	7	3
	-30a-2	19	1	3	54	8	5
	-31a-4	60	3	3	66	9	6
	-34a-5	21	2	2	31	9	8
	-38a-1	30	5	1	33	9	6
	-38b-2	35	3	3	16	7	*
	-39c-3	33	2	4	56	8	7
	-48a-2	22	2	2	50	7	4
Total	17		48	42		122	67

* Although ring formation was not observed in these families, new translocations were detected from the test-cross results (Table VI).

TABLE V. The relationship between partial sterility in X_1 and X_2, and the frequency of detectable translocations in X_3.

Translocation stock	Number of partially sterile X_1 spikes	X_2 families segregating for fertility		X_3 families in which translocations were detected		
		Numbers	Percent of X_1 partially sterile spikes	Numbers	Percent of X_1 partially sterile spikes	Percent of X_2 families segregating for fertility
T(1-7)(2-3)a	17	7	41.2	1	5.9	14.3
T(3-4)(6-7)b	7	2	28.6	1	14.3	50.0
T(2-5)(3-4)a	11	5	45.4	2	18.2	40.0
T(1-5)(2-4)a	10	2	20.0	1	10.0	50.0
T1-5c	26	10	38.4	1	3.8	10.0
T2-4a	5	2	40.0	1	20.0	50.0
5903	62	27	43.5	10	16.1	37.0
Total	138	55	39.8	17	12.3	30.9

progenies. Only one (5903-39c) was found to carry a new translocation.

Considerable range in fertility was observed in the partially sterile X_1 spikes (3 to 74 per cent) and X_2 plants (16 to 73 per cent), but there was no relationship between the per cent fertility and the presence of translocations (Tables III and IV). Therefore, the actual degree of sterility could not be used in selection for translocations. Considering the fact that fertility in a translocation heterozygote is dependent on such factors as type of orientation at MI, length of the interstitial segment, and the frequency of cross-overs (18), these results are not surprising.

Theoretically, a translocation heterozygote on selfing produces half fertile and half partially sterile progenies, the latter being characterized by a ring formation at MI. The data presented in Table IV are in agreement with this expectation. Out of a total of 122 X_3 plants examined cytologically, 67 plants showed a 04, and 55 plants showed 7^{II}. The deviation from 1:1 ratio was found statistically to be nonsignificant (Chi-square = 1.18). Similarly in the X_2 families segregating for fertility, the ratio of partially sterile to fertile plants was approximately 1:1, the Chi-square being 0.4 and nonsignificant.

In Table V it can be seen that out of a total of 138 X_2 families grown, 55 families (39.8 per cent) were marked as segregating for fertility. Partial sterility in the remaining 83 spikes, therefore, could be due to other nonheritable factors (49). A total of 17 out

of the 55 families selected, representing 12.3 per cent of the X_2 families grown and 30.9 per cent of the families selected, was found to carry a new translocation. These results confirm the usefulness of partial sterility as a marker for possible translocations. Out of the 17 families, ring formation was observed only in 15 families (Tables III and IV), whereas in two families T(1-5)(2-4)a-5-3 and 5903-38b-2, evidence that a translocation occurred, was obtained from the test-cross results involving fertile plants of these families and Montcalm (Table VI). On this basis, although partial sterility in the remaining 38 families could be due to causes other than translocations, such as viable deficiencies and genic mutations, it appears that at least some of these families do carry a translocation, which could not be detected cytologically by a ring formation.

A considerable difference exists in the frequency of new translocations between different stocks (Table V). In the stocks T1-5c and T(1-7)(2-3)a, 10 and 14.3 per cent respectively of the X_2 families segregating for fertility were found to carry a new translocation, whereas in the remaining stocks the frequency of translocations ranged from 37 to 50 per cent. Many factors such as genotype, age, oxygen and moisture content of seeds have been reported to affect radiosensitivity of seeds (88,89). Although these factors were not controlled, it is doubtful if pronounced differences would have been present in these stocks, since they had been stored under the same conditions and the seed sources were of the same age. Therefore, no definite reasons could be attributed

TABLE VI. Metaphase I Configurations in the F_1's of crosses between fertile X_3 or X_4 plants, Montcalm and the appropriate parental translocation stock.

Fertile plant designation Parent stock	X_1	X_2	X_3	X_4	MI Configuration in crosses with Montcalm	Parent stock	Interpretation
T(1-7)(2-3)a	- 14 -	1 -	1 -		$204 + 3^{II}$ (3)*	-	Homozygous for the original translocations
		2 -	1		$204 + 3^{II}$ (1)	7^{II} (5)*	" "
		4 -	1		$204 + 3^{II}$ (3)	7^{II} (1)	" "
		5 -	1		$204 + 3^{II}$ (2)	7^{II} (2)	" "
	- 16 -	1 -	1		-	7^{II} (2)	" "
		2 -	1		$204 + 3^{II}$ (2)	7^{II} (2)	" "
		3 -	1		-	7^{II} (1)	" "
		4 -	1		$204 + 3^{II}$ (1)	-	" "
	- 18 -	4 -	5		$204 + 3^{II}$ (1)	7^{II} (1)	" "
		4 -	9		$204 + 3^{II}$ (2)	7^{II} (1)	" "
	- 21 -	1 -	2		-	7^{II} (1)	" "
		1 -	5		$204 + 3^{II}$ (1)	7^{II} (1)	" "
	- 24 -	2 -	1		$304 + 1^{II}$ (2)	$04+5^{II}$ (2)	Homozygous for a new translocation
		6 -	1		$204 + 3^{II}$ (3)		Homozygous for the original translocations
		3 -	2		$304 + 1^{II}$ (2)	$04+5^{II}$ - (2)	Homozygous for a new translocation
		3 -	3		$204 + 3^{II}$ (1)	-	Homozygous for the original translocations
	- 25 -	6 -	1		$204 + 3^{II}$ (1)	-	" "
		6 -	2		$204 + 3^{II}$ (4)	7^{II} (2)	" "
	- 26 -	6 -	11		$204 + 3^{II}$ (3)	7^{II} (3)	" "
T(3-4)(6-7)b	- 1 -	1 -	1		-	7^{II} (1)	" "
		4 -	1		$204 + 3^{II}$ (1)	7^{II} (2)	" "
		5 -	1		$204 + 3^{II}$ (2)	7^{II} (2)	

TABLE VI CONTINUED

T(3-4)(6-7)b	- 1 - 6 - 1	204 + 3^{II} (1)	-	Homozygous for the original translocations
	- 6 - 1 - 6	08 + 3^{II} (2)	-	Homozygous for a new translocation
	- 1 - 1	-	7^{II} (1)	Homozygous for the original translocations
	- 1 - 5 - 1	204 + 3^{II} (2)	-	" "
	- 1 - 5 - 5	08 + 3^{II} (2)	04+5^{II} (2)	Homozygous for a new translocation
	- 1 - 5 - 8	08 + 3^{II} (2)	04+5^{II} (2)	" "
T(2-5)(3-4)a	- 4 - 1 - 1	04 + 5^{II} (2)	06+04+2^{II}(7)	Unexpected
	- 3 - 1	04 + 5^{II} (6)	06+04+2^{II}(7)	"
	- 4 - 2	04 + 5^{II} (4)	06+04+2^{II}(3)	"
	- 6 - 1	04 + 5^{II}(4)	06+04+2^{II}(8)	"
	- 6 - 3 - 2	304 + 1^{II} (1)	04+5^{II} (2)	Homozygous for a new translocation
	- 3 - 3	304 + 1^{II} (2)	04+5^{II} (1)	" "
	- 8 - 2 - 1	06+04+1^{II}(1)	04+5^{II} (1)	" "
	- 10 - 1 - 1	204 + 3^{II} (2)	7^{II} (2)	Homozygous for the original translocations
	- 5 - 2	204 + 3^{II} (3)	7^{II} (2)	" "
	- 5 - 6	204 + 3^{II} (2)	-	" "
	- 11 - 2 - 1	204 + 3^{II} (1)	7^{II} (1)	" "
	- 4 - 2	204 + 3^{II} (2)	-	" "
	- 6 - 1	204 + 3^{II} (2)	7^{II} (1)	" "
T(1-5)(2-4)a	- 5 - 2 - 1	204 + 3^{II} (2)	7^{II} (1)	" "
	- 6 - 1	-	7^{II} (1)	" "
	- 3 - 1	06+04+2^{II}(6)	04+5^{II} (2)	Homozygous for a new translocation
	- 3 - 2	204 + 3^{II} (1)	-	Homozygous for the original translocations
	- 3 - 3	204 + 3^{II} (2)	-	" "
	- 6 - 3 - 1	204 + 3^{II} (2)	7^{II} (2)	" "
	- 4 - 1	204 + 3^{II} (2)	7^{II} (2)	" "
	- 5 - 1	204 + 3^{II} (3)	7^{II} (1)	" "
Tl-5c	- 3 - 1 - 1	04 + 5^{II} (2)	-	" "
	- 3 - 4	06 + 4^{II} (2)	04+5^{II} (2)	Homozygous for a new translocation

TABLE VI CONTINUED

T1-5c	- 3 - 3 - 5	$06 + 4^{II}$ (2)	$04+5^{II}$ (2)	Homozygous for a new translocation
	- 3 - 8	$06 + 4^{II}$ (2)	$04+5^{II}$ (1)	" "
	- 3 - 9	$04 + 5^{II}$ (2)	-	Homozygous for the original translocation
	- 4 - 4 - 1	$04 + 5^{II}$ (1)	-	" "
	- 6 - 1 - 1	$04 + 5^{II}$ (1)	-	" "
	- 3 - 1	$04 + 5^{II}$ (1)	-	" "
	- 5 - 1	$04 + 5^{II}$ (3)	-	" "
	- 7 - 1 - 1	$04 + 5^{II}$ (2)	-	" "
	- 12 - 1 - 1	$04 + 5^{II}$ (1)	-	" "
	- 4 - 1	$04 + 5^{II}$ (1)	-	" "
	- 5 - 1	$04 + 5^{II}$ (2)	-	" "
	- 14 - 3 - 1	$04 + 5^{II}$ (1)	-	" "
	- 4 - 1	$04 + 5^{II}$ (1)	-	" "
	- 15 - 1 - 1	$04 + 5^{II}$ (2)	-	" "
	- 5 - 1	$04 + 5^{II}$ (1)	-	" "
	- 16 - 4 - 1	$04 + 5^{II}$ (1)	-	Homozygous for the original translocation
	- 4 - 5	$04 + 5^{II}$ (3)	-	" "
T2-4a	- 1 - 3 - 2	-	7^{II} (1)	" "
	- 5 - 1	$04 + 5^{II}$ (2)	-	" "
	- 6 - 1	$04 + 5^{II}$ (1)	7^{II} (2)	" "
	- 2 - 1 - 1	$04 + 5^{II}$ (1)	7^{II} (1)	" "
	- 2 - 1	-	7^{II} (1)	" "
	- 6 - 1	-	7^{II} (2)	" "
	- 5 - 3	$204 + 3^{II}$ (2)	-	Homozygous for a new translocation
	- 5 - 1 - 1	$204 + 3^{II}$ (2)	-	" "
	- 5 - 5 - 1	$204 + 3^{II}$ (2)	-	" "
	- 5 - 5 - 2	$204 + 3^{II}$ (2)	-	" "

TABLE VI CONTINUED

5903	- 2b - 2 - 1	08 + $3^{II}_{II}(3)$	0	Loss of a translocation
	- 3 - 1	010 + $2^{II}_{II}(2)$	0	Homozygous for the original translocations
	- 4 - 1	08 + $3^{II}_{II}(2)$	0	Loss of a translocation
	- 6 - 1	010 + $2^{II}_{II}(1)$	0	Homozygous for the original translocations
	- 1 - 4	010 + $2^{II}_{II}(2)$	0	" "
	- 1 - 12	010 + $2^{II}(2)$	0	" "
	- 3a - 2 - 1	010 + $2^{II}_{II}(3)$	0	" "
	- 3 - 1	010 + $2^{II}(3)$	0	" "
	- 5 - 1	-	7^{II} (2)	" "
	- 4 - 15	010 + $2^{II}_{II}(3)$	0	" "
	- 4 - 16	010 + $2^{II}_{II}(2)$	0	" "
	- 4 - 21	010 + $2^{II}_{II}(3)$	0	" "
	- 4 - 27	010 + $2^{II}(3)$	0	" "
	- 7a - 3 - 1	010 + $2^{II}_{II}(3)$	0	" "
	- 4 - 1	012 + $1^{II}_{II}(2)$	0	Homozygous for a new translocation
	- 2 - 3	010 + $2^{II}_{II}(2)$	0	Homozygous for the original translocations
	- 2 - 4	010 + $2^{II}(2)$	0	" "
	- 24a - 6 - 1	010 + $2^{II}(1)$	0	" "
	- 30a - 1 - 1	010 + $2^{II}_{II}(2)$	0	" "
	- 3 - 1	010 + $2^{II}_{II}(3)$	0	" "
	- 4 - 1	010 + $2^{II}_{II}(2)$	0	" "
	- 2 - 1	012 + $1^{II}_{II}(2)$	0	Homozygous for a new translocation
	- 2 - 2	012 + $1^{II}_{II}(2)$	0	" "
	- 2 - 143	012 + $1^{II}(3)$	0	" "
	- 31a - 2 - 1	012 + $1^{II}_{II}(4)$	0	" "
	- 3 - 1	010 + $2^{II}_{II}(3)$	0	Homozygous for the original translocations
	- 6 - 1	010 + $2^{II}_{II}(2)$	0	" "
	- 4 - 3	010 + $2^{II}_{II}(3)$	0	" "
	- 4 - 152	010 + $2^{II}_{II}(2)$	0	" "
	- 4 - 156	012 + $1^{II}(3)$	0	Homozygous for a new translocation
	- 34a - 1 - 1	010 + $2^{II}(1)$	0	Homozygous for the original translocations

TABLE VI CONTINUED

5903	- 34a - 2 - 1	010 + 2^{II} (3)	0	Homozygous for the original translocations	
	- 3 - 1	010 + 2^{II} (2)	0	"	"
	- 3 - 2	010 + 2^{II} (2)	0	"	"
	- 3 - 3	010 + 2^{II} (2)	0	"	"
	- 3 - 5	010 + 2^{II} (3)	0	"	"
	- 35a - 1 - 1	010 + 2^{II} (1)	0	"	"
	- 38a - 5 - 1	08+04+1^{II} (3)	0	Homozygous for a new translocation	
	- 1 - 2	010 + 2^{II} (2)	0	Homozygous for the original translocations	
	- 1 - 3	010 + 2^{II} (1)	0	"	"
	- 1 - 188	010 + 2^{II} (2)	0	"	"
	- 38b- 4 - 1	012 + 1^{II} (3)	0	Homozygous for a new translocation	
	- 39c- 1 - 1	010 + 2^{II} (3)	0	Homozygous for the original translocations	
	- 2 - 1	010 + 2^{II} (3)	0	"	"
	- 4 - 1	010 + 2^{II} (3)	0	"	"
	- 5 - 1	010 + 2^{II} (3)	0	"	"
	- 3 - 1	012 + 1^{II} (3)	0	Homozygous for a new translocation	
	- 3 - 2	012 + 1^{II} (2)	0	"	"
	- 48a- 1 - 1	010 + 2^{II} (3)	0	Homozygous for the original translocations	
	- 4 - 1	010 + 2^{II} (3)	0	"	"
	- 2 - 2	010 + 2^{II} (2)	0	"	"
	- 2 - 3	010 + 2^{II} (1)	0	"	"

* The bracketed numbers represent the number of F_1 plants cytologically examined.

0 Because of difficulty in crossing, these crosses could not be obtained (page 39)

for these differences. In view of the small population studied, these differences may have been even fortuitous.

(b) Identification of lines homozygous for new translocations

The X_3 or X_4 fertile plants were crossed with Montcalm and the appropriate parental stock to identify lines homozygous for new translocations. In general, crossing was quite successful, except where 5903 or lines derived from it were used as a female parent. In the fall of 1962, a total of 150 spikes from the X_3 or X_4 fertile plants of 5903 were crossed with Montcalm and 5903, but only one cross was successful. Crossing had to be repeated, this time using Montcalm as a female parent, and therefore analysis of the fertile plants had to be based on results obtained in crosses with Montcalm alone (Table VI). A similar failure was encountered by Wang (144) in crosses using a line 4256-1-6-1 (later designated as 161), as a female parent, and was considered due to the damage caused to the pistil in the process of emasculation. As mentioned earlier, 5903 was obtained from the above line by irradiation. The explanation by Wang was not considered satisfactory, and therefore other possible reasons were subsequently investigated. This information, and the conclusions reached will be presented under general discussion.

The F_1's of all crosses between fertile X_3 or X_4 plants, Montcalm and the parental stock were grown, and examined cytologically at MI. The results and interpretations are presented in Table VI. It can be seen from this table that out of a total of 17 translocations induced

(Table IV) 14 were established in a homozygous condition. These lines are listed in Table VII and given a new designation. The MI configurations in the F_1's of crosses between some of these lines and Montcalm are illustrated in Figures 2 to 11. In the remaining three cases (Table VI) viz. 5903-3a-, 5903-34a- and 5903-48a-, configurations other than already existing ($0\text{I}0+2^{\text{II}}$) were not observed, although the number of fertile plants tested was seven, six and four, respectively. The exact reason for this is not known. A possible reason, however, may be that in these cases a translocation occurred between the chromosomes already involved in the translocation complex, which was detectable in the form of a ring in the PMC's. Evidently such a translocation will not affect the existing level of translocation complex, and therefore would not be effective in the synthesis of a complete translocation stock. That this occurred, could be confirmed by crossing these lines with 5903 and studying PMC's in the hybrids. A $0\text{I}4+5^{\text{II}}$ configuration at MI would prove this prediction, since normally a 7^{II} would be expected in such a hybrid. Unfortunately these crosses, though attempted, were not successful as mentioned earlier.

The results in Table VI in general, are as expected, and therefore only the few exceptions will be discussed here. The lines T(2-5)(3-4)a-4-1-1, T(2-5)(3-4)a-4-3-1, T(2-5)(3-4)a-4-4-2 and T(2-5)(3-4)a-4-6-1 showed a $0\text{I}4+5^{\text{II}}$ in crosses with Montcalm, and $0\text{I}6+0\text{I}4+2^{\text{II}}$ in crosses with the parental stock at MI instead of the expected configurations of $2\text{I}4+3^{\text{II}}$ and 7^{II} respectively. This could indicate that a back translocation occurred in both the original translocations in such a way

TABLE VII. Lines which have been identified to be homozygous for new translocations.

Line designation		Homozygous for	New designation proposed*
T(1-7)(2-3)a	- 24 - 2 - 1	304 + 1^{II}	6201
	- 3 - 2	304 + 1^{II}	
T(3-4)(6-7)b	- 6 - 1 - 6	08 + 3^{II}	6202
	- 1 - 5 - 5	08 + 3^{II}	
	- 1 - 5 - 8	08 + 3^{II}	
T(2-5)(3-4)a	- 6 - 3 - 2	304 + 1^{II}	6203
	- 3 - 3	304 + 1^{II}	
	- 8 - 2 - 1	06 + 04 + 2^{II}	6204
T(1-5)(2-4)a	- 5 - 3 - 1	06 + 04 + 2^{II}	6205
T(1-5)c	- 3 - 3 - 4	06 + 4^{II}	6206
	- 3 - 5	06 + 4^{II}	
	- 3 - 8	06 + 4^{II}	
T(2-4)a	- 2 - 5 - 3	204 + 3^{II}	6301
	- 5 - 1 - 1	204 + 3^{II}	
	- 5 - 5 - 1	204 + 3^{II}	
	- 5 - 5 - 2	204 + 3^{II}	
5903	- 2b - 2 - 1	08 + 3^{II}	6302
	- 4 - 1	08 + 3^{II}	
	- 7a - 4 - 1	012 + 1^{II}	6303
	- 30a - 2 - 1	012 + 1^{II}	6304
	- 2 - 2	012 + 1^{II}	
	- 2 - 143	012 + 1^{II}	
	- 31a - 2 - 1	012 + 1^{II}	6305
	- 4 - 156	012 + 1^{II}	
	- 38a - 5 - 1	08 + 04 + 1^{II}	6306

TABLE VII CONTINUED

- 38b	- 4 - 1	$012 + 1^{II}$	6307
- 39c	- 3 - 1	$012 + 1^{II}$	6308
	- 3 - 2	$012 + 1^{II}$	

* Lines, originating from the same partially sterile X_1 spike should be identical in chromosome constitution, and therefore have been proposed a common designation. In new designations, the first two digits represent the year of synthesis.

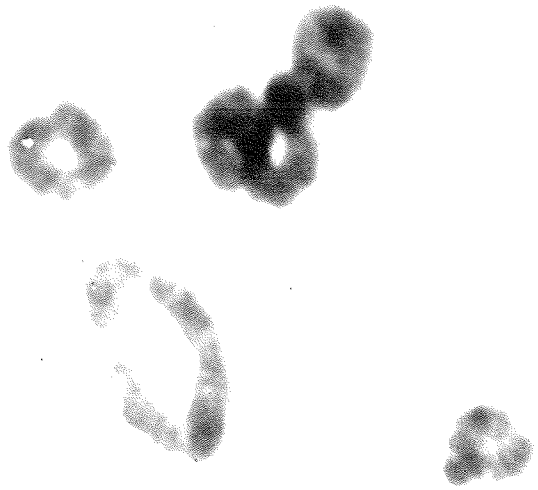

FIGURE 2: A 0_4+5^{II} at metaphase I in the hybrid from the cross T1-5c-3-3-4 x T1-5c.

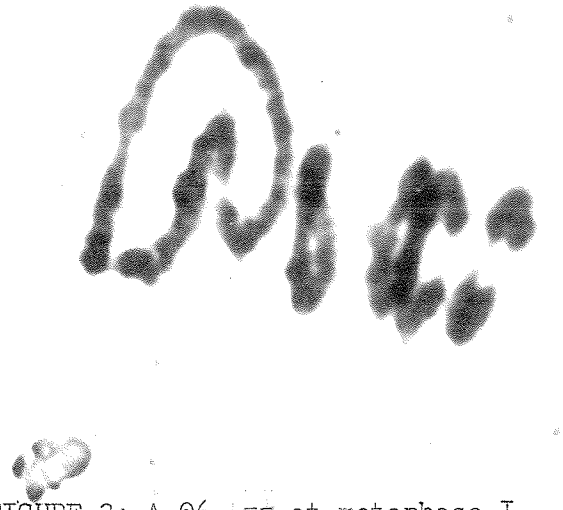

FIGURE 3: A 0_6+4^{--} at metaphase I in the hybrid from the cross T1-5c-3-3-4 x Montcalm.

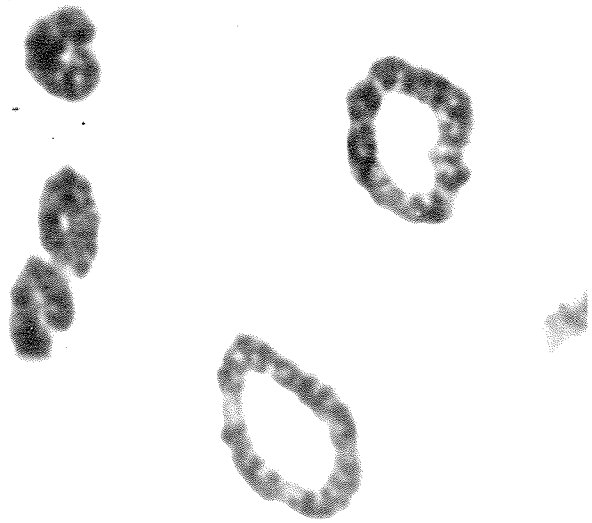

FIGURE 4: A 20_4+3^{II} at metaphase I in the hybrid from the cross T2-4a-2-5-3 x Montcalm.

FIGURE 5: A 0_8+3^{II} at metaphase I in the hybrid from the cross 5903-2b-2-1 x Montcalm.

FIGURE 6: A $\odot 6+\odot 4+2^{II}$ at metaphase I in the hybrid from the cross T(1-5)(2-4)a-5-3-1 x Montcalm.

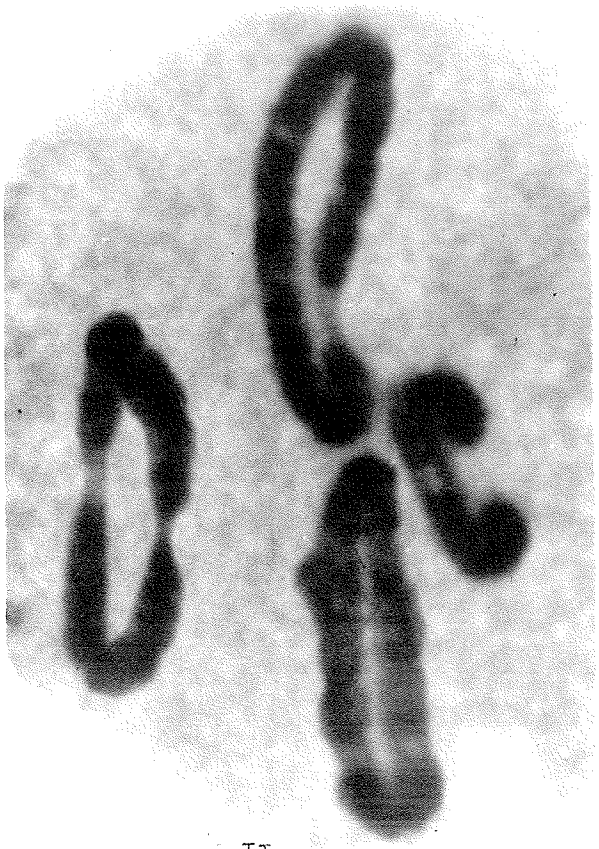

FIGURE 7: $3\odot 4+1^{II}$ at metaphase I in the hybrid from the cross T(1-7)(2-3)a-24-3-2 x Montcalm.

FIGURE 8: A $\odot 8+\odot 4+1^{II}$ at metaphase I in the hybrid from the cross 5903-38a-5-1 x Montcalm.

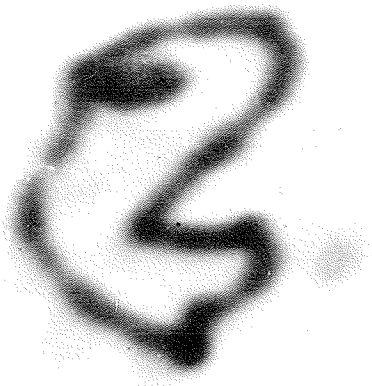

FIGURE 9: A $\odot 12+1^{II}$ at metaphase I in the hybrid from the cross 5903-30a-2-1 x Montcalm.

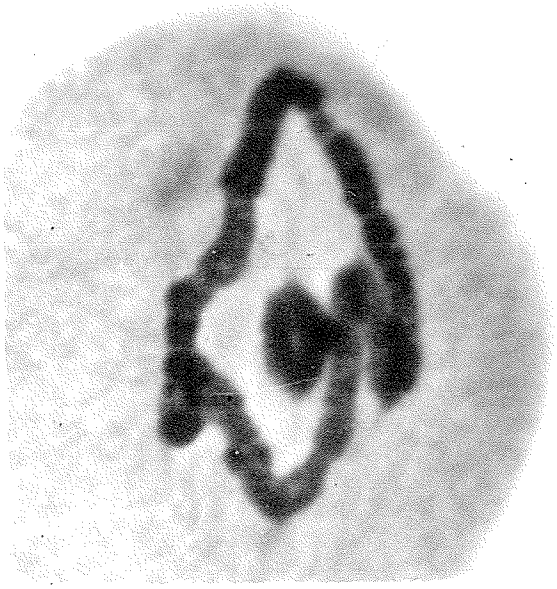

FIGURE 10: A $\odot 12+1^{II}$ at metaphase I in the hybrid from the cross 5903-31a-2-1 x Montcalm.

FIGURE 11. A $\odot 12+1^{II}$ at metaphase I in the hybrid from the cross 5903-38b-4-1 x Montcalm.

that in one of them both the translocated chromosomes were restored to the normal karyotype, whereas in the other translocation, one chromosome was restored to normal and the other chromosome exchanged a segment with a chromosome not involved in the translocations. Such a change would result in loss of both the original translocations, and synthesis of a new translocation. If this is true, it is necessary to visualize simultaneous breaks in five chromosomes. Further, these breaks would have to occur in both homologues at identical positions, since none of the four X_2 fertile families tested were homozygous for the original configurations. These conditions, and also the fact that ring formation was not observed in the corresponding X_3 families segregating for fertility (Table III), (although it's possibility cannot be ruled out entirely because only two plants were examined cytologically), indicate that such a change is unlikely to have occurred. The possibility of an error in crossing or in planting the material was also ruled out by repeating the crosses and confirming the results. Therefore, no definite explanation could be suggested at this stage. It appears that some error in handling the material in an earlier stage of the program may have been responsible for the results obtained.

The lines T(1-5)(2-4)a-5-3-1 and 5903-38b-4-1 showed synthesis of a new translocation, being homozygous for $0{+}0{+}4{+}2^{II}$ (Figure 6) and $0{+}2{+}1^{II}$ (Figure 11) respectively. This was unexpected since ring formation was not observed in the corresponding X_3 families segregating for fertility (Table IV). This is particularly true for line 5903-38b-2, where seven plants were cytologically examined. The exact reason for a failure

of the new translocation to show as a ring at MI is not known. A possible reason, however, could be an incorrect identification of the partially sterile X_2 plant used for growing X_3 progenies, which were cytologically examined. In addition, this could also result from an environmental effect since the two populations were grown in different environments and environment is known to affect chiasma frequency (105).

The lines 5903-2b-2-1 (Figure 5) and 5903-2b-4-1 were found to be homozygous for $08+3^{II}$ instead of $010+2^{II}$ as expected. This indicates that a back translocation of the type reported by Wang (144) and Chang (26) occurred in the original translocation complex. A more or less similar case of back translocation occurred in the line 5903-38a-5-1, identified to be homozygous for $08+04+1^{II}$ (Figure 8), whereas the parental stock was homozygous for $010+2^{II}$. Here one chromosome in the translocation complex apparently exchanged a segment with a third chromosome. This would require simultaneous breaks and exchanges among three chromosomes, two of which were already involved in the translocation complex. This would effectively restore one of the chromosomes to normal.

(c) Identification of the chromosomes involved in the new translocations

Out of the 14 newly synthesized translocation stocks (Table VII), four have been identified with respect to the chromosomes involved in the translocations. In addition, the chromosomes involved in the translocation complex of the stock 5903 were also identified. The results and inter-

pretations are presented in Table VIII (a,b,c,d,e).

The stock 5903 was homozygous for $\bigcirc 10+2^{II}$ (Figure 12). In the hybrids between 5903 and the testers T5-6a and T3-4a, two types of MI configurations ($\bigcirc 10+2^{II}$ and $\bigcirc 8+3^{II}$) were observed (Table VIIIa), indicating that chromosomes 3,4,5 and 6 were involved. The test-cross results involving the testers T1-7a, T1-5d and T1-5f indicated that chromosome 7 was involved. Therefore, it is concluded that 5903 is homozygous for translocations involving chromosomes 3-5-7-4-6.

The stock 6201 (Table VIIIb) was found to be homozygous for $3\bigcirc 4+1^{II}$ (Figure 7), and the known chromosomes involved in the translocations were (1-7) and (2-3). On crossing this line with the testers T1-6a and T1-5b, the hybrids showed $\bigcirc 8+\bigcirc 4+1^{II}$ configurations at MI, indicating that chromosomes 5 and 6 were involved in the new translocation, since chromosome 1 was already involved in one of the existing translocations. The results involving other testers support this conclusion. On this basis, line 6201 was found to be homozygous for translocations between chromosomes (1-7)(2-3) and (5-6).

The known chromosomes involved in the stock 6203 (Table VIIIc) were (2-5) and (3-4). The hybrid between this stock and the tester T1-6a at MI showed $2\bigcirc 4+3^{II}$ configuration, indicating that chromosomes 1 and 6 were involved in the new translocation. The results with other testers also support this conclusion. Therefore, 6203 is homozygous for translocations between chromosomes (1-6)(2-5) and (3-4).

The line 6204 (Table VIIId) was identified to be homozygous for $\bigcirc 6+\bigcirc 4+2^{II}$ (Figure 13), and the known chromosomes involved were (2-5) and

TABLE VIII(a,b,c,d,e). Metaphase I configurations in the test-cross hybrids involving the stocks 5903, 6201, 6203, 6204 and 6307, and different tester stocks.

a. Stock 5903, homozygous for $\odot 10+2^{II}$. The known chromosomes involved were 3-5-7.

Tester	MI Configuration	Translocated Chromosomes	Number of plants examined	Interpretation
T1-5d	$012 + 1^{II}$	either '1' or '5'	3	
T1-5f	$012 + 1^{II}$	" "	4	
T5-6a	$010 + 2^{II}$) $08 + 3^{II}$)	both '5' and '6'	2	
T1-7a	$012 + 1^{II}$	either '1' or '7'	3	Chromosomes involved are 3-5-7-4-6
T3-4a	$010 + 2^{II}$) $08 + 3^{II}$)	both '3' and '4'	3	
T2-3c	$012 + 1^{II}$	either '2' or '3'	3	
T2-3d	$012 + 1^{II}$	" "	2	
T2-4a	$012 + 1^{II}$	either '2' or '4'	2	

b. Stock 6201, homozygous for $304+1^{II}$. The known chromosomes involved were (1-7)(2-3)

T1-6a	$08+04+1^{II}$	both '1' and '6' (in two different rings)	2	
T1-5b	$08+04+1^{II}$	both '1' and '5' (in two different rings)	2	Chromosomes involved are (1-7)(2-3)(5-6)
T2-4a	06+04+04	either '2' or '4'	2	
T1-7a	$204 + 3^{II}$	both '1' and '7' (in the same ring)	2	
T3-4a	06+04+04	either '3' or '4'	2	

c. Stock 6203, homozygous for $304+1^{II}$. The known chromosomes involved were (2-5)(3-4)

T1-6a	$204 + 3^{II}$	both '1' and '6' (in the same ring	4	
T1-5b	$08+04+1^{II}$	both '1' and '5' (in two different rings)	3	Chromosomes involved are (2-5)(3-4)(1-6)

TABLE VIII CONTINUED

—C—

T2-4a	08+04+1^{II}	both '2' and '4' (in two different rings)	3	
T1-7a	06+04+04	either '1' or '7'	2	
T3-4a	204 + 3^{II}	both '3' and '4' (in the same ring)	2	

d. Stock 6204, homozygous for 06+04+2^{II}. The known chromosomes involved were (2-5)(3-4)

T1-5b	08+04+1^{II}	either '1' or '5'	2	
T2-4a	010 + 2^{II}	both '2' and '4' (in two different rings)	3	Chromosomes involved are (2-5-7) (3-4)
T1-7a	08+04+1^{II}	either '1' or '7'	3	
T3-4a	06 + 4^{II}	both '3' and '4' (in the same ring)	2	

e. Stock 6307, homozygous for 012+1^{II}. The known chromosomes involved were 3-5-7-4-6

T1-6a	014	either '1' or '6'	4	Chromosomes involved are 2-3-5-7-4-6
T1-2a	014	either '1' or '2'	2	

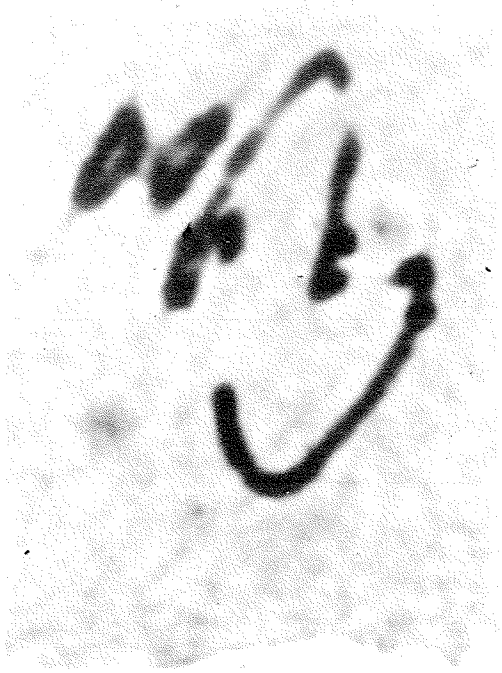

FIGURE 12: A $\odot 10+2^{II}$ at metaphase I in the hybrid from the cross 5903 x Montcalm.

FIGURE 13: A $\odot 6+\odot 4+2^{II}$ at metaphase I in the hybrid from the cross T(2-5) (3-4)a-8-2-1 x Montcalm.

FIGURE 14: A $\odot 10+\odot 4$ at metaphase I in the hybrid from the cross 5903 x T1-2a.

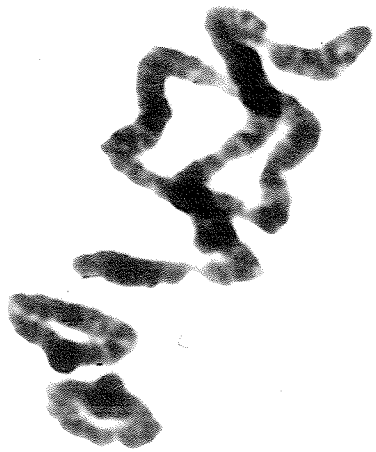

FIGURE 15: A $\odot 6+\odot 4+2^{II}$ at metaphase I in the hybrid from the cross (T3-5-7a x T1-2a)-1-9 x Montcalm.

(3-4). The test-cross results involving the tester T1-5b excluded the possibility of chromosome 1 being involved, since chromosome 5 was already involved in one of the original translocations. Results involving the tester T1-7a indicate that chromosome 7 was involved. Further, results using the tester T3-4a suggest that the new translocation was added to the translocation involving chromosomes 2 and 5. On this basis this stock has been identified as involving chromosomes (2-5-7) and (3-4).

The known chromosomes involved in the stock 6307 (Table VIIIe) homozygous for $0\frac{1}{2}+1^{II}$ (Figure 11), were 3-5-7-4-6, and therefore either chromosome 1 or 2 was expected to be added to the existing translocation complex. The test-cross results involving the testers T1-6c and T1-2a indicate that chromosome 2 was involved in the translocation. Therefore, this line is homozygous for translocations involving chromosomes 2-3-5-7-4-6.

Chang (26) pointed out that using a standard tester set for identification of multiple translocations, no information is obtained on their interchange pattern. Accordingly in the present study identification of the stocks 5903, 6204 and 6307 does not provide any information on their interchange pattern.

II. Intercross Method

(a) Direct approach by crossing 5903 and T1-2a

A total of 12 hybrid seeds, obtained from crossing 5903 and T1-2a were planted in September 1961. Meiosis was checked in two plants and

as expected a MI configuration of 010+04 (Figure 14) was observed.

1. Back crossing

Attempts to backcross the F_1 hybrid to 5903 were not successful. In the beginning, a reason for this failure was considered to be the absence of good pollen produced by the F_1 hybrid. But in later work, as mentioned earlier on page 39 difficulty was encountered in crosses using 5903 as a female parent, and therefore appears to be responsible for failure of this method.

2. F_2 analysis

The 12 hybrid plants obtained from crossing 5903 and T1-2a were grown to maturity, and as expected (91,124) were found to be almost completely sterile. A total of only 15 F_2 seeds were obtained. Mitotic chromosome numbers of these seeds were checked in root tips. Eight seedlings gave a normal chromosome count (2n=14), four were found to be trisomics (2n=15),and one seed did not germinate. Chromosome counts could not be obtained in the remaining two seeds, but the plants from these were almost dwarf and completely sterile, indicating that perhaps they also were aneuploids.

Meiosis in the PMC's of the eight normal plants was studied; four of these showed 010+04, two showed $04+5^{II}$ and two showed 7^{II} configurations at MI. Theoretically, these different types are expected to be in equal frequency but the population was too small to verify the hypothesis. On test-crossing the plants with 7^{II} and the fertile progenies from those with $04+5^{II}$, none was found to carry the desired chromosome constitution.

(b) Bridging approach

Approximately 30 hybrid seeds, obtained from crossing T3-5-7a and T1-2a were planted in the fall of 1961. Meiosis was studied in two plants, and as expected $\circ 6+\circ 4+2^{II}$ configuration at MI was observed.

A total of 50 seeds from a single F_1 hybrid plant was planted. Among these, ten plants were found to be dwarfs and/or sterile, and perhaps were aneuploids. The frequency distribution of MI configurations in the remaining 40 plants is given in Table IX.

TABLE IX. Frequency distribution of MI configurations in the F_2 population of the cross T3-5-7a x T1-2a.

MI Configurations	Number of Plants
7^{II}	9
$\circ 4 + 5^{II}$	8
$\circ 6 + 4^{II}$	5
$\circ 6 + \circ 4 + 2^{II}$	18
Dwarfs and/or sterile	10
Total	50

Theoretically, the different types of MI configurations are expected to be in equal frequency, but data in Table IX indicate a considerably higher frequency of the F_1 type plants ($\circ 6+\circ 4+2^{II}$). A similar trend was indicated in the results obtained from the 5903 x T1-2a hybrid (page 53). Burnham et al. (21) reported 20 plants with $\circ 8+3^{II}$, 9 plants

with 7^{II} and one plant with $0\!4\!+\!5^{II}$ in the F_2 population of a hybrid with $0\!8\!+\!3^{II}$. These results appear to indicate the existence of some type of competition between normal chromosome genotypes and translocated chromosome genotypes either at the gametic or at the zygotic level, and also the better competitive ability of the translocated chromosome genotypes. The limited information available in the present study, however, does not warrant any such conclusion, but this phenomenon appears to be worthy of further study.

The F_2 plants, which showed 7^{II} at MI were completely fertile. The MI configurations of hybrids involving these plants and Montcalm are presented in Table X. It can be seen from this Table that plants (T3-5-7a x T1-2a)-1-6, (T3-5-7a x T1-2a)-1-8 and (T3-5-7a x T1-2a)-1-9 gave a configuration of $0\!6\!+\!0\!4\!+\!2^{II}$ (Figure 15) at MI, and therefore were homozygous for both the translocations. These plants were expected to be identical in chromosomal constitution. This was confirmed by making crosses between them and studying meiosis in the hybrids. As expected a configuration of 7^{II} was observed at MI.

One of these lines (T3-5-7a x T1-2a)-1-9, designated as 6309 was selected, and crossed with 5903. A total of 11 hybrid plants were grown. Meiosis was studied in the PMC's of one of these plants, and as expected at MI $0\!6\!+\!0\!4\!+\!2^{II}$ configuration was observed. The data on fertility of these plants are presented in Table XI. It can be seen from this table that the fertility of individual plants varies from 3.5 to 33.3 per cent with an average fertility of 13.9 per cent. This shows a considerable improvement in fertility as expected (62), over the hybrid 5903xT1-2a,

TABLE X. MI configurations in the F_1 hybrids, obtained from crossing F_2 fertile plants of the cross T3-5-7a x T1-2a and Montcalm.

F_2 fertile plant designation	MI configuration	Number of Plants Cytologically Examined	Interpretation
T3-5-7axT1-2a-1-6	$0\bar{6}+0\bar{4}+2^{II}$	2	Homozygous for both translocations.
-1-7	7^{II}	3	Homozygous for normal chromosome constitution.
-1-8	$0\bar{6}+0\bar{4}+2^{II}$	2	Homozygous for both translocations.
-1-9	$0\bar{6}+0\bar{4}+2^{II}$	2	" "
-1-10	$0\bar{4} + 5^{II}$	4	Homozygous for one translocation.
-1-25	$0\bar{4} + 5^{II}$	4	" "
-1-29	7^{II}	3	Homozygous for normal chromosome constitution.
-1-35	$0\bar{6}+ 4^{II}$	1	Homozygous for one translocation.
-1-42	$0\bar{4}+ 5^{II}$	5	" "

TABLE XI. Percent fertility of F_1 hybrid plants obtained from crossing the stock 6309 with 5903.

F_1 hybrid plant designation	Per cent Fertility
(6309x5903)- 1	6.6
- 2	33.3
- 3	3.5
- 4	16.7
- 5	20.8
- 6	13.3
- 7	5.1
- 8	7.7
- 9	12.8
-10	33.3
-11	23.3
Average	13.9

which is almost completely sterile (see also Figure 16).

Approximately 130 F_2 seeds have been obtained from these hybrid plants, which will be analyzed further in the hope of obtaining a line homozygous for 010+04.

III. Fertility of Translocation Heterozygotes

Results in terms of per cent fertility of translocation heterozygotes according to their MI configurations are presented in Table XII. In this table, the average per cent fertility of translocation heterozygotes reported by Shih and Shebeski (124) have also been included for comparison. The results obtained in the present study could not be considered very reliable, since the material was grown at different times and in different environments.

Results in Table XII indicate a wide range in fertility between translocation heterozygotes involving the same number of chromosomes as well as between plants within the same translocation heterozygote. Burnham (16) pointed out that in a species with alternate chromosome separation, fertility is dependent on the amount of crossing-over, which occurs in the interstitial segment. Further, in barley fertility is extremely sensitive to environment, and therefore, these results are not surprising.

In general, the results obtained in the present study are in agreement with those reported by Shih and Shebeski (124). However, there are a few exceptions. For example, the fertility of plants with $06+4^{II}$ and 06+04+04 was considerably higher in the present study than

	1	2	3
Per cent fertility	0.0	10.0	33.3

FIGURE 16: Improvement in fertility in the 6309 x 5903 hybrid over the 5903 x T1-2a hybrid. (In the above figure the empty florets have been removed to increase contrast). The hybrid 5903 x T1-2a (1) was almost completely sterile, whereas the hybrid 6309 x 5903 shows improvement in fertility (2 and 3).

TABLE XII. Percent fertility of translocation heterozygotes at different levels of chromosome participation.

Source		Number of plants evaluated	Fertility Average	Fertility Range
Metaphase I Configuration : 0_4+5^{II}				
T(3-4)(6-7)b-6-1-5*	X T(3-4)(6-7)b	6	52.5	37.0 - 70.0
T(2-5)(3-4)a-4 *	X Montcalm	13	62.2	38.5 - 75.0
T(2-5)(3-4)a-8-2-1	X T(2-5)(3-4)a	4	60.5	45.8 - 81.8
T(1-5)(2-4)a-5-3-1	X T(1-5)(2-4)a	2	46.3	40.7 - 51.8
T1-5c-3-3*	X T1-5c	4	37.6	28.0 - 53.3
T1-5c-3-3-9	X Montcalm	3	51.6	49.0 - 54.9
(4256-1	X C1310)-F_2's	8	59.6	35.5 - 77.7
Plants with $0_4 + 5^{II}$		40	54.7 (54.7)+	37.6 - 62.2 (38.5 - 73.6)+
Metaphase I Configuration : 0_6+4^{II}				
T(2-5)(3-4)a-8-2-1	X C1432	2	44.6	44.0 - 45.2
T1-5c-3-3*	X Montcalm	8	38.4	30.0 - 48.8
(4256-1	X C1310)-F_2's	3	48.2	41.6 - 63.6
Plants with $0_6 + 4^{II}$		13	44.6 (24.7)+	38.4 - 48.2 (15.8 - 37.8)+
Metaphase I Configuration : 2_04+3^{II}				
T(1-7)(2-3)a-24-3-2	X C1358	2	30.8	18.4 - 41.8
T(2-5)(3-4)a-6-3-3	X C1432	4	39.4	25.0 - 51.2
" " "	X C1483	4	30.0	8.3 - 38.4
T(1-5)(2-4)a-6-4-1	X Montcalm	3	33.3	16.7 - 55.5
T2-4a-2-5*	X Montcalm	8	18.4	3.6 - 39.3
Plants with 2_04+3^{II}		21	31.6 (33.4)+	18.4 - 39.4 (15.6 - 51.1)+
Metaphase I Configuration $0_6+0_4+2^{II}$				
T(2-5)(3-4)a-4*	X T(2-5)(3-4)a	13	25.6	20.0 - 37.0
T(2-5)(3-4)a-8-2-1	X Montcalm	1	20.6	-
T(1-5)(2-4)a-5-3-1	X Montcalm	4	22.7	14.3 - 31.2
161	X Montcalm	4	11.7	7.7 - 13.6
(4256-1	X C1310)-F_2's	8	28.5	12.8 - 38.5
6309	X 5903	11	13.9	5.1 - 33.3
Plants with $0_6+0_4+2^{II}$		41	21.9 (20.6)+	11.5 - 28.5 (16.7 - 27.9)+

TABLE XII CONTINUED

Metaphase I Configuration: $0{+}3^{II}$				
T(3-4)(6-7)b-6-1*	X Montcalm	5	13.6 (21.3)+	3.6 - 21.0 (14.3 - 29.8)+
Metaphase I Configuration: $0{+}2^{II}$				
T(2-5)(3-4)a-8-2-1	X C1420	1	3.3	-
5903	X Montcalm	2	4.3	(1.1 - 8.3)
$5903\text{-}X_3$ fertile families	X Montcalm	48	2.4	(0 - 10.6)
Plants with $0 + 2^{II}$		51	3.9 (14.6)+	2.4 - 4.3 (12.1 - 16.7)+
Metaphase I Configuration: $0{+}0{+}1^{II}$				
T(1-7)(2-3)a-24-3-2	X C1483	2	19.6	13.6 - 26.3
" " "	X C1385	2	13.6	4.8 - 21.7
T(2-5)(3-4)a-6-3-3	X C1420	3	13.1	9.5 - 22.2
T(2-5)(3-4)a-8-2-1	X C1385	4	5.7	2.0 - 7.8
" " "	X C1358	4	6.3	4.7 - 8.6
Plants with $0{+}0{+}1^{II}$		15	12.7 (8.8)+	5.7 - 19.6 (6.9 - 10.8)+
Metaphase I Configuration: 06+04+04				
T(1-7)(2-3)a-24-3-2	X C1420	1	25.0	-
" " "	X C1432	3	19.7	13.3 - 25.8
T(2-5)(3-4)a-6-3-3	X C1358	1	5.0	-
Plants with 06+04+04		5	17.2 (3.3)+	5.0 - 25.0 (0.9 - 5.8)+
Metaphase I Configuration: 010 + 04				
5903	X C1310	4	1.86	0 - 4.8
Metaphase I Configuration: $012 + 1^{II}$				
5903	X C1384	2	0.0	-
"	X C1336	3	0.0	-
"	X C1358	4	0.5	0 - 1.0
"	X C1420	3	1.1	0 - 1.6

TABLE XII CONTINUED

5903	X XT12	4	2.2	0 - 3.1
"	X Ert 47	4	4.3	1.6 - 5.6
5903-30a-2*	X Montcalm	12	0.0	-
5903 - 39c - 3 -2	X Montcalm	4	0.0	-
5903-38b-4	X Montcalm	4	0.0	-
Plants with $\bigcirc 12+1^{II}$		40	1.0	0 - 4.3

Metaphase I Configuration: ⊖14

5903-38b-4	X C1483	4	0.0	-
" "	X C1310	4	0.0	-
Plants with ⊖14		8	0.0	-

* Data from different lines have been pooled together

\+ These figures represent the average percent fertility reported by Shih and Shebeski (124).

reported by these authors. On the contrary, fertility of plants with $0\bar{1}0+\bar{2}^{II}$ was found to be considerably lower than that reported by Shih and Shebeski (124). These discrepancies can be explained on the basis that the material studied in the two cases was different as was the environment. The hybrids between the line T(1-7)(2-3)a-24-3-2 (homozygous for $30\bar{4}+1^{II}$) and other tester stocks gave uniformly higher fertility. Unfortunately, data on fertility of this line in the heterozygous condition are not available. A possible reason, however, could be that this line is a two-rowed type and it has been observed at the University of Alberta, by Kasha*, that two-rowed type translocation heterozygotes are relatively more fertile than six-rowed types.

Shih and Shebeski (124), and Nishimura (91) reported that the fertility of translocation heterozygotes decreases with increasing complexity of the translocation, i.e. the number of chromosomes involved and the size of ring or rings. The results presented in Table XII are in agreement with this conclusion. For example, plants with $0\bar{8}+\bar{3}^{II}$ were more sterile than $0\bar{6}+0\bar{4}+\bar{2}^{II}$ plants. Similarly $0\bar{1}0+\bar{2}^{II}$ plants were less fertile than either $0\bar{8}+0\bar{4}+1^{II}$ or $0\bar{6}+0\bar{4}+0\bar{4}$ plants. The sterility of $0\bar{8}+0\bar{4}+1^{II}$ plants was higher than that of $0\bar{6}+0\bar{4}+0\bar{4}$ plants. In addition, the present results also indicate that of the two factors viz. the number of chromosomes involved and the size of ring or rings, the latter would be more important in its effect on fertility. The relationship between MI configuration and fertility in translocation heterozygotes has been illustrated in Figure 17.

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* Quoted by Smith (128)

FIGURE 17. The relationship between MI configuration and fertility in translocation heterozygotes of barley. (In the above figure, the empty florets have been removed to increase contrast). With increasing complexity in the translocation, there is a gradual decrease in fertility (2 to 7), until complete or almost complete sterility is reached in plants with a $\odot 12+1^{II}$, $\odot 10+\odot 4$, and $\odot 14$ MI configurations (8,9,10). Fertility seems to be more dependent on the size of the ring or rings than the number of chromosomes involved (compare 4,6 and 7,8).

FIGURE 17.

MI configuration.	1	2	3	4	5	6	7	8	9	10
	7^{II}	0_4+5^{II}	0_6+4^{II}	2_04+3^{II}	$0_6+0_4+2^{II}$	0_8+3^{II}	$0_8+0_4+1^{II}$	$0_{12}+1^{II}$	$0_{10}+0_4$	0_{14}
Per cent fertility	100.0	58.8	38.1	25.0	17.5	15.8	7.8	0.0	0.0	0.0

The average per cent fertility of plants with 010+04 and 012+1^{II} was 1.9 and 1.0 respectively, and the range in fertility varied from complete sterility to 5.6 per cent. These results are in agreement with those of Nishimura (91).

GENERAL DISCUSSION

I. Comparison of Different Methods

The synthesis of complex translocation stocks in barley, reported in the present study has been accomplished using two methods viz. irradiation of homozygous translocation stocks, and intercrossing two interchange stocks. Using the irradiation method, a total of 17 translocations have been induced (Table IV) of which 14 (Table VII) have been established as homozygotes. This represents a fairly high frequency and indicates the success of this method.

The irradiation method was modified in the present study to minimize cytological work. A second selection on the basis of reduced fertility was conducted in X_2 and the cytological detection of a translocation was carried out in the X_3 progenies rather than in the X_2 as is usually done. Considering the fact that sterility in the immediate X-rayed populations could be due to factors other than reciprocal translocations (49) it seems logical to assume that sterility in X_2 population should be more indicative of possible translocations rather than in X_1. Results presented in Table V support this assumption. Out of a total of 138 X_2 families grown, only 55 families were found to be segregating for fertility, of which 17 carried a translocation. Thus considerable material could be discarded in X_2 without resorting to cytological analysis. The ultimate synthesis of a translocation, however, will be delayed by one generation. Since it takes less than three months to grow a generation, particularly when adequate greenhouse space is available, the time loss is negligible in relation to the efficiency of the method.

Although the irradiation method has been used successfully in barley for the synthesis of larger rings (26,91,143,144) and the present results are also in agreement, there has been some indication (26,144) that instead of inducing a new translocation, the original translocation could be lost. Based on the evidence in the literature on the differential sensitivity of certain parts of chromosomes to irradiation (24,117,118,120,138) this was attributed to a process of back translocation by Wang (144). If such an event occurs frequently it would be theoretically more and more difficult to make further progress in a translocation stock with increasing complexity of its translocation. In the present study, however, out of the 17 translocations induced, a back translocation apparently occurred only in two lines 5903-2b- and 5903-38a-5-1 (page 47). Indeed, in the line 5903-38a-5-1 a new translocation was also synthesized. Such a low frequency of breaks at the same position could be due to chance alone and therefore, should not be a problem in the synthesis of a complete translocation stock. Further support to this conclusion is obtained by analyzing the frequency of desired interchanges induced in 5903 on a probability basis, assuming no difference in radiosensitivity between different chromosomes. The probability of inducing desired interchanges in 5903 would be $10_{c1} \cdot 4_{c1} / 14_{c2} = 40/91$ for seed treatment, and the actual results are in agreement with this expectation. Out of the ten translocations induced in this stock (Table IV), six were considered to be desirable, since in three cases translocations appeared to have occurred within the original complex (page 40) and a back translocation

occurred in one case (page 47). This is close to the expected frequency (4.4) and the difference was found to be statistically nonsignificant (Chi-square = 0.58).

The success of the 'intercross' method is dependent on the occurrence of a cross-over in the differential segment (the region between the break points of two translocations involving a common chromosome), its subsequent detection, and fertility of the hybrid between two translocation stocks. Although this method has been used successfully in Campanula (30), einkorn wheat (150) and maize (62), attempts in barley did not succeed (73). The failure has been considered either due to inadequacy of the method of selection or to the low frequency of crossing-over in the differential segment. Therefore in the present study the method was modified to overcome these limitations. Firstly, it was used to synthesize a stock homozygous for 01O+04 to avoid the necessity of a cross-over. Such a stock could be irradiated to unite the two translocations. Secondly, two methods of overcoming sterility were attempted, namely, direct synthesis by back-crossing, and a two-step bridging approach to carry out the synthesis. The back-cross method, although expected to partially overcome the problem of sterility, was not successful, because of the inability to use 5903 as a female parent. However, this method cannot be ruled out, since in later work by a modified method of emasculation, it was possible to produce hybrid seed using 5903 as the female parent. The results obtained using the bridging approach indicate that this method

would be successful to complete the synthesis, but the amount of time required would be almost twice that required when the synthesis is done in one step.

In conclusion, it would appear that the irradiation method is more desirable than the intercross method for the synthesis of complex translocations in barley particularly at higher levels of chromosome participation. It appears possible to synthesize a complete translocation stock exclusively using irradiation without causing any appreciable break-down of a pre-existing translocation complex. Further, a ring produced by irradiation is expected to be more stable than that built by selecting cross-overs in the differential segment as pointed out by MacDonald (73).

If the complete translocation stock is to be used in the diploidization of autotetraploids, the irradiation method would have an additional advantage. Cyclic irradiation of a translocation homozygote is expected to induce cryptic structural changes besides the cytologically detectable translocations. Gradual accumulation of such structural changes would result in greater differentiation of the karyotype, which may be necessary for inducing diploidization of autotetraphids.

II. Practicability of the Oenothera Method

The fertility level of a hybrid obtained from crossing a complete translocation stock with a normal stock, is an important factor in determining the success of the Oenothera method. In barley the problem

of high sterility has been reported to be a major obstacle in application of the Oenothera method (91,94,124). The results obtained in the present study (Table XII) also indicate a similar trend.

A possible reason for the high sterility of translocation heterozygotes with increasing complexity in interchange appears to be the present procedure followed for the isolation of translocations. In translocation studies, partial sterility has been used as a marker for possible translocations. This means that only those translocations, which are phenotypically apparent by partial sterility are isolated, and therefore high sterility in larger rings should be expected. According to Burnham (16) in species with alternate segregation such as barley, the degree of sterility depends on the frequency of crossovers in the interstitial segments. Based on this Hanson (57) suggested that interchanges ranging from complete fertility to 50 per cent sterility should be expected in barley, but no /extensive attempt has been made so far to detect interchanges with complete or almost complete fertility. That such translocations occur, could be tested by irradiating a relatively small population and examining cytologically progenies from every plant in the next generation.

Although a wide range in sterility of translocation heterozygotes involving the same number of chromosomes has been reported (91,124) and was also observed in the present study (Table XII), no selection for fertility has been made in using interchanges for the synthesis of larger rings. In the present study, the hybrids with a MI configuration of

08+04+1^{II} and 06+04+04, obtained from crossing the two-rowed line T(1-7)(2-3)a-24-3-2 (homozygous for 304+1^{II}) with other tester stocks showed uniformly high fertility up to 25 per cent (Table XII). It has been observed at the University of Alberta by Kasha* that two-rowed heterozygous translocation lines are more fertile than six-rowed and therefore such a line would be more desirable for inducing further translocations.

In conclusion, it appears that the present method of synthesis of larger translocation rings without any consideration of fertility may have been detrimental to the success of the Oenothera method of gamete selection. The potential value of such a method, therefore, cannot be adequately tested until a concerted effort has been made to improve the fertility of complex translocation heterozygotes following the suggestions outlined above. Yamashita (150) reported a fertility of 13.72 per cent in the translocation heterozygote involving all the chromosomes of einkorn wheat, which is similar to barley in chromosome number and other aspects.

III. Difficulty in Crosses Involving the Stock 5903 or Lines Derived from it as a Female Parent

Wang (144) reported difficulty in using a line designated "161" as a female parent in a hybridization program. The stock 5903 was obtained from this line by irradiation. As mentioned earlier (page 39) in the present study crosses involving 5903 or lines derived from it as the female were not successful. In order to find the cause of this

* Quoted by Smith (128)

failure, various possibilities were considered and tested. The results are discussed below.

Since the line was fertile when naturally selfed, non-synchronization of pollination with ovary-maturation was ruled out. However, when the same line was emasculated and selfed manually, no seed was obtained even though spikes were emasculated at different stages of maturity, and pollinated at different intervals in relation to the time of emasculation. On close examination of crossed heads, it was found that fertilization occurred in a number of florets but the embryos began to degenerate three to four days after fertilization. It was considered that perhaps some substance essential for the normal development of embryos was absent in the hybrid embryos and that possibly this substance would be normally supplied by awns and/or flag leaf, which usually were removed in the process of emasculation. To test this hypothesis, two heads were emasculated from the side without removing any part of the spike, and then pollinated. Surprisingly, a good seed set was obtained in both spikes. This indicated that in the stock 5903, presence of awns was essential for the normal development of the embryos. To test this further, in one head the awns were removed approximately 24 to 36 hours after natural pollination. Seeds developed normally indicating that even 24 hours after pollination awns were not essential for the development of the embryos. In order to obtain additional information, a few more heads were emasculated in different ways, such as flag leaf removed but awns left intact, awns removed from one side of the head but left intact on the other, awns removed and left on

alternate florets and so on. This work was done on a very limited scale with the few plants still available for crossing, and therefore it was not possible to draw many conclusions. Seeds were obtained in all cases where some awns were left on the spike indicating that it is not necessary for the entire spike to remain intact. Line 5903 is apparently very sensitive to the emasculation process and the factors causing sterility when awns are removed should be studied in detail.

PART B

DIPLOIDIZING EFFECT OF COMPLEX INTERCHANGES AT TETRAPLOID LEVEL IN BARLEY

LITERATURE REVIEW

I. Fertility of Autotetraploids

Though the first artificial polyploid in higher plants was produced by Winkler (148) in 1916, the usefulness of polyploidy in plant breeding was not realized until 1937, when Blakeslee and Avery (10), and Nebel (87) independently demonstrated that chromosome doubling in plants can be induced by means of the drug colchicine. Since then a large number of autotetraploids have been produced artificially in many crop species (35,76,103). Autotetraploids have proven more useful in crops where seed is not an economic product (76,134). Examples of such crops are sugarbeet, turnip, alsike clover, red clover, berseem, grapes, watermelon, snapdragons, etc. In seed crops, however, lowered seed set has been the greatest deterrent in the utilization of autotetraploids. The only examples of success in seed crops are rye (11) and toria (99,100). Other cereal and oil seed crops, such as corn, flax, barley, and oil seed rape show the usual agronomic advantages of chromosome doubling, but their lowered seed fertility outweighs the increased vigor.

According to Smith (125) in cultivated barley, the first autotetraploid strain was produced by Müntzing in 1936 by means of heat shocks. Since then considerable numbers of autotetraploids have been obtained with colchicine (27,33,34,37,47,70,82,95,107,127,128,141). The extensive literature on various aspects of barley autotetraploids has been summarized by Smith (127). Recently Reinbergs (106) outlined the present stage of autotetraploid breeding in barley. Tetraploid

barley produces larger seeds than the diploids, but the number of seeds per spike is lower (97,111,127,128,141,142). Some of these seeds are underdeveloped and fail to germinate (55,127). Aneuploid plants with chromosome numbers ranging from 26 to 31 have been reported in the progeny of euploids (27,34,60,108,111,112,125,145). Most of these aneuploids are associated with dwarfism, and in others the fertility is substantially reduced in comparison with euploids. All these factors result in considerably lower yields of the tetraploids as compared with the diploids from which they were derived. Müntzing (82) found that the average yields of ten tetraploid strains of barley were from 60 to 79 per cent lower than the yields of the corresponding diploids. Several authors (82,97,107,127,128,145) reported lower fertility in tetraploid barley, and that fertility varies with the variety and environment.

The factors causing sterility in autotetraploids are not completely understood. Darlington (29) considered that the sterility in autotetraploids is due to the formation of multivalents and irregular disjunction of chromosomes at anaphase I, resulting in inviable, unbalanced gametes. Kostoff (64) postulated that species with small chromosomes would have a lower frequency of quadrivalents, and thus would be less sterile. Müntzing (81) however, did not accept this view and soon it was discovered that this is not always true (103). Morrison and Rajhathy (78) in a study of meiosis of various autotetraploid cereals and grasses found no such relationship between chromosome length and

quadrivalent formation. They proposed that in all autotetraploids, approximately two-thirds of the chromosomes form quadrivalents, irrespective of their size differences.

Strong evidence in support of Darlington's view was from the numerous reports of aneuploid plants in the progency of autotetraploids (1,2,11,27,34,60,77,82,83,108,111,112,122,125,145). Considerable differences have been found in the frequency of aneuploids between different crop species and between different varieties of the same species. Thus Alexander (2) reported that in maize, seed set may be reduced as much as 39 per cent through aneuploidy. In autotetraploid rye, Müntzing (83) reported 23 per cent aneuploid plants. Reinbergs and Shebeski (108) reported 21.9 to 38.7 per cent aneuploids in different populations of autotetraploid barley. Most of these aneuploids were dwarfs and completely sterile. In addition, 8 to 77.8 per cent of the euploid plants were also found to be dwarfs. These were considered to be unbalanced euploids of the type reported by Smith (125). Rommel (111) also found a similar relationship between aneuploidy, seed set and sterility in autotetraploid barley. Wang (145) reported that environment has also a significant effect on the frequency of aneuploids in barley. Thus aneuploidy appears to be particularly disturbing to development in barley. Armstrong and Robertson (3) found no aneuploid plants in autotetraploid alsike clover, and considered that if aneuploid gametes occurred, the zygote formed from their union apparently failed to function.

There are three opposing views as to the relationship between meiotic irregularity and fertility. A number of workers (11,27,46,61,85,

86,137,141,142) presented evidence that high quadrivalent frequency and other meiotic irregularities are associated with sterility. A second group (3,113) found a positive correlation between per cent seed set and quadrivalent frequency. Roseweir and Rees (113) working with rye stated that selection for high fertility should be based on increasing the quadrivalent frequency and reducing the univalent and trivalent frequency. They warned that this surprising positive correlation may not be true of other autotetraploids, since in rye the chiasma/ta are distal and relatively few, allowing a regular separation of quadrivalents in anaphase I. Armstrong and Robertson (3) also noted stable meiosis in tetraploid alsike clover, characterized by a high frequency of quadrivalents, which separated regularly at anaphase I. However, they failed to associate cytological behaviour with fertility. A third group (3,77,79,104,129,145) failed to establish any relationship between meiotic behaviour and fertility.

Various suggestions have been made that the sterility of autotetraploids may have a genetical basis (1,3,60,77,79,83,100,104,107, 114,122,128). Others proposed that the disturbed genetic relationship brought about by chromosome doubling manifests itself as a physiological imbalance causing sterility (36,66). Parthasarathy and Rajan (98) suggested that fertility is governed by a system of polygenes, which is a balanced state in the diploids and this balance is upset by chromosome doubling.

Schwanitz (121) postulated that the sterility in autotetraploids was caused mainly by physiological disturbances in the plants. This

view has been shared by several other workers (31,32,55,56,77). Some of these workers suggested genotypic control of these physiological disturbances.

Stebbins (134) concluded that the most common defects in induced autotetraploids are slower growth and reduced fertility, and since both these defects are physiological in nature, genetically controlled physiological imbalance is usually responsible for sterility in autotetraploids rather than irregular chromosome behaviour. But Reinbergs and Shebeski (108) on the basis of the observed high frequency of aneuploids, concluded that the main contributing factor to sterility was irregular chromosome distribution.

Selection has been used as a method of improving fertility of autotetraploids, and has been effective in cross-pollinated crops, such as rye, toria, maize, red clover, alsike clover, and buckwhest (103), but not in self-fertilized crops such as barley (106,107,128); although the first attempts seemed to be encouraging (82,95,96,97). Reinbergs and Shebeski (82) pointed out that selection for increased fertility should be made in the heterogeneous populations of autotetraploid hybrids. Earlier experimental evidence in support of this view was provided by Müntzing (82), and Smith (128). These authors found that the tetraploids obtained from variety hybrids were superior to those from standard varieties, and that selection fer fertility in such hybrid populations was somewhat effective. On the basis of these results it appears possible to improve fertility of autotetraploid barley by suitable hybridization between widely divergent stocks and subjecting the populations to

repeated selection, but further research is required to test the ultimate success of the method.

II. Diploidization

Allopolyploids in general have regular meiosis, characterized by diploid pairing and consequently satisfactory fertility. Autopolyploids, on the other hand have irregular meiosis, characterized by multivalent formation and uneven distribution of chromosomes, and consequently reduced fertility (52). Although, no definite relationship has been established between the extent of multivalent formation and seed fertility in experimentally produced autotetraploids, still there are indications that fertility must be dependent to a certain extent on meiotic regularity. Thus it should be possible to increase the fertility of autotetraploids by induction of normal chromosome pairing and distribution, so that the autotetraploid will behave cytologically as a diploid. This process has been referred to as "diploidization" (133).

In nature, diploidization has played an important role in the evolution of most of the polyploid species. Earlier autopolyploids were thought to be of a wide occurrence. Later it was realized that autopolyploidy by itself rarely produces morphologically distinct species, and most of the earlier examples turned out to be allopolyploids (132). The spontaneous mutation process followed by natural selection resulted in gradual diploidization of autopolyploids towards allopolyploids.

Experimental evidence on diploidization in literature is rather scanty. Gilles and Randolph (46) were the first to demonstrate the occurrence of a gradual shift from a multivalent to a bivalent type of synapsis in induced autotetraploids of maize over a period of ten years. Similar results were obtained by Swaminathan and Sulbha (137) in toria. They studied 'raw' (C_1,C_2) and 'evolved' (C_{17},C_{18},C_{19}) autotetraploids of toria. The latter were obtained by a mass pedigree system of selection. Their results were even more striking than those of Gilles and Randolph (46). In autotetraploid rye, Bremer and Bremer-Reinders (11) and Hilpert (61) reported that selection for fertile plants was accompanied by meiotic regularity.

Two methods have been suggested by which diploidization of an autopolyploid might have occurred in nature (133), i.e., accumulation of structural chromosome changes, and mutations controlling chromosome pairing. Many examples in support of the first method have been provided by Stebbins (133). The principle behind this method originates from the hypothesis of preferential pairing due to differential affinity among the chromosomes proposed by Darlington (29). Evidence in support of the second method that chromosome pairing is under genetic control, has been presented by Müntzing and Prakken (84), Rees (105) and Riley and Chapman (109). Swaminathan and Sulbha (137) consider that these two methods may not be mutually exclusive, and might be operating simultaneously.

Stebbins (134) suggested the possibility of experimentally inducing diploid pairing in autotetraploids by irradiation. In the same year

Shebeski (123) suggested the use of a complete translocation stock in inducing diploidization. McCollum (75) using irradiation, was able to increase preferential pairing in the tetraploid, obtained from crossing two subspecies of Dactylis glomerata, in which pollen of the male parent was irradiated to produce chromosomal aberrations.

Hagberg and Åkerberg (52) outlined the different ways in which diploidization can be induced experimentally in autotetraploid populations as follows:

(1) Repeated treatment by mutagenic agents, intercrossing, and selection in many distantly related autotetraploid populations.

(2) Accumulation of structural mutations at the diploid level, and then doubling the plants heterozygous for maximum aberrations.

(3) Systematic hybridization of different translocation types covering all the chromosomes of the species, and then doubling the chromosome set of maximum heterozygosity.

MATERIALS AND METHODS

Preliminary work on the development of autotetraploids* in barley from hybrids involving homozygous translocation stocks was started in February 1961. Initially, a translocation stock 5903, homozygous for translocations involving ten chromosomes and a normal variety O.A.C.21 were crossed. The origin of 5903 has been mentioned earlier on page 14. O.A.C. 21 was selected because of its relatively high fertility at the tetraploid level (107). Subsequently in the summer of 1961 four more homozygous translocation stocks were included in the study to test the diploidizing effect at different levels of translocation participation in the resulting tetraploids. The hybrids obtained from crossing these stocks are listed in Table XIII along with their expected metaphase I configurations. In this table, the new system of designating the translocation stocks has been used and the old designation is given in parentheses. The origin of the stocks T1-5d and T1-2a has been mentioned earlier (Table II). The stocks T(1-6)(2-5)a and T(3-5-7)(4-6)a were synthesized in the Plant Science Department. The stock T(1-6)(2-5)a was obtained by intercrossing the stocks T1-6a and T2-5a, which were derived from the variety Mars at the University of Minnesota and from the variety Bonus at Svalöf respectively. T(3-5-7)(4-6)a was isolated from an X-rayed population of a stock T(3-5-7)a obtained from the variety Mars at the University of Minnesota.

The hybrid seeds obtained from different crosses as well as seeds of the variety Montcalm were treated with colchicine to produce

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* Hereafter referred to as tetraploids.

tetraploids. The following procedure was adopted.

TABLE XIII. The F_1 hybrids obtained from crossing different homozygous translocation stocks and their expected metaphase I configuration.

Hybrid	Expected MI Configuration
5903 x 0.A.C.21	$0\bar{1}0 + 2^{II}$
5903 x T1-5d (C1384)+	$0\bar{1}2 + 1^{II}$
T(3-5-7)(4-6)a(161)+ x T1-2a (C1310)+	$0\bar{6} + 0\bar{4} + 0\bar{4}$
5903 x T1-2a (C1310)+	$0\bar{1}0 + 0\bar{4}$
5903 x T(1-6)(2-5)a(II4)+	$0\bar{1}4$

+ Figures within parentheses represent old designation.

Seeds were germinated in petri dishes on moist filter paper at room temperature for 48 to 60 hours. The seedlings were then placed in a shallow layer of 0.1 per cent aqueous colchicine solution for three hours. After treatment, the seedlings were rinsed in water and planted in a flat pan containing soil and sand mixture. A second colchicine treatment was given just before the initiation of flower primordia. In barley this occurs when the seedlings are in the three-leaf stage (Melnyk, personal communication). Seedlings were carefully removed from the containers. The roots were thoroughly washed to remove the soil and immersed in 0.1 per cent solution for one hour. After thoroughly washing the roots in water, the seedlings

were transplanted in pots. These treated seedlings were designated as the Co generation.

The screening for tetraploidy was done cytologically by chromosome counts in root tips of seedlings in the C_1 generation. The following procedure was followed. The root tips were pretreated in tap water at 0 to 2°C for 21 to 24 hours and fixed in Farmers fixative (3:1 of ethyl alcohol and acetic acid respectively). Squashes were made using the standard Feulgen technique. The hybrids 5903 X T1-5d, 5903 x T(1-6)(2-5)a and 5903 x T1-2a were highly sterile and therefore all the available seeds were cytologically examined, whereas in case of the hybrids 5903 x O.A.C. 21 and T(3-5-7)(4-6)a x T1-2a, and the variety Montcalm a maximum of 15 seeds from each C_0 plant were cytologically analysed. Seedlings with mitotic chromosome numbers from 26 to 32 were planted in the greenhouse in March 1962 (C_1 generation). The number of dwarf and sterile plants within each population was noted. Fertility of the remaining plants was determined according to the method used by Reinbergs and Shebeski (107) as follows: Fifteen spikelets attached to the five lower internodes of a spike were cut and removed. The next 30 spikelets were counted and the part of the spike above them was removed. These 30 spikelets were threshed by hand and the number of seeds counted. Per cent fertility was determined by the following formula:

$$\frac{\text{Number of seeds obtained}}{30} \times 100$$

Seeds from individual C_1 plants were harvested separately. The C_2 generation was planted in the field during the summer of 1962. Unfortunately the material was badly damaged by hail and therefore data on fertility could not be recorded. The tetraploid population from the cross 5903 x T(1-6)(2-5)a was completely destroyed. The available seeds from the remaining four tetraploid populations were harvested in bulk.

During the summer of 1963, all the available C_3 seeds from the tetraploid populations of the hybrids 5903 x T1-5d, T(3-5-7)(4-6)a x T1-2a and 5903 x T1-2a and approximately 120 seeds from each tetraploid of the hybrid 5903 x O.A.C.21 and O.A.C. 21 were germinated and chromosome counts made in root tips following the procedure described earlier. The seedlings were transplanted in the field for fertility comparisons. Unfortunately the material was heavily infected by stem rust and therefore it was not possible to record observations on fertility.

Throughout the study, the diploid variety Montcalm was used as a control and the tetraploid variety O.A.C. 21 was used as a standard variety for fertility comparisons.

RESULTS AND DISCUSSION

I. Induction of Tetraploidy

The results of colchicine treatment for inducing tetraploidy in various barley hybrids and the variety Montcalm are presented in Table XIV. It is apparent from these results that the treatment was quite effective in inducing tetraploidy in all the hybrids, and completely ineffective in the case of Montcalm. Among the hybrids, almost complete success was obtained in the hybrids 5903 x O.A.C. 21, 5903 x T1-5d, 5903 x T1-2a and 5903 x T(1-6)(2-5)a, whereas in the hybrid T(3-5-7)(4-6)a x T1-2a, 41.2 per cent of the plants treated and 20.6 per cent of the C_1 seeds examined were doubled.

Although a number of workers (27,33,70,107) noticed differences between barley varieties in response to colchicine, no one has reported as high a frequency as obtained in the present study. Smith (128) obtained 4 and 18 per cent doubled seeds in the treated populations of barley varieties and hybrids respectively. This difference was statistically significant. On this basis he concluded that it was easier to obtain tetraploids by treating F_1 diploid seeds than seeds of pure varieties. Since the hybrids in the present study were obtained from crossing translocation homozygotes, it would appear that the translocation complex of the hybrids tends to favor the action of colchicine in inducing chromosome doubling. An alternate explanation could be the existence of genotypic differences in

TABLE XIV. The effect of colchicine treatment in inducing tetraploidy in various barley hybrids and the variety Montcalm.

Hybrid or variety	Number of seeds treated	Number of C_o plants seeds of which were examined cytologically	C_o plants doubled		Number of C_1 seeds cytologically examined	C_1 seeds doubled*	
			Number	Percentage		Number	Percentage
5903 x O.A.C. 21	73	19	19	100.0	164	139	84.8
T(3-5-7)(4-6)a x T1-2a	27	17	7	41.2	126	26	20.6
5903 x T1-5d	35	9	9	100.0	34	34	100.0
5903 x T1-2a	34	7	7	100.0	19	19	100.0
5903 x T(1-6)(2-5)a	60	7	6	85.7	20	17	85.0
Montcalm	20	8	0	0.0	68	0	0.0

* Represents euploids as well as aneuploids.

the material with respect to the action of colchicine. These possibilities could be explored by analyzing colchicine treated populations of translocation stocks of varying complexity as well as pure varieties obtained from diverse sources.

Rommel (110) obtained euploid and aneuploid hexaploid seedlings in the progeny of tetraploid barley plants and explained their origin from unreduced gametes fertilized by reduced gametes. In the present study, two seedlings with 2n=42 chromosomes were obtained in a C_1 progeny of the hybrid 5903 x O.A.C. 21. They lived approximately three weeks.

II. Fertility Comparison in the C_1 Generation Between Tetraploid Barley Populations.

The fertility means and range in fertility in the C_1 generation of the tetraploid populations (euploid as well as aneuploid plants) are presented in Table XV. The diploid variety Montcalm, used as a control was completely fertile and therefore, fertility corrections were not necessary. As is evident from this table, a considerable difference in fertility between different tetraploid populations as well as between plants of the same population exists. These results are in agreement with the conclusion of earlier workers (82,107,127, 128, 145) that fertility of tetraploid barley is dependent on variety and environment. None of the hybrid tetraploids was better than the O.A.C. 21 tetraploids used as a standard for comparison. Comparing the hybrid populations, tetraploids of the hybrid 5903 x O.A.C. 21 gave relatively better performance, whereas tetraploids of the hybrid 5903 x

T(1-6)(2-5)a gave the poorest performance.

TABLE XV. Average per cent fertility and range in fertility of the tetraploid populations in the C_1 generation.

Hybrid or variety	Average fertility (per cent)	Number of plants tested	Range in fertility (per cent)
5903 x O.A.C. 21	26.1	69	3.3 to 63.3
T(3-5-7)(4-6)a x T1-2a	21.0	15	9.5 to 53.3
5903 x T1-5d	17.7	18	1.1 to 43.3
5903 x T1-2a	10.6	8	3.3 to 40.0
5903 x T(1-6)(2-5)a	4.9	4	3.3 to 8.3
O.A.C. 21	57.3	6	19.0 to 73.3

In general, the euploid plants within each tetraploid population gave a higher mean per cent fertility than the aneuploid plants. However, a few aneuploid plants were more fertile than some euploid plants. Aneuploid plants with 2n=29 chromosomes gave relatively higher fertility than plants with 2n=27 chromosomes while those with other chromosome numbers (2n=26,30,31,32) were almost completely sterile. These results are in agreement with those of Reinbergs and Shebeski (108) and Rommel (111).

III. The Occurrence of Aneuploid and Dwarf Plants in the Tetraploid Barley Populations

A number of aneuploid and dwarf plants were noted in the tetra-

ploid barley populations. The results are presented in Tables XVI and XVII. Table XVI gives the number and percentage of aneuploid plants in the C_1 and C_3 generations, whereas Table XVII represents the number and percentage of dwarf plants within each group of plants with different chromosome numbers in the C_1 generation.

TABLE XVI. Number and per cent of aneuploid seedlings in the C_1 and C_3 generation of the tetraploid barley populations.

Hybrid or variety	Generation	Number of seedlings examined	Aneuploid seedlings (2n=26 to 32 chromosomes) Number	Per cent of seedlings examined
5903 x O.A.C.21	C_1	139	81	58.2
	C_3	104	56	53.8
T(3-5-7)(4-6)a x T1-2a	C_1	26	15	57.7
	C_3	36	23	63.9
5903 x T1-5d	C_1	34	21	61.7
	C_3	94	57	60.6
5903 x T1-2a	C_1	19	12	63.2
	C_3	13	12	92.3
5903 x T(1-6)(2-5)a	C_1	17	14	82.3
	C_3+			
O.A.C.21	C_1*			
	C_3	103	25	24.3
Total or mean (excluding O.A.C.21)	C_1	235	143	60.8
	C_3	247	148	59.9

+ This hybrid was destroyed by hail in the summer of 1962.
* Not examined.

TABLE XVII. Number and per cent of dwarfs within each group of plants with different chromosome numbers in the C_1 generation of the tetraploid barley hybrids.

Hybrid	Plants with 28 chromosomes		Plants with 27 chromosomes		Plants with 29 chromosomes		Plants with 26,30, 31 & 32 chromosomes		Total	Per cent of total plants
	Number of dwarfs	Per Cent of total plants	Number of dwarfs	Per Cent of total plants	Number of dwarfs	Per Cent of total plants	Number of dwarfs	Per Cent of total plants	Number of dwarfs	
5903 x O.A.C.21	20	37.7	16	53.3	16	45.7	11	78.6	63	47.7
T(3-5-7)(4-6)a x T1-2a	5	45.4	3	42.8	1	16.6	1	100.0	10	40.0
5903 x T1-5d	3	23.1	4	57.1	2	28.6	4	66.7	13	39.4
5903 x T1-2a	2	28.6	3	75.0	3	60.0	3	100.0	11	57.9
5903 x T(1-6)(2-5)a	2	66.7	3	75.0	4	66.7	4	100.0	13	76.6
Total and average	32	36.8	29	55.8	26	44.1	23	82.1	110	48.6

It can be seen from the Table XVI that the frequency of aneuploid plants in the two generations C_1 and C_3 was comparable. The frequency of aneuploid plants in the hybrid tetraploid populations was much higher (53.8 to 92.3 per cent) than in the tetraploid variety O.A.C.21. Reinbergs and Shebeski (108) reported 37.0 per cent aneuploid plants in the C_4 generation of four tetraploid varieties, whereas in the present study 60.8 and 59.9 per cent of the plants were aneuploids in the C_1 and C_3 generation respectively. The tetraploid hybrid populations differ in percentage of aneuploid plants, and in general, corresponds to the percentage of dwarf plants present in each population (Tables XVI and XVII). For example 58.2 per cent aneuploid and 47.7 per cent dwarf plants were present in the C_1 generation of the tetraploid hybrid 5903 x O.A.C.21, whereas in the tetraploid hybrid 5903 x T(1-6)(2-5)a, these figures were 82.3 and 76.6 per cent respectively. This is not surprising, since dwarfism has been reported to be associated with aneuploidy in barley (108,111,145).

In general, the proportion of dwarf plants in different populations was relatively higher (39.4 to 76.6 per cent) than that reported by Reinbergs and Shebeski (108). Their estimates were as low as 23.4 per cent in the variety O.A.C.21 and as high as 81.4 per cent in Montcalm, based on the average of the C_2 to C_5 generations. Comparing different chromosome groups, the highest percentage of dwarfs was noted in aneuploid plants with chromosome numbers 26,30,31 and 32 followed by 27 and 29 chromosome groups. These results are in agreement with those of Reinbergs and Shebeski (108), and Rommel (111). However, in

the present study the percentage of dwarfs in the 28 chromosome plants was much higher than that reported by Reinbergs and Shebeski (108). These dwarf plants probably represent unbalanced euploids of the type reported by Smith (125).

GENERAL DISCUSSION

Although the factors causing sterility in tetraploid barley are not completely understood, meiotic irregularity resulting in a high frequency of aneuploids in tetraploid populations is considered to be a major factor (60,108,111). Therefore, attempts to stabilize meiosis in autotetraploid barley are expected to improve fertility. The present investigation was undertaken to obtain preliminary information on the possibility of inducing "diploidization" in autotetraploid barley using complex translocation stocks. The diploidizing effect was judged by its effect on improving fertility.

The success of this method in inducing diploidization would depend primarily on the nature of control of chromosome pairing. This method would be successful only if chromosome pairing is primarily dependent on chromosome homology. The question whether chromosome pairing is dependent on chromosome homology or is under genetic control has not been settled. Recently evidence is accumulating in favor of genetic control of chromosome pairing (45,105,109,113). The most convincing evidence is that of Riley and Chapman (109) who showed that diploid chromosome pairing of hexaploid wheat is under genetic control. Stebbins (133) favours the view that chromosome pairing is primarily dependent on chromosome homology, and cites many examples to support his conclusion. Based on Stebbins (134) suggestion, McCollum (75) using irradiation was able to induce preferential pairing in the tetraploid obtained from crossing two subspecies of Dactylis glomerata. A second important factor determining the success of this method would

be the extent to which the chromosomes have been structurally altered to increase preferential pairing in the tetraploid.

The results presented in Table XV indicate that the five tetraploid hybrids obtained from crossing homozygous translocation stocks were considerably lower in fertility (4.9 to 26.1 per cent) in comparison with the tetraploid variety O.A.C.21. Although in the present study meiosis was not studied, the high frequency of aneuploids in hybrid tetraploids (approximately 60 per cent, Table XVI) in comparison to that in tetraploid O.A.C.21 as well as in the material studied by other workers (108,111) may indicate that the process of meiosis was more irregular in the hybrid tetraploids. The relatively higher frequency of dwarf plants among the eutetraploid plants (Table XVII) than that reported by Reinbergs and Shebeski (108) supports this conclusion, since increased meiotic irregularity will result in a higher number of unbalanced euhaploid gametes and thereby increase the frequency of unbalanced eutetraploid plants. The increased meiotic irregularity may indicate that preferential pairing was not induced in these tetraploid hybrids and that chromosomes were associated randomly resulting in more complex configurations than quadrivalents.

In the present study the diploidizing effect of translocation complexes cannot be evaluated with certainty for two reasons. Firstly, the data are too limited and secondly the tetraploids of parent stocks used for producing tetraploid hybrids were not available for comparison. The performance of hybrid tetraploid populations was judged in relation

to that of tetraploid O.A.C.21. Since in tetraploid barley fertility varies considerably with variety and environment (107,128,145), this comparison may not represent the true picture. Further investigations on extensive scale, therefore, are necessary to test the ultimate success of the method, but the present evidence does not appear to be encouraging.

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