THE INDUCTION, DETERMINATION AND IDENTIFICATION OF RECIPROCAL TRANSLOCATIONS IN BARLEY

by

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ABSTRACT

A homozygous reciprocal translocation stock for chromosomes a-d, 7031, of the barley variety Montcalm and six other multiple translocation stocks, II-4 (a-f)(b-g), II-8 (a-f)(b-g), III-13 (d-g)(c-e), III-15 (d-g)(c-e), III-22 (d-g)(c-e), and 4256-1 (a-c-d) were used in a study for inducing further translocations by means of X-irradiation. Partially sterile plants or spikes were selected in the X_1 for locating new translocations. Cytological examination for ring formation was made on X_2 plants. Progeny of plants in which ring formation had been found were crossed with suitable tester stocks in an attempt to identify the new translocations.

No new translocations were found in any line of 7031, but in a number of lines the original translocation was lost. One line was identified as a-b instead of the original translocation a-d. Back-translocation was also found in four X_3 lines of III-22. Studies of ring formation in crosses of these four lines with tester stocks showed that one of the original translocations, c-e, was no longer present. A new reciprocal translocation was added to the a-c-d of 4256-1. The new translocation has been partially identified. It is either b-f or b-g, or e-f or e-g.

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INTRODUCTION

The 'Oenothera' method of obtaining homozygous lines from hybrids as proposed by Burnham (4) consists of crossing a homozygous multiple translocation stock with a promising hybrid, selfing the F_1 and isolating essentially homozygous plants with a normal chromosome complement from the F_2. He suggested that if the 'Oenothera' type complex could be developed in corn or barley, then gametic selection would be possible. In order for this method to be applied in practice, the first essential step is to develop a stock or line homozygous for reciprocal translocations involving all chromosomes so that when the homozygous reciprocal translocation stock is crossed with a normal stock, a complete ring of all chromosomes will result at meiosis in the F_1.

Two methods have been suggested for increasing the number of homozygous multiple translocations in an existing translocation stock. One method is by intercrossing two homozygous translocation stocks in which the translocations involve a common chromosome and in which the breaks are far enough apart to furnish a differential segment. A crossover in the differential segment will make it possible to combine the two translocations in the same gamete. The other method is by cyclic irradiation of existing homozygous translocation stocks seeking further translocations

and gradually building the number of translocations to the point that not only will all chromosomes be involved in translocation but that in the heterozygous condition, one large ring will result.

As indicated by Burnham (4), the 'Oenothera' method should be particularly applicable to crops which possess a relatively small number of chromosomes and which have a relatively high level of fertility when heterozygous for translocations. Barley is one of the important economic crops providing these advantages and hence was used in this study.

The chief objectives of this study were :

To induce further translocations in a homozygous reciprocal translocation stock of the Montcalm mutant '7031' and/or in any of six multiple translocation stocks;

To fix the new translocations in a homozygous condition;

To identify the chromosomes involved in the new translocations.

LITERATURE REVIEW

The earliest report involving translocations was that by Belling (2). Although he tried to account for the 50 per cent rate of aborted pollen in the F_2 of a velvet bean cross by assuming a two gene difference, he suggested that abnormal chromosome behavior also could account for the results. That translocations were responsible was not interpreted until 1925, when he explained this breeding behavior on the basis of segmental interchanges between non-homologous chromosomes (3). Since then, the phenomenon of reciprocal translocations has been widely studied in a number of plants. Some of the chromosomal interchanges in plants occurred spontaneously (33,44). Other have resulted from irradiation of polleh or seeds (9,29,48,52,55).

Since X-rays are highly potent and widely available for genetic experimentation, most of the voluminous literature on the induction of mutations deals with the effects of X-rays rather than other forms of irradiation. Many mutations induced by X-rays show altered morphological nature. As reported by Smith (50), Stadler observed that 95 per cent of the seedling mutants were chlorophyll variants, the remaining five per cent consisted of a wide range of morphological types. Different types of barley mutants have been observed and fully reported by Gustafsson (17). Of the viable mutants in barley, the dense-headed mutant known as erectoides

is very common and has been reported by many workers (15, 21,22,23,24,25,26,27,31,35,39,43,54). Chromosomal interchanges in barley, induced by irradiation, have been studied quite extensively (9,24,28,30,54). Caldecott and Smith (9) claimed that a ring of four chromosomes was the most common chromosome aberration induced by X-rays.

The dosage of X-rays required to induce mutation, including chromosomal aberration, varies with different species. For common cereals such as wheat, barley, oats and rye, an X-ray dose of 10,000 to 20,000 r units is the most suitable (16). Some workers (13,14,45,46) have indicated that the frequency of chromosomal aberrations per r unit increases with such factors as increased age and moisture content of the treated seed, on increase in chromosome number of the plant or with lapse of time between irradiating and planting.

One effect of irradiation is the reduction of fertility in plants grown from X-rayed seeds (11,17,19,20, 21). Gustafsson (17) pointed out that sterile individuals often appear in the progeny of X_1 plants. He suggested that the sterility is due partly to structural heterozygosity (chromosomal sterility) and partly to recessive factors (deficiencies, genic mutations). The two sterility types can be distinguished from each other to a certain extent.

The meiosis-disturbing factors often bring about complete sterility whereas translocation sterility in barley reduces fertility by 20 to 25 per cent. As reported by Smith (49), White and Burnham observed 27 different heterozygous interchange stocks in barley in which the pollen abortion varied from 15 to 40 per cent whereas one line with two separate rings of four had about 51 per cent aborted pollen. Ovule sterility in plants with a ring of four varied from 25 to 29 per cent. Smith (51) found that plants with a ring of four chromosomes had about 28 per cent pollen and ovule sterility. Hanson and Kramer (30) found that progenies of plants heterozygous for an interchange resulted in about 23 per cent ovule abortion. Nishimura (37) reported that the fertility of barley plants with a ring of four is 65 per cent, a ring of six or two rings of four is about 45 per cent, a ring of eight or a ring of six plus a ring of four is about 28 per cent and a ring of eight plus a ring of six is 12 per cent.

The degree of sterility would be expected to vary in different interchanges depending upon the manner of separation and the frequency of crossing-over between the centromere and the point of interchanges (5,6,30). In maize, Burnham (5,7) found that non-disjunction (i.e., the chromosomes with homologous centromeres go to the same pole) occurs only when there is no crossing-over in the

interstitial segmental region, and there is always a low frequency of non-disjunction separation regardless of whether or not the frequency of crossing-over is low or high. Burnham (6) reported that in certain species in which alternate chromosomes in the interchange complex pass to the same pole 70 to 90 per cent of the time, ovule and pollen abortion is usually low. Barley is probably one of the crops with higher frequency of alternate separation. Hanson (29) observed the sterility in translocation barley stocks averaged about 25 per cent, indicating that alternate separation may be predominant. It was assumed that plants with zigzag rings lead to alternate separation and produce fertile plants (5,29,54).

There are many diverse views on where breakages occur. From studies of chromosomal breakage induced by X-rays, Sax (40) concluded that breaks in a chromosome do not occur at random in Tradescantia. According to Sax and Mather (42) there is a tendency for more breaks to occur near the centromere rather than near the free end in pollen grains. A similar observation was reported by Camara et al.(10) who found that breakage and reunion of the chromosomes induced by X-rays occurred near or at the centromere in Triticum. On the contrary, Swanson (53) found that in pollen tubes, the breaks are more frequent near the free ends than near the centromere in Tradescantia,

and in Drosophila melanogaster, it was found that the breaks are randomly distributed along the chromosome and that, if mitotic length is considered, euchromatic and heterochromatic regions are equally breakable (1,34). However, Darlington and La Cour (12) gave evidence that in Trillium roots, the heterochromatin is unbreakable at least when it is charged with thymonucleic acid. Sax (41) compared the frequency of chromatid breaks in centric chromosomes with the frequency in acentric fragments and found that the frequency of chromatid breaks per unit length in the fragments was only a tenth of that in the centric chromosomes. Hagberg and Burnham (25) studied the frequencies of breakage in different chromosomes of the six rowed barley variety Mars, and suggested that breakage occurs more often in chromosome b than in others.

As mentioned in the introduction, two methods for the production of multiple translocation stocks were suggested by Burnham (4). One is dependent on a suitable crossover in a common differential segment involving two different translocations to combine the translocations into one stock, and the other is by cyclic irradiation of a homozygous translocation stock to induce new translocations.

By using the first method, Burnham (4) suggested that the longer the differential segments are, i.e., the

section between the breakpoints of two translocations involving a common chromosome, the greater the chance of obtaining a crossover in this segment and hence a combination of the two translocations in the same gamete. Recently, Inman (32) obtained a ring of six in maize by this method. He stated that the translocation break point localized near the ends of the chromosome in producing the large ring of homozygous lines is more useful, since crossing-over is more likely to occur if the differential segment is as long as possible. For this reason, combinations of translocations with short differential segments were avoided.

By the second method, Burnham obtained a ring of 10 in maize (6). Nishimura et al. (37,38) by means of cyclic X-irradiation, obtained different types of ring formation in barley. They suggested a ring of eight plus a ring of six may be synthesized in a shorter time than a complete ring of 14. The expected fertility of the complete ring would be approximately 12 per cent.

1. Induction and Recognition of Translocations

One thousand seeds of 7031 were irradiated with 10,000 r units of X-rays in the spring of 1956 and approximately two hundred seeds of each of the six multiple translocations stocks were irradiated at the same rate in the spring of 1957.

The X_1 plants of 7031 were grown in the field during the summer of 1956. Plants were spaced at least two inches apart within rows and rows were spaced one foot apart. Non irradiated seed of 7031 was planted at 10-row intervals as check material. The X_I of the six multiple translocation stocks with parental checks were grown during the summer of 1957.

In both years, partial-sterility was used as a marker for possible translocations. In 1956, individual plants were used as a basis for selection but in 1957 individual spikes were used. Although selections in 1956 were made on a plant basis, the individual spikes or tillers were kept separate because, as pointed out by Stadler (52), each tiller may come from a different cell initial and mutation in one primordium may not be duplicated in other primordia.

All the selected plants or spikes were numbered in order and the percentage of fertility recorded.

In the late fall of 1956 working with the selections

MATERIALS AND METHODS

A dense-headed barley mutant '7031' obtained from the variety Montcalm by irradiation with cobalt-60 (35), and subsequently identified as a homozygous translocation stock for chromosomes 'a' and 'd' (31), was used in this study. In addition, six multiple translocation barley stocks II-4, II-8, III-13, III-15, III-22, and 4256-1 were included. The chromosomes involved in each of the translocation stocks and other revelant information is listed in Table I.

Table I. The designation, pedigree, and originating station of the seven translocation stocks used for induction of further translocations.

Line designation	Chromosomes involved in translocation	Parent varieties or hybrids	Originating station
7031	a-d	Montcalm	University of Saskatchewan
4256-1	a-c-d	Mars	University of Minnesota
II-4	(a-f)(b-g)	XT18xC1483*	University of Manitoba
II-8	"	"	"
III-13	(d-g)(c-e)	XT2xC1432*	"
III-15	"	"	"
III-22	"	"	"

* XT18 and XT2 were derived from the variety Bonus by X-ray at Svalöf. C1432 and C1483 were derived from the variety Mars by X-ray at University of Minnesota.

from irradiated 7031 and in the fall of 1957 working with the selections from the irradiated multiple translocation stocks, six seeds from each selected spike were sown in a 6-inch pot, and the X_2 grown in the greenhouse. Young spikes of X_2 plants were collected and fixed in Carnoy's fixative solution of 95 per cent ethanol, chloroform and glacial acetic acid in a ratio of 6:3:1. Cytological examination of pollen mother cells for ring formation was made using the acetocarmine smear technique described by Smith (47).

2. Determination of Lines Homozygous for New Translocation

The first step in determination of lines homozygous for new translocations was to check the fertility of each progeny (X_2) of X_1 plants in which ring formation had been found. Theoretically, the X_2 plants from a single partially sterile plant in which a translocation had been noted are expected to fall into three categories as follows:

¼ homozygous for the new translocation and fully fertile,

½ heterozygous and hence partially sterile,

¼ homozygous normal and fully fertile.

Only the fully fertile plants, half of which should be homozygous for the new translocation were harvested. The partially sterile plants were discarded.

In all stocks in which ring formation was found, fifteen of the remnant seeds from the parent partially sterile spike were sown in the greenhouse for classification of fertility of the resulting progeny. According to Mather (36) the probability would be very high (p lies between 0.980 and 0.990), that fifteen seeds would give rise to at least one plant homozygous for the new translocation.

All X_3 plants from the fully fertile plants found in the X_2 of 7031 and the six multiple translocation stocks were grown in the field during the summers of 1957 and 1958 respectively. The rows which showed segregation for fertility were marked. Plants from each fertile progeny row and also the fertile plants from within the segregating rows were crossed with Montcalm and with tester stocks whenever possible. All the tester plants were used as female parents. Table 2 lists the tester stocks, the chromosomes involved in reciprocal translocations, parental source, and originating station.

All the F_1's from crosses with Montcalm were grown in the greenhouse and examined cytologically for ring formation to determine lines homozygous for new translocations.

Table 2. Designation, pedigree and originating station of the tester stocks used in identifying new translocations

Tester stocks	Chromosomes involved in translocation	Parental source	Originating station
Montcalm	___	(Michigan 31604x4307M.C) x Mandscheuri 1807M.C.	Macdonald College
XT15	d-f	Bonus	Sval8f
XT17	d-g	Bonus	"
Ert.7	a-b	Gull	"
Ert.47	c-f	?	"
C1432	c-e	Mars	University of Minnesota
C1483	b-g	"	"
C1346	b-e	"	"
C1384	a-b	"	"
C1317	b-d	"	"
C1336	C-f	"	"
7031	a-d	Montcalm	University of Saskatchewan
II-8	(a-f)(b-g)	XT18 x C1483	University of Manitoba
III-22	(d-g)(c-e)	XT2 x C1432	"

3. Identification of Translocations

Once a new translocation was determined by means of cytological examination of the F_1 from crosses with Montcalm, the F_1s of the crosses involving the same

lines with testers which had been made concurrently were grown in the greenhouse and examined cytologically to identify the new translocation. In order to save time, the determination and identification of translocations were carried out simultaneously.

RESULTS AND DISCUSSION

1. Induction of Translocations

A total of 59 partially sterile plants was selected from the X_1 population of 7031 in the late summer of 1956. Owing to space limitations in the greenhouse, the X_2 of only 44 of the X_1 plants were grown. Although six seeds were planted from each X_1 plant, not all germinated and hence it was not possible to examine cytologically six F_2 plants per X_1 for ring formation in meiosis.

The first eight plants selected were threshed on a plant basis but in all remaining plants the individual spikes were threshed separately and only those spikes that had less than 60 per cent fertility were included for examination.

The plant and spike designation of the X_1, their fertility, and the number of X_2 plants examined for ring formation as well as the results of cytological examination are presented in Table 3.

A ring of four configuration was found in meiosis in the progeny of 15 of the 44 X_1 plants and this certainly represents a high frequency of translocations using low fertility as a marker. In only one of the 15 X_1 plants was ring formation found in more than one spike.

Table 3. The fertility of the selected X_1 plants or spikes of 7031 in per cent, and ring formation found in the X_2.

Plant No.	Spike No.	Fertility of selected X_1 spikes in per cent	No. of X_2 plants examined	No. of plants with a ring of 4 chromosomes
7031-1*			4	1
-2*			4	
-3*			3	2
-4*			4	
-5*			3	
-6*			5	
-7*			5	2
-8*			5	2
7031-9	(a)	50	5	
	(b)	5	2	
7031-10	(a)	5	2	
	(b)	50	3	
	(c)	50	3	
7031-11	(a)	50	3	1
	(b)	10	3	
	(c)	10	4	
	(d)	40	4	
7031-12	(a)	50	5	
	(b)	40	5	
	(c)	40	3	
7031-13	(a)	40	6	3
7031-14	(a)	50	6	
	(b)	50	3	
	(c)	50	6	
7031-15	(a)	50	6	
	(b)	5	6	
	(c)	50	5	2
7031-16	(a)	50	6	2
	(b)	50	2	
7031-17	(a)	40	3	
	(b)	50	5	
	(c)	25	6	

Table 3 continued.

Plant No.	Spike No.	Fertility of selected X_1 spikes in per cent	No. of X_2 plants examined	No. of plants with a ring of 4 chromosomes
7031-18	(a)	30	6	
	(b)	5	4	
7031-19	(a)	40	5	
	(b)	50	6	1
	(c)	40	2	
7031-20	(a)	25	6	3
	(b)	50	6	1
7031-21	(a)	30	5	
	(b)	30	5	
7031-22	(a)	40	5	
7031-23	(a)	10	4	
	(b)	25	4	1
7031-24	(a)	50	5	
7031-25	(a)	10	5	
	(b)	50	6	
7031-26	(a)	10	4	
	(b)	10	2	
7031-27	(a)	50	1	
	(b)	40	1	
	(c)	50	5	
7031-28	(a)	10	2	
	(b)	40	2	
	(c)	25	5	
7031-29	(a)	45	3	
	(b)	50	5	
	(c)	10	5	
7031-30	(a)	25	6	
	(b)	30	6	
7031-31	(a)	40	6	
	(b)	30	6	
7031-32	(a)	50	5	
	(b)	40	4	

Table 3 continued.

Plant No.	Spike No.	Fertility of selected X_1 spikes in per cent	No. of X_2 plants examined	No. of plants with a ring of 4 chromosomes
7031-33	(a)	10	4	2
	(b)	50	2	
	(c)	35	6	
7031-34	(a)	40	6	3
	(b)	50	2	
	(c)	50	1	
7031-35	(a)	40	5	
	(b)	40	5	
7031-36	(a)	10	1	
	(b)	60	2	
7031-37	(a)	25	6	
	(b)	40	6	
7031-38	(a)	50	6	
	(b)	50	6	4
	(c)	40	1	
7031-39	(a)	50	5	
	(b)	40	6	
	(c)	25	4	
7031-40	(a)	35	5	3
	(b)	20	6	
	(c)	25	6	
7031-41	(a)	45	2	
	(b)	45	5	
7031-42	(a)	50	5	
	(b)	60	5	
	(c)	50	6	
7031-43	(a)	10	1	
	(b)	40	5	
	(c)	25	4	
7031-44	(a)	35	3	
	(b)	40	2	
Total			406	33

* Spikes of these plants were mixed.

As mentioned previously, selection in the X_1 of the six multiple translocation stocks was made strictly on the basis of partially sterile spikes, and no attempt was made to maintain plant identity. A total of 54 spikes were selected. Their pedigree, fertility and the cytological analysis for translocations in their progeny is presented in Table 4. Ring formation was found only in three of the six multiple translocation stocks, i.e. III-15, III-22, and 4256-1. A total of four new translocations were obtained out of the 54 selected X_1 spikes.

The partial sterility of the heads in which no ring formation was found is likely due to chromosomal disturbances at meiosis such as deletions or genic mutations other than translocations. As shown in Tables 3 and 4, there seems to be no apparent correlation between partially sterile spikes and chromosomal interchanges, since only 12 out of 87 spikes of 7031* and 4 out of 54 spikes in the six multiple translocation stocks gave rise to ring formation.

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* Spikes for plant numbers 1 to 8 of 7031 were mixed, therefore, the frequency of ring formation was not included in this discussion.

Table 4. The fertility of selected X_1 spikes in per cent and ring formation in the X_2 of six multiple translocation stocks.

Line No.	Spike No.	Fertility of selected X_1 spikes in per cent	No. of X_2 plants examined	No. of plants with a ring of 4 chromosomes
III-13	1	45	6	
	2	50	2	
	3	40	2	
	4	45	2	
	5	30	6	
	6	50	6	
	7	50	3	
III-15	1	50	6	
	2	40	6	
	3	10	6	1
	4	50	4	
	5	30	5	
	6	10	4	
	7	25	4	
	8	45	4	
	9	40	4	
	10	35	4	
	11	30	1	
	12	45	1	
	13	50	6	
	14	50	6	
	15	50	3	
	16	40	3	
	17	35	4	
	18	40	6	
	19	40	3	
	20	45	1	
	21	10	1	
	22	50	1	
III-22	1	50	6	
	2	10	5	3
	3	50	5	
	4	10	6	
	5	50	5	1
II-4	1	50	4	
	2	50	4	
	3	45	6	
	4	10	6	
	5	50	4	

Table 4. continued.

Line No.	Spike No.	Fertility of selected X_1 spikes in per cent	No. of X_2 plants examined	No. of plants with a ring of 4 chromosomes
II-8	1	50	5	
	2	40	5	
	3	50	2	
	4	25	6	
	5	25	1	
4256-1	1	10	6	
	2	40	6	
	3	40	1	
	4	45	6	
	5	30	6	
	6	40	6	3
	7	40	3	
	8	45	4	
	9	55	4	
	10	10	3	
Total			225	8

All translocations that were observed involved only two chromosomes since no configurations were noted other than a ring of four plus five bivalents at diakinesis. Since the quadrivalents always formed a closed ring and no chain appeared, it was concluded that the translocated segments were relatively large.

Micro-nuclei were found in a few of the quartets, indicating that one chromosome of the ring had lagged at anaphase, and had been excluded at the second division to form a micro-nucleus. Chromosomal bridges plus fragment formation were also found in certain cells at anaphase, suggesting the presence of inversions.

A number of seedling variants were noted in the X_2 of both experiments. Of the chlorophyll variants albino and xantha plants were produced in a much higher frequency than other chlorophyll mutants. A few short and curly plants were also found.

2. Fixation and Identification of New Translocations

Eight of the fifteen of 7031 in which translocations were found were selected for further work. In each line plants had to be developed that were homozygous for the new reciprocal translocation and then the translocation would be identified. Fifteen remnant seeds from the original X_1 spikes of the eight selected lines were sown in the greenhouse to produce F_2 populations that should segregate in a 1:1 ratio of fertile:partially sterile plants. Half of the fully fertile plants theoretically should be homozygous for the new reciprocal translocation.

Unfortunately, fertility of even the normal check plants was not high under the greenhouse growing conditions and therefore it was not possible to determine with any degree of certainty which were the fully fertile X_2 plants. Therefore seed from each X_2 plant was sown in the field and the fertility of the X_3 generation was used to determine the homozygosity or heterozygosity for translocation of the X_2 plant.

Several of the X_2 plants were almost completely sterile and these were assumed to be heterozygous for the translocation and therefore their progenies were not grown in the field.

The ratio of progenies fully fertile to segregating for fertility as well as the ratios of fertile to partially sterile plants in the segregating rows are presented in Table 5.

The ratio of fertile plants and partially sterile plants in the segregating rows was approximately 1:1 which was considered indicative that the X_2 plants had been heterozygous for a translocation and consequently segregated for fertility. Fifty per cent of these fertile plants were expected to be homozygous for the new translocation. In addition fifty per cent of the X_3 rows that were fully fertile also were expected to be true breeding lines for the new translocations.

Crosses between Montcalm and at least one or two plants in every X_3 row of 7031 were made because it was impossible to determine which were the fully fertile progenies and which were heterogeneous for fertility at the time that crossing had to be completed. In addition, as many crosses with tester stocks as

Table 5. Segregation for fertility in the X_3 generation of eight selected potentially new translocation stocks of 7031.

X_1 designation	Fertility in X_3		Classification for fertility in the Segregation rows	
	Non-segregating rows	Segregating rows	Fertile plants	Partially sterile plants
7031-13(a)	2	5	28	30
7031-15(a)	5	3 (2)*	6	4
(b)	3	0		
(c)	5	3	22	15
7031-16(a)	4	2 (4)	13	13
7031-20(a)	3	1 (4)	8	7
(b)	3	1 (5)	1	2
7031-23(a)	7	0		
7031-33(a)	2	0		
(b)	7	0		
(c)	2	1 (3)	4	5
7031-34(a)	5	0 (2)		
(b)	6	1 (5)	2	2
(c)	2	1 (2)	4	5
7031-38(a)	12	0		
(b)	9	0		
(c)	3	1 (2)	3	4

* ( ) The bracketed numbers represent the number of X_2 plants that was highly sterile and considered heterozygous for the translocation. If their progenies had been grown, they would segregate.

could possibly be made were completed. Later, the crossed seed was harvested only from the rows that were homozygous for full fertility or from plants that were determined to be fully fertile.

The F_1's of all crosses were grown in the greenhouse during the winter of 1957. Cytological examination for ring formation was first made on the F_1 of the crosses with Montcalm and the data are presented in the first column of Table 6. In these crosses with Montcalm, the new translocation stocks were expected to give a ring of six or two rings of four chromosomes and the normal stocks to give a ring of four chromosomes. It was rather surprising therefore to find that the two types obtained were either seven bivalents or a ring of four chromosomes plus five bivalents. At first it was thought that the seven bivalents were due to faulty crossing techniques and actually were the result of selfing rather than crossing. Selfing was ruled out when the crosses with the other tester stocks were examined. The configurations from these crosses are also summarized in Table 6.

In every cross with Montcalm where seven bivalents were obtained, only single rings of four were obtained with tester stocks, indicating clearly that

Table 6. Meiotic configurations in the F_1 of crosses between X_3 lines of 7031 with Montcalm and with other tester stocks.

X_3 of 7031	Testers								
	Mont-calm	7031 (a-d)	XT15 (d-f)	XT17 (d-g)	Ert.47 (e-f)	Ert.7 (a-b)	C1346 (b-e)	C1432 (c-e)	C1483 (b-g)
13(a)-3	7II					⊙4			
15(a)-1	7II	⊙4							
-2	7II					⊙4			⊙4
15(c)-1	7II						⊙4	⊙4	⊙4
-2	⊙4								
-3	7II								
-5	7II		⊙4	⊙4		⊙4	⊙4		⊙4
-6	7II			⊙4		⊙4			
16(a)-1	⊙4	7II							
-3	⊙4								
-4	⊙4								
20(a)-2	⊙4								
-4	⊙4								
20(b)-1	⊙4								
-4	⊙4								
33(a)-2	7II	⊙4							⊙4
-3	⊙4								
34(a)-2	⊙4								
-4	⊙4	⊙6	2⊙4						
-5	⊙4			2⊙4					⊙6
38(b)-4	7II	⊙4			⊙4	⊙4	⊙4	⊙4	
-5	7II		⊙4				⊙4		
-6	7II	⊙4				⊙4	⊙4		
-7	7II		⊙4		⊙4	⊙4	⊙4	⊙4	
-8	7II	⊙4	⊙4						
38(c)-1	⊙4								
-3	⊙4								

the ring of four configuration was derived from the tester and therefore seven bivalents would be expected from crosses with Montcalm.

In line 16(a)-1 where a ring of four chromosomes was obtained in the cross with Montcalm, seven bivalents resulted in the cross with 7031. This could indicate that no new translocation had occurred and that line 16(a)-1 carried the original translocation present in the non-irradiated 7031.. However, in line 34(a)-4 where a ring of four was obtained in the cross with Montcalm, a ring of six was obtained in the cross with 7031.

Since the X_3 lines 34(a)-2, 34(a)-4, and 34(a)-5 were all derived from the same original X_1 spike and each gave a ring of four chromosomes in crosses with Montcalm, the data from crosses with tester stocks were combined for the identification of the new translocation. These are presented in Table 7, and as indicated in the footnote, the translocation was identificatied as reciprocal between chromosomes a and b.

There are two possible explanations for the results obtained from crossing X_3 lines of 7031 with Montcalm and other tester stocks. The first is that a mis-labelling of seed lots occurred and that a normal

seed lot had originally been irradiated rather than 7031 itself. If this had happened then all X_3 lines that gave seven bivalents with Montcalm were normal and all that gave a ring of four configuration in crosses with Montcalm were the result of a translocation induced by X-irradiation. One of these marked 16(a)-1 appeared to be a-d and the other, 34(a), was identified as a-b.

Table 7. Meiotic configurations in the F_1 hybrids from crosses between 34(a) lines and tester stocks.

Tester parent	Tester chromosomes	Cytological configuration	Translocated chromosomes in 34(a)	No. of plants examined
7031	a-d	⊙6+4II	either'a'or'd'	2
XT15	d-f	2⊙4+3II	not'd'or'f'	3
XT17	e-g	2⊙4+3II	not'e'or'g'	2
C1483	b-g	⊙6+4II	either'b'or'g'	4

It can be readily noted from Table 7 that in the crosses of 34(a) with XT15 and XT17, chromosomes 'd' and 'g' were not involved in the new translocation or a ring of six chromosomes would have been obtained. Since a ring of six chromosomes was obtained in the crosses of 34(a) with 7031 and C1483, it was concluded that chromosomes 'a' and 'b' were involved and thus the new translocation is a-b.

The other possible explanation was that back-mutation occurred in all lines that gave seven bivalents in crosses with Montcalm, and that with the exception of 34(a), which will be discussed later, where the ring of four configuration was obtained in crosses with Montcalm, no new translocation had occurred.

At the time these results were obtained and analyzed, the first possibility (that of mis-labelled seed) seemed the more plausible. Therefore, in an attempt to obtain at least one new translocation in existing translocation stocks, new seed lots were used for X-irradiation, and these were the six multiple translocation stocks described in Table 2.

Remnant seed was available from the X_1 spikes of III-22-2, III-22-5, and 4256-1-6 but not from III-15-3. The X_2 was grown in the spring of 1958 in the greenhouse and because of good growing conditions, X_2 plants were easily classified for fertility*. The ratio of fertile to partially sterile plants was approximately 1:1. During the summer of 1958, progenies of both fertile and partially sterile plants were grown in the field.

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* All plants with more than 90 per cent fertility classified as fully fertile.

The X_3 from plants which had been classified as fertile in X_2 were homozygous for full fertility. The X_3 from plants classified as partially sterile in X_2 segregated for fertility. The results of the classification for fertility in X_3 is presented in Table 8.

Table 8. Inter-line and intra-line segregation for fertility in the X_3 of III-22-2, III-22-5 and 4256-1-6.

X_1 designation	Inter-line fertility in X_3		No. of plants in intra-line segregation	
	Fertile lines	Segregating lines	Fertile plants	Partially sterile plants
III-22-2	6	5	39	41
III-22-5	6	7	50	44
4256-1-6	7	5	49	54

Crossing was concentrated between plants from the fertile non-segregating X_3 lines as female parents and Montcalm and tester stocks as male parents. Generally speaking crossing was quite successful except where C1336 was used as a male parent or where 4256-1-6 was used as a female parent. No successful crosses were completed with C1336. Two crosses were eventually successfully completed with 4256-1-6 when it was used

as a male parent. A possible explanation for the lack of success in crossing with 4256-1-6 as a female is that its pistil can be easily damaged during the process of emasculation.

The F_1's were grown in the greenhouse in the fall of 1958. Cytological examination for ring formation was made and the results are presented in Table 9.

In the crosses of Montcalm with X_3 lines derived from III-22, two types of configurations were expected. If no new translocation was present, two rings of four would be obtained. If a new translocation had been added to the existing two, either three rings of four or a ring of four plus a ring of six would result.

As shown in Table 9, six of the ten lines originating from III-22 appeared to be homozygous normal for the original translocations on the basis of the configuration obtained in their hybrids with Montcalm. Line III-22-2 was crossed with a sufficient number of testers to identify it positively as being (d-g)(c-e).

The remaining four lines, however, instead of producing more than two rings of four in crosses with Montcalm, produced only one ring of four, indicating that the four lines were homozygous for only one reciprocal translocation.

Table 9. Types of ring formation obtained in the F_1 of crosses between tester stocks and X_3 lines of III-22-2, III-22-5, and 4256-1-6.

X_3 lines	Tester Stocks								
	Mont-calm	C1483 (b-g)	C1317 (b-d)	C1432 (c-e)	C1384 (a-b)	C1346 (b-e)	7031 (a-d)	II-8 (a-f) (b-g)	III-22 (d-g) (c-e)
III-22-2-1	04	06	06(.05)or 204(.95)		204			06+04	04
-2	204	06+04	204(.75)or 06+04(.25)		304	06+04	06+04	204+06	
-7	204	06+04	204		304				
-8	204				304				
-10	204	06+04	204(.50)or 06+04(.50)		304				
III-22-5-6	204		204(.90)or 06+04(.10)						
-8	204		204(.50)or 04+06(.50)						
-11	04			204					
-12					204				
-13	04	06							
4256-1-6-1	06+04					206			

It is exceedingly unlikely that mis-labelling of seed occurred again and therefore it is logical to assume that back translocation to the original chromosomes took place not only in these lines but also the 'suspect' lines of 7031. In the three lines derived from the X_1 spike of III-22-5, and the one line derived from III-22-2, the back translocation occurred in chromosome 'c' and 'e' leaving the reciprocal translocation (d-g). This would account for the ring of six chromosomes in the hybrid from the cross with C1483 (b-g) and would satisfactorily explain all other configurations obtained except for the discrepancy in the cross III-22-2-1 with C1317 in which 95 per cent of the cells showed two rings of four rather than the expected ring of six. However, this same type of irregularity occurred in all the crosses with C1317.

It is reasonable to assume that the length of the 'd' chromosome in the b-d of C1317 differs from the length of the 'd' chromosome in the d-g of III-22, which would account for the low frequency of a ring of six. But in addition, in many cells in the crosses involving C1317, open bivalents frequently were found, indicating that minor chromosomal aberrations were associated with this tester. Therefore the tester is considered unreliable.

A new reciprocal translocation was found to be present. When the parent line, 4256-1, is crossed with Montcalm, a ring of six chromosomes plus four bivalents is seen at meiotic metaphase (see figure 1). When line 4256-1-6-1 was crossed with Montcalm, a ring of six plus a ring of four and two bivalents were observed at meiotic metaphase (see figure 2). The ring of four chromosomes indicated that the new translocation did not involve chromosomes a, c, or d. In the cross of 4256-1-6-1 with C1346 (b-e), two rings of six and one bivalent was observed (see figure 3). Therefore, the new translocation must include either chromosome 'b' or chromosome 'e' but not both. Unfortunately, because of the difficulty in completing crosses with 4256-1-6-1, no other crosses were available for study. Therefore, the new translocation could be one of the following four types: b-f, b-g, e-f, e-g.

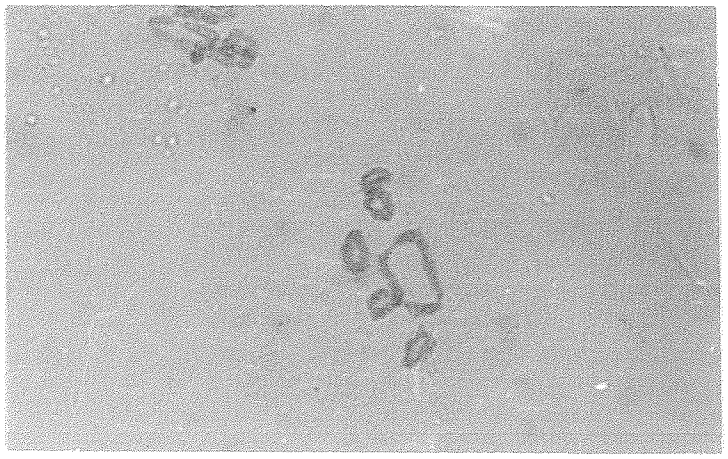

Figure 1. One ring of six and four bivalents at metaphase from F_1 of cross between line 4256-1 and Montcalm.

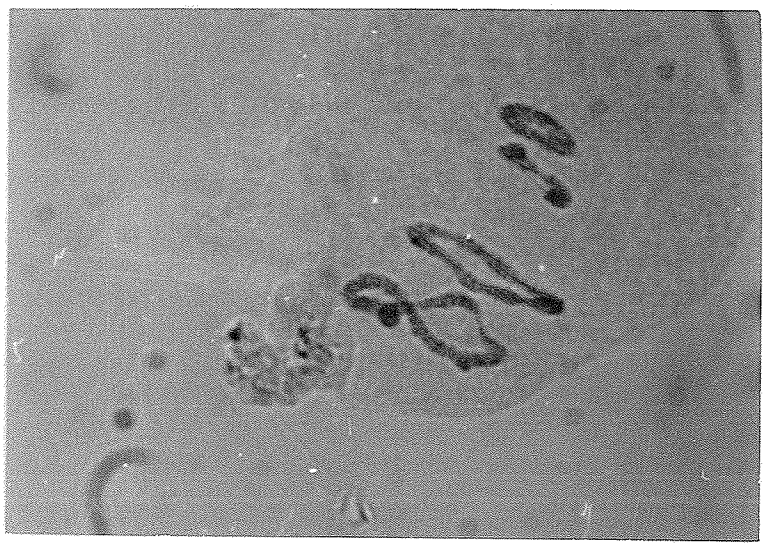

Figure 2. A ring of six plus a ring of four and two bivalents at metaphase from F_1 of cross between line 4256-1-6-1 and Montcalm.

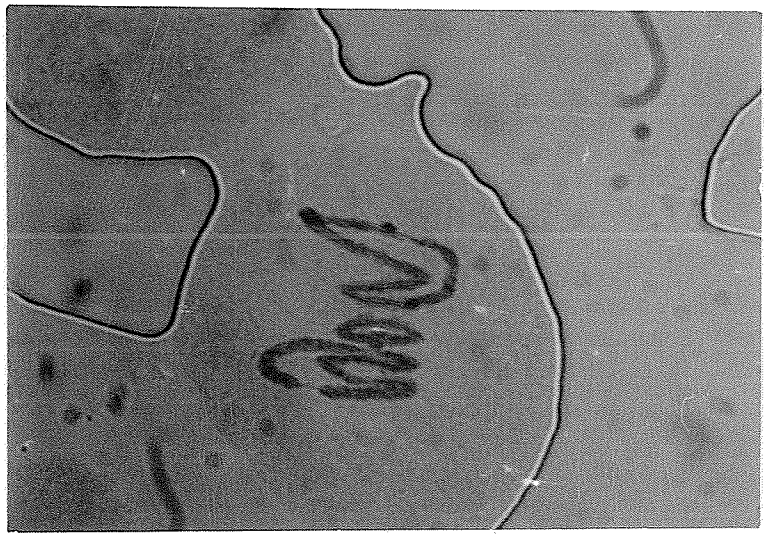

Figure 3. Two rings of six and one bivalent at metaphase from F_1 of cross between line 4256-1-6-1 and C1346.

GENERAL DISCUSSION AND CONCLUSIONS

The induction of translocations in crop plants by means of X-irradiation has been investigated by a number of workers. Based on the published results, this project was undertaken with the expectation that translocations would be induced in barley if seeds were X-irradiated at a dosage of 10,000 r units. New translocations would appear as heterozygotes in the X_1 and would be recognizable by the partial sterility of the X_1 spikes. The results in this study certainly confirm that it is relatively easy to induce translocations and that partial sterility is an excellent marker for the presence of translocations. Although not all partially sterile spikes were found to possess translocations, indicating other types of mutations as causing partial sterility, the proportion of partially sterile spikes possessing translocations was sufficiently high to give satisfactory results. No other obvious character that could be used as an indicator of translocations was noted.

Theoretically, one-half of the fully fertile X_2 plants derived from an X_1 spike heterozygous for a single reciprocal translocation would be homozygous for the new translocation. Results obtained in this study are in

agreement with the theoretical expectations. Although the size of X_2 populations derived from specific X_1 plants heterozygous for translocations was too small to warrant conclusions, the combined results of all X_3 crosses with Montcalm (Tables 6 and 9) show twenty parental translocation types as compared with eighteen non-parental translocation types.

A most interesting aspect of the non-parental translocation types was, with only one exception, that rather than a new translocation being developed an existing translocation lost. Although the possibility of seed admixtures cannot be entirely ruled out, these unusual results are attributed to a process of back-translocation. There has been published evidence that certain regions are more susceptible to chromosome or chromatid breakage than others. This has been referred to earlier in the review of literature (10,25,40,42,53). Therefore it seems reasonable to assume that when seeds of existing reciprocal translocation stocks are X-irradiated to induce new translocations, the selected stocks have, by the very fact that translocations are present, weak regions that could easily break again and either recombine in their original normal condition or combine to maintain their translocation identity.

A change from a-d to a-b, as was obtained in 7031-34(a)-4, could be explained by the expected breaks in the known weak positions of 'a' and 'd' and in addition a break in chromosome 'b'. The 'd' segments would recombine to give a normal 'd' and the 'a' and 'b' segments would combine to produce the heterozygote a-b. Hagberg and Burnham (28) indicated that chromosome 'b' breaks more frequently than any other chromosome.

Data certainly are too limited to more than suggest this unusual phenomenon of back-translocation. This work will have to be repeated on a larger scale with a wide variety of existing translocation stocks for verification.

If the phenomenon of back-translocation occurs fairly often, it increases the difficulty of adding new translocations to existing translocation stocks. This would be overcome by increasing the size of populations used.

One of the difficulties encountered in this study was the disappointing results obtained from the use of two of the recommended translocation tester stocks. Although most of the tester stocks provided a good source of pollen, no seed was obtained in any of the crosses using C1336 as a male parent. This result may have been fortuitous, but good seed sets were obtained using other testers.

Although excellent seed sets were obtained in crosses with C1317, it proved unsatisfactory because of irregular chromosome configuration found in the F_1 of its hybrids.

In 4256-1-6, approximately 120 heads from different lines had been emasculated and pollinated with pollen from various testers, but no seed was obtained from any of these crosses. This accounts for the inability to completely identify the new translocation in this line. Fortunately it was possible to make a few crosses with 4256-1-6-1 as a male parent and to positively determine that a homozygous multiple reciprocal translocation stock has been developed. Its identity has been narrowed down to be a-c-d plus any of the following: b-f, b-g, e-f, e-g.

Further crosses with appropriate tester stocks will have to be made to complete the identity of this new translocation. However, the line will be well suited for further cyclic irradiation in the program of trying to develop a stock homozygous for reciprocal translocations involving all chromosomes.

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