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.