A NEW TECHNIQUE FOR THE PRODUCTION OF A-B TRANSLOCATIONS AND THEIR USE IN GENETIC ANALYSIS[1]

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TRANSLOCATIONS between A (chromosomes of the basic set) and B (supernumerary chromosomes) chromosomes in maize were first produced by ROMAN (1947) by irradiation. ROMAN (1947) established that the nondisjunction of the B centromere in the division of the generative nucleus of the microspore results in a pollen grain with two sperm nuclei that differ in their constitution with respect to the translocated A segment (one will be deficient—hypoploid, and the other will have an extra segment—hyperploid). Depending upon how these sperm nuclei unite with the egg and the polar fusion nucleus of the embryo sac, two classes of seeds will result: 1) seeds with an embryo hypoploid for the segment translocated to the B centromere and an endosperm hyperploid for this segment, and 2) seeds that are hyperploid in the embryo and hypoploid in the endosperm for this segment. Subsequent investigations have demonstrated the usefulness of these aberrations in genetic studies (ROMAN and ULLSTRUP 1951; BIANCHI 1961; BIANCHI *et al.* 1961; BELLINI, BIANCHI and OTTAVIANO 1961; PETERSON and WERNSMAN 1964; ROBERTSON 1964a.)

Until recently (BECKETT 1967, 1968) no new A-B translocations beyond those originally produced by ROMAN and ULLSTRUP 1951) have been reported. This paper describes a technique for producing new A-B translocations of a predetermined constitution by crossing over between existing A-B translocations and reciprocal A translocations and considers their usefulness in genetic studies.

MATERIALS AND METHODS

Crosses were made between female parents carrying an A-B translocation (designated A_1-B, Figure 1a) and male parents having a reciprocal A translocation (designated A_1-A_2, Figure 1b). The A chromosome (A_1) common to both translocations has a distal break point in the A_1-A_2 translocation and a proximal break point in the same arm in the A_1-B translocation. If pairing of the translocated chromosomes in the F_1 is followed by a crossover in the segment between the proximal and distal break points (Figure 1c) and if adjacent I disjunction occurs, a new A-B translocation will be produced. The A_1B and A_2A_1 members of the exchange are unchanged, but the BA chromosome now consists of a B centromere, a proximal segment of the B chromosome, a portion of the A_1 chromosome, and a portion of the A_2 chromosome ($B^{A_1A_2}$ Figure 1d).

Pollen from F_1 plants was used on normal female parents carrying a recessive endosperm gene in the region corresponding to the translocated segment of the A_2 chromosome (Figure 1,
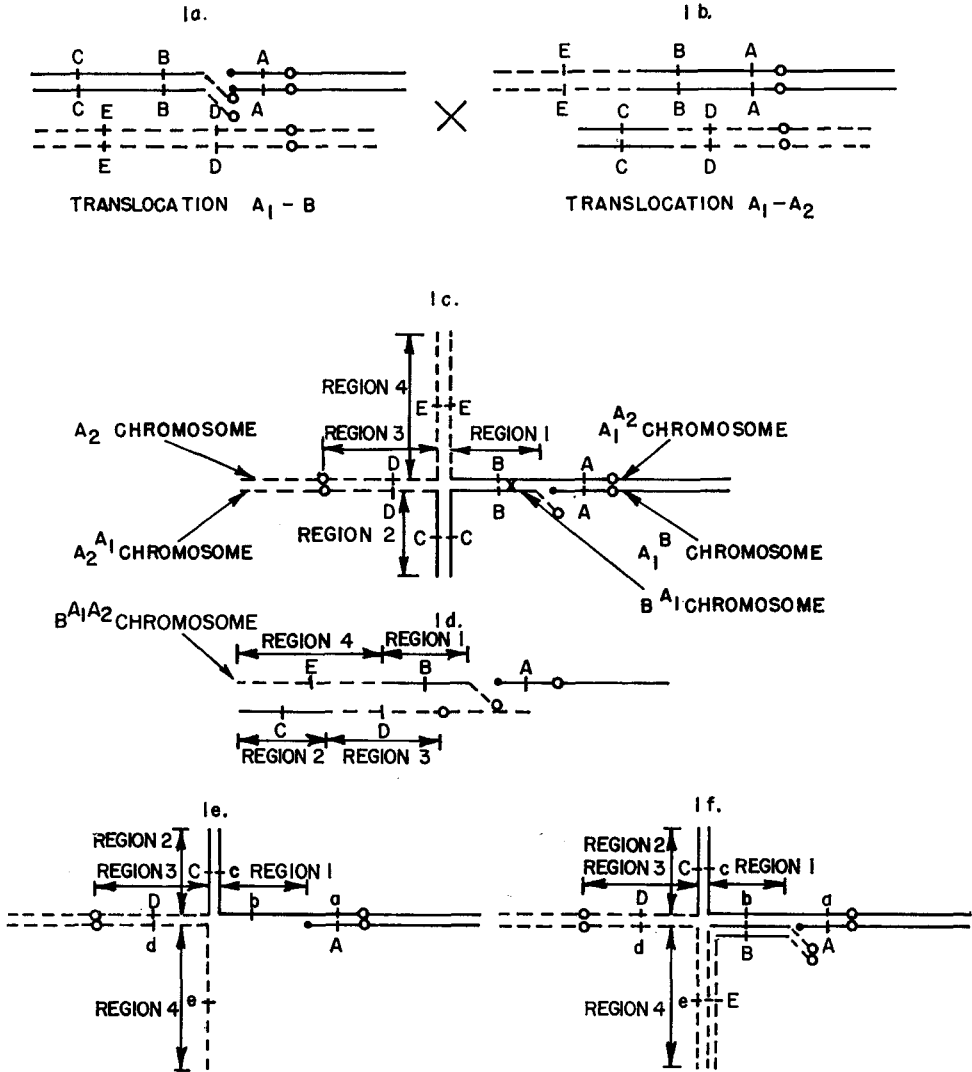
FIGURE 1.—Diagram of an A_1–B translocation female parent (1a) and the reciprocal A_1–A_2 translocation parent (1b) which will produce an F_1 (1c) in which crossing over (in region 1) followed by adjacent I segregation will result in the production of a new A–B translocation with the configuration shown in 1d. Nondisjunction of the B centromere in the division of the generative nucleus of a pollen grain carrying the new translocation will result in one sperm deficient (hypoploid) for BA_1A_2 chromosome and one sperm hyperploid for the chromosome. Figure 1e shows the hypoploid condition that results when the deficient sperm functions and Figure 1f the hyperploid condition that results when the hyperploid sperm functions in fertilizing a normal plant carrying recessive alleles $(a, b, c, d$ and $e)$ in all regions.

region 4). When a deficient sperm from pollen grains carrying the new A–B translocation unites with the polar fusion nucleus, the resulting hypoploid endosperm will show the recessive trait (Figure 1e). The same kernel will contain an embryo hyperploid for the new B^A chromosome (Figure 1f). The reciprocal fertilization would result in a hyperploid endosperm and a deficient

embryo. In this instance, if the female parent carried a recessive seedling or plant trait it would be uncovered. Thus the new translocation can be used to locate endosperm, seedling and mature plant traits in new chromosomal segments that were not involved in the original A–B translocations. In the isolation of these new translocations only recessive endosperm markers were used since seeds that showed the trait would have a hyperploid embryo which would permit the propagation of the new translocation.

Once a new translocation was produced it was then used to locate genes with respect to the break points of the translocations involved. Any gene located in the region distal to the break point of the original B translocation but proximal to the break point of the reciprocal A translocation (Figure 1, region 1), will be uncovered by the new translocation. Any gene in the portion of the A_1 chromosome transferred to the A_2 centromere (Figure 1, region 2) will not be uncovered by the new translocation. The original A–B translocation uncovered genes in this region but the new translocation will not. Any gene in the A_2 segment that is transferred to the B centromere by crossing over (Figure 1, region 4) will be uncovered by the new translocation. Any gene proximal to the break point of the reciprocal A translocation in the chromosome not involved in the original A–B translocation (Figure 1, region 3) will not be uncovered by the new translocation. Since all these break points have been mapped cytologically, it is possible to use the new A–B translocations to place genes to known physical segments of chromosome.

Seeds with the recessive phenotype on the testcross ears, indicated the presence of a new A–B translocation. These seeds were planted and the resulting plants self-pollinated. The segregation of the dominant phenotype on the self-pollinated ear indicated that the original seed showing the recessive phenotype was due to the functioning of the new A–B translocation and that it was not the result of contamination. A plant from a contaminated seed would be homozygous for the recessive trait. The new A–B translocation was used to locate known genes; one of two procedures was followed: 1) at the same time plants produced from the selected seeds were self-pollinated, they were crossed as male parents to the genes to be tested, or 2) the plants from the selected seeds were self-pollinated and outcrossed to a standard line. The outcross seeds were then used as a source of the new A–B translocation for crosses with the tester stocks.

Table 1 lists the reciprocal A translocations used in this study, the B translocations with which they were crossed and the genes used to test for the presence of the new translocations. The reciprocal A translocations were selected so that the break points in the arm involved in the B translocations were well out on the arm, to maximize the distance between the B and A translocation break points and to ensure the greatest possible distance for crossing over.

TABLE 1

Stocks involved in eight F_1 combinations showing reciprocal A translocations with their break points, A-B translocations with their break points, and the genes used to test for the production of new A-B translocations

A translocation	A translocation break point*	A–B translocation	A–B translocation break point†	Genes used to test for new A–B translocation
$T1\text{-}2_c$	1S.77, 2L.33	TB-1b	1S.05	w_3
$T1\text{-}2_{4464}$	1S.53, 2L.28	TB-1b	1S.05	w_3
$T1\text{-}4_{4692}$	1L.46, 4L.15	TB-1a	1L.20	c_2
$T2\text{-}3_{7285}$	2L.26, 3L.39	TB-3a	3L.10	w_3
$T2\text{-}3_{6270}$	2S.46, 3L.60	TB-3a	3L.10	al
$T4\text{-}7_{4698}$	4L.08, 7L.74	TB-7b	7L.30	c_2
$T4\text{-}9_{6222}$	4L.03, 9S.68	TB-9b	9S.40	c_2
$T4\text{-}9_{6504}$	4L.09, 9S.83	TB-9b	9S.40	c_2

* Break points by LONGLEY (1961).
† Break points as given by ROMAN and ULLSTRUP (1951).

TABLE 2

List of genes tested with the new A-B translocations and their location on the chromosome map

Gene	Chromosome location*	Crosses used to produce new A–B translocations
al (albescent, white endosperm-variable white and green seedling)	Short arm 2 (4)	$TB\text{-}3a \times T2\text{-}3_{6270}$
w_3 (white endosperm-albino seedling, viviparous)	Long arm 2 (111)	$TB\text{-}1b \times T1\text{-}2_c$
		$TB\text{-}1b \times T1\text{-}2_{4464}$
		$TB\text{-}3a \times T2\text{-}3_{7285}$
sh_1 (shrunken endosperm)	Short arm 9 (29)	$TB\text{-}9b \times T4\text{-}9_{6222}$
		$TB\text{-}9b \times T4\text{-}9_{6504}$
gl_1 (glossy seedling)	Long arm 7 (36)	$TB\text{-}7b \times T4\text{-}7_{4698}$
gl_2 (glossy seedling)	Short arm 2 (30)	$TB\text{-}3a \times T2\text{-}3_{6270}$
gl_3 (glossy seedling)	Long arm 4 (118)	$TB\text{-}1a \times T1\text{-}4_{4692}$
		$TB\text{-}7b \times T4\text{-}7_{4698}$
		$TB\text{-}9b \times T4\text{-}9_{6222}$
		$TB\text{-}9b \times T4\text{-}9_{6504}$
gl_4 (glossy seedling)	Long arm 4 (86)	$TB\text{-}1a \times T1\text{-}4_{4692}$
		$TB\text{-}7b \times T4\text{-}7_{4698}$
		$TB\text{-}9b \times T4\text{-}9_{6222}$
		$TB\text{-}9b \times T4\text{-}9_{6504}$
c_2 (aleurone color factor, recessive colorless)	Long arm 4 (123)	$TB\text{-}1a \times T1\text{-}4_{4692}$
		$TB\text{-}7b \times T4\text{-}7_{4698}$
		$TB\text{-}9b \times T4\text{-}9_{6222}$
lg_1 (liguleless)	Short arm 2 (11)	$TB\text{-}3a \times T2\text{-}3_{6270}$
yg_2 (yellow-green plant)	Short arm 9 (7)	$TB\text{-}9b \times T4\text{-}9_{6222}$
		$TB\text{-}9b \times T4\text{-}9_{6504}$
vp_5 (white endosperm-albino seedling, viviparous)	Short arm 1 (1)	$TB\text{-}1b \times T1\text{-}2_c$
		$TB\text{-}1b \times T1\text{-}2_{4464}$
lw_1 (white endosperm-albino seedling)	Long arm 1 (128)	$TB\text{-}1a \times T1\text{-}4_{4692}$
v_4 (virescent seedling)	Long arm 2 (83)	$TB\text{-}1b \times T1\text{-}2_c$
		$TB\text{-}1b \times T1\text{-}2_{4464}$
		$TB\text{-}3a \times T2\text{-}3_{7285}$

* Number in parentheses refers to the map position on the respective chromosomes as given by NEUFFER, JONES and ZUBER (1968).

Table 2 lists the genes followed in tests of the new translocations and their locations on the linkage map.

A test was considered positive for an endosperm gene when numerous mutant seeds were observed on the testcross ears. Although ears usually either did or did not segregate for the trait, infrequently an ear would be found on which an occasional mutant seed occurred. Such ears were not counted as a positive test, since they could be the result of self-contamination. In scoring for seedling traits a minimum of fifty seeds was planted. The only tests recorded as positive were those segregating for numerous mutant seedlings. In these tests, an infrequent family was found in which an occasional mutant seedling occurred. Such families were considered to be the result of self-contamination. When w_3, lw_1, vp_5 and *al* were used as testers, self-contaminant seeds have the white-albino phenotype of white endosperm albino seedlings. Such seeds are readily distinguishable from the two classes expected when a white-albino gene is uncovered by an A–B translocation, namely: 1) white seeds with green seedlings, if the hyperploid sperm fertilizes the egg nucleus and the hypoploid sperm the polar fusion nucleus, and 2) yellow seeds with albino seedlings, if the reciprocal fertilization takes place.

NOMENCLATURE

In choosing a symbol for the new translocations, information was utilized from both of the parents. For example, from the cross between TB-$9b$ and $T4$-9_{6222} a new translocated chromosome was produced that included part the long arm of chromosome 4 (the new piece transferred to the B^A chromosome) and the proximal portion of the old A–B translocation, which involved the short arm of chromosome 9. The symbol $TB\ 4L,\ 9S_{6222}$ is used to designate this new translocation. The "4L" next to the letters TB indicates that the new segment involved in this translocation came from the long arm of chromosome 4. The "9S" indicates that the original A–B translocation involved the short arm of chromosome 9. The subscript (6222) identifies the reciprocal A translocation that gave rise to the new translocation. Thus the symbol specifies the chromosomal arms involved in the new translocation and the reciprocal A translocation and A–B translocation that gave rise to it. Similarly, the translocation produced from the cross between TB-$1b$ and $T1$-2_c will be $TB\ 2L,\ 1S_c$.

RESULTS AND DISCUSSION

The first four translocations involved the transfer of the long arm of chromosome 4 to the B^A chromosome. The F_1's between the A-B translocation and the reciprocal A translocations were testcrossed on c_2 tester plants (genotype $A_1A_1A_2A_2C_1C_1c_2c_2RR$). Crossing over in the F_1, followed by adjacent I segregation and nondisjunction of the B centromere in the division of the generative nucleus, would result in seeds with colorless aleurone when the deficient sperm united with the polar fusion nucleus (see Figure 1e). Such seeds are expected to have embryos that would be hyperploid for the new "hybrid" translocation (see Figure 1f).

TRANSLOCATION $TB\ 4L,\ 9S_{6222}$

This translocation was produced from the cross between $T4$-9_{6222} and TB-$9b$. The F_1 testcrossed on the c_2 stock yielded ears that had various numbers of yellow seeds (without anthocyanin color), ranging from 2–9 seeds per ear. Some of the testcross seeds had purple scutellum. For scutellar color, C_2 as well as other aleuron and scutellar genes are needed (SPRAGUE 1932a). The presence of yellow seeds with colored scutellum indicated that the seeds with deficient endosperm must have embryos with C_2 presumably carried by the new B^A chromosome in the hyperploid condition.

Plants from the yellow seeds were used in crosses with stocks homozygous for sh_1 (chromosome 9), gl_3, gl_4 (chromosome 4) and heterozygous for yg_2 (chromosome 9) to locate these genes with respect to the translocation break points. At the same time, the plants from the yellow seeds were self-pollinated to confirm the presence of the new translocation in the hyperploid condition (i.e., the segregation of seeds with colored aleurone). The results of these crosses are given in Table 3.

Among the outcrosses of plants segregating for purple seeds upon self-pollination, all of the four tester genes were observed to be segregating; indicating that these genes are located in the regions translocated to the B centromere. Since yg_2 and sh_1 are located on the short arm of chromosome 9 the results indicate that both are proximal to the break point of the reciprocal A translocation (Figure 1,

TABLE 3

Results obtained from self-pollinating and testcrossing
TB 4L, $9S_{6222}$ *with* sh_1, yg_2, gl_3 *and* gl_4.*

Number of plants	Self-pollination Segregated for C_2	Testcrosses			
		Segregated for sh_1	Segregated for yg_2	Segregated for gl_3	Segregated for gl_4
3	+	+	+	+	0
1	+	+	+	0	+
11	+	+	+	0	0
2	+	+	0	0	+
4	+	0	+	0	0
1	+	0	0	+	0
3	+	+	0	0	0
1	+	—	—	—	0
3	+	—	—	0	—
1	+	—	—	0	0
2	+	0	—	0	0
1	+	—	0	0	0
1	—	—	—	0	0
1	—	—	0	0	0
1	+	+	—	0	—
1	+	+	—	0	0
1	+	+	0	0	—

* + = segregation, — = no segregation, 0 = no test.

region 1). McCLINTOCK (1944) located yg_2 in or near the first chromomere of chromosome 9, which would suggest a position well out on the short arm, distal to the reported break point of the translocation (9S.68). McCLINTOCK's placement of yg_2 was based on observable deficiencies and is undoubtedly more accurate than that determined by a translocation break point. Nonhomologous pairing in a translocation heterozygote frequently hampers the accurate placement of the break points. The evidence suggests that in this case the break point of the translocation is probably considerably distal to the reported position in chromosome 9.

The positive tests with gl_3 and gl_4, although limited, indicate that these genes are in the distal .97 of the long arm of chromosome 4 (Figure 1, region 4). Since gl_4 is closest to the centromere, the results indicate that this gene is distal to 4L.03.

Eight plants listed in Table 3 were observed to segregate purple seeds upon self-pollination but they did not possess the A–B translocation as indicated by the outcrosses. These plants could be the result of heterofertilization (SPRAGUE 1929, 1932b) in which a deficient sperm from a pollen grain carrying the new translocation united with the polar fusion nucleus and a sperm from a pollen grain without the new translocation fertilized the egg nucleus. They also could result from the loss of chromosome 4 from the sperm fertilizing the polar fusion nucleus but not from the sperm uniting with the egg nucleus. Some environmental or genetic background factors that interfered with the development of color in the aleurone of these seeds that had only one dose of C_2 could also produce a spurious noncorrespondence of embryo and endosperm. Seeds with colorless endosperms and

Cc embryos which did not carry the new translocation were observed in all the crosses involving the c_2 locus.

The two seeds that segregated neither for C_2 upon self-pollinating nor for any of the test genes upon outcrossing were due to contamination. The last three sets of crosses recorded in Table 3 give unexpected results. The source of these anomalies is not known and additional testing of them will be necessary.

TRANSLOCATION $TB\ 4L,\ 9S_{6504}$

For the production of this translocation, $T\ 4\text{-}9_{6504}$ and $TB\text{-}9b$ were used. The number of yellow seeds ranged from 5 to 12 per ear in the testcross of the F_1 with the c_2 tester. The results of crosses with the same testers as were used with the previous translocation are given in Table 4. The data do not differ from those obtained for the former translocation. Since the chromosome 9 break point in translocation $T4\text{-}9_{6222}$ is 9S.83, the results indicate that yg_2 is proximal to this point. Again the crosses to gl_3 and gl_4 are limited, but both were uncovered and are therefore located in the distal 91% of the long arm of chromosome 4. Thus gl_4 is distal to 4L.09.

The last two sets of crosses of Table 4 give anomalous results. Further tests of these will be required.

TRANSLOCATION $TB\ 4L,\ 1L_{4692}$

Translocations $T1\text{-}4_{4692}$ and $TB\text{-}1a$ were used to produce this translocation. Again a c_2 tester was used to select for the new translocation. The number of yel-

TABLE 4

Results obtained from self-pollinating and testcrossing
TB 4L, $9S_{6504}$ *with* sh_1, yg_2, gl_3 *and* gl_4*

Number of plants	Self-pollination Segregated for C_2	Testcrosses Segregated for sh_1	Segregated for yg_2	Segregated for gl_3	Segregated for gl_4
3	+	+	+	0	0
1	0	+	+	0	(+)
2	+	+	0	0	+
2	+	+	0	0	(+)
1	+	0	+	0	+
7	+	0	+	0	0
1	+	0	0	+	0
1	+	+	0	0	0
1	+	—	—	0	0
2	+	—	0	0	—
3	+	—	0	0	0
4	—	—	—	0	0
1	+	+	—	0	0
1	—	—	+	0	0

* + = segregation, (+) = segregation for a few recessive seedlings but not sufficient to rule out contamination, — = no segregation, 0 = no test.

TABLE 5

Results obtained from self-pollinating and testcrossing
TB 4L, $1L_{4692}$ *with* lw_1, gl_3, *and* gl_4 *

Number of plants	Self-pollination Segregated for C_2	Segregated for lw_1	Testcrosses Segregated for gl_3	Segregated for gl_4
8	+	—	0	+
1	+	—	+	0
17	+	—	0	0
1	+	0	0	+
2	+	0	+	0
1	0	—	0	+
7	+	—	0	—
1	+	—	—	0
3	—	—	0	0
1	—	0	0	—

* $+$ = segregation, $—$ = no segregation, 0 = no test.

low seeds ranged from 1–5 per year. Many of these yellow seeds had a purple scutellum, which indicates the presence of a hyperploid embryo carrying C_2 and a hypoploid endosperm deficient for C_2. The plants from the yellow seeds were self-pollinated and used as a pollen source for crossing with plants carrying lw_1, which is known to be in the long arm of chromosome 1, and also with plants carrying gl_3 and gl_4. Table 5 shows the results of these crosses.

The gene lw_1 maps well out in the long arm of chromosome 1 (NEUFFER, JONES and ZUBER 1968). Since it is not uncovered by TB 4L, $1L_{4692}$, it must be distal to the break point of $T1$-4_{4692} in the long arm of chromosome 1 (L.46) (Figure 1, region 2). Both gl_3 and gl_4 are uncovered by the new translocation, placing gl_4 distal to the break point of the reciprocal translocation (4L.15).

TRANSLOCATION TB 4L, $7L_{4698}$

The cross between T 4-7$_{4698}$ and TB-7b was the source of this new translocation. Yellow seeds in the cross of the F_1 to c_2c_2 plants, ranging in number from 5–12 per ear, indicated the production of a new translocation. The plants from the yellow seeds were self-pollinated and used as pollen parents in crosses to plants homozygous for gl_1 (long arm, chromosome 7) and gl_3 and gl_4 (long arm, chromosome 4). The results of these crosses are given in Table 6.

The occurrence of purple seeds on the self-pollinated ears of plants from the yellow seeds confirms the production of a new translocation. The segregation of gl_1 seedlings when this new translocation was crossed to plants carrying this gene indicates that gl_1 is located between .30 and .74 of the long arm of chromosome 7 (Figure 1, region 1).

The absence of gl_3 or gl_4 seedlings when this translocation was crossed to plants of these genotypes was unexpected since the break point for the reciprocal A translocation was reported as 4L.08 and these two genes had been uncovered by

TABLE 6

Results obtained from self-pollinating and testcrossing
TB 4L, 7L$_{4698}$ *with* gl$_1$, gl$_3$ *and* gl$_4$*

Number of plants	Self-pollination Segregated for C_2	Segregated for gl_1	Testcrosses Segregated for gl_3	Segregated for gl_4
1	+	+	—	0
8	+	+	0	—
38	+	+	0	0
7	+	0	—	0
12	+	0	0	—
1	0	+	0	0
1	+	—	—	—
6	+	—	0	—
12	+	—	0	0
1	—	+	0	0

* $+$ = segregation, $-$ = no segregation, 0 = no test.

the previous three translocations, which have break points at 4L.03, 4L.09 and 4L.15. The negative results cast some doubt on the reported cytological position of the break point in chromosome 4 of *T4-7$_{4698}$*. The genetic results would place the break point in the long arm of chromosome 4 between gl_3 and c_2.

The last plant tested in Table 6 gave unexpected results. Further testing of this plant is indicated.

TRANSLOCATION *TB 2L, 1S$_c$*

This translocation and the three following translocations have been briefly described by ROBERTSON (1964b).

The *TB 2L, 1S$_c$* translocation was synthesized by crossing *T1-2c* and *TB-1b*. The w_3 gene on the long arm of chromosome 2 was used to test for the formation of the new B^A chromosome. In crosses to w_3, white-dormant seeds were found with the number per ear ranging 1 to 14. On the same ears there were also found yellow viviparous seeds ranging from 1 to 6 per ear. The number of yellow viviparous kernels probably does not represent the total number of deficient (hypoploid) embryos since w_3 is not always viviparous.

The plants from the white seeds were self-pollinated and used as male parents in crosses to standard lines. All the self-pollinated ears segregated for yellow seeds, as expected if a new translocation had been produced.

Plants from seeds of the outcrosses to standard lines were self-pollinated and used as pollen parents in crosses to stocks carrying w_3, v_4 (located in the long arm of chromosome 2) and vp_5. The vp_5 gene is located in the short arm of chromosome 1 and is uncovered by *TB-1b*.. Table 7 shows the results of self-pollinating the plants from the cross of standard $\times \dfrac{TB\ 2L,\ 1S_c}{w_3}$ as well as the crosses with the test genes.

TABLE 7

Results obtained from self-pollinating and testcrossing plants from the cross of standard × TB 2L, 1S$_c$/w$_3$ with w$_3$ v$_4$ and vp$_5$

Number of plants	Self-pollination Segregation for w$_3$	Cross to w$_3$ Segregation for w$_3$	Cross to w$_3$ Hypoploid† test for w$_3$	Cross to v$_4$ Segregation for v$_4$	Cross to vp$_5$ Segregation for vp$_5$
1	—	..	+	—	+
1	—	..	+	0	+
2	—	..	+	0	0
1	—	..	—	—	+
4	—	..	—	0	+
5	—	..	—	0	—
2	—	..	—	—	0
3	—	..	—	0	0
2	—	..	—	—	—
5	+	+	..	—	—
2	+	+	..	—	—
1	+	+	..	—	+
1	+	+	..	0	—
2	+	—	..	—	+
1	+	—	..	—	0
1	+	—	..	0	+
1	+	—	..	0	—
1	+	+	..	0	+
3	+	+	..	0	0

* + = segregation or positive hypoploid test, — = no segregation or negative hypoploid test, 0 = no test.
† A positive hypoploid test is indicated by the segregation of white seeds that produce green seedlings and/or the segregation of yellow seeds with albino or viviparous seedlings.

Since the original hyperploid plant used in the outcross to standard was heterozygous for w$_3$, most, if not all, the plants with the new translocation also should not carry w$_3$ because it is in the nontranslocated chromosome. Most, if not all, the plants heterozygous for w$_3$ should not have the new translocation. The data in Table 7 support these expectations. Since w$_3$ and vp$_5$ are lethal traits, segregating families were used in the testcross. Thus a negative hypoploid test in crosses to these families does not necessarily indicate a negative test for the presence of the translocation but rather may be the result of having crossed to a plant that did not carry the tester gene. Crosses to homozygous v$_4$ plants, though limited, indicate that this gene is not uncovered by the translocation and thus is proximal to the break point of *T1–2c* in chromosome 2 (2L.33). ROBERTSON (1961) placed vp$_5$ on chromosome 1, 1.8 crossover units proximal to the break point of *T1-2c* (1S.77). The data from Table 7 agree with this position.

In Table 7 some of the tested plants segregated for w$_3$ in both the self- and outcross and at the same time gave a hypoploid test for vp$_5$ indicating the presence of an A–B translocation involving the short arm of chromosome 1. Such results are expected if, when the standard × $\dfrac{TB\ 2b,\ 1S_c}{w_3}$ cross was made, a crossover in

region 1 (Figure 1) of the new "hybrid" A–B translocation had occurred followed by adjacent I segregation. These events will produce a gamete with the reconstituted original A–B translocation (*TB-1b*) and carrying w_3 (Figure 2). The occurrence of at least five such crossovers in the data recorded in Table 6 suggests a rather high reversion rate in this translocation, although the sample is too lim-
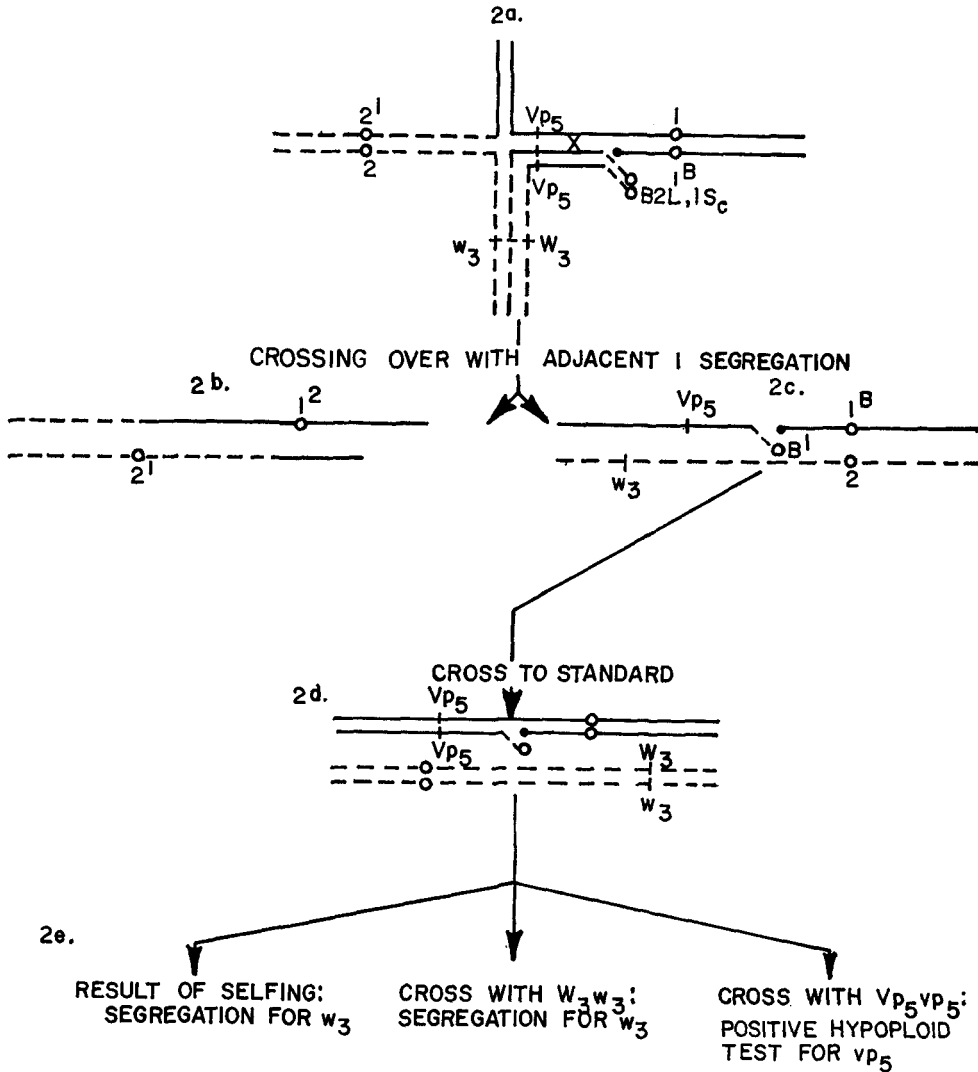

FIGURE 2.—Steps involved in the reversion of *TB 2L, 1S$_c$* to the original *TB-1b* and results expected in crosses to tester genes. (2a) The new "hybrid" A–B translocation showing the crossover responsible for reversion. (2b and 2c) Gametes resulting from crossing over followed by adjacent I segregation. (2d) The constitution of the plant to be self-pollinated and crossed with the tester genes. (2e) Results expected upon self-pollination and crossing to plants heterozygous for w_3 and vp_5.

ited to get an accurate estimate of the true rate. Some of these five palnts could be the result of crossing over in region 4 (Figure 1) between w_3, and the break point. If this occurred, w_3 would be transfered into the new A–B translocation.

TRANSLOCATION $TB\ 2L, 1S_{4464}$

The cross of $T1$-2_{4464} and TB-$1b$ was used to produce this new A–B translocation. The same testing procedure was used with this translocation as with the preceding one. Table 8 gives the results of self-pollinating and testcrossing plants from the cross standard $\times \dfrac{TB\ 2L, 1S_{4464}}{w_3}$ with plants carrying w_3, v_4 and vp_5. Table 9 gives additional data from crosses involving this translocation. The male parents used in the testcrosses reported in this table were derived from seed produced on self-pollinated ears out of the cross standard $\times \dfrac{TB\ 2L, 1S_{4464}}{w_3}$. Only self-pollinated ears not segregating for w_3 were selected since they are more likely to carry the translocation.

TABLE 8

*Results obtained from self-pollinating and testcrossing plants from the cross of standard $\times$ TB 2L, $1S_{4464}/w_3$ with w_3, v_4 and vp_5**

Number of plants	Self-pollination Segregation for w_3	Cross to w_3 Segregation for w_3	Hypoploid† test for w_3	Cross to v_4 Segregation for v_4	Cross to vp_5 Segregation for vp_5
2	—	. .	+	+	+
1	—	. .	—	+	+
2	—	. .	—	0	+
1	—	. .	+	0	+
3	—	. .	+	+	—
2	—	. .	+	0	—
2	—	. .	+	0	0
1	—	. .	—	—	—
1	—	. .	—	+	0
3	—	. .	—	0	—
1	—	. .	—	0	0
1	—	. .	0	0	—
1	+	+	. .	—	+
2	+	+	. .	+	—
2	+	+	. .	—	—
1	+	+	. .	0	—
1	+	—	. .	0	—
2	+	0	. .	—	—
2	+	+	. .	0	0
2	+	—	. .	0	0
1	+	0	. .	0	—

* + = segregation or positive hypoploid test, — = no segregation or negative hypoploid test, 0 = no test.
† A positive hypoploid test is indicated by the segregation of white seeds that produce green seedlings and/or the segregation of yellow seeds with albino or viviparous seedlings.

TABLE 9

Results obtained from self-pollinating and testcrossing plants carrying
TB 2L, $1S_{4464}$ *with* w_3, v_4 *and* vp_5 *(see text for orgin of* TB)*

Number of plants	Self-pollination Segregation for w_3	Cross to w_3 Hypoploid† segregation test for w_3	Cross to v_4 Hypoploid† segregation for v_4	Cross to vp_5 Hypoploid† test for vp_5
1	—	+	+	+
3	—	+	+	—
2	—	+	0	—
2	—	+	0	0
1	—	—	+	—
4	—	0	+	—
1	—	—	0	—
1	—	—	0	0

* + = segregation or positive hypoploid test, — = no segregation or negative hypoploid test, 0 = no test.

† A positive hypoploid test is indicated by the segregation of white seeds that produce green seedlings and/or the segregation of yellow seeds with albino or viviparous seedlings.

The data from Tables 8 and 9 indicate that this translocation uncovers v_4 while the previous one did not. This means that v_4 is located in the region between the break points for these two translocations, 2L.28 and 2L.33, respectively. This translocation also uncovers vp_5, which would place this gene proximal to 1S.53.

Three classes of exceptional plants are observed in Table 8: 1) One plant is observed that segregated for w_3 upon selfing and outcrossing but does not segregate for v_4 when crossed to this stock. This plant gave a positive hypoploid test with vp_5. These results are expected if the plant carried the original A–B translocation produced by crossing over as suggested for translocation *TB 2L, 1S_c.* 2) One plant is found that does not segregate for w_3 upon selfing or outcrossing nor for v_4 or vp_5 when tested against those stocks. However, it is semisterile indicating a translocation is present. The reciprocal crossover product to that used to explain the class 1 exception (Figure 2b) would give the observed results. 3) Two exceptional plants are present that segregate for w_3 upon selfing and v_4 when crossed to stocks carrying this gene. Such plants would be expected if a crossover had occurred that transferred w_3 into the new A–B translocation. Since these plants have the new A–B translocation, they would be expected to be semisterile. One was semisterile as expected but the other was normal. A bookkeeping error is the most likely explanation for the results obtained with the normal plants.

TRANSLOCATION *TB 2L, 3L_{7285}*

This translocation was derived from a cross between *T2-3_{7285}* and *TB-3a*. Testcrossing F_1 plants on those carrying w_3 resulted in some white endosperm seeds that gave rise to green plants. These plants were self-pollinated and crossed to a standard line. Plants from the cross standard $\times \dfrac{TB\ 2L,\ 3L_{7285}}{w_3}$ were self-pollinated

TABLE 10

Results obtained from self-pollinating and testcrossing plants from the cross of standard $\times$ TB 2L, $3L_{7285}$/w_3 with w_3 and v_4 *

	Self-pollination	Cross to w_3		Cross to v_4
Number of plants	Segregation for w_3	Segregation for w_3	Hypoploid† test for w_3	Segregation for v_4
3	—	. .	+	+
16	—	. .	+	0
1	—	. .	—	+
23	--	. .	—	0
1	+	+	. .	—
2	+	—	. .	—
8	+	+	. .	0
1	+	—	. .	0

* + = segregation or positive hypoploid test, — = no segregation or negative hypoploid test, 0 = no test.

† A positive hypoploid test is indicated by the segregation of white seeds that produce green seedlings and/or the segregation of yellow seeds with albino or viviparous seedlings.

and crossed to w_3 and v_4. The data are given in Table 10. Additional crosses involving this translocation were made with plants derived from seeds of self-pollinated ears from the cross standard $\times$ $\dfrac{TB\ 2L,\ 3L_{7285}}{w_3}$. Self-pollinated ears not segregating for w_3 were selected. The data from these crosses are given in Table 11.

This translocation uncovers v_4. This is in agreement with the results from the previous translocation *TB 2L, 1S* $_{4464}$ which also uncovered v_4. The break point in

TABLE 11

Results obtained from self-pollinating and testcrossing plants carrying TB 2L, $3L_{7285}$ with w_3 and v_4 (see text for origin of TB) *

	Self-pollination	Cross to w_3	Cross to v_4
Number of plants	Segregation for w_3	Hypoploid† test for w_3	Segregation for v_4
2	—	+	+
3	—	+	0
1	—	—	+
3	—	0	+
1	0	+	+
8	—	—	0
7	—	—	—
1	—	0	—
1	0	—	—

* + = segregation or positive hypoploid test, — = no segregation or negative hypoploid test, 0 = no test.

† A positive hypoploid test is indicated by the segregation of white seeds that produce green seedlings and/or the segregation of yellow seeds with albino or viviparous seedlings.

chromosome 2 is 2L.28 in *TB 2L, 1S₄₄₆₄* and 2L.26 in *TB 2L, 3L₇₂₈₅*. The plants in Table 11 that did not segregate for w_3 and /or v_4 when tested against these genes were homozygous for the normal chromosomes derived from the standard line to which the translocation stock had originally been crossed.

TRANSLOCATION *TB 2L, 3L₆₂₇₀*

The cross between plants carrying *T2-3₆₂₇₀* and *TB-3a* was the source of this translocation. The F_1 progeny of this cross were used as pollen parents in crosses with plants carrying *al*. The occurrence of white endosperm seed in this cross indicated that a new hybrid translocation had been produced. This was confirmed by planting the white seeds, which gave green plants that segregated for yellow seeds when self-pollinated. At the same time these plants were self-pollinated they were crossed to a standard line. The results of self-pollinating and outcrossing plants from this cross (standard $\times \dfrac{TB\ 2S,\ 3L_{6270}}{al}$) to *al, lg₁* and *gl₂* are given in Table 12. Crosses with the new translocation to *lg₁* and *gl₂* uncover these genes indicating that they must be in the distal 54% of the short arm of chromosome 2.

Two classes of exceptional plants are found in Table 12. Four plants occur that segregated for *al* when self-pollinated and *lg₁* and *gl₂* when crossed with stocks carrying these genes. A crossover transferring *al* into the new A-B translocation would explain these results. As expected, since these plants carry the translocation, they were all observed to be semisterile. The other exceptional class consists of five plants that did not segregate for *al* when self-pollinated and upon outcrossing to stocks carrying *al, lg₁* and *gl₂* failed to segregate for one or more of these traits. These plants could be the result of transferring the normal allele of *al*

TABLE 12

Results obtained from self-pollinating and testcrossing plants from the cross of standard $\times$ TB 2S, 3L₆₂₇₀/al *with* al, lg₁ *and* gl₂*

Number of plants	Self-pollination Segregation for al	Cross to al		Cross to lg₁ gl₂ Segregation for lg₁ and gl₂
		Segregation of al	Hypoploid† test for al	
2	—	. .	+	+
9	—	. .	0	+
1	—	. .	+	0
1	—	. .	—	—
2	—	. .	—	0
2	—	. .	0	—
8	+	+	. .	—
7	+	+	. .	0
4	+	0	. .	+
14	+	0	. .	—

* + = segregation or positive hypoploid test, — = no segregation or negative hypoploid test, 0 = no test.

† A positive hypoploid test is indicated by the segregation of white seeds that produce green seedlings and/or the segregation of yellow seeds with albescent seedlings.

from the translocated chromosome into the normal chromosome 2 resulting in normal plants without *al*. All these plants had normal pollen and seed set, as expected.

CONCLUSIONS

The foregoing experiments have established that it is possible to produce new A–B translocations by crossing over between a reciprocal A $(A_1–A_2)$ and an A–B translocation $(A_1–B)$. These new translocations have attached to the B centromere a proximal portion of the B chromosome, a segment of the A_1 chromosome and all of the A_2 chromosome that was translocated in the original $A_1–A_2$ translocation. The other components of the translocation consist of the $A_1{}^B$ chromosome from the original A–B translocation and $A_2{}^{A_1}$ member of the reciprocal A translocation.

These new "hybrid" translocations can be used to locate genes on segments of the genome that were not involved in the original A–B translocations produced by ROMAN (ROMAN and ULLSTRUP 1951). Also when several "hybrid" translocations involving the same chromosomes are tested against genes carried on the $B^{A_1A_2}$ chromosomes of the different aberrations, it is sometimes possible to locate the genes with respect to the break points and thereby place them on the cytological map. Thus, v_4, which maps near the centromere in the long arm of chromosome 2, was uncovered by *TB 2L, 1S$_{4464}$* and *TB 2L, 3L$_{7285}$* and hence must be distal to the breakpoints of these translocations in chromosome 2 (2L.28 and 2L.26, respectively). It was not uncovered by *TB 2L, 1S$_c$* with a break point at 2L.33 and consequently v_4 must be proximal to this break point. Assuming the correct break point determination, this places v_4 between 2L.28 and 2L.33. Also, genes in region 1 (Figure 1) can be placed with a fair degree of accuracy if this region is not too long. For example in translocation *TB 4L, 7L$_{4698}$* gl_1 is located cytologically between 7L.30 and 7L.74.

The precision of this cytological mapping depends upon the accuracy of the break point determination. In these experiments no attempt was made to confirm the break points reported by LONGLEY (1961). On occasion genetic results were not in agreement with reported break point positions (e.g., the uncovering of yg_2 by *TB 4L, 9S$_{6222}$* and the failure of *TB 4L, 7L$_{4698}$* to uncover gl_3 or gl_4). The genetic results can serve as a check on the cytological information. When discrepancies are found, such as those indicated above, reexamination of the cytological material is indicated.

The crossing of four translocations to standard lines before further testing provided the opportunity for crossing over to occur. Evidence for two classes of crossovers was obtained: 1) Those that reconstituted the original parental translocations (see Figure 2) were observed with *TB 2L, 1S$_c$* and *TB 2L, 1S$_{4464}$* and 2) crossovers that involved the A_2 segment transferred to the B^{A_1} chromosome (Figure 1, region 4) were observed with *TB 2L, 1S$_{4464}$* and *TB 2S, 3L$_{6270}$*. If the exceptional classes Tables 7, 8 and 12 are the result of crossing over, their frequency would indicate that this is not a rare event. If so, the $B^{A_1A_2}$ chromosome must be pairing with the homologous segments of the A_1 and A_2 chromosomes with an appreciable frequency in these hyperploid plants. This is in contrast to what

ROBERTSON (1967) found in hyperploid *TB-9b* plants. About 96% of the time the two B^A chromosomes of these plants paired and as a consequence very few cross-over progeny were observed. The more complex rearrangement of the "hybrid" A–B translocation might in some way be responsible for the more frequent pairing of the $B^{A_1A_2}$ chromosome with the homologous regions of the A_1 and A_2 chromosomes. Further tests will be necessary to determine how crossing over in these "hybrid" A–B translocations compares with that of the original A–B translocations.

Because of the danger of losing these new translocations through crossing over it will be essential that they be continually checked against the appropriate tester genes whenever they are propagated. To eliminate such loss, homozygous lines should be established as quickly as possible.

SUMMARY

A technique utilizing crossing over between an A–B translocation (A_1–B) and a reciprocal A translocation (A_1–A_2) to produce new "hybrid" A–B translocations is described. The B^A element of these translocations consists of a portion of the original B^{A_1} chromosome plus the A_2 segment of the reciprocal A translocation ($B^{A_1A_2}$). The remaining elements of the translocation consist of the $A_1{}^B$ and $A_2{}^{A_1}$ chromosomes. Eight new "hybrid" translocations are described. Three incorporate the long arm of chromosome 2, one the short arm of chromosome 2 and four the long arm of chromosome 4 as the A_2 segment of the $B^{A_1A_2}$ chromosome. These new translocations were used successively to locate genes in chromosome regions corresponding to the translocated A_2 segments. Their usefulness in the cytological mapping of genes was also demonstrated.

LITERATURE CITED

BECKETT, J. B., 1967 Two new B-type translocations. Maize Genet. Coop. News Letter **41**: 139. ——, 1968 A–B-type translocation involving the short arm of chromosome 3 and a translocation complex involving chromosomes 5, 6 and a supernumerary. Maize Genet. Coop. News Letter **42**: 132.

BELLINI, G., A. BIANCHI and E. OTTAVIANO, 1961 The use of interchanges involving B type chromosomes in studying artificial mutagenesis in maize. Z. Vererbl. **92**: 85–99.

BIANCHI, A., 1961 Duplication of specific chromosome segments to increase kernel weight in maize. Genetics **96**: 851–852.

BIANCHI, A., G. BELLINI, M. CANTIN and E. OTTAVIANO, 1961 Nondisjunction in presence of interchanges involving B-type chromosomes in maize, and some phenotypical consequences of meaning in maize breeding. Z. Vererbl. **92**: 213–232.

LONGLEY, A. E., 1961 Breakage points for four corn translocation series and other corn chromosome aberrations maintained at the California Institute of Technology. U. S. Dept. Agric. Res. Serv. Publ. ARS 34–16.

McCLINTOCK, B., 1944 The relations of homozygous deficiencies to mutation and allelic series in maize. Genetics **29**: 478–502.

NEUFFER, M. G., L. JONES and M. S. ZUBER, 1968 *The Mutants of Maize.* Crop Sci. Soc. Am. Madison, Wisconsin.

PETERSON, P. A. and E. A. WERNSMAN, 1964 A monosomic type approach in maize breeding. Crop Sci. **4**: 533–535.

ROBERTSON, D. S., 1961 Linkage studies of mutants in maize with pigment deficiencies in endosperm and seedling. Genetics **46**: 649–662. ——, 1964a Transfer of intact segments of maize chromosomes: A possible Method. J. Heredity **55**: 107–114. ——, 1964b The production of new A–B translocations by crossing over with reciprocal A translocations. Maize Genet. Coop. News Letter **38**: 82–84. ——, 1967 Crossing over and chromosomal segregation involving the B⁹ elements of the A–B translocation B–9b in maize. Genetics **55**: 433–449.

ROMAN, H., 1947 Mitotic nondisjunction in the case of interchanges involving the B-type chromosomes in maize. Genetics **32**: 391–409.

ROMAN, H. and A. J. ULLSTRUP, 1951 The use of A–B translocations to locate genes in maize. Agron. J. **43**: 450–454.

SPRAGUE, G. F., 1929 Hetero-fertilization in maize. Science **69**: 526–527. ——, 1932a The inheritance of scutellum colors in maize. U.S. Dept. Agric. Tech. Bull. 292. ——, 1932b The nature and extent of heterofertilization in maize. Genetics **17**: 358–368.