THE CHROMOSOMAL LOCATION OF Rf_1, A RESTORER GENE FOR CYTOPLASMIC POLLEN STERILE MAIZE[1, 2]

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Received April 17, 1961

COMPLETE restoration of pollen fertility to cytoplasmically pollen sterile maize in Texas (T) cytoplasm was shown by JONES (1951) to be mediated by at least one major dominant gene, and was later shown by DUVICK (1956) to require the simultaneous presence of at least two dominant genes, plus modifiers. The two major genes have been designated Rf_1 and Rf_2. Rf_2 is present in homozygous dominant form in most United States inbred lines of corn, whereas Rf_1 usually is homozygous recessive. BLICKENSTAFF, THOMPSON and HARVEY (1958) have reported that a major gene for full restoration of T steriles is located on chromosome 3 and that it has the following linear order and map distances with regard to anthocyanin-1 (A_1) and liguleless leaf-2 (Lg_2), two marker genes of chromosome 3:

$$A_1—36.2—Lg_2—20.8—Rf$$

In preliminary reports SNYDER (1955) and ANDERSON and DUVICK (1956) have also reported that a major gene for restoration of pollen fertility to T steriles is located on chromosome 3. SNYDER used recessive marker genes, and ANDERSON and DUVICK used endosperm-marked reciprocal chromosome translocations as described by ANDERSON (1952).

The present article amplifies the preliminary reports of SNYDER and of ANDERSON and DUVICK, presents additional data, and identifies the gene located by both methods as Rf_1. All genes referred to as Rf_1 and rf_1 in this presentation have been shown by means of appropriate testcrosses to be allelic to the recessive fertility restorer gene (designated rf_1) in the inbreds SK2, C106, and KA8. The restorer genetics of these inbreds has been described by DUVICK (1956).

PART I. USE OF CHROMOSOMAL INTERCHANGES FOR LOCATING POLLEN RESTORATION GENES. MATERIALS AND METHODS

Several reciprocal translocation stocks from among those maintained at the California Institute of Technology were crossed to WG3, BH2 and KY21, three

[1] Contribution No. 3179 of the Maryland Agricultural Experiment Station; Scientific Article No. A869.

[2] This work was supported, in part, by a contract with the U. S. Atomic Energy Commission (At-04-3-41).

inbred lines which are homozygous for the dominant forms of Rf_1 and Rf_2. All translocations were closely linked to *wx*, on chromosome 9. The translocation stocks used were as follows:

1-9a	5-9a	8-9a
1-9c	6-9e	9-10b
1-9 $_{4698}$	6-9c	
2-9b	7-9 $_{4363}$	
3-9c	7-9a	
3-9g		
4-9g		

The crosses (translocation × restorer) were then testcrossed as males to a T-sterile inbred of the waxy genotype *wx wx* rf_1 rf_1 Rf_2 Rf_2. The inbred contained all needed modifiers to Rf_1, so that all Rf_1—Rf_2—plants were fully fertile. All translocation stocks were found to be rf_1 rf_1, but were not tested for identity of the rf_2 gene. The tests with translocations were set up to identify linkages between translocations and rf_1.

The testcrosses were grown in both Iowa and California, or in Iowa only. They were classified as "fertile" or "sterile" and also as "normal" or "translocation heterozygote." The first pair of classifications was based on anther examination and refers to fertility restoration of cytoplasmic pollen sterility. The second pair of classifications was based on examination of open-pollinated ears and refers to the semisterility of eggs and pollen caused by a heterozygous translocation. Criteria for classification were as follows:

Fertile: Anthers normal, with 95–100 percent of pollen normal-appearing except in case of translocation heterozygotes, all of which (in the absence of cytoplasmic male sterility) have anthers with uniformly pebbly surface and about 50 percent of aborted pollen grains.

Sterile: Includes all completely pollen-sterile plants plus all grades of partial pollen fertility except that due to translocation heterozygotes as described above.

Normal: Full seed set on open-pollinated ears.

Translocation heterozygote: Scattered seed set, about 50 percent of possible kernels.

In most seasons, hot dry weather reduced the fertility of partial fertiles and it was relatively easy to divide plants into fertile and "sterile" classes. In one season, however, to be discussed further, partial fertility was sufficient to cause some problems in classification.

RESULTS AND DISCUSSION

Both of the 3-9 translocations showed a close linkage with rf_1. No linkage was found between rf_1 and any of the other translocations. Table 1 summarizes the tests with the 3-9 translocations. Progenies within a particular testcross have been combined, for none differed significantly within a testcross pedigree. Chi-

TABLE 1

Summary of testcrosses involving chromosome 3–9 reciprocal translocations and Rf_1

Pedigree	Location	Year	Classification: Parental NF* No. plants	Parental TS No. plants	Recombination NS No. plants	Recombination TF No. plants	Percent N	Percent F	Percent recombinations
Waxy ^{T}(T3-9g × WG3)	Iowa	1955	37	43	5	4	46	52	10
Waxy ^{T}(T3-9c × BH2)	Iowa	1956	50	39	2	0	57	55	2
	Calif.	1956	30	26	0	1	53	54	2
Waxy ^{T}(T3-9c × WG3)	Iowa	1956	259	277	7	9	48	49	3
	Calif.	1956	118	109	1	2	52	52	1
Waxy ^{T}(T3-9c × KY21)	Iowa	1958	186	213	14	10	47	46	6

* NF—normal; fertile. TS—translocation heterozygote; sterile.
NS—normal; sterile. TF—translocation heterozygote; fertile.
See text for further explanation of classification types.

square tests showed that none of the values shown for percent normals and for percent fertiles differed significantly from the expected 50 percent.

The small population involving the 3-9g translocation had about ten percent of recombinants. The testcrosses involving translocation 3-9c had about one to three percent of recombinants except for that made with KY21 as source of restoration. Here, the percentage of recombinations was 5.7. This was significantly different ($\chi^2<.01$) from the pooled value for the other 3-9c tests. The other values did not differ significantly among themselves. The reason for the high recombination figure for the KY21 testcross is not clear. It differed from the others in several ways: (1) The initial cross to 3-9c was made at a different time, although going back to the same source; (2) the final testcross was made at a different time and place and was grown in a different season; (3) the number of partial fertiles in the testcross was higher than in the other testcrosses, and their fertility was so high that in some cases it was hard to know with certainty where to draw the line between full fertiles and "steriles." The standard used, that of calling only completely normal-appearing tassels "fertile" and all others "sterile" was arbitrary (in the absence of progeny tests of the classified plants), even though it did give an equal distribution of the two fertility classes.

Cytological studies by LONGLEY (1958) place the breakage points of the two 3-9 translocations as follows:

3-9g	3L.40	9L.14
3-9c	3L.09	9L.12

All chromosome translocations tested involved chromosome 9 but since only those also involving chromosome 3 showed linkage with rf_1, it is apparent that rf_1 is on chromosome 3, rather than 9. The close linkage with 3-9c indicates that rf_1 is reasonably close to the centromere, probably on the long arm.

The close linkage with 3-9c also indicates that rf_1 probably is near tassel seed-4 (ts_4) at a maximum distance of about eight units since the 3-9c breakpoint has been shown to be closely linked (about 2.4 units) to ts_4 (E. G. ANDERSON, unpublished data). One must remember of course that suppression of crossing over

in the region of the translocation may have occurred, so that the genetic map distance could be greater than is indicated by this method.

PART II. USE OF MARKER GENES FOR LOCATING POLLEN RESTORATION GENES.

MATERIALS AND METHODS

The original cytoplasmic male sterile lines utilized in these tests were obtained from DR. D. F. JONES. The marker lines were obtained from the Maize Genetics Cooperative. The following recessive marker genes were used to test for linkage of restorer genes with chromosome 3: ts_4, lg_2, and dwarf-1 (d_1). The crosses made are indicated in Table 2, which shows the genotypes of the parents and of the F_1 progeny, as well as F_1 phenotypes and numbers of F_1 plants observed. The marker genes slashed (*sl*) and glossy seedling-1 (gl_1) were used to test for linkage of restorer genes with chromosome 7, and marker genes brachytic-1 (br_1), brown midrib-2 (bm_2) and anther ear-1 (an_1) were used to test for linkage with chromosome 1. Table 3 shows in detail the crosses made with these two sets of marker genes.

RESULTS AND DISCUSSION

Rf_1 was shown to be linked with all of the marker genes on chromosome 3 (Table 4) with the closest linkage being to ts_4. Each of the allelic sets studied gave evidence of normal segregation. The F_2 ratios did not differ significantly from expected (Table 5). The linkage data reported earlier (SNYDER 1955), and shown in this report as Pedigree F, had indicated that ts_4 and Rf_1 were about one unit apart according to the recombinations between these two genes directly, but that they were about ten units apart by difference estimates involving linkage of each of these two genes with the other marker genes used. Linkage values derived from Pedigree F could have been inaccurate since (1) the cross was made in the repulsion phase, (2) the linkages of the marker genes are with a member of a segregating complementary pair of *Rf* genes, and (3) the F_2 population was quite small. In Pedigrees A through E, by way of contrast, one member of the complementary pair of *Rf* genes was homozygous dominant in both parents so that Rf_1 linkage values are based on 3:1 instead of 9:7 fertility segregations.

The more extensive data from Pedigrees A through E indicate that the estimate of ten units between Rf_1 and ts_4 was probably the most nearly correct. Differences between Rf_1-ts_4 recombination values for Pedigrees A through E (Table 4) were not statistically significant and it was determined by a 4×5 contingency table that the classification data are homogeneous. Therefore, the data were combined and a recombination value of about 10.5 percent was calculated from a total of 1570 F_2 plants. Similarly, the Rf_1-lg_2 and Rf_1-d_1 recombination values for Pedigrees B and D are not significantly different and it was found that these data are homogeneous. The combined data from a total of 510 F_2 plants gave recombination values of about 34.5 percent for the Rf_1-lg_2 linkage and 26.5 percent for the Rf_1-d_1 linkage. It is interesting to note that the sum of the recombination values for Rf_1-ts_4 (10.5%) and ts_4-lg_2 (23.6%) is approximately

TABLE 2

Parental and F_1 data of cyto-sterile × chromosome 3 marker line crosses

Pedigrees	Parents entering crosses	Genotypes of parents	F_1 plants	F_1 genotypes	F_1 phenotypes
A	C106^{T*} × (Coop 49–26)^{TR}†	$\frac{+\ rf_1}{+\ rf_1}\frac{+}{+} \times \frac{ts_4\ +}{ts_4\ +}\frac{+}{+}$	30	$\frac{+\ rf_1}{ts_4\ +}\frac{+}{+}$	all fertile
B	(Coop 49–26)^{TR} × (Coop 55–1220–7)–1	$\frac{ts_4\ +}{ts_4\ +}\frac{+}{+} \times \frac{+\ rf_1}{+\ rf_1}\frac{+}{+}$	30	$\frac{+\ rf_1}{ts_4\ +}\frac{+}{+}$	all fertile
C	(Coop 49–26)^{TR} × (Coop 55–1220–1)–1	$\frac{ts_4\ +}{ts_4\ +}\frac{+}{+} \times \frac{+\ rf_1}{+\ rf_1}\frac{+}{+}$	30	$\frac{+\ rf_1}{ts_4\ +}\frac{+}{+}$	all fertile
D	C106^T × (Coop 49–26)–3	$\frac{+\ rf_1}{+\ rf_1}\frac{+}{+} \times \frac{ts_4\ +}{ts_4\ +}\frac{+}{+}$	30	$\frac{+\ rf_1}{ts_4\ +}\frac{+}{+}$	all fertile
E	C106^T × (Coop 49–26)–2	$\frac{+\ rf_1}{+\ rf_1}\frac{+}{+} \times \frac{ts_4\ +}{ts_4\ +}\frac{+}{+}$	30	$\frac{+\ rf_1}{ts_4\ +}\frac{+}{+}$	all fertile
F	C106^T × (Coop 49–26)–1	$\frac{+\ rf_1}{+\ rf_1}\frac{+}{+} \times \frac{ts_4\ +}{ts_4\ +}\frac{rf_2}{rf_2}$	30	$\frac{+\ rf_1}{ts_4\ +}\frac{rf_2}{+}$	all fertile

* "Texas" type cyto-sterile.
† Restored cyto-sterile tassel-seeded line.

TABLE 3

Parental and F_1 data of cyto-sterile × chromosome 7 and chromosome 1 marker line crosses

Pedigrees	Parents entering crosses	Genotypes of parents	F_1 plants	F_1 genotypes	F_1 phenotypes
G	Wf9^T × (Coop 55–1220–7)–2	$\frac{+}{+}\frac{rf_1}{rf_1}\frac{rf_2}{rf_2} \times \frac{sl}{sl}\frac{+}{+}\frac{+}{+}$	30	$\frac{+}{sl}\frac{+}{rf_1}\frac{+}{rf_2}$	all fertile
H	Wf9^T × (Coop 55–1220–7)–3	$\frac{+\ +}{+\ +}\frac{rf_1}{rf_1}\frac{rf_2}{rf_2} \times \frac{gl_1}{gl_1}\frac{sl_1}{sl_1}\frac{+}{+}\frac{+}{+}$	30	$\frac{+\ +}{gl_1\ sl}\frac{+}{rf_1}\frac{+}{rf_2}$	all fertile
I	C106^T × (Coop 50–32–5)–1	$\frac{+\ +}{+\ +}\frac{rf_1}{rf_1}\frac{+}{+} \times \frac{br_1}{br_1}\frac{bm_2}{bm_2}\frac{+}{+}\frac{rf_2}{rf_2}$	30	$\frac{+\ +}{br_1\ bm_2}\frac{+}{rf_1}\frac{+}{rf_2}$	all fertile
J	C106^T × (Coop 51–80)–1	$\frac{+\ +}{+\ +}\frac{rf_1}{rf_1}\frac{+}{+} \times \frac{br_1}{br_1}\frac{an_1}{an_1}\frac{+}{+}\frac{rf_2}{rf_2}$	30	$\frac{+\ +}{br_1\ an_1}\frac{+}{rf_1}\frac{+}{rf_2}$	all fertile

TABLE 4

F_2 Crossing over values among chromosome 3 marker genes and between marker genes and Rf_1

Pedigrees	ts_4–lg_2 Percent	ts_4–d_1 Percent	lg_2–d_1 Percent	Rf_1–ts_4 Percent	Rf_1–lg_2 Percent	Rf_1–d_1 Percent
A	.....	.....	.....	8.0±2.8	.....	.....
B	19.5±1.7	23.0±2.1	35.0±2.5	15.5±4.1	33.0±3.6	25.5±3.8
C	.....	.....	.....	9.0±3.3	.....	.....
D	26.5±2.3	33.5±2.6	49.5±3.2	12.0±4.2	36.5±3.7	27.0±3.9
E	.....	.....	.....	0.0±6.5	.....	.....
Combined pop.	23.5±1.5	28.0±1.6	41.5±2.0	10.5±1.7	34.5±2.6	26.5±2.7
F	24.5±2.3	30.0±2.6	52.0±3.5	1.0±354.0	36.0±10.5	21.0±17.4

TABLE 5

P values for 3:1 and 9:7 fertility and 3:1 chromosome 3 marker gene F_2 segregations

Pedigrees	Number plants	3 fertile 1 sterile	3 Ts_4 1 ts_4	3 Lg_2 1 lg_2	3 D_1 1 d_1
A	553	0.10	0.95	...	...
B	260	0.10	0.80	0.50	0.60
C	400	0.70	0.30	...	...
D	250	0.80	0.95	0.85	0.15
E	107	0.80	0.02	...	...
Combined pop.	1570	0.02 9 Fertile 7 Sterile	0.75	0.60	0.60
F	213	0.20	0.98	0.40	0.08

equal to the Rf_1-lg_2 recombination value (34.5%). The Rf_1-lg_2 and Rf_1-d_1 values for population F are in reasonably close agreement with those for populations A through E, combined.

All recombination values were calculated by the product method (IMMER 1930). The Rf_1-ts_4 value was verified by the maximum likelihood method for the combination of data from several populations (ALLARD 1956).

No linkage was demonstrated between fertility restoration and marker genes on either chromosome 1 or 7. Chi-square tests of the recombination data indicated independent assortment between restoration and each of the marker genes employed. Thus, it is indicated that the second major restorer gene tested here (possibly Rf_2, although it has not been tested for allelism with the gene designated rf_2 in Wf9) is not located on the short arm of chromosome 7 nor on a considerable length of the long arm of this chromosome. It also does not appear to be located on a considerable length of the long arm of chromosome 1.

Discussion concerning linkage data obtained by the use of marker lines: Difficulty in obtaining backcross linkage data from crosses made in the coupling phase has arisen from the fact that pollen restoration is dependent upon two complementary *Rf* genes which can be heterozygous in both the cyto-sterile and marker lines. At the time these studies were conducted, two *Rf* genotypes were available,

namely, C106^{T} ($rf_1\ rf_1\ Rf_2\ Rf_2$) and Wf9^{T} ($rf_1\ rf_1\ rf_2\ rf_2$). The other homozygous genotype ($Rf_1\ Rf_1\ rf_2\ rf_2$) needed for efficient testing of the *Rf* genotypes of marker plants is now available (DUVICK 1959). In addition, restored cyto-steriles of the $Rf_1\ Rf_1\ Rf_2\ Rf_2$ genotype have been isolated. One such line is designated C106^{TR}.

In the future it is planned to make the Cyto-sterile × Marker line crosses in the coupling phase to facilitate the collection of backcross linkage data. To accomplish this and be certain which *Rf* gene is under test for linkage, the genotypes entering into the crosses will be as follows:

(1) For testing Rf_1—Marker linkage

C106^{TR} ($Rf_1\ Rf_1\ Rf_2\ Rf_2\ MM$) × Marker ($rf_1\ rf_1\ Rf_2\ Rf_2\ mm$)

(2) For testing Rf_2—Marker linkage

C106^{TR} ($Rf_1\ Rf_1\ Rf_2\ Rf_2\ MM$) × Marker ($Rf_1\ Rf_1\ rf_2\ rf_2\ mm$)

The rf_1 and rf_2 genotypes of the marker lines will be determined by testcrossing each marker line plant to cyto-sterile versions of (1) the $Rf_1\ Rf_1\ rf_2\ rf_2$ and (2) the $rf_1\ rf_1\ Rf_2\ Rf_2$ genotype. The F_1 progenies of these paired crosses will be all fertile and all sterile respectively, if the marker plant was $rf_1\ rf_1\ Rf_2\ Rf_2$. They will be all sterile and all fertile respectively, if the marker plant was $Rf_1\ Rf_1\ rf_2\ rf_2$.

GENERAL DISCUSSION

The marker gene studies and the translocation studies have shown close agreement in placing the rf_1 locus in the proximal portion of the long arm of chromosome 3, not more than about 11 units from ts_4. The marker gene studies indicate further that the locus is to the left of ts_4, that is, between ts_4 and d_1. The *Rf* gene studied by BLICKENSTAFF *et al.* (1958) also was located in this general region, and it too may well be Rf_1, although published data do not list any crosses which definitely establish its allelism with the rf_1 locus as defined in this report. However, the linkage of *Rf*—lg_2 reported by BLICKENSTAFF *et al.* is 20.8 ± 2.3, whereas that of Rf_1—lg_2 reported here is 34.5 ± 2.6, which leaves an area of disagreement of about 14 units. It is possible that the vagaries of sampling, or unknown influences on recombination frequencies could be responsible for the different estimates or it is also conceivable that the *Rf* gene studied by BLICKENSTAFF *et al.* is at a different locus. An obvious next step is to cross one of the Rf_1 lines used in our studies with the one used by BLICKENSTAFF *et al.* and then testcross the F_1 to an $rf_1\ rf_1$ tester such as C106^{T}. This is the procedure used to establish identity of the various Rf_1 genes studied by the two methods described in the present paper. An alternative would be to cross C106, SK2 or KA8 to one of the two cytoplasmic male sterile single crosses BLICKENSTAFF *et al.* used. If the three-way cross were male sterile, this would indicate that their *rf* source was indeed rf_1.

SUMMARY

Rf$_1$, one of two major dominant genes needed to restore pollen fertility to cytoplasmically pollen sterile maize (in Texas cytoplasm) has been shown to be on the proximal end of the long arm of chromosome 3, by means of tests involving chromosomal translocations and tests involving marker genes. Both methods place *Rf*$_1$ not more than about 11 crossover units from *ts*$_4$. The marker gene method gives the following map distances and gene order: *d*$_1$—27—*Rf*$_1$—11—*ts*$_4$—24—*lg*$_2$.

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