Linkage and Inheritance Studies Involving an Annual Pollen Restorer and other Genetic Characters in Beta vulgaris L.

Theron E. Roundy

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LINKAGE AND INHERITANCE STUDIES INVOLVING AN ANNUAL
POLLEN RESTORER AND OTHER GENETIC CHARACTERS

IN BETA VULGARIS L.

by

Theron E. Roundy

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1972
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Theron E. Roundy
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ABSTRACT

Linkage and Inheritance Studies Involving an Annual Pollen Restorer and other Genetic Characters in Beta vulgaris L.

by

Theron E. Roundy, Master of Science

Utah State University, 1972

Major Professor: Dr. DeVere R. McAllister
Thesis Director: Dr. J. Clair Theurer
Department: Plant Science

A pollen-restorer sugarbeet inbred, developed by four generations of selection of highly fertile plants from a CMS X table beet cross, was studied to determine if a change from sterile to fertile cytoplasm had occurred. Data showed that the fertility expressed by the restorer inbred was the result of genetic factors and not cytoplasmic reversion. Linkage tests with the \( R^f \) gene showed independence of the YRB group, \( m \) and \( vi_4 \). A yellow-leaf mutant was inherited as a simple recessive factor. A partial pollen-restorer character, found in the yellow-leaf material, was inherited as a single dominant gene. The restorer factor was independent of \( yl \) and \( m \), while the \( yl \) gene showed independence of \( m \) and \( B \).

(40 pages)
INTRODUCTION

Cytoplasmic male sterility (CMS) is one of the most valuable tools that a plant breeder can utilize in his work because it eliminates the time consuming work of emasculation or roguing. One of the greatest benefits of cytoplasmic male sterility is its usefulness in the economic production of commercial crops. To utilize this sterility in commercial production, breeding lines which carry genes for complete pollen restoration must be available in crops grown for seed. Restorer genes are necessary in sugarbeets only when a cytoplasmic male-sterile line is used to produce the single-cross pollinator involved in a four-way hybrid.

Good pollen-restorer genes have been developed in some crops such as maize and sorghum. However, strong pollen-restorer genes are very scarce in sugarbeets. In the 1940's F. V. Owen made an effort to select and develop pollen-restorer lines in sugarbeets. He did not find a line, in the curly-top resistant material with which he was working, which could completely restore pollen fertility. From that time until the 1960's very little work was undertaken in this area of investigation. Since 1960 a renewed effort has been exerted, particularly at the U.S. Department of Agriculture, Crops Research Laboratory at Logan, Utah, to develop pollen-restorer lines for commercial use. A good fertility-restorer gene (R^f) was discovered and isolated from a CMS sugarbeet X table beet cross in 1966. Selection of the most fertile plants in the most fertile populations and selfing
through four generations yielded an annual sugarbeet line in which all plants were completely fertile.

The objectives of this study were to determine whether the fertility developed in this restorer inbred was due to genetic factors or to reversion to normal cytoplasm. In addition, linkage tests were conducted with some common marker genes and the $R^f$ gene to determine if a linkage association existed between them. A chlorophyll mutant which carried a partial pollen-restorer factor was also studied for inheritance and linkage. It is hoped that the information acquired from this study will not only add to the basic knowledge of the crop, but also be of use to those involved in its commercial breeding.
REVIEW OF LITERATURE

Cytoplasmic male sterility in sugarbeets is complex in nature and has been found to be affected by several factors. Owen (17) reported that sterility is conditioned by sterile cytoplasm in association with two complementary genes in the homozygous recessive state (Sxxzz). He further noted that when the dominant alleles of either or both genes were present, a partial restoration of fertility occurred. Plants having normal cytoplasm (N) were fertile regardless of the different alleles of x and z which were present. He found that certain hermaphrodites when crossed to male-sterile lines gave progeny which were completely male sterile. He concluded that these normal beets were of constitution Nxxzz and designated them as O-types. Bliss and Gableman (5) conducted an inheritance study utilizing cytoplasmic male-sterile sugarbeets and table-beet pollinators. They found that the dominant allele of the X gene restored fertility completely while the gene Z in a dominant condition restores partial fertility to plants with sterile plasm. Hogaboam (9) indicated that a new gene locus (Sh) enhances the pollen-restoring ability of the X and/or Z genes without being a pollen-restorer gene itself. Other investigators have presented data which concur with these experimental results (11, 12, 15, 31, 33). In addition to these major genes, phenotypic expression may be altered by minor modifying factors either in the cytoplasm or the chromosomes (15, 16, 22).

Environmental effects exert a significant influence on the expression of male sterility in sugarbeets. Owen (17) noted variation in the
expression of male sterility when plants were subjected to slightly different environmental conditions especially with regard to temperature. Rohrbach (22) reported that shorter day lengths promote increased sterility while fertility increases with longer days. He noted that semi male-sterile plants were much more susceptible to environmental changes than were sterile or fertile plants. Cortessi (8) also found that temperature affects the expression of cytoplasmic male sterility. Her work indicated that there is an apparent interaction between temperature and day length with regard to sterility expression. Other investigators have reported that lines intermediate in fertility are greatly influenced by the environment (13, 15). Cleij (7) found that exposure of germinated seed of CMS lines to temperatures up to 55°C resulted in the appearance of male-sterile plants.

Explanations other than environment or modifying factors have been given to account for the variation observed in progeny from crosses using a cytoplasmic male-sterile line. Stein, Gableman, and Struckmeyer (27) reported a change in fertility of progeny from crosses between cyto-sterile sugarbeets and fertile table beets. These plants appeared to be typical male-sterile lines at first, but developed a low percentage of fertile pollen in their later flowering stages. Some environmental differences existed but these had only a minor influence on the change. They concluded that the sterile cytoplasm had reverted to a normal condition.

A procedure for the production of four-way hybrids in sugarbeets utilizing cytoplasmic male-sterile and pollen-restorer lines has been suggested (32). Some success has been made in isolating strong restorer lines. Theurer and Ryser (33) isolated an inbred which carried strong pollen-
restorer genes from the variety US 201. A few other researchers have also reported studies involving pollen-restorer genes (5, 11, 12).

In 1961 Owen (18) crossed a cytoplasmic male-sterile line of sugarbeets, SLC 03 CMS, to the Ruby Queen variety of table beet. All the plants in the F₁ generation were fertile while the F₂ segregated both fertile and sterile plants. The material was then carried through four selfed generations by selecting the most fertile lines each time. This process of selection resulted in increased average fertility in each generation and ultimately yielded a line in which all plants had over 90% stainable pollen. This data showed that the table-beet variety carried strong pollen-restorer genes. Theurer (31) concluded that the pollen-restorer factor was inherited as a single dominant gene.

Although studies have shown the inheritance patterns of a number of genes in sugarbeets, relatively few of these have been placed into linkage groups. The YRB group has been the most extensively studied group. Included in this group are the genes for yellow pigment (Y), hypocotyl color (R), colored leaf (C₁), trout leaf (Tr), colored vein (Cᵥ), annual growth habit (B), variegated foliage (v₁), curly-top resistance (C), and crinkled foliage (cr) (1, 3, 19, 29).

Additional linkage associations have been determined between monogerm seed (m), late bolting (lb), and another gene for curly-top resistance. Mendelian sterility (a₁), lutescens (lu₂), and russet root (ru) have been proposed as marker genes for three of the seven remaining groups. They have shown independence of each other and other genes with the exception of lu₂ versus ru and m versus ru (29).
Chlorophyll deficiencies which result in white, light green, or variegated foliage are common among higher plants (20). These defects may be caused by alteration of the genome, plasmone, or plastome (34). Considerable work with chlorophyll deficiencies in various crops and other higher plants has been done to determine inheritance patterns and the causes of these defects. Some chlorophyll mutants in sugarbeets, such as variegated foliage \( (v_1) \), lutescens \( (lu) \), virescens \( (vi) \), and chlorina \( (ch) \) have been studied in relation to inheritance and linkage associations (25, 26, 28).

Hogaboam (9) indicated that linkage may exist between the \( X-x \) locus for pollen restoration in male-sterile cytoplasm and the \( M-m \) locus for monogermness. Bordonos (6) also suggested that pollen sterility is linked with the monogerm character. Kinoshita and Takahashi (12) conducted an experiment in which they found linkage between a pollen-restoration gene and the monogerm gene. In a different experiment, Kinoshita (11) reported a pollen-restorer factor which was governed by two complementary genes. He noted a linkage relationship between the male sterility and monogerm characters.
MATERIALS AND METHODS

Sources of Genetic Material

All the material which was used in this study was obtained from Dr. J. Clair Theurer, Research Geneticist, Agricultural Research Service, U.S. Department of Agriculture, Logan, Utah.

The line RB 55163 was the restorer inbred selection chosen for this study. It was the S₅ generation of the cross SLC O3 CMS X Ruby Queen table beet. In addition to the Rᵣ gene, this line carried the B gene for annual growth habit which was transferred to all subsequent progeny.

The other characters used in linkage analysis were obtained from stock sources of the respective genes. The lines utilized were as follows: 0534-(m); 953 CMS-(R); M9529-(R₅); M9522-1-(Tr); and M9536-(v₁₄).

A yellow leaf mutant selected from Great Western stock by company breeders was sent to Logan with other material in 1967. An S₂ line, M9520-6, homozygous for the yellow-leaf character was found to be carrying a pollen-restorer factor. This line was utilized in inheritance and linkage studies.

Description of Genetic Characters

B--Annual growth habit (1, 2, 4). Plants which carry the B allele initiate seed stalk production when subjected to warm temperatures and 18-24 hour light periods. This factor is affected by some minor modifiers which tend to alter expression under different conditions.
m--Monogerm seed character (23, 24). The homozygous recessive genotype is expressed in the development of single-germ seeds on the respective plants. The multigerm phenotype may be conditioned by a series of four different alleles (M, M^1, M^Br, M^2). This character is also altered by non-allelic factors which produce some double-germ fruits on a basically monogerm inflorescence.

R--Red pigment (2, 10, 19). Plants carrying the dominant allele produce a red pigmentation while the homozygous recessives express green coloration. This gene is made up of a series of different alleles (R, R^t, R^p, R^cs, r) which affect the coloration of hypocotyl, root and foliage. R alleles produce red hypocotyls in seedlings and red crown buds in older beets. R^p produces pink petioles and hypocotyls. R^t is expressed by strong red stripes extending into the petioles and also the seed stalk. R^cs plants show a brilliant pink coloration of crown and petioles. The character is also expressed in the seed stalk much like the R^t allele but lighter in color.

R^f--Restores fertility to CMS plants. This gene is expressed by the development of normal anthers and pollen in plants. The F_1 plants from a CMS X restorer cross produce yellow anthers and varying amounts of viable pollen. The phenotypic expression may be altered by environment and minor modifying factors.

Tr--Trout or spotted leaf (19). This character is expressed as red spots in the leaf with R and yellow spots with Y. Pigmentation is the most intense in the first true leaves. There is considerable variation from plant to plant in the expression of this factor.

vi_4--Virescens (26). This character is inherited as a simple recessive factor. Seedlings of vi_4 plants are normal but there is
a delay in chlorophyll production in the development of the early true leaves. There is considerable variation between plants in the time necessary for normal pigmentation to develop. Recessive and dominant plants are indistinguishable in later growth.

*y1*—Yellow leaf (Figure 1). Cotyledons and first true leaves are normal in coloration and development regardless of the genotype. Phenotypic expression does not occur until after approximately 2 months of growth. Older leaves begin turning light green first with the younger leaves developing lighter coloration later. The leaf coloration of the developed character corresponds to Calliste Green in Ridgeway's "Color Standards" (21). This light green color remains for 4 to 6 weeks and then the leaves gradually turn dark again until normal coloration is almost reached.

**Methods of Procedure**

The material used in this study was grown in the greenhouse during 1971 and the first part of 1972. The temperature was kept fairly constant at 70 F in the winter but it increased in the summer in relation to the outside temperature varying over the range 60-90 F. High light requirements were met by supplementing natural light with incandescent light for 8 hours at night and also on overcast days.

Evaluation of the restorer inbred cytoplasm was accomplished by crossing the emasculated inbred line to an O-type pollinator. Crosses for linkage studies were made by emasculating the restorer inbred, followed by hand pollination with appropriate marker lines serving as the pollen parent. Plants were emasculated in the early bud stage before the anthers were ready to dehisce. The sepals were gently spread
Figure 1. Sugarbeets showing normal (left) and yellow leaf (right) phenotypes
apart to remove the anthers and then carefully pushed back together again. Some difficulty was experienced during emasculation because the flowers are very small and susceptible to injury. Damage to the stigma or floral bracts results in permanent dessication of the stigma. Flowers were ready for pollination approximately 2 or 3 days after emasculation. Pollinations were made by breaking anthers over the stigmas and gently spreading the pollen on them.

When the marker stocks carried either cytoplasmic or Mendelian sterility, the restorer inbred was used as the pollinator in making the crosses for the linkage tests. These crosses were made by either exchanging bags on the inflorescences of the parents or by bagging the two plants together in the same snowfibre bag.

Seed from the original crosses was surface sterilized with a dilute solution of sodium hypochlorite for about 20 minutes, washed for 3 hours in running water, and then planted in small medicine cups filled with vermiculite. The F₁ plants were transplanted into soil-filled pots, classified, and selfed. Backcrosses were made to recessive parental lines with the F₁ plants used as the pollen parents. The F₂ seed was bulked for use in the classification of the F₂ segregates. Both F₂ and BC₁ plants were classified several times in reference to the characters being tested. Fertility observations were made on the F₂ and BC₁ material. In addition, the fertile F₂ segregates were crossed to the annual tester line SLC 03 CMS to determine if restorer genes were present in the material. This was necessary only with the virescens test in the backcross material because the other recessive parental lines were carrying sterile cytoplasm. Progeny from these
test crosses were planted in ground beds in the greenhouse for fertility classification.

Classification for fertility was made on the basis of anther color, pollen dehiscence, and the percentage of stainable pollen from aceto-carmine preparations of anthers and pollen. These fertility readings were made on each plant when approximately 15 to 20 flowers were open. Plants which exhibited very low fertility were observed for an additional 3 weeks. During this time pollen readings were made at weekly intervals to determine if there was any change in the fertility.

The $F_2$ progeny from the cross, normal X yellow-leaf mutant, were germinated in pots filled with vermiculite. The seedlings were then transplanted, two plants to a pot, and placed in growth chambers. The temperature was kept at 65 F and high intensity light provided for only 8 hours a day. This was done to prevent the plants from bolting as the gene for annual habit was present in the material and the yellow-leaf character was easier to classify on vegetative plants. The plants were watered as needed with a one-half normal Hoagland's solution. This insured adequate soil fertility so that classification would be made for the mutant character and not a mineral deficiency. After classification for the yellow-leaf character, the plants were placed in a well-lighted greenhouse for seed stalk production. Observations were then made for annual growth habit, monogermness, and percent fertility.

The data collected in each test was analyzed by $\chi^2$ statistics to determine the goodness of fit to expected genetic ratios. Analysis was made on plants from the $F_1$, $F_2$ and $BC_1$ generations. Mather's
formulas (14) for partitioning the total \( \chi^2 \) into its three components were utilized in ascertaining linkage relationships.

**Description of Crosses**

The crosses which were made to determine inheritance patterns and linkage associations are described in detail in this section.

The following cross was used to evaluate the nature of the cytoplasm of the restorer inbred.

\[
\text{RB 55163 (emas.)} \times \text{953 (S } R^f R^f r^r B^B) \quad \text{ (N } r^f r^f R^R B^b) \\
\]

The following crosses were made to study linkage relationships between the restorer gene and the R gene for red hypocotyl color.

0830 is the \( F_2 \) generation of the cross

\[
\text{953 CMS} \times \text{RB 55163 (S } r^f r^f R^R B^b) \quad (S R^f R^f r^r B^B)
\]

0832 is the \( BC_1 \) generation of the cross

\[
\text{03 CMS} \times \text{0811 (S } r^f r^f r^r B^B) \quad (S R^f R^f R^R B^b)
\]

The following crosses were used to determine if linkage existed between the restorer factor and the candy-stripe character.

0828 is the \( F_2 \) generation of the cross

\[
\text{RB 55163 (emas.)} \times \text{M9529} \quad (S R^f R^f c^c s^s r^r B^B) \quad (r^f r^f c^c s^s c^c B^b)
\]

0831 is the \( BC_1 \) generation of the cross

\[
\text{03 CMS} \times \text{0813 (S } r^f r^f c^c s^s r^r B^B) \quad (S R^f R^f c^c s^s c^c B^b)
\]

The following crosses were made to study the linkage association of the restorer gene with the gene for trout leaf.
0826 is the F₂ generation of the cross

RB 55163 (emas.) × M9522-1
(S R⁵ R⁵ trtrrrBB) (r⁵ r⁵ TrTrRRbb)

0844 is the BC₁ generation of the cross

03 CMS × 0812
(S r⁵ r⁵ trtrrrBB) (S R⁵ r⁵ TrtrRrBb)

The following crosses were used to study the linkage association of the restorer gene with the gene for the monogerm character.

0836 is the F₂ generation of the cross

RB 55163 (emas.) × 0534
(S R⁵ R⁵ MMrrBB) (r⁵ r⁵ mmRRbb)

0846 is the BC₁ generation of the cross

B4137 × 0821
(S r⁵ r⁵ mmrrBB) (S R⁵ r⁵ MmRrBb)

The following crosses were made to study the linkage association of the restorer gene with the gene for virescens.

0838 is the F₂ generation of the cross

M9536 × RB 55163
(r⁵ r⁵ vi₄ vi₄ a₁ a₁ RRbb) (R⁵ R⁵ vi₄ vi₄ A₁ A₁ rrBB)

0837 is the BC₁ generation of the cross

M9536 × 0822
(r⁵ r⁵ vi₄ vi₄ a₁ a₁ RRbb) (R⁵ r⁵ vi₄ vi₄ A₁ a₁ RrBb)

The following cross was made to study the inheritance of the yellow-leaf mutant. In addition to this, linkage associations were determined with the genes for pollen restoration, monogermness, and annual growth habit.
0845 is the $F_2$ generation of the cross

\[
\begin{array}{ccc}
03\text{ CMS} & \times & M9520-6 \\
(S\ yly1r\ r^{MMBB}) & & (yly1R\ R^{mmbb})
\end{array}
\]
RESULTS AND DISCUSSION

Studies with the Restorer Inbred

Evaluation of the restorer cytoplasm

The segregation of the restorer gene in the $F_2$ line 0827 is summarized in Table 1. $F_1$ plants were very homogeneous, exhibiting excellent pollen dehiscence and high fertility. The $F_2$ generation yielded 261 fertile and 107 sterile plants. As previously noted, Theurer (31) concluded that the restorer factor was inherited as a single dominant gene. Based on this hypothesis, the data fit the expected ratio with a 0.07 probability. This indicated that the cytoplasm of the inbred had remained in a sterile condition. If it had reverted to normal cytoplasm, there would not have been any segregation in the $F_2$ generation but all progeny would have been fertile.

The variation in the percent fertility is presented in Figure 2. An explanation of the phenotypic expression of the first three classes is essential for complete understanding of the data. The MS I class consisted of plants which had white or brown, shrunken anthers. There was no yellow coloration associated with the anthers. Pollen was not produced by plants in this class. Plants in the MS II class had completely yellow anthers or brown anthers containing streaks of yellow. The pollen produced by such plants did not stain with aceto-carmine and was considered inviable at the first reading. However, one-half of the plants in this class did produce some viable pollen during later flowering stages. There was no anther dehiscence observed in this class. The T classification refers to the appearance
Table 1. Evaluation of normal versus sterile cytoplasm in the annual pollen restorer line RB 55163

<table>
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<tr>
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<th>Fertile</th>
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<th>Sterile</th>
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<th>$\chi^2$</th>
<th>P*</th>
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<td>Obs.</td>
<td>Exp.</td>
<td>Obs.</td>
<td>Exp.</td>
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<tr>
<td>$F_1$</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_2$</td>
<td>261</td>
<td>276</td>
<td>107</td>
<td>92</td>
<td>3.26</td>
<td>.05-.10</td>
</tr>
</tbody>
</table>

* Expected on basis of 3:1 segregation in the $F_2$
Figure 2. Fertility variation of 368 plants in the F₂ of RB 55163 X 953.
of a trace of stainable pollen. This classification was based on the observance of one stainable pollen grain to 100 of the nonstainable ones. Anthers were yellow in color and may have dehisced.

Classification of plants into these low fertility groups was difficult, particularly for the MS II and T classes. The phenotypic expression was affected to a great extent by environment as well as by minor genetic factors. Nielson (15) reported similar difficulty in classification, during extensive testing for O-type pollinators, because minor genes tend to modify the fertility of progeny from various CMS crosses. He also observed that environmental interactions caused a considerable amount of variation among different genotypes. Oldemeyer (16), Theurer (30, 33) and Hogaboam (9) also reported considerable variability in phenotypic expression due to modifying factors.

The large amount of variation in fertility that was expressed in this experiment is evidenced by the data in Figure 2. Approximately one-fourth of the plants had over 70% stainable pollen. Another one-fifth of the progeny were in the range of 20 to 60%, while the balance of the plants exhibited very low fertility. This variation was very similar to that which Theurer found in his work (31). The partial male fertiles were observed over a period of 3 weeks during which time many of them expressed a change in fertility. Most of them increased in fertility, some remained the same, but none decreased in fertility. Because of this information, the plants in the MS II and T classes were considered to be of the fertile genotype. On the other hand, the plants in the MS I class did not show any change in fertility regardless of the length of time involved. Furthermore, when these steriles were crossed back to an O-type pollinator, the resulting
progeny were completely male sterile. Thus the fertility variation was attributed to genetic factors.

**Linkage relationships of the \( R_f \) gene**

**YRB group.** Chi-square tests for linkage and deviation in Mendelian ratios of the \( R_f \) gene with the YRB linkage group are given in Table 2. X and Y denote the genetic factors under consideration in the cross. The designations under the heading linkage phase refer to the phase in which the genes entered the cross; i.e. repulsion or coupling; and the generation involved. Therefore, \( RF_2 \) represents material in the \( F_2 \) generation and the genes entered the cross in repulsion.

The overall \( \chi^2 \) value is not given but has been partitioned into its three components according to Mather. \( \chi^2_X \) values are for 3:1 ratios for the first gene listed. \( \chi^2_Y \) gives the values for 3:1 ratios for the second gene while \( \chi^2_L \) gives the value attributable to linkage between the two genes. Each \( \chi^2 \) has one degree of freedom.

The data given in Table 2 are from the tests involving the \( R_f \) gene with the red hypocotyl, candy stripe, and trout leaf characters. In every instance, both in \( F_2 \) and \( BC_1 \), there was no indication of linkage between the genes tested. Three of the values in the table were significant. However, these deviations were the result of the failure of the respective genes to fit the expected 3:1 ratios.

Test crosses were made with \( F_2 \) plants from the candy stripe and trout leaf tests. Fertile \( F_2 \) segregates crossed to a CMS tester line gave progeny which were either completely fertile or segregating for fertility. Sterile segregates from the \( F_2 \) when crossed with an 0-type pollinator, produced completely male-sterile offspring. This proved
Table 2. $\chi^2$ tests for linkage and deviation in Mendelian ratios of the $R^f$ gene and genes in the YRB linkage group

<table>
<thead>
<tr>
<th>Genes $(X Y)$</th>
<th>Linkage Phase</th>
<th>No. of families</th>
<th>Number of individuals</th>
<th>Total</th>
<th>$\chi^2_x$</th>
<th>$\chi^2_y$</th>
<th>$\chi^2_L$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R^f R$</td>
<td>$R F_2$</td>
<td>20</td>
<td>167 84 48 17</td>
<td>316</td>
<td>3.308</td>
<td>8.168**</td>
<td>2.031</td>
</tr>
<tr>
<td></td>
<td>$R BC_1$</td>
<td>11</td>
<td>18 26 24 15</td>
<td>83</td>
<td>0.301</td>
<td>0.12</td>
<td>3.481</td>
</tr>
<tr>
<td>$R^f R^{cs}$</td>
<td>$R F_2$</td>
<td>8</td>
<td>72 35 33 16</td>
<td>156</td>
<td>3.418</td>
<td>4.923*</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>$R BC_1$</td>
<td>6</td>
<td>19 27 17 10</td>
<td>73</td>
<td>4.945*</td>
<td>0.013</td>
<td>3.082</td>
</tr>
<tr>
<td>$R^f Tr$</td>
<td>$R F_2$</td>
<td>10</td>
<td>63 25 34 7</td>
<td>129</td>
<td>3.165</td>
<td>0.002</td>
<td>2.24</td>
</tr>
<tr>
<td></td>
<td>$R BC_1$</td>
<td>7</td>
<td>20 22 22 22</td>
<td>86</td>
<td>0.046</td>
<td>0.046</td>
<td>0.046</td>
</tr>
</tbody>
</table>

* 5% point of significance
** 1% point of significance
that these sterile plants were not carrying restorer genes and that the sterility was cytoplasmically inherited, not Mendelian. Thus, the fertile F₂ plants carried restorer genes while the sterile segregates did not carry the Rf gene.

Monogerm—m. Data for the linkage test between the restorer gene and the monogerm factor is given in Table 3. The $\chi^2$ for the F₂ generation showed significance at the 3% level for both linkage and deviation of the monogerm factor from expected ratios. The significance of the m gene is probably due to error in classification. Savitsky (23) observed variation in homozygous mm plants in which some produced bigerm flowers on the basal portion of the main axis as well as the lateral branches. He attributed this development to modifying factors not associated with m. Some of the plants observed in this experiment produce nearly all bigerm flowers. Close examination of these plants revealed some monogerm flowers toward the terminal ends of the branches. However, no multigerm flowers were observed on these plants. Consequently, these plants were assigned to the homozygous recessive class. Some of the error observed in this test may be due to placing a number of these plants in the dominant class.

Significance of the $\chi^2$ for linkage indicated that linkage may have been present, although the actual probability of 3% is just less than the rejection point of 5%. This indicated that if linkage was present, it was quite weak. A recombination value of $0.402 \pm 0.037$ was calculated using the product method. This figure compares favorably with the values $0.362 \pm 0.017$ and $0.400 \pm 0.015$ which Kinoshita found in his work. The standard error of this experiment was twice as great as in Kinoshita's
Table 3. $\chi^2$ tests for linkage and deviation in Mendelian ratios of the $R^f$ gene with the $m$ and $vi_4$ genes

<table>
<thead>
<tr>
<th>Genes (X Y)</th>
<th>Linkage Phase</th>
<th>No. of families</th>
<th>Number of individuals</th>
<th>Total</th>
<th>$\chi^2_x$</th>
<th>$\chi^2_y$</th>
<th>$\chi^2_L$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R^f m$</td>
<td>C F2</td>
<td>16</td>
<td>201 42 47 20</td>
<td>310</td>
<td>1.896</td>
<td>4.133*</td>
<td>4.658*</td>
</tr>
<tr>
<td></td>
<td>C BC1</td>
<td>17</td>
<td>24 19 23 32</td>
<td>98</td>
<td>1.469</td>
<td>0.163</td>
<td>2.000</td>
</tr>
<tr>
<td>$R^f vi_4$</td>
<td>C F2</td>
<td>30</td>
<td>20 14 14 5</td>
<td>53</td>
<td>3.327</td>
<td>3.327</td>
<td>0.756</td>
</tr>
<tr>
<td></td>
<td>C BC1</td>
<td>2</td>
<td>5 6 4 3</td>
<td>18</td>
<td>0.888</td>
<td>0.000</td>
<td>0.222</td>
</tr>
</tbody>
</table>

* 3% point of significance
work, which was probably due to the fact that his populations contained approximately 300 to 500 more plants.

While the $F_2$ generation failed to fit the expected ratios, the $BC_1$ showed no significance due to linkage or to deviation of either gene. Classification was not a problem with the backcross material as the plants definitely expressed one phenotype or the other in regard to monogermness. In addition, no partial male fertiles were observed, but all plants were either sterile or had over 30% stainable pollen. The backcross clearly gave no indication of linkage between the two genes. Although the $F_2$ data showed significance, the recombination value of 40.2% was close to the 50% figure for independence. The difficulty in classification could have produced enough error to account for the significance observed. These results failed to confirm earlier investigations which showed that linkage did exist between the two characters. This was probably due to the fact that the same material was not used and the pollen restoration was conditioned by different genes.

**Virescens--vi$_4$.** Also included in Table 3 are the results of the test involving the vi$_4$ gene and the restorer gene. The data show no significance in either the $F_2$ or $BC_1$ generations. Little difficulty was experienced in classification of the chlorophyll mutant. Test cross progeny also showed good segregation into the different fertility classes. When plants were found to be carrying restorer genes, they expressed a fairly high degree of fertility. There were very few partial male fertiles.
Studies Involving a Yellow-Leaf Mutant

Inheritance of $R_f$ factor from the yellow-leaf mutant

During the process of determining the inheritance of the yellow-leaf character, observations showed that the mutant stock was carrying a pollen-restorer factor. In light of the study already in progress, a test was implemented to ascertain the inheritance pattern of this restorer character. Table 4 gives the results of this study. The observations made in the $F_2$ generation showed a 3:1 segregation of the fertile and sterile plants. This gave a good fit to the expected value. Most of the plants observed were very low in fertility. The MS II class comprised approximately one-third of the total population. Subsequent observations showed the same fertility changes as were noted previously. At the same time, the male-sterile plants expressed no alteration in fertility. Therefore, it appears that this fertility restoration factor is dependent on a single dominant gene. Its effectiveness in restoring fertility appears to be rather weak or limited, especially in comparison to the $R_f$ gene reported above. In this case, modifying factors seem to have a much greater influence on the phenotypic expression. The ability to restore fertility is apparently controlled by different genes in the respective lines.

Inheritance of the yellow-leaf character

Inheritance of the yellow-leaf mutant was determined by utilizing the same material as in the previous inheritance test. Table 5 summarizes the data from the yellow-leaf observations. Classification of the $F_1$ plants was made by comparing them with normal plants grown
Table 4. Inheritance of fertility restoration factor found in the yellow leaf material

<table>
<thead>
<tr>
<th></th>
<th>Fertile</th>
<th></th>
<th>Sterile</th>
<th></th>
<th></th>
<th>( \chi^2 )</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obs.</td>
<td>Exp.</td>
<td>Obs.</td>
<td>Exp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₁</td>
<td>18</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₂</td>
<td>209</td>
<td>219.75</td>
<td>84</td>
<td>73.25</td>
<td>2.103</td>
<td>.10-.20</td>
<td></td>
</tr>
</tbody>
</table>

* Expected on basis of 3:1 segregation in the F₂
Table 5. The inheritance of yellow leaf character in F$_2$ of a cross between the yellow leaf mutant and a normal line.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Exp</th>
<th>Yellow</th>
<th>Exp.</th>
<th>$\chi^2$</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obs.</td>
<td></td>
<td>Obs.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_1$</td>
<td>18</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_2$</td>
<td>339</td>
<td>340.50</td>
<td>115</td>
<td>113.50</td>
<td>.0264</td>
<td>.80-.90</td>
</tr>
</tbody>
</table>

* Expected on basis of 3:1 segregation in the $F_2$
concurrently under the same conditions. Plants homozygous for the \( y_l \) gene show a light green coloration in the top part of the inflorescence when bolting. The \( F_2 \) mutant plants showed some varying degrees of expression. Some of them expressed an almost complete yellowing of all the leaves. Others developed yellow spots which at first appeared to be the beginning of necrosis. However, these spots gradually enlarged to encompass the entire leaf area with no death of cells. After the plants were placed in the greenhouse, the mutant segregates regained some green coloration. They were barely distinguishable from the normal plants until seed stalk initiation. Chi-square analysis gave an excellent fit to the expected 3:1 ratio.

**Linkage relationships**

**Fertility-restorer factor from yellow-leaf mutant.** Linkage of this restorer gene was tested with the \( y_l \) and \( m \) genes. Data from the \( y_l \) and \( m \) tests are summarized in Table 6. Neither test showed any significance in regard to linkage between the factors. Therefore, the \( R_f^e \) gene is inherited independently of the genes for yellow leaf and monogermness.

**Yellow-leaf character--\( y_l \).** Table 6 also contains the data collected in testing the linkage of \( y_l \) with \( B \) and \( m \). No significant deviations were observed among the plants classified. Thus the \( y_l \) gene is inherited independently of the annual habit and monogerm characters.
Table 6. $\chi^2$ tests for linkage and deviation in Mendelian ratios of the $R^f$ gene, from the yellow leaf material, and the $y_l$ gene with the characters annual growth habit and monogermness.

<table>
<thead>
<tr>
<th>Genes (X Y)</th>
<th>Linkage Phase</th>
<th>No. of Families</th>
<th>Number of individuals</th>
<th>Total</th>
<th>$\chi^2_X$</th>
<th>$\chi^2_Y$</th>
<th>$\chi^2_L$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R^f$ yl</td>
<td>R F$_2$</td>
<td>18</td>
<td>X Y 166 Xy 43 xY 66 xy 18</td>
<td>293</td>
<td>2.103</td>
<td>2.731</td>
<td>0.0003</td>
</tr>
<tr>
<td>$R^f$ m</td>
<td>R F$_2$</td>
<td>18</td>
<td>X Y 157 Xy 52 xY 60 xy 24</td>
<td>293</td>
<td>2.103</td>
<td>0.137</td>
<td>0.519</td>
</tr>
<tr>
<td>yl m</td>
<td>C F$_2$</td>
<td>18</td>
<td>X Y 175 Xy 57 xY 42 xy 19</td>
<td>293</td>
<td>2.713</td>
<td>0.137</td>
<td>0.910</td>
</tr>
<tr>
<td>yl B</td>
<td>C F$_2$</td>
<td>18</td>
<td>X Y 257 Xy 86 xY 78 xy 33</td>
<td>454</td>
<td>0.073</td>
<td>0.355</td>
<td>0.940</td>
</tr>
</tbody>
</table>
SUMMARY

A test was conducted to determine if a change from sterile to normal cytoplasm had occurred in a pollen-restorer sugarbeet inbred that showed increased fertility each generation of selection. Results showed that the cytoplasm has not reverted but had remained in a sterile condition. The fertility developed in the inbred, through four generations of high fertility selection and selfing, was attributable to genetic factors.

Linkage tests of this restorer gene with the $R$, $R^{CS}$, and $Tr$ genetic characters showed independence of the YRB linkage group. Further tests with the $m$ and $v_{14}$ characters showed independence of the $R^f$ gene. The conclusions by other scientists that fertility restorer factors were associated with the monogerm character was not confirmed.

A factor, which restores partial fertility in sterile cytoplasm was found in material expressing a yellow-leaf mutant. Inheritance and linkage studies were conducted with both characters. The pollen-restorer factor was found to be inherited as a single dominant gene. However, results showed that it was not a strong restorer gene. The yellow-leaf character was conditioned by a single recessive gene. Linkage tests with the restorer gene showed independence of $yl$ and $m$. Independence of $m$ and $B$ was determined for the $yl$ gene.
LITERATURE CITED


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