Evaluation of Combining Ability in Self-Fertile Lines of Sugar Beets Using Male-Sterile Testers

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The discovery of cytoplasmic male sterility in sugar beets by Owen (4)[2] stimulated interest in developing improved varieties of sugar beets through hybridization on a commercial scale. Although male sterility in open-pollinated material can be used to produce top-cross varieties, it is desirable to develop and use inbred lines to obtain maximum benefit from hybrid combinations. Since *Beta vulgaris* L. is normally a highly self-sterile species, the development of inbred lines is difficult without using the self-fertile gene reported by Owen (3) in 1942.

Using self-sterile material Stewart, Gaskill and Coons (8), and Doxtator and Skuderna (2) found that hybrid vigor can be expected with sugar beet crosses particularly in gross sugar per acre. In both instances the information on sucrose content was inconclusive but they agreed that possibly in some specific combinations increased sucrose percentage could be obtained. However, the general belief among sugar beet breeders has been that sucrose content is conditioned by additive factors with no expression of heterosis or dominance for sucrose content. Coons in 1936 (1) reported heterosis for yield in sugar beets and stated that in most instances among 41 F_1 hybrids the sucrose percentages approached the means of the parents. More recently, Powers, ET AL. (6) and Peterson and Dickenson (5) have reported the expression of heterosis for sucrose content among single crosses.

This study was undertaken to obtain additional information on the use of male-sterile lines to produce test crosses for evaluating combining ability of self-fertile inbred lines and to study hybrid vigor in regard to yield and sucrose content.

Materials and Methods

The test crosses to the male-sterile lines were made in 1956 using space isolations. One self-fertile pollen parent was planted in each of 18 isolations with the respective male-sterile tester parents. The tester parents were rogued prior to and during flowering to remove possible pollen producers. Seed was harvested individually from each tester and pollinator parent.

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[2] Numbers in parentheses refer to literature cited.

Table 1.—Root Yields and Sucrose Percentages of Parental Lines.

Tester Parents	Yield Tons/Acre	Sucrose %
113H3	24.88	13.25
E322	23.55	14.22
E21	23.64	14.12
A2-90H0	20.22	13.92
175H15	18.00	14.90
Average	22.06	14.08
LSD .05	3.49	0.55
.01	4.70	0.74
Male Parents		
5-189	22.85	14.53
5-236	23.07	13.79
A2	20.30	13.97
5-235	19.05	13.92
5-185	17.23	14.20
5-207	15.15	14.19
5-181	14.33	13.17
5-197	13.76	14.31
5-180	13.64	13.78
5-231	13.05	13.90
5-238	13.03	13.91
5-239	12.30	13.32
5-237	11.56	13.88
5-204	11.15	12.54
5-205	10.28	13.92
5-208	10.28	13.72
5-188	9.96	13.84
5-194	9.54	13.97
Average	14.58	13.83
LSD .05	2.86	0.52
.01	3.86	0.70

The male-sterile testers used were as follows:

113H3[3]—A male-sterile line derived from US 22/3.

E322—An F_1 male sterile resulting from a cross between two self-sterile parents, 225H0[3] x C-51-1.

E21—An F_1 male sterile resulting from a cross between two self-fertile inbred lines, A2-90H0 x 19.2[3].

A2-90H0[3]—A male-sterile equivalent of a multigerm inbred line.

175H15[3]—A male-sterile equivalent of a monogerm inbred line.

The pollinator lines, with the exception of A2, were self-fertile inbred lines of diverse pedigrees. Fifteen of the lines were inbred three generations and two of them, 5-194 and 5-197, were inbred four generations. Lines 5-235 through 5-239 were closely

[3] These lines were developed by the USDA, ARS, Crops Research Division, Sugar Beet Section, Salt Lake City, Utah.

related, each descending from a different root selection from the same segregating F_2 material. A2 was an open-pollinated variety.

The experimental design for the progeny test was a randomized block with 12 replications. The parents were randomized within male and female groups and kept together as sub-blocks within each replication. Because of limited seed supplies of some lines and crosses, the plots were limited to single rows 40 ft. long. The field test was grown in 1957 at Nampa, Idaho.

Results

Parents:

A wide range in vigor was found among the pollen parents as shown by their respective yields in Table 1. Although they were planted in blocks apart from the more vigorous test crosses, biases were probably introduced because of competition between the single row plots used in the test. The range among the tester lines was not as great.

Table 2.—Gross Sugar per Acre for Test Crosses Between 18 Pollinator Lines and 5 Cytoplasmic Male-Sterile Testers. Figures Given Are Pounds per Acre.

Pollen Parents	Male-Sterile Testers					
	113H3	E322	E21	A2-90H0	175H15	Avg.
5-231	7840	8030	7300	7510	7500	7640 [1]
5-181	7900	7230	7980	7990	7010	7630 [1]
5-239	7910	7650	7620	8250	6350	7560
5-236	7500	7350	7720	7650	6720	7390
5-189	7600	7820	7910	7870	5570	7360
5-197	6800	7720	7990	8210	5900	7320
A2	7360	7370	7260	7060	7450	7300
5-207	6630	6840	7820	7350	7650	7260
5-194	6910	6720	7830	8350	6220	7220
5-235	7320	7610	7350	7370	6360	7210
5-180	7600	7240	7270	7220	6110	7090
5-185	7120	6540	7580	7240	6990	7090
5-238	6790	6640	7130	7560	6770	6990
5-204	7180	6740	7530	7110	6250	6970
5-188	7530	7020	7290	7350	5260	6910
5-208	7560	6610	6540	6920	6470	6820
5-205	6730	6710	7160	6730	6690	6810
5-237	6580	7190	7020	7240	5790	6780 [1]
Avg.	7270	7180	7460 [1]	7500 [1]	6510 [1]	7190
LSD .05	910	890	850	780	840	390
.01	N.S.	1200	N.S.	1060	1140	510

LSD between averages of testers .05 = 200, .01 = 270

Gross sugar per acre for check varieties: US22/3 = 6750, US35/2 = 6240

[1] Differ significantly from test average, .05.

Differences in sucrose content among the inbred lines were significant (.01) but the range was comparatively less than for yield. Omitting line 5-204 because the exceptionally low sucrose content is questionable, the two extremes differed by only 1.36 percent sucrose. The lack of a negative correlation between sucrose content and yield is noticeable among the self-fertile lines.

Test Crosses:

In evaluating the desirability of crosses, the greatest return per acre is the primary factor to consider. Gross sugar per acre is the best indirect measure of this but high gross sugar as a result of high sucrose content is preferred to high gross sugar due to high root yields.

The average of the five test crosses for each line was considered a measure of the general combining ability for the respective lines. Similarly, the measure of general combining ability for the testers was the average of the 18 crosses with the respective pollinator lines.

Table 3.—Sucrose Percentage of Test Crosses Between 18 Pollinator Lines and 5 Cytoplasmic Male-Sterile Testers.

Pollen Parents	Male-Sterile Testers					
	113H3	E322	E21	A2-90H0	175H15	Average
5-188	14.72	14.42	14 78	14.80	15.26	14.80[1]
5-231	14.89	14.22	14.65	14.75	15.21	14.74[1]
5-207	14.55	15.00	14.56	14.80	14.52	14.68
5-208	14.39	14.94	14.54	14.64	14.67	14.64
5-239	14.32	14.40	14.82	14.62	14.99	14.63
5-189	14.49	14.53	14.59	14.71	14.81	14.62
A2	14.45	14.52	14.62	14.59	14.76	14.59
5-197	14.35	14.61	14.72	14.62	14.43	14.55
5-204	13.88	14.55	14.46	14.36	14.93	14.44
5-238	14.31	14.28	14.31	14.21	14.93	14.41
5-205	13.93	14.28	14.00	14.09	15.55	14.37
5-236	13.92	14.46	14.41	14.41	14.63	14.36
5-237	13.62	14.44	14.17	14.33	15.09	14.33
5-194	13.90	14.15	14.41	14.22	14.98	14.33
5-235	13.91	14.12	14.62	13.99	14.80	14.29
5-181	14.59	14.02	14.20	14.00	14.52	14.27
5-180	14.13	14.48	14.28	14.22	14.25	14.27
5-185	14.02	14.14	14.34	13.96	14.18	14.13[1]
Average	14.24[1]	14.42	14.47	14.41	14.81[1]	14.47
LSD .05	0.68	N.S.	N.S.	0.57	0.62	0.28
.01	N.S.	N.S.	N.S.	0.75	0.81	0.37

LSD between averages of testers .05 = 0.15, .01 = 0.20

[1] Differ significantly from test average.

% Sucrose of check varieties: US22/3 = 13.78, US35/2 (824) = 14.51

Table 4.—Root Yields of Test Crosses Between 18 Pollinator Lines and 5 Cytoplasmic Male-Sterile Testers. Yields Are Given as Tons per Acre.

Pollen Parents	Male-Sterile Testers					
	113H3	E322	E21	A2-90H0	175H15	Average
5-181	27.1	25.8	28.1	28.5	24.1	26.73[1]
5-231	26.3	28.2	24.9	25.5	24.7	25.92
5-239	27.6	26.6	25.7	28.2	21.2	25.85
5-236	26.9	25.4	26.8	26.5	23.0	25.73
5-235	26.3	26.9	25.1	26.3	21.5	25.23
5-194	24.8	23.8	27.2	29.4	20.8	25.19
5-197	23.7	26.4	27.1	28.1	20.4	25.16
5-189	26.2	26.9	27.1	26.8	18.8	25.16
5-185	25.4	23.1	26.4	26.0	24.6	25.09
A2	25.5	25.4	24.8	24.2	25.2	25.02
5-180	26.9	25.0	25.5	25.4	21.4	24.85
5-207	22.8	22.8	26.8	24.8	26.3	24.71
5-238	23.7	23.2	24.9	26.6	22.7	24.24
5-204	25.8	23.2	26.0	24.8	20.9	24.14
5-205	24.2	23.5	25.6	23.9	21.5	23.71
5-237	24.2	24.9	24.8	25.3	19.2	23.66
5-188	25.6	24.4	24.7	24.8	17.2	23.33[1]
5-208	26.2	22.1	22.5	23.6	22.0	23.31[1]
Average	25.52	24.88	25.78[1]	26.04[3]	21.98[1]	24.83
LSD .05	2.9	2.8	2.7	2.5	2.6	1.25
.01	N.S.	3.8	N.S.	3.3	3.5	1.69

LSD between averages of testers .05 = 0.66, .01 = 0.89

Yields of check varieties: US22/3 = 24.5, US35/2 (824) = 21.5

[1] Differ significantly from test average, .05.

Tables 2, 3, and 4 give the results of the test crosses for gross sugar, percent sucrose, and root yields. Six lines were among the better general combiners both for gross sugar per acre and percent sucrose. These lines are 5-231, 5-239, 5-189, 5-197, 5-207, and A2.

Lines 5-181 and 5-236 were good combiners for yield and gross sugar per acre, but their hybrids were low in percent sucrose. Line 5-188 produced crosses that were high in sugar content but low in yield. These lines are not included in the following discussion.

The question arises as to the performance of the individual crosses among the six lines considered to have the best general combining ability. To study the effect of specific combining ability, the means of the individual crosses were adjusted by adding to or subtracting from them the deviation of the means of all respective crosses of each parent from the test averages. For example, the root yield of the cross 113H3 x 5-231 is adjusted as follows:

113H3 x 5-231 yielded 26.3 tons per acre
Test average = 24.83 T/A
All 5-231 crosses averaged 25.92 T/A
All 113H3 crosses averaged 25.52 T/A

Adjusted mean for 113H3 x 5-231 is as follows:

$$\overline{X}_a = 26.3 - (25.92 - 24.83) - (25.52 - 24.83)$$
$$= 24.5 \text{ tons per acre.}$$

The adjustment removes the general effect of both parents so that if specific effects did not occur the adjusted means of the individual crosses should equal the mean of the test . The deviations from the mean of the test then can be attributed to specific combining ability.

Table 5 gives the adjusted means of the test crosses for the six better general combining lines. It is noticeable with many of the crosses that a favorable specific reaction for yield is accompanied by an unfavorable reaction for sucrose percentage and vice versa. Crosses 113H3 x 5-231, E322 x 5-231. 175H15 x 5-207 and E322 x 5-207 are examples. Seven of the 30 crosses numeri-

Table 5.—Means of Crosses Involving the Better General Combining Lines Adjusted to Remove the General Effects of the Parents.

Pollen Parents	Male-Sterile Testers				
	113H3	E322	E21	A2-90H0	175H15
Yield, Tons/A					
5-231	24.5	27.1[3]	22.9	23.2	*26.5*[3]
5-239	25.9[3]	25.6[3]	23.8	*26.0*[3]	23.1
5-189	*25.2*[1]	26.6[3]	25.9[3]	*25.3*[1]	21.4
5-197	22.7	*26.1*[3]	*25.9*[3]	*26.6*[3]	23.0
5-207	22.2	22.9	26.0[3]	23.7	29.3[3]
A2	24.6	25.2[1]	23.7	22.8	27.9[3]
Test Mean = 24.8					
% Sucrose					
5-231	14.85[3]	14.00	14.38	14.54[1]	*14.60*
5-239	14.39	14.29	14.66[3]	*14.52*[1]	14.49[1]
5-189	*14.57*[1]	14.43	14.44	*14.62*[2]	14.32
5-197	14.50[1]	*14.58*[1]	*14.64*[3]	*14.60*[2]	14.01
5-207	14.57[1]	14.85[3]	14.35	14.65[3]	13.97
A2	14.56[1]	14.45	14.50[1]	14.53[1]	14.30
Test Mean = 14.47					

Italicized numbers are those that numerically exceed the test means for both yield and percent sucrose.

[1] Numerically above the test mean.

[2] Exceeds test mean at .05 level.

[3] Exceeds test mean at .01 level.

cally exceeded the test means for both yield and sucrose percentage (italicized in Table 5), with most differences being significant at .05 level of probability. In no instance did a cross which showed a large positive deviation from the test mean for yield also show a large positive deviation from the test mean for percent sucrose. The cross E21 x 5-197 gave deviations significant at .01 level of probability; however, its adjusted mean for yield was 3.3 tons per acre below the highest adjusted mean and 0.21 percent below the highest mean for percent sucrose.

Because there appeared to be a tendency for the higher yielding crosses to be down in sucrose, correlation coefficients were computed for yield vs. sucrose content using individual test cross means. Correlation coefficients were computed within testers and combined. The r values obtained were .11, —.36, —.13, —.22, —.39 and —.45 for crosses with 113H3, E322, E21, A2-90H0, 175H15 and combined, respectively. Only the combined value —.45 is significant (.05). This can be explained as being due to tester 175H15 whose crosses were high in sugar and low in yield in comparison to the others. However, within 175H15 the r value was not significant.

Although there is a negative trend in the yield-percent sucrose relationship it is not a strong association. Therefore, it should be possible to obtain high sugar-high yield combinations such as A2-90H0 x 5-197 and A2-90H0 x 5-239.

Relative Importance of General and Specific Combining Ability:

To gain further information on the relative importance of general and specific combining ability in this test, the sums of squares in the analysis of variance table (Table 6) for test crosses were divided into the female, male, and female x male components. The variance components were then computed as indicated in Table 6. The variance attributable to the males and females was considered an index of that part of the over-all variation among the test crosses due to the general combining ability of the parents. The interaction male x female variance was considered an index of that part of the over-all variation due to specific combining ability.

The variances obtained for the female parents s_F^2, show that the differences in general combining ability of the female parents were responsible for the greater part of the variation among the 90 test crosses, for both yield and sucrose content. Previously it was pointed out that 175H15 gave high sugar, low yielding crosses in comparison to the other male-sterile parents. Therefore, this parent probably contributed a large portion to the s_F^2 values.

Table 6.—Analysis of Variance Tables Showing a Breakdown of the Components of Mean Squares.

Source of Variation	D. F.		M. S.	M. S. Expectation	Variance Components
Yield					
Replications	11		708.9		
Test Crosses	89		1081.1		
Females		4	10,470.2	$S^2+12S_{FM}^2+216S_F^2$	$S_F^2 = 45.88$
Males		17	955.1	$S^2+12S_{FM}^2+60S_M^2$	$S_M^2 = 6.58$
Females × Males		68	560.3	$S^2+12S_{FM}^2$	$S_{FM}^2 = 29.47$
Error	979		206.7	S^2	
Total	1079				
% Sucrose					
Replications	11		4.2397		
Test Crosses	89		1.4473		
Females		4	9.2332	$S^2+12S_{FM}^2+216S_F^2$	$S_F^2 = 0.039$
Males		17	2.1889	$S^2+12S_{FM}^2+60S_M^2$	$S_M^2 = 0.023$
Females × Males		68	0.8039	$S^2+12S_{FM}^2$	$S_{FM}^2 = 0.018$
Error	979		0.5847	S^2	
Total	1079				

The general combining ability of the pollen parents caused comparatively less variation among the crosses in yield than in percent sucrose.

For yield the variance attributable to the interaction, s_{FM}^2, shows that specific combining ability was responsible for a greater portion of the variation than the male parents. This was not so in regard to sucrose. This would indicate that specific combining ability was of greater importance for yield than for sucrose content. For sugar content, general combining ability was of greater importance than specific combining ability of the parents.

Expression of Dominance and Heterosis:

The test crosses as a group are compared to the performance of the parents in Table 7. The averages of the test crosses for yield and percent sucrose were significantly above the averages

Table 7.—Average of 90 Test Crosses Compared to the Average Performance of the Male and Tester Parents.

	Yield, Tons/A	% Sucrose
Avg. of test crosses	24.83	14.47
Avg. of male parents	14.58	13.83
Avg. of tester parents	22.06	14.08

for either male or tester parents. In the absence of dominance or heterosis, the average of the test crosses should be approximately midway between the averages of the male and tester parents. Since this was not the case, it is assumed that dominance and/or heterosis exist for both yield and sucrose content.

To further study the parent-test cross relationship, individual test crosses were compared to the means of their respective parents, $\frac{P_1 + P_2}{2}$,and to the high parent. This was done for both yield and percent sucrose. If a test cross significantly exceeded the mean of its parents, it was considered to be an expression of dominance; but if the test cross was significantly above the high parent, it was considered to be an expression of heterosis. The remaining crosses were divided into two classes, those numerically above and those numerically below the mean of their respective parents. Table 8 shows the number of test crosses found in each category.

Table 8.—Classification of the 90 Test Crosses in Relation to the Performance of their Respective Parents.

	Yield of Roots	Percent Sucrose
Number showing heterosis	36	18
Number showing dominance	43	16
Of the remaining crosses:		
Number above $\frac{P_1 + P_2}{2}$	10	49
Number below $\frac{P_1 + P_2}{2}$	1	7

Most of the crosses showed either dominance of heterosis for yield of roots with only one cross yielding less than the mean of its parents. Furthermore, in checking the yields of the male parents (Table 1) with the averages of their respective test crosses (Table 4) little, if any, relationship was found between the vigor of the inbreds and the yield of their respective test crosses.

All but seven of the crosses were above the averages of their respective parents, $\frac{P_1 + P_2}{2}$, for percent sucrose. In addition, fewer crosses showed dominance or heterosis for sucrose content than for yield. Again, as with yield, there was little relationship between the sucrose content of the pollen parents (Table 1) and the sucrose content of their test crosses (Table 3).

These results emphasize the need for test crossing. In this test, parental performance would have provided a poor evaluation of the value of the inbreds for use in hybrid combinations.

Seven single crosses showed heterosis for both percent sucrose and yield. Of these, six were crosses to A2-90H0 and one to 175H15. However, only three of these crosses produced high gross sugar per acre. This means that heterosis PER SE does not insure a high yield of sugar per acre. In some cases, however, it may be an important factor in obtaining high gross sugar per acre from beets of high sucrose content.

Discussion:

The male-sterile testers used in this study were not consistent in evaluating the inbred lines for combining ability. The results indicate that a single tester line is inadequate for the determination of combining ability. However, if several testers are used the number of entries for a progeny test becomes unwieldy.

A possible solution would be to produce test crosses in a similar manner but to increase the quantity of single-cross seed produced. A preliminary test could then be made having a single entry representing each line to be tested. The one entry would consist of a blend of equal quantities of seed from all the test crosses for a particular line. After this preliminary screening, the remnant seed of the individual single crosses could be used for an additional progeny test to gain more precise information of the combining ability of the lines performing the best in the composite test.

It would also be desirable to use male-sterile testers that show promise for commercial use since specific combining ability may be relatively important particularly for yield. Thus desirable combinations found among the test crosses could be of immediate commercial value.

Based upon the results of Rounds, Rush ET AL. (7), percent sucrose should be given due emphasis when evaluating combining ability of sugar beet inbred lines since it is desirable to obtain high gross sugar per acre from beets that are high in sucrose content. The results of this test indicate that it is possible to isolate and identify such lines.

Specific combining ability for yield and the expression of heterosis or dominance for sucrose content may be important factors in obtaining hybrid combinations that will produce high gross sugar per acre with high sucrose content. Even so, knowing that they are factors to be considered, breeding programs can be arranged to place more emphasis on test crossing as a means of evaluating inbred lines and less on the performance of the lines themselves. The poor relationship between parental performance and the combining ability of the parental lines in this test also emphasizes the need for test crossing.

Summary

Seventeen self-fertile inbred lines and one open-pollinated variety of sugar beets were crossed to five cytoplasmic male-sterile tester lines. The crosses were planted in a field test with the parents included. The results of the test showed that there were differences among the inbred lines for general combining ability and that specific combining ability was important particularly in regard to yield. Heterosis and phenotypic dominance were found for both yield and sucrose content. Parental performance showed little association with combining ability indicating the necessity of making test crosses in evaluating inbreds.

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