The Evaluation of and the Use of the Top-Cross Test as a Method of Selecting Inbred Lines of Sugarbeets for General Combining Ability

R. K. OLDEMEYER[1], W. H. DAVIS[2], H. L. BUSH[1] AND A. W. ERICHSEN[1]

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The performance of top-cross hybrids remains the primary initial method for screening inbreds for superior general combining ability in cross-pollinated crops. Plant breeders generally agree that no one tester strain allows perfect evaluation of inbred lines.

Part I of this paper presents the genetic evidence, collected by the plant breeders of The Great Western Sugar Company, used for choosing the German red beet as a top-cross tester variety for inbred lines of sugarbeets. This evidence is derived from a study of the association of results using different tester parents, an examination of the relative top-cross performance of inbreds from different open-pollinated sources and the most critical evidence, from the single-cross performance of inbreds of known top-cross performance.

Part II deals with the details of the use of the top-cross and the interpretation and use of the results.

Part I The German red beet as a top-cross tester variety

Literature Review

Plant breeders have generally chosen tester varieties with all or some of the following characteristics: (a) of different origin than the material being tested, (b) of relatively broad genetic base, whether it is a double cross hybrid, synthetic of inbreds or an open-pollinated variety, and (c) inherently poor in performance. Several investigators have reviewed the literature concerning the selection of tester varieties in corn (4, 5, 8, 9)[3].

Oldemeyer concluded that the German red beet variety was as reliable a top-cross tester as was a sugarbeet variety (6), and

[1] Director of Agricultural Research, Statistician-Agronomist, Plant Breeder respectively, Agricultural Experiment Station, The Great Western Sugar Company, Longmont, Colorado.
[2] Formerly, Plant Breeder, with Agricultural Experiment Station, The Great Western Sugar Company, Longmont, Colorado; presently with L. Teweles Seed Company, Milwaukee, Wisconsin.
[3] Numbers in parentheses refer to literature cited.

the red beet was preferable because it had a dominant marker gene which made it useful for identifying hybrids in all psuedo-self fertile inbred lines of sugarbeets. Using a limited number of parents, represented by inbreds, heterozygous strains and tetraploid strains, Finkner et al. (3) concluded performance of parents *per se* and diallel data were more accurate measures of combining ability than performance of hybrids with the red beet.

Materials and Methods

The use of a tester variety with a dominant red color marker for testing lines of sugarbeets was outlined in a previous paper, as was the use of the red hypocotyl marker (7). All hybrids between inbred lines from which data were taken for this investigation were made using the red hypocotyl dominant character. Selective thinning to red hypocotyl plants produced homogeneous populations of hybrids.

Performance trials from which the data were collected comprised two-row plots, 18 feet long, generally in four replications. A control block (2) or a lattice experimental design was used.

After machine harvest, all beets were uniformly crowned, washed, weighed and rasped. The sugar content was determined on the rasped brei by the standard Sachs-LeDocte cold digestion technique.

The results, using the German red beet, a sugarbeet variety, and a newly synthesized red beet, were compared. This new red beet was developed by J. O. Gaskill, U. S. Department of Agriculture, Fort Collins, Colorado; it was derived by introducing dark red color from the garden beet into the U. S. variety "Synthetic Check" by backcrossing. Yield of roots and sugar percentage of individual top-cross hybrids were converted to a percent of check which was GW359 $\times$ German red beet in case of German red beet hybrids and GW359 $\times$ Gaskill's red beet in the case of Gaskill's red beet hybrids; GW359 was the check variety in GW359 hybrids.

Results and Discussion

Tester Comparison

The association of the performance of the top-cross hybrids of a series of inbreds using three different top-cross hybrid tester parents was determined from performance trials conducted in 1958. Correlation coefficients were calculated from the data which were in percent of check, and these are summarized in Table 1.

The correlation coefficients, all significant at the 1% level, are within the range which might be expected from similar

Table 1.—Correlation coefficient of the performance of top-cross hybrids using (A) German red beet, (B) Gaskill's red beet and (C) GW359.

	Number of pairs	Yield of roots	Sugar %
r_{ab}	49	.58**	.70**
r_{ac}	34	.64**	.68**
r_{bc}	34	.72**	.54**

** Significant at the 1% probability level.

tester parents, considering the precision of the trials from which the data were drawn. One could, by considering rather insignificant differences, deduce that tester C (GW359) gives a more accurate estimate of combining ability for weight. The GW359 tester correlates closer with both tester A (German red beet) .64 and tester B (Gaskill's red beet) .72 than A with B, .58. For sugar percent, A perhaps gives the best estimate, $r_{ab} = .70$, $r_{ac} = .68$ while $r_{bc} = .54$. The trends are not strong enough for a choice of testers to be made solely on the basis of these data, and there is the possibility that the closer relationships are meaningless.

The Great Western Sugar Company uses an arbitrary classification, based upon top-cross performance, for determining the disposition of inbred lines. Inbred lines are classified according to their top-cross performance as measured by gross sugar yield (root yield $\times$ sugar percentage) expressed in percent of check. With some exception for lines having extremely high sugar percentage or purity, lines having a top-cross below 105% of the check in total sugar are discontinued, those with 105-115% of the check are retested, and those having 115% or more of check are multiplied for more extensive and precise testing. Table 2 summarizes the possible disposition of a series of inbreds comparing the three different tester parents.

Table 2.—Comparison of the disposition of a series of inbred lines based upon top-crossing with (A) German red beet, (B) Gaskill's red beet and (C) GW359 sugarbeet and using total sugar as the base character.

	n		n		n		n		n
A = B	38	A > B	4	B > A	4	A >> B	1	B >> A	2
A = C	25	A > C	5	C > A	7	A >> C	0	C >> A	2
B = C	22	B > C	4	C > B	7	B >> C	0	C >> B	1

N = M when both are 105% or less, both are 105-115% or when both are 115% or more of the check variety.

N > M when N is 105-115% while M is less than 105% or when N is 115+% and M is 105-115% of the check variety.

N >> M when N is more than 115% while M is less than 105% of the check variety.

None of the tester parents appears better than another when disposition of inbreds is considered. The proportion of lines classified differently is about the same for all three testers. (Table 2). A χ^2 (Chi square) goodness of fit test did not indicate that the ratios deviated more than expected, p = .5-.7, and indicated that such distributions could be expected from random sampling.

Inbred Source Comparison

It was observed in the early years of testing, and continues to be observed, that the average top-cross performance in Colorado of inbred lines developed from varieties synthesized by Great Western is better than for inbreds developed from varieties obtained elsewhere. The proportion of superior lines identified and selected, after subsequent testing, from Great Western sources has also been greater.

The inbred lines which were tested in 1957, using the German red beet as a tester parent, were divided as to source and then as to performance of their top-cross hybrids (gross sugar yield) in 10% classes from 70 to 130% of check. These data are summarized in Table 3.

Table 3.—The top-cross performance of inbred lines divided in percent of check classes and as to having been developed from Great Western sources or other sources.

% of Check class	Other	GW
70-90	10	1
80-89	35	8
90-99	21	63
100-109	15	105
110-119	1	43
120-129	0	9
Totals	82	229

Goodness of fit $\chi^2 = 118.92$ from which $P < .001$.

It is clear that the level of performance is much lower for other sources, mode 80-89%, as compared to Great Western sources, mode 100-109%. The proportion of superior lines, above 110%, is far lower for other sources also.

When tested in Colorado, Great Western varieties always perform better than varieties developed in other areas of the world. Inasmuch as the top-cross performance of inbreds seems to be correlated with the performance of the varieties from which they are derived, it can be deduced that the top-cross hybrid performance of an inbred from within a source reflects the relative value of the inbred as a hybrid parent or its combining ability.

The Relationship Between Single-Cross and Top-Cross Performance

The true measure of whether a particular tester accurately measures the general combining ability of inbred lines is whether there is a positive relationship between the performance of the top-cross of a line and the average performance of specific hybrids involving that line. The single-cross trials of 1959 and 1960 contained hybrids of inbreds selected for their different levels of performance in top-crosses having the German red beet as the tester parent. Single-cross hybrid performance was the average performance in three trials in northern Colorado, each consisting of four replications of plots, two rows $\times$ 18 feet. The top-cross data were taken from only one trial of four replications of plots of two rows $\times$ 18 feet. Single-cross test A results are from an average of two years; while for test B, they are from one year. Test A contained 47 single-crosses involving 34 inbred parents, while test B contained 26 single-crosses involving 19 parents.

The relationship between single-cross performance and top-cross performance as expressed in correlation coefficients is summarized in Table 4. Columns one and two are multiple correlations in which the top-cross performance of p_1 and p_2 are compared to the performance of the single-cross $p_1 \times p_2$. Only moderately high multiple correlations existed for tonnage and sugar percentage, while the correlation in test A for gross sugar was not significant. Using the average top-cross performance of the two parents in a simple correlation (third column), a much higher correlation is obtained.

Table 4.—Correlation between top-cross and single-cross performance.

	Test A 1959-60	Test B 1960	Test B 1960
	$R_{P_1P_2.SC}$	$R_{P_1P_2.SC}$	$r_{\bar{X}\ P_1P_2.SC}$
Tonnage	.50*	.51**	.89**
% Sugar	.59**	.33*	.95**
Total sugar	.25 NS	.49*	.86**

*, ** Statistically significant at the 5% and 1% level of probability, respectively.

A clearer delineation of the relationship between top-cross and single-cross performance is obtained from a trivariate frequency table of the same data (Tables 5 and 6). Parental top-cross performances were arbitrarily divided into five classes, according to relationship to check variety, GW359 $\times$ German red beet. Plus signs indicate the inbred performed better than the check and minus signs poorer with double plus signs, indicating the best performing lines and the double minus signs

Table 5.—Comparison of top-cross performance, column and row headings, with single-cross performance, rank in variety trial in boxes, Test A, 1959-60.

Female / Male	++	+	=	—	— —	Ratio[a]
			Weight of roots			
++	(1) (3) (4) (12)	(28)	(6)(8)(17)(22) (23)(24)(26)(41)	(7)(10)(11) (16)(18)	(2)(5)(9) (14)	18:22
+			(35)(36)(37) (46)	(15)(32)		1:6
=			(21)(25)(29)(40) (40)(42)(43)	(30)(38)(39) (44)(45)	(19)(36)	2:14
—			(20)(30)		(47)(48)	1:4
Ratio[a]	4:4	0:1	7:21	6:12	5:8	
			Sugar content (%)			
++	(5)	(1)(2)(4)(10) (14)	(12)(21)(42)	(3)(9)(15) (24)(39)		11:14
+		(6)(8)(17)(19) (23(25)(38)	(7)(28)(35) (40)(45)	(11)(13)(18)(22) (27)(33)(36)(43) (47)(48)		10:22
—			(20)(29)(32) (41)	(26)(30)(31) (44)(46)(49)		1:10
Ratio[a]	1:1	10:12	4:12	7:21		
			Total sugar (Weight of roots × sugar content)			
++	(22)	(3)(7)(18)(25)(28) (29)(37)(38)(46)	(1)(4)	(19)(21)(26)(27) (34)(40)		8:18
+		(13)(24)	(2)(5)(6)(30) (31)	(10)(17)(23)(29) (42)(45)(47)(48)		7:15
=			(16)	(8)(11)(12)(14) (15)(20)(35)		7:8
—				(33)(39)(41)(43) (44)		0:5
Ratio[a]	1:1	4:11	6:8	11:26		

[a] Number of crosses which exceed the mean of the trial compared to the total in the class.

Table 6.—Comparison of top-cross performance, column and row headings, with single-cross performance, rank in variety trial in boxes, Test B, 1960.

Female / Male	++	+	=	—	Ratio[a]
			Weight of roots		
++	(3)	(1)(2)(6)(7)	(4)(16)	(9)(19)(20)	6:10
+		(14)	(5)(10)(13)(25)(27)	(18)(22)(23)(24)	1:10
=			(12)		0:1
—			(11)(15)(17)(20)	(26)	0:5
Ratio[a]	1:1	4:5	4:12	1:8	
			Sugar content (%)		
++		(1)	(2)(24)	(13)	3:4
+		(3)(4)(5)(6)(9)(16)	(7)(11)(17)(19)(20)(26)	(8)(10)(12)(13)(24)	11:17
=			(18)(21)(23)	(22)(27)	0:5
Ratio[a]		6:7	3:11	5:8	
			Total sugar (Weight of roots × sugar content)		
++		(1)(3)(4)(6)(7)(10)(18)(27)	(8)(26)	(5)(16)(19)(24)	7:14
+		(2)(11)	(14)(17)	(12)(15)(20)(22)	1:8
—			(13)(21)(23)	(25)	0:4
Ratio[a]		6:10	1:7	1:9	

[a] Number of crosses which exceed the mean compared to the total in the class.

the poorest. The top-cross performance of the female is subdivided across the top row; of the male, along the left column. Numbers in the boxes are the rank in the trial of a single-cross having parents whose top-cross performance is indicated in the left of the row and top of the column. If the top-cross hybrid performance is related to single-cross performance, the low rank numbers should be on the top left and the high rank numbers in the lower right.

Yield of roots is predicted well, sugar content could not be expected to be predicted better, while total sugar was predicted less well than its two components, yield and sugar content. These data are consistent with the correlation coefficients.

Any one or all of four factors may account for the above associations not being closer:

First, the trials upon which the performances were determined were not very precise, i.e., four replicates of 36 feet of row for top crosses. This lack of precision, no doubt, is a major cause for the relationship appearing looser than it probably is.

Second, genotype × year interaction may have been reflected because the testing of the top crosses and single crosses was done in different years.

Third, specific combining ability could exist between the inbreds and the red beet testers. However, the comparison presented in Table 3 would indicate that it is not likely to be a significant factor unless Great Western lines are more likely to combine specifically with the red beet than others.

And fourth, specific combining ability between inbred lines in the single crosses used would result in the general combining ability measure (red beet hybrids) appearing erratic. In trials since 1960, our experience has been that the variation due to specific combining ability is very minor in comparison to general combining ability.

Conclusions

The evidence presented in this paper does not conflict with evidence presented before (7) as to the use of the German red beet as a top cross tester variety. The German red beet appears to be as reliable as Gaskill's red beet and a sugarbeet variety in assessing combining ability of inbred lines; as evidence for this, tester hybrids with it perform best when the inbred lines are from adapted material, and single-cross performance agrees reasonably well with top-cross performance using the red beet tester.

Part II Use of the Top-Cross Technique for Selecting Superior Lines

Inbred lines are initiated by self pollination of plants from an open-pollinated variety with or without selection of the parental roots for sugar content, and purity and/or disease resistance. Intense intraline and interline selection for agronomic type is made in the first and second selfed generations. A sugar content and purity (pressed juice) selection is made in the first selfed generation at which time only the very poor lines and roots are discarded.

Once an inbred has been established by self pollination as determined by its morphological uniformity, the S_2 generation or beyond, roots are selected for top-crossing with the German red beet. Some of the roots of each line are simultaneously selfed another generation. Top crossing is accomplished by planting rows of inbred lines alternately with rows of red beets. Seed is harvested from several roots of each inbred line.

To obtain a complete stand in top-cross hybrid trials, the following germination procedure is used:

Two grams of each top-cross lot of seed are germinated in a

ragdoll of paper toweling after standard washing. Strong light is shielded from the germination cabinet to suppress the development of pigment in the normally occurring red hypocotyl sprouts so that a clear distinction can be made between the red beet color and normal hypocotyl color.

Hybrids per two grams are counted during a 10-day period; the seeding rate is adjusted to 12 hybrids per foot which results in very satisfactory stands. A shortage of red beet hybrids occurs only with inbreds containing genes for self fertility, although, even then, many lines have enough hybrids for testing.

Trials, generally, consist of two 18-foot rows replicated four times arranged in a 7×7 simple lattice design. Top-cross seed lots, about which there is doubt as to whether they contain enough hybrids to establish a stand, are planted in separate trials which are smaller lattices or control block designs. Each trial contains three entries of a top-cross check. The check variety is different for each of the three different growing areas of The Great Western Sugar Company, being the standard open-pollinated variety crossed to the red beet for Colorado, Montana and Ohio areas. Components measured in the trials are calculated to percent of check for year to year and trial to trial comparison. Diseases caused by *Cercospora* and *Aphanomyces* cause such major reduction in productivity in all top crosses in Ohio except those involving highly resistant inbreds that a visual elimination of many lines can be made. In this case, only plots containing vigorous top crosses are harvested for comparison to the check top cross. Because only selected plots are harvested, a control block design has been used in Ohio. Further, one-row plots in eight replications are used because of the existence of high field variability.

Characters which are measured are yield of roots, sugar percentage, purity and bolting percent. In addition; *Cercospora* infection readings are made in Ohio. Yield of roots and sugar percentage are considered components of yield while apparent thin juice purity is the measure of processing quality. Apparent thin juice purity is determined on a pressed juice which has been purified by liming and phosphating followed by filtration in a technique outlined previously (1). Recoverable sugar is computed by multiplying yield of roots by sugar percentage and reducing the product by a factor which takes into account loss in 60 purity molasses and a constant processing loss of 0.3% on beets.

The averages for 1965 and 1966 of the statistical least significant differences for weight and sugar content and the coefficient of variation are summarized in Table 7. These statistics

Table 7.—Average coefficients of variability and least significant differences (% of check) for various characters in 1965 and 1966.

Year	No. trials	Wt. roots		Sugar %		Purity %		Recoverable sugar yield	
		CV	LSD*	CV	LSD*	CV	LSD*	CV	LSD*
1965	9	8.88	14.71	5.31	7.78	1.35	2.00	—	—
1966	10	8.51	13.04	4.08	6.08	1.46	2.17	10.21	15.53

* Significant at the 5% probability level.

can be compared to the arbitrary limits which have been chosen for use in disposing of lines which have been top-crossed. As indicated in the section *Tester Comparison,* lines which have 105% or less total sugar than the check are discontinued, with 105-115% retested, and with more than 115% are considered for multiplication.

No least significant difference was calculated for recoverable sugar yield in 1965, but considering the greater variability of the components, weight of roots and sugar percentage, the LSD for sugar yield in 1965 would have been considerably greater than 15.5. Using an arbitrary figure of 15% greater than the check for selection, some lines selected as superior might actually be no better than the check. These lines will be eliminated on further testing with the only loss being testing effort. It is unlikely that many highly superior lines become discontinued. Results with lines in the 105-115% of check class indicate that only a few exceed 115% of the check on retesting; it would then be expected that only rarely would one of the lines with 105% or less have a retest of 115% or more.

To maintain the genetic purity of inbred lines of cross pollinated crops, the lines must be uniform enough for outcrossed plants and/or plants resulting from seed mixture to be identified and rogued. In practice, Great Western selects no line for multiplication unless it has reached a degree of uniformity equivalent to an inbred in the fourth selfed generation. (Visual uniformity, within lines and between sublines, is used rather than a stated number of selfings to determine when a line may be sib increased, because in the first and second selfed generation, roots arising from outcrossing are unavoidably selected for carrying the line.)

Early testing is practiced to eliminate poor combining lines and to allow concentration of testing effort on the better lines. Superior lines in the S_2 and S_3 generations, as well as others, which are too variable to increase, are top crossed again along with the lines which have 105-115% of the check in total sugar. Two and sometimes three sublines are top crossed if a retest is indicated.

In recent years, fewer lines with only average performance are being found in the single cross trials. This increased reliability of selection of lines for general combining ability can be attributed to an increased amount of top-cross data being available for a line before it is multiplied. Data from several sublines and data from several years' trials allows a more critical selection.

Sugar content is generally used only as a yield factor in the selection of lines for multiplication. However, if a choice has to be made between lines of equal recoverable sugar yield, the line with the low sugar content will be discontinued because of the increased cost of harvest and delivery. Conversely, lines with high sugar content are favored.

The all inclusive character, apparent thin juice purity, is the quality selection criterion and is related to the proportion of total sugar that can be extracted in the factory process. Prior to 1961, thin juice apparent purity was not determined on top-cross hybrids because it was believed that high sugar lines also had high purity. As more experience was gained in testing, it became apparent that sugar content and purity were not necessarily related and, more often than not, lines with high root yields had lower than average purity. Lines having a purity significantly below the check are discontinued regardless of the yield of total sugar.

Complete pedigrees of the lines are at hand whenever a selection is made in order to minimize the selection of too many lines derived from the same "mother" root and same source. It has been necessary to accept from some sources lines with poorer agronomic characteristics and with lower top-cross performance than lines from other sources to insure that all sources are represented in the advanced testing program.

It is estimated that 75% of the lines are lost before top-cross testing due to self incompatibility. (Most occurs in selfing the original plant from the source.) Discarding of lines because of poor agronomic type, e.g. large crowns, bolting, sprangles and hairiness, accounts for another 50% loss. Top-cross testing eliminates at least 90% of the remaining lines so that fewer than 1.5% of all roots originally selfed result in lines being carried to the stage of advanced testing.

Top-cross testing, using the German red beet, is considered an efficient and accurate tool for selecting inbred lines of sugarbeets for superior general combining ability.

Summary

A comparison of top-cross results from a series of inbred lines, using the German red beet, Gaskill's red beet and GW359,

indicated little choice as to the tester which gives the most accurate estimate of general combining ability. Top-cross performance of lines from a source was found to be related to the performance of the source *per se*. Top-cross performance of a line, using the German red beet tester, was found to be related to single-cross performance. In the breeding program of The Great Western Sugar Company, more than 90% of the inbred lines which are top-cross tested are eliminated on the basis of the top-cross test.

Literature Cited

(1) BROWN, ROBERT J. and ROBERT F. SERRO. 1954. A method for determination of thin juice purity from individual mother beets. Proceedings Am. Soc. Sugar Beet Technol. 8 (2): 274-278.

(2) BUSH, H. L. and R. K. OLDEMEYER. 1961. Comparison of statistical designs for a large number of entries of sugar beet strains. J. Am. Soc. Sugar Beet Technol. 11 (4): 306-308.

(3) FINKNER, R. E., H. S. REDABAUGH and C. W. DOXTATOR. 1966. Correlation coefficient studies of testers and parents for combining ability in sugar beets. J. Am. Soc. Sugar Beet Technol. 14 (3): 238-247.

(4) GREEN, J. M. 1948. Relative value of two testers for estimating top-cross performance in segregating maize progenies. J. Am. Soc. Agron. 40: 45-57.

(5) KELLER, K. R. 1949. A comparison involving the numbers of, and relationship between testers in evaluating inbred lines of maize. Agron. J. 41: 323-331.

(6) NANDA, DEVENDER K. 1966. Evaluation of eight inbred lines of maize (*Zea mays L.*). Crop Sci. 6: 67-69.

(7) OLDEMEYER, R. K. 1954. General combining ability of sugar beet inbreds as determined with two different top cross testers. Proc. Am. Soc. Sugar Beet Technol. 8 (2): 59-63.

(8) RAWLINGS, J. O. and D. L. THOMPSON. 1962. Performance level as criterion for choice of maize testers. Crop Sci. 2: 217-220.

(9) SINGH, R. D. 1958. Evaluation of second cycle inbreds of maize by different types of related and unrelated testers. Indian J. Gen. Plant Breeding 18: 199-205.