Reciprocal Recurrent Selection for the Development of Improved Sugarbeet Hybrids*

R. J. Hecker

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INTRODUCTION

Superiority of sugarbeet (*Beta vulgaris* L.) hybrids over open-pollinated cultivars had been demonstrated clearly by 1946 (3, 11, 14). The potential and practicality of producing hybrids using cytoplasmic male sterility (CMS) were reported first by Owen in 1946 (10). The first such multigerm hybrids were produced in 1954, followed by monogerm (one embryo per seed ball) hybrids in 1957 (15). All cultivars currently marketed in the U.S. and most of western Europe are monogerm hybrids (primarily three-way hybrids) produced by using Owen's CMS.

Continued yield improvement of hybrids is dependent upon the accuracy and ease with which superior combining genotypes can be identified, isolated, and utilized. Since root yield has been shown to be conditioned primarily by nonadditive gene action (4, 6, 7, 8, 13) reciprocal recurrent selection (RRS) should be an effective method of developing parental lines for hybrids. RRS is a breeding scheme involving simultaneous crossing, progeny testing, and selection within two different genetically variable populations. Each population serves as the combining ability tester for making selections from the other. If there is sufficient additive and nonadditive genetic variance present and the testing methods are sufficiently precise, each cycle should result in an improved version

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of each population cross. Hecker (5), using RRS, reported improved general combining ability of lines isolated from populations relatively low in either root yield or sucrose content. However, specific hybrid combinations among the derived populations were not tested. The objective of this study was to measure the performance of the specific hybrids among those RRS-derived populations.

MATERIALS AND METHODS

Reciprocal recurrent selection was started with a heterogeneous monogerm open-pollinated sugarbeet cultivar, 'American Crystal No. 2 Mono' (population A) and 'GW 359', a diverse multigerm open-pollinated cultivar (population B). Both sources were classified as self sterile, but most plants could be forced to self pollinate under bags at Fort Collins, Colorado. The study was started by selecting from each population 280 plants of desirable root and crown shape from about 600 competitive plants. These 560 pollen-fertile plants were randomly interpollinated in a polycross plot arranged so that each plant was adjacent on all four sides to plants of the other source. Two branches on each flowering plant were bagged. Self- and open-pollinated (OP) seed was harvested individually from each plant. Based on separate tests of random green hypocotyl (rr) A plants interpollinated with pink (R-) B plants, about 95% of the progeny from the A plants were A X B hybrids. In similar reciprocal test crosses, about 55% were B X A hybrids. This difference probably resulted from the greater pollen production of the multigerm B plants than the monogerm A plants.

After progeny tests of 169 population A and 225 population B plants (plants that produced selfed seed and enough polycrossed seed for a replicated progeny test) at one location for one year, the A C1 (first-cycle) population was made by natural interpollination of 69 S_1 plants from seed of 13 mother plants that had produced polycrossed progeny significantly superior for recoverable sucrose production. The B C1 population was developed similarly from 150 S_1 plants from seed of 22 mother plants.

The second cycle of RRS was started by selecting 100 roots from the A C1 population and 125 from the B C1. The roots were selected for acceptable root and crown shape and were above the mean of their respective population for root weight, sucrose content, and thin juice purity. This phenotypic selection, as well as that in the first cycle, was too mild to have had a significant genetic effect. These selections were from about 1,000 plants of each C1 population. Sucrose concentration (percentage of fresh weight) was determined using standard procedures of beet pulp extraction and polarization of the filtrate. Juice purity was determined by a modified method of Brown and Serro (1). Each selected root was cut longitudinally in half, then one half was cut into two quarters. The A and B halves were planted into separate A and B crossing plots. The halves were surrounded by random quarters from the other source. Two flowering branches were bagged, and self- and open-pollinated seed were harvested from each half-root derived plant. Sufficient self- and open-pollinated seed was produced on 92 A C1 plants and 113 B C1 plants to be included in progeny tests. These 205 progenies were evaluated for recoverable sucrose, fresh root weight, and sucrose concentration in a six replication lattice test at one location for one year. Three separate C2 populations from each C1 were synthesized for high recoverable sucrose, root yield, and sucrose concentration. These six C2 populations were synthesized using 39, 41, and 56 S_1 plants from seed of 12, 17, and 15 A C1 mother plants respectively, and using 53, 35, and 32 S_1 plants from 14, 15, and 9 B C1 mother plants. S_1 plants from some of the mother plants were common to two, or rarely all three, of the resulting A C2 and B C2 populations. These six C2 populations were random mated one generation by natural interpollination of about 100 plants within each population. These six C2 populations, each segregating for green and pink hypocotyl, were used to make six A C2 X B C2 test hybrids, not including A(suc.)C2 X B(recov. suc.)C2, A(root yield)C2 X B(recov. suc.)C2, and A(suc.)C2

X B(root yield)C2, since these combinations of selection characters were among the six hybrids tested. Hybrid plants from these crosses were identified by using homozygous recessive green hypocotyl segregants as females and dominant pink hypocotyl segregants as pollinators. A CO X B CO hybrids were identified in the same manner. Previous research established that there was no relationship between hypocotyl color and sucrose content or root yield (9). In the hybridizations in this study the multigerm B C2 populations were always used as males because of their greater pollen production. Maternal effects on sucrose production never have been detected in diploid sugarbeets.

The experiments to evaluate the combining ability of these A C2 and B C2 populations with each other were grown as irrigated summer crops at Ft. Collins, Colorado, in 1980 and 1982 in single-row 6.7 m plots with 56 cm between rows in lattice designs with six replications. Plants were thinned to 25 cm, leaving only hybrid (Rr) plants. Recoverable sucrose was calculated from percentage sucrose, percentage purity, and fresh root weight.

RESULTS AND DISCUSSION

Analysis of the data (populations fixed and years random) showed a significant difference between years for recoverable sucrose, root weight, percentage sucrose, and percentage juice purity. There were no significant entry X year interactions except for percentage sucrose which was significant at P=0.05. Variances between years were homogeneous. Hence, the data from both years were combined for analyses.

C2 RRS Populations

Although performance of the A C2 and B C2 populations per se was not the most important aspect of the study, these means nonetheless provide some useful information. In Table 1 the B CO means were significantly higher (t-test, not shown) than the A CO means for recoverable sucrose and root yield, but the A CO means were significantly higher than B CO for percentage sucrose and percentage purity. The higher recoverable sucrose production of B CO

was due entirely to its superior root yield. A CO was a high sucrose, high purity cultivar, but it had relatively low root yield.

The means in Table 1 show that all three A C2 populations producted significantly more recoverable sucrose than A CO from which they were derived. Highly signifi-

Table 1. Means of sugarbeet sucrose yield and its components for RRS source populations and second cycle populations, 1980 and 1982.

RRS population and its selection emphasis	Recoverable sucrose	Root yield	Sucrose concent.	Thin juice purity
	----- Kg ha^{-1} -----		------- % -------	
A(recov. suc.)C2	6,673**	53,554**	16.3	89.3
A(root yield)C2	5,909**	50,706**	15.5*	88.3**
A(suc.)C2	5,702**	43,373	16.5*	90.0
A CO	4,833	39,440	16.0	89.5
LSD (0.05)	595	4,910	0.48	0.87
B(recov. suc.)C2	5,909	48,450	16.1*	89.0*
B(root yield)C2	5,931	52,027	15.0	88.1
B(suc.)C2	6,008	48,227	15.9*	89.4**
B CO	5,550	48,768	15.4	88.0
LSD (0.05)	606	4,980	0.47	0.90

*,** Significantly higher or lower (P=0.05 and 0.01, respectively) than the respective source population.

cant root yield improvement was made in A(recov. suc.)C2 and A(root yield)C2 while no significant root yield improvement was shown for A(suc.)C2. On the other hand a significant improvement of sucrose concentration was made in A(suc.)C2, but there was a significant decrease of sucrose in A(root yield)C2. The latter also had a highly significant decrease in thin juice purity.

In the case of the three B C2 populations, no significant increase was made in recoverable sucrose or in root yield, however, significant increases were made in the sucrose content and purity of B(suc.)C2 and B(recov. suc.)C2.

The performance of these six C2 populations indicated that advances were made in recoverable sucrose and root yield in the A population (the relatively low root yield and high sucrose source), particularly when root yield and

recoverable sucrose were the selection criteria. Sucrose was advanced in the A C2 population only when sucrose concentration was the selection criterion. In the B population (the relatively high root yield and lower sucrose source), the only advances made were for sucrose and purity when sucrose and recoverable sucrose were the respective selection criteria.

The changes effected in these A C2 and B C2 populations should be primarily due to additive gene effects. Partitioning of genetic variances for root yield and sucrose content (4, 6, 7, 8, 13) has shown that root yield is primarily conditioned by non-additive gene effects, whereas sucrose content is primarily additive. Since source A had relatively low root yield, genotypes with additive effects for root yield apparently were selected more easily than in the higher root yield source B. This would indicate that genotypes with greater additive effects for root yield were selected in A populations when B was used as a tester parent, than vice versa. A similar condition existed in the case of sucrose content where more progress was made in the low sucrose B populations than in the higher sucrose A populations. The performance of these A C2 and B C2 populations had the same relationship as those reported previously (5).

Hybrids Among C2 RRS Populations

The results of 2 years of field testing of A C2 X B C2 hybrids are shown in Table 2. All six of these hybrids produced more recoverable sucrose than the A CO X B CO hybrid, but none of the six hybrids was significantly different from another.

For root yield, only those hybrids involving A(root yield)C2 or B(root yield)C2 were significantly more productive than A CO X B CO. Similarly, for sucrose concentration, RRS apparently successfully selected genotypes that produced hybrids with significantly higher sucrose than the A CO X B CO hybrid only when the selection was for superior sucrose concentration. The thin-juice purity of the six A C2 X B C2 hybrids was superior to that of the

Table 2. Means for sucrose yield and its components for sugarbeet hybrids among RRS sources and second cycle populations, 1980 and 1982.

Hybridization	Recoverable sucrose	Root yield	Sucrose concent.	Thin juice purity
	----- kg ha^{-1} ------		-------- % --------	
A(recov. suc.)C2 X B(recov. suc.)C2	6,113*	47,266	16.3**	89.8*
A(root yield)C2 X B(root yield)C2	6,343**	53,402**	15.7	88.9
A(suc.)C2 X B(suc.)C2	6,023*	43,673	16.4**	89.3
A(recov. suc.)C2 X B(root yield)C2	6,292**	53,027**	15.7	88.5
A(recov. suc.)C2 X B(suc.)C2	6,243**	49,376	16.3**	89.4
A(root yield)C2 X B(suc.)C2	6,292**	49,899*	16.1*	89.5
A CO X B CO	5,379	44,995	15.5	88.7
LSD(0.05)	579	4,695	0.5	0.94

*,** Significantly higher or lower (P=0.05 and 0.01, respectively) than A CO X B CO.

hybrid of the two original populations only in the case of A(recov. suc.)C2 X B(recov. suc.)C2.

Although the test hybrids in Table 2 were 100% hybrids, the progeny tests of population A plants and population B plants in cycles 1 and 2 were about 95% and 55% hybrids, respectively. It was impossible using source populations A and B, neither of which had male sterility, to achieve 100% hybridization in the cycle 1 and cycle 2 test crosses. Since test crosses of population A selections in both cycles had higher hybridization percentages, they would be expected to be more precise tests for combining ability than the population B test crosses, thus limiting combining ability improvement in B populations.

The greatest merit of RRS should be in selecting genotypes from the two sources that combine well due to the presence of nonadditive as well as additive gene effects.

The results of the hybridizations among A C2 and B C2 populations presented in Table 2 indicate that the method as used was successful in selecting sugarbeet genotypes with improved combining ability. The greatest amount of recoverable sucrose was produced by A(root yield)C2 X B(root yield)C2, 6,343 kg ha^{-1}. However, this increased productivity came about solely from the advance in root yield. The same condition existed for A(recov. suc.)C2 X B(root yield)C2 that produced 6,292 kg ha^{-1} recoverable sucrose. A more desirable hybrid combination was A(root yield)C2 X B(sucrose)C2 (6,290 kg ha^{-1}), the increased recoverable sucrose coming from significant increases in both root yield and sucrose content. It is not possible from these experiments to partition the effects in the hybrids into additive and nonadditive genetic components.

Problems in Using RRS in Sugarbeet

The ultimate product of RRS would be a single-cross hybrid between two inbred lines, one derived from population A and one from population B. In theory, these two inbreds would contain the genes from their respective sources that maximize specific combining ability (SCA). In an applied RRS breeding program in sugarbeets, it would be necessary that the two lines be useful as parents of a single-cross hybrid before significant homozygosity had been reached because the loss of vigor associated with homozygosity in sugarbeet would preclude economic seed production of a commercial single-cross hybrid.

The ultimate derivation of two lines by RRS would require that one of the lines be monogerm, CMS, and have an isogenic O-type (maintainer) line. If the initial source of that line were monogerm and O-type, or at least segregating for O-type, only a backcross derived CMS and its O-type maintainer would need to be developed from the RRS derived population. A problem with the application of RRS in sugarbeet is the necessity to achieve 100% hybridization in the test crosses A X B and B X A, each cycle. This could be achieved only by having genetic male sterility in the two populations and using and maintaining it

as outlined by Doney and Theurer (2). Significant preliminary time would be required to incorporate this genetic male sterility into two adequate source populations. However, breeding is a long term process, and although the development of such breeding populations would require several years, it would be relatively inexpensive.

Another problem in the use of RRS is the preservation of maternal genotypes in each cycle. The incorporation of the gene for self fertility (S^f) accompanied by genetic male sterility would resolve this problem (2). However, S^f populations ultimately derived from such an RRS program would have to be maintained as heterozygotes for genetic male sterility in order to prevent a rapid approach to homozygosity within the RRS derived populations. This would have to be done in both the maintainer (O-type) line developed from one source and the pollinator line developed from the other source. Modern methods of cloning (12) would appear to offer a solution to this problem. Cloning as a substitute for selfing would also allow greater opportunity to more nearly achieve truly random pollination in the A X B and B X A test crosses each cycle. Even though cloning would obviate the incorporation of the S^f gene, Mendelian male sterility would need to be incorporated in each of the sources to insure 100% hybridization in the test crosses each cycle. Nearly 100% hybridization in these test crosses each cycle would be essential to maximize progress by RRS, because SCA effects must be measured accurately to rapidly concentrate the genes responsible for high SCA between populations. These test crosses also should be evaluated in an array of environments in an effort to have wide adaptation in resulting hybrids. Choice and condition of the original source populations also would be critical. Necessary disease resistance, adaptation, etc., would need to exist in the original source populations and would need to be maintained in succeeding cycles as outlined by Doney and Theurer (2) or would have to be incorporated by backcrossing after the desired number of cycles of RRS had been completed.

In this study, two cycles of RRS produced populations that exhibited improved combining ability compared with the original sources. This combining ability enhancement occurred in spite of shortcomings in this RRS study, namely, only two cycles were completed, frequency of hybrids in the test crosses may have been as low as 55%, test crosses were not a product of completely random pollination, test crosses were evaluated at only one location, and preservation of maternal genotypes by selfing may not have retained all the genes for higher combining ability. Methods now are available to alleviate these technical obstacles, and in a large sugarbeet breeding program it would be practical to carry on one or more RRS breeding schemes.

SUMMARY

Sugarbeet (*Beta vulgaris* L.) sucrose yield improvement, conditioned by additive and nonadditive gene action, depends on the successful selection of superior combining genotypes. Reciprocal recurrent selection (RRS) was tested as a means of developing populations that combine well together. Two cycles of RRS, with separate emphasis on recoverable sucrose, root yield, and sucrose concentration resulted in three populations from each source. The original population A (A CO) was a high sucrose and relatively low root yield cultivar; population B (B CO) was a cultivar with lower sucrose but high root yield. The three A C2 (second cycle) populations generally had higher recoverable sucrose and root yield per se than the original A population, while the B C2 populations were improved for sucrose and juice purity, compared to the original B. These results indicated that RRS successfully selected additive gene effects for higher root yield in the low-yield A populations and for higher sucrose in the low-sucrose B populations. The combining ability of A C2 and B C2 populations with each other was superior or equal, never inferior, to A CO X B CO. It was not possible to partition the combining ability of population crosses into components, but the net effect of two cycles of RRS was signifi-

cantly greater sucrose production in the six A C2 X B C2 hybrids that were tested, compared with the A CO X B CO hybrid. This study indicated that RRS may be an effective breeding method for improving sucrose production of sugarbeet hybrids.

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