Line x tester analysis in sugarcane (Saccharum officinarum)

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ABSTRACT

Combining ability variances and effects were estimated for important agronomic and biochemical traits in sugarcane using line x tester mating design. Five commercially grown varieties and one line from germplasm collection were involved in this study of which three were used as females (lines) and three as males (testers). The six agronomic characters studied were number of millable stalk per stool, stalk diameter, stalk height, weight of millable stalk, refroctometre brix and plant volume. The five bio-chemical characters studied include polatronic reading (pol), fibre, purity, brix and pure obtainable cane-sugar (POCS). Among the lines, Aiwa and Mana and among the testers Vatu and Mentor were found to be the best general combiners for most of the agronomic and bio-chemical characters. Four out of nine crosses namely Aiwa x Mentor, Kaba x Vatu, Kaba x Yasawa and Mana x Yasawa were found to be the best crosses. Analysis of Variance for bio-chemical characters showed that only one character (% fibre) was significant. The line x tester interaction was not significant for any of the characters studied. Estimates of variance due to gca and sca and their ratio revealed predominantly non-additive gene action for these characters. Non-additive gene action can be exploited in F_1 generation. Development of new hybrid varieties with improved agronomic and bio-chemical characters is therefore suggested.

1 INTRODUCTION

Sugarcane is a member of the grass family and is classified in the genus *Saccharum*, tribe Andropogoneae and family Poaceae. Sugarcane is grown as an important cash crop in many tropical and subtropical countries. The study of the characters of agronomic and commercial interest in the progeny resulting from the crossings in sugarcanes is of great importance. This is because parents can be identified for hybridization program. The effectiveness of the selection of the progeny can also be measured through such studies. Choice of parents can be determined by factors such as short – and long – term goals, available materials, the flowering time and breeding behavior of parents in specific cross combinations, and by the amount of information available on any parent or cross combinations.

One of the methods used to select the parental material for hybridization from the germplasm to identify their genetic worth is Line x Tester analysis. This mating design provides information about the general and specific combining ability of parents and estimates of other genetic parameters. The mating plan involves "l" lines and "t" testers. All of these "l" lines are crossed to each of the "t" testers and thus, "l" x "t" full sib progenies are produced. These progenies resulting from line x tester matings, along with or without the parents, can be tested in a replicated trial using suitable field design (Comstock and Robinson 1948; Singh and Chaudhary 1985).

Therefore in order to gather the information on various accessions in the germplasm collection the present study was undertaken to estimate the variance components and effects due to general and specific combining ability in the progenies produced by line x tester crosses of selected clones.

2 MATERIALS AND METHODS 2.1 SELECTION OF PARENTS

The material for the study comprised of morphophysiologically diverse three female parents (lines) namely; Aiwa, Kaba and Mana and three male parents

(testers) namely; Mentor, Vatu and Yasawa. The above male and female parents except for Mentor are commercial varieties cultivated in Fiji. The selection of these parents was based on flowering and performance as commercial varieties.

2.2 CROSSING PROCEDURE

Sugarcane is cross-pollinated by wind; thus, pollen is generally controlled to ensure progeny of known parentage. Selected female varieties pollinated with the pollen from selected male varieties to produce (crosses). Line x tester matings among three lines and three testers resulted in nine F1 crosses namely Aiwa X Mentor, Aiwa X Vatu, Aiwa X Yasawa , Kaba X Mentor, Kaba X Vatu, Kaba X Yasawa, Mana X Mentor, Mana X Vatu and Mana X Yasawa. The above crosses were made during the 1995 crossing season. For each cross combination, two stalks of male flowers and two stalks of female flowers were tied together and kept in buckets containing acid solution. It was necessary to change the acid solution in bucket in-order to keep the female parents in freshly prepared solution all the time. This enabled the flowers to remain healthy and fresh for a longer time. The solution was changed every three days until such time the flowers continued to open.

2.3 RIPENING, DRYING AND STORAGE OF SUGARCANE SEEDS (FUZZ)

Ripening of sugarcane seed was carried out in the maturing shed. After the completion of anthesis and hence pollination, female inflorescences were bagged using mosquito netting before the fuzz started to shed. The stalks were kept in the acid solution for further two weeks before the tassels were cut and tied to metal tie-bars under the maturing shed. Mature fuzz (seeds) has moisture content of 12 to 13% at the time of harvesting and will germinate readily above 20°C if adequate moisture is available. Therefore the bags containing the seeds were air dried and left in the maturing shed for three weeks before the bags were removed and the seeds were separated from the main

axis and larger lateral axis. The seeds of each cross were packed separately in plastic bags containing silica gel. The dried seeds collected from the respective crosses were stored in the refrigerator at subfreezing temperatures.

2.4 SOWING, GERMINATION AND HARDENING OF SEEDLINGS

For this study, painted tin trays measuring 50 cm in length x 35 cm in width x 10 cm in depth with holes were filled with sieved-sterilized river bank soil to grow the sugarcane seeds in the glasshouse. The soil was sterilized in bulk under plastic covers sealed tightly at the ground. The fuzz was sown in tin trays with holes measuring 50 cm in length, 35 cm in width and 10 cm in depth. The trays were filled with fumigated soil and the fuzz was spread evenly on top of the soil. To flatten the fuzz to the soil surface water was sprinkled lightly. The trays were then labeled and kept in the glasshouse for seed germination. After 5 – 6 days the seeds started to germinate and the trays were kept in the glasshouse for further one week before it was transferred to the hardening frame. The trays were removed from the glasshouse after two weeks and kept in the hardening frame. The seedlings in the hardening frame were watered frequently. including NPK were supplied for rapid growth of the seedlings.

2.5 TRANSPLANTING OF SEEDLINGS IN FIELD AND EXPERIMENTAL TRIAL

The seedlings were transplanted into the 3"x 5" plastic bags filled with soil in three replications during mid September 1996. From each cross and parent 45 seedlings were transplanted in each replication. The seedlings were watered regularly and fertilizer was applied every two weeks for two months. The leaves of the seedlings were clipped off to produce thicker and stronger seedlings. The seedlings were raised in the nursery for three months before planting in the field. The trial design for the experiment was randomized complete block design with three replications. The site where the trial was established was on flat land. The initial soil analysis results at this site were pH = 6 - 7, P = 13.3 ppm, K = 68 ppm and Ca = 3302ppm. At all stages of the experiment the objective was to keep the experimental error within each group as small as possible. The trial was planted on the 24 December 1996. Each row in each replication represented one family. The parents Aiwa, Mana, Kaba, Mentor, Vatu and Yasawa were also planted in the trial for comparison. The seedlings were removed from the plastic bags and held upright in the furrows. The roots of the seedlings were pressed firmly in the furrows and covered with soil. Twenty seedlings were selected and planted in three replications. The seedlings were planted with a row spacing of 1.37 m and with intra-row spacing of 0.6 m. In this trial, weeds were controlled manually. All recommended cultural practices were followed for raising the crop.

Harvesting

The trial was harvested in November 1997 when the age of the crop was 11 months.

2.6 CHARACTERS STUDIED

Data on the following characters were recorded in November 1997.

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Agronomic Characters

i) Number of millable stalks per stool

Individual stalks per stool for each of the crosses in all the three replications were counted and the data were recorded.

ii) Stalk thickness (cm)

For each stalk in each of the stools, the thickness was measured approximately in the middle of the stalk using a vainer caliper. The mean stalk thickness was then calculated.

iii) Stalk height (cm)

For each stalk in each of the stools, the height of the stalk was measured using the measuring tape. The measurement was taken from the root of the stalks to the third dewlap. The mean stalk height was then calculated.

iv) Refractometer brix

Field brix was measured from each of the stalks per stool using the refractormeter. The mean brix was then calculated.

v) Weight of the millable stalks (kg)

All the number of millable stalks per stool was weighed to give the weight of the millable stalks per stool.

vi) Weight per stalk (kg)

Weight per stalk was calculated by dividing weight of millable stalks by number of millable stalks.

vii)Plant volume (cm³)

Plant volume was calculated by using the formula:

$$V = \pi D^2 x L x N$$

Where: $V = plant \ volume \ (an index \ of \ vigor),$

D = stalk diameter (cm),

L = stalk length (cm)

N = number of millable stalks per stool.

2.7 BIO-CHEMICAL CHARACTERS AND THEIR MEASUREMENTS

- Percent pol = Pol reading is the reading obtained from polarimeter
- Percent purity: = (%cane sugar in cane) / (% soluble solids in cane) = Pol / 100 Brix
- Percent fibre
- Percent POCS = (% cane sugar in cane) 2 (% impurities in cane)
- Brix

3 STATISTICAL ANALYSIS

The following statistical analysis were carried out:

• *Line x tester: Analysis of variance (anova):*

The first step in the line x tester analysis was to perform analysis of variance as per design used to calculate F values among the genotypes including crosses and parents. Similarly analysis of variance for pooled data was carried out for parents and crosses.

- Line x tester analysis for genetic components: Line x tester analysis was carried out following methodology described by Singh and Chaudhary 1985.
- Estimation of general combining ability effects

 Effects due to general and specific combining abilities
 were estimated using formula presented by Singh and
 Chaudhary 1985.

• Estimation of specific combining ability effects

Estimates of specific combining ability effects were calculated using formula given below (Singh and Chaudhary 1985): Sij (xij/r) - (xi/tr) - (xj/lr) - (x../ltr) Where: Sij = specific combining ability effects for cross 1, xij = grand total for cross 1, xij = grand total of lines for cross 1, xij = grand total of testers for cross 1, xij = grand total crosses, 1 = number of lines, t = number of testers and r = number of replications

4 RESULTS

4.1 AGRONOMIC CHARACTERS

Analysis of variance (Table 1) showed that genotypic differences were significant for most of the agronomic characters studied.

Table 1. Analysis of variance for line x tester design for agronomic characters.

Source	d.f	•	Mean Squar	re Values for D	ifferent Chara	cters	•	•
		No. of Millable Stalks	Stalk Thickness (cm)	Stalk Height (cm)	Plant Volume (cm ³)	Weight Per Stalk (kg)	Weight of Millable Stalks (kg)	Refractor Meter Brix
Replication	2	3.400	0.680	639.980**	5.0×10^9	0.005	5.260	8.150**
Genotypes	14	8.780**	3.600**	969.390**	9.9x10 ⁹ **	0.1357**	24.570**	4.690**
Lines	2	1.790	4.140*	146.760	$3.3x10^9$	0.023	0.400	1.260
Testers	2	1.130	2.060	821.970*	6.1×10^9	0.063	1.890	6.390**
Line x Testers	6	1.540	0.210	238.460	2.0x10 ⁹	0.040	0.810	1.170
Error	28	1.130	0.790	190.810	1.5×10^{10}	0.020	4.340	0.930
σ^2 sca		0.370	0.440	3.990	66932.800	0.205	1.090	0.280
σ^2 gca		0.050	0.280	2.660	8755.950	0.054	0.100	0.270
σ^2 sca : σ^2 gca		8.220	1.550	1.500	7.640	3.796	11.310	1.040

^{*} P = 0.05 ** P = 0.01

Estimates of variance due to specific combining ability $(\sigma^2 sca)$ and general combining ability $(\sigma^2 gca)$ and their ratio (Table 1) revealed that the former was higher than the latter for all characters studied. This indicated a predominantly non-additive gene action for these characters. General combining ability of males and females are presented in Table 2. Among the female parents, Mana was a good general combiner for all agronomic characters, except for millable stalks, while the variety Aiwa contributed only to the number of millable stalks. Mana was also found to be the best contributor of volume (plant vigor). The other female, Kaba, was found to be good combiner for stalk thickness. Kaba was also observed to be a poor combiner for most of the characters studied.

Among the testers (Table 2), Vatu was observed to be a good general combiner for number of millable stalks, stalk height, plant volume and weight of stalks. Another male parent, Mentor was found to be a good general combiner for stalk thickness, plant volume, and weight per stalk. Yasawa was found to be a poor combiner for most of the characters studied but was found to be a good combiner for brix only.

Specific combining ability effects are presented in Table 3. The five crosses Aiwa x Mentor, Aiwa x Vatu, Kaba x Vatu, Mana x Yasawa and Kaba x Yasawa were found to be the best specific combinations for most of the characters studied having very high SCA effects for yield attributes. Mana x Mentor had a high SCA effect for number of millable stalks and plant volume. The crosses Mana x Vatu, Kaba x Yasawa, Kaba x Mentor and Aiwa x Yasawa were found to be the best specific combinations for brix. All the crosses except for Aiwa x Yasawa, Kaba x Mentor and Mana x Vatu had a high SCA effect for plant volume.

The proportional contributions of lines (females) and testers (males) and their interactions to the total variance for different characters presented in Table 4 revealed that the maximum contribution to the total variance of stalk thickness were made by female parents and for the other two characters (stalk height and brix) contribution to the total variance was made by made parents. The line x tester interactions contributed towards the number of millable stalks.

Table 2. Effects of general combining ability of the parents for agronomic characters.

Character / Parent	No. of Millable Stalks	Stalk Thickness (cm)	Stalk Height (cm)	Plant Volume (cm³)	Weight Per Stalk (kg)	Weight of Millable Stalks (kg)	Refractor- Meter Brix
Females:							
Aiwa	0.460	-0.780	-0.620	-12504.990	-0.054	0.120	0.420
Kaba	-0.430	0.470	-3.930	-7786.690	-0.831	-0.240	-0.120
Mana	-0.030	0.310	4.550	20291.680	0.038	0.130	-0.310
SE	0.354	0.296	4.6	41365.580	0.048	0.694	0.321
Males:							
Mentor	-0.320	0.480	-1.840	9874.430	0.048	-0.050	-0.360
Vatu	0.380	0.000	10.350	17840.780	0.049	0.480	-0.670
Yasawa	-0.060	-0.480	-8.500	-27715.200	-0.095	-0.430	0.960
SE	0.354	0.296	4.600	41365.580	0.048	0.694	0.321

Table 3. Effects of specific combining ability of the crosses agronomic characters.

Character/Parent	No. of Millable Stalks	Stalk Thickness (cm)	Stalk Height (cm)	Plant Volume (cm ³)	Weight Per Stalk (kg)	Refractor- meter Brix
Aiwa x Mentor	-0.540	0.350	8.810	233.690	0.090	-0.300
Aiwa x Vatu	0.860	-0.140	0.420	18363.100	-0.040	-0.120
Aiwa x Yasawa	-0.330	-0.190	-9.230	-20697.000	-0.050	0.410
Kaba x Mentor	-0.050	-0.140	-2.150	-14261.000	-0.030	0.370
Kaba x Vatu	-0.180	0.180	2.460	8647.260	0.030	-0.540
Kaba x Yasawa	0.220	-0.040	-0.320	5613.560	0.002	0.190
Mana x Mentor	0.590	-0.210	-6.670	11927.100	-0.060	-0.070
Mana x Vatu	-0.680	-0.030	-2.90	-27010.000	0.008	0.680
Mana x Yasawa	0.090	0.250	-9.550	15083.300	0.050	-0.590
SE (±)	0.164	0.513	7.980	71647.300	0.083	0.557

Table 4. Proportional contributions of lines and testers and their interaction variances for various characters.

Character/ Contributions of	No. of Millable Stalks	Stalk Thickness (cm)	Stalk Height (cm)	Plant Volume (cm³)	Refractor- Meter Brix
Lines	29.770	61.600	11.260	24.980	12.610
Testers	18.850	30.600	56.160	45.690	63.960
Lines and Testers	51.380	7.800	32.580	29.330	23.420

Note: Higher values indicate stronger effects

4.2 BIO-CHEMICAL CHARACTERS

Analysis of variance (Table 5) showed that genotypic difference was significant at (P = 0.01) for percent fibre of the five bio-chemical characters studied. The line x tester interactions was not significant for all characters studied. Estimates of variance (σ^2) due to SCA and GCA and their ratio (Table 5) revealed that the σ^2 sca was higher than the σ^2 gca for all characters studied.

The effects of GCA of males and females are presented in Table 6. Among the female parents, Aiwa was found to be good general combiner of percent pol, percent purity and percent fibre, which are important factors in determining percent POCS. The female parent Kaba was observed to be a poor combiner for all the bio-chemical characters under study.

Table 5. Analysis of variance for line x tester design for some bio-chemical characters.

Source	d.f	Mean Square Values for Different Characters							
		% Pol	% Purity	% Fibre	%POCS	Brix			
Replications	2	211.730	87.140	7.870**	13.730	12.910			
Genotypes	14	76.600	15.710	3.530**	3.890	1.850			
Lines	2	25.990	8.700	0.930	1.130	0.039			
Testers	2	254.220	116.850	5.700**	15.250	7.370			
Line x Testers	6	41.510	20.240	0.110	2.130	1.148			
Error	28	102.510	95.080	0.900	4.590	2.310			
ÕSCA		4.510	4.990	0.510	1.070	0.622			
ÕGCA		1.660	1.090	0.300	0.410	0.266			
ÕSCA : ÕGCA		2.717	4.578	1.700	2.610	2.340			

^{*} P = 0.05 **P = 0.01

Table 6. Effects of general combining ability of the parents for bio-chemical characters.

Character/Parent	% Pol	% Purity	% Fibre	%POCS	Brix
Females:					
Aiwa	1.880	0.920	0.330	0.370	0.070
Kaba	-1.430	-1.040	-0.310	-0.340	- 0.050
Mana	-0.450	0.120	-0.040	0.020	- 0.030
SE	3.370	3.250	0.317	0.714	0.507
Males:					
Mentor	-4.250	-4.160	-0.250	-1.130	0.310
Vatu	-1.710	1.900	0.890	-0.280	-0.720
Yasawa	5.960	2.250	-0.064	1.420	1.010
SE	3.370	3.250	0.317	0.714	0.507

Among the testers (Table 6) Yasawa was observed to be a good general combiner for percent pol, percent purity, and percent POCS. Mentor was a poor combiner for all the bio-chemical characters studied.

Table 7. Effects of specific combining ability of the crosses for biochemical characters.

Character/Parent	% Pol	% Purity	%Fibre	%POCS	Brix
Aiwa x Mentor	0.210	1.200	0.220	0.270	-0.410
Aiwa x Vatu	-1.790	-1.180	-0.190	-0.440	0.070
Aiwa x Yasawa	1.570	-0.020	-0.020	0.150	0.360
Kaba x Mentor	-1.650	-3.130	-0.040	-0.690	0.430
Kaba x Vatu	-1.480	0.940	0.020	-0.130	-0.620
Kaba x Yasawa	3.130	2.200	0.030	0.810	0.200
Mana x Mentor	1.430	1.940	-0.160	0.400	0.000
Mana x Vatu	3.270	0.260	0.180	0.550	0.560
Mana x Yasawa	-4.710	-2.190	0.000	-0.960	-0.540
SE (±)	5.850	5.630	0.550	1.240	0.877

Specific combining ability effects are presented in Table 7. Two crosses namely Kaba x Yasawa and Mana x Vatu were found to be the best specific combinations for all five bio-chemical characters studied having a very high SCA effect for percent pol, percent fibre, percent purity and percent POCS. Aiwa x Yasawa and Mana x Mentor also had a high SCA effects for percent pol and percent purity, respectively.

The proportional contributions of lines (females) and testers (males) and their interactions to the total variance for different characters in this crop are shown in Table 8. It is evident that male parents made the maximum contributions to the total variance for the bio-chemical characters under study.

5 DISCUSSION

5.1 AGRONOMIC CHARACTERS

It is evident from Table 1 that genotype differences for all the seven characters studied were significant at p = 0.01. Punia (1986) reported similar results in sugar cane line x tester analysis. The line x tester interaction analysis in the present study was not significant for any of the characters studied. This situation could be explained in

terms of different environmental conditions and different varieties of sugarcane used as parents in the experiment. Punia (1986) found that the line x tester interactions were also significant at p=0.01 for all the characters studied. Thus, this indicated that the testers should have markedly different combining ability effects.

Table 8. Proportional contributions of lines and testers and their interaction to total variance for various characters.

Character / % Contributions of	% Pol	% Purity	% Fibre	%POCS	Brix
Lines	7.150	5.240	13.600	5.450	0.397
Testers	69.990	70.380	83.260	73.940	75.940
Lines and Testers	22.860	24.380	3.140	20.610	23.64

Note: Higher values indicate stronger effects.

Estimates of variance (σ^2) due to SCA and GCA and their ratios (Table 1) revealed that SCA were higher then GCA for all the characters studied. Similar results were obtained by Yang and Chu (1962), Miller (1977), Punia (1986), Coleman *et al.* (1962). This indicated a predominantly non-additive gene action for these characters. This type of gene action could be exploited in hybrids (F_1 s) since in sugarcane most of the varieties are F_1 hybrids between different selected parents. Hogarth (1971) and Hogarth *et al.* (1981) estimated genetic variance in sugarcane hybrids to select parents for further hybridization.

The effects of GCA of males and females are presented in Table 2. Aiwa and Mana appeared to be the best general combiners for number of millable stalks and stalk height, which are important factors in cane yield. Together these two parents contributed a large number of favourable genes for high cane yield and other related characters. Kaba was found to be a good combiner for stalk thickness. Hence, the two female parents, Aiwa and Mana could be used in future programs for the improvement of the characters such as millable stalks, stalk thickness, stalk height, weight of stalks and brix.

In testers, Vatu appeared to be a good general combiner for number of millable stalks, stalk height, plant volume and weight of stalks. This parent may be presumed to have a relatively large number of favourable alleles for these characters. Mentor was found to be a good general combiner for stalk thickness, plant volume and weight per stalk while Yasawa was found to be a good general combiner for brix only.

Specific combining ability effects are presented in Table 3 Aiwa, which was the best general combiner for brix, showed high SCA effect only in one cross-combination (Table 3), indicating that a parent having a good GCA effect need not necessarily produce better hybrids. A parent with poor GCA, however, might produce better hybrids (Table 3).

Mana x Mentor, and Aiwa x Mentor which were good x good and poor x good general combiners for plant volume (Table 2), also appeared to be the best specific

combiners for this trait (Table3). For number of millable stalks and plant volume, the best cross was Kaba x Yasawa (Table 3) involving poor x poor general combiners. The superiority of poor x poor general combiners to others might be owing to over dominance and epistasis.

There was maximum contribution from the female parents to the total variance of the character stalk thickness. On the other hand, maximum contribution to the total variance for the characters such as stalk height, plant-volume and brix was made by the male parents. The line x tester interactions contributed towards the number of millable stalks. Punia (1986) also reported that the maximum contribution to the total variance of stalk thickness was made by the female parents and the line x tester interactions contributed towards the number of millable stalks.

5.2 BIO-CHEMICAL CHARACTERS

It is evident from Table 4 that genotypic difference was significant (p = 0.01) for only one character of the five biochemical characters studied that is percent fibre. The line x tester interactions was not significant for any of the characters studied. This indicated that the testers did not show markedly different combining ability effects.

Estimates of variance (σ^2) due to SCA and GCA and their ratios (Table 5) revealed that the σ^2 SCA was higher than the σ^2 GCA for all characters studied. This indicated a predominantly non-additive gene action for these characters. Aiwa, Mana and Yasawa appeared to be the best general combiners for percent pol, percent purity and brix (Table 6), which are important factors in the determination of percent POCS. Together the above parents contribute a large number of favourable genes towards percent POCS and other related characters. Kaba and Mentor were observed to be poor combiners for all the bio-chemical characters studied. Hence, the parents Aiwa, Mana and Yasawa could be used in future programs for the improvement of the characters that are percent pol, percent purity and brix.

Tester Yasawa, was the best general combiner for brix and percent POCS showed high SCA effect (Table 7) in two out of the three cross combinations indicating that this parent could be used in future programs to improve characters such as percent POCS and brix. Kaba x Yasawa having poor x good general combiners for brix, percent pol, percent purity, percent POCS (Table 6), appeared to be the best specific combiners for the above traits (Table7).

It is evident (Table 8) that the maximum contributions to the total variance for all the bio-chemical characters under study were made by the male parents.

The information available for quantitative characters plays a vital role in planning for breeding strategies for a particular character. Chen et al. (1986) studied inheritance of quantitative characters in sugarcane and concluded that it is imporant for a breeder to evaluate parents before attempting hybridization programme. In the present study SCA (indicating non-additive genetic variance) has generally been greater than GCA for both cane yield as measured by plant volume and sugar content. Similar results were obtained by Yang and Chu (1962), Miller (1977), Rao and Ethirajan (1983) in sugarcane cross evaluation.

Selection of parents for sugar content could be successful, but progeny testing would seem to be necessary for yield of cane (Heinz 1987). Some success could be achieved by selecting parents for cane yield since there is substantial additive genetic variance. In view of the magnitude of non-additive genetic variance, predictions based on the yields of parent varieties would not be very good. Consequently, it would be desirable to test a large number of crosses for SCA for yield of cane.

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