

THE USE OF TOPCROSSES TO SELECT FOR SUPERIOR MALE STERILE LINES IN SORGHUM

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ABSTRACT

Grain sorghum [*Sorghum bicolor* (L.) Moench] topcrosses produced by crossing 40 randomly selected male sterile A-lines with a genetically broad-based pollen parent, a mixture of 100 fertility restore R-lines, were evaluated, for general combining ability (GCA) effects, heritability estimates, and phenotypic and genotypic correlations in two different production environments. Significant differences were detected between environments, among topcrosses, among fertile female B-lines, and between topcrosses and B-lines for grain yield, harvest index, growth rate, plant height, test weight, heading date, and panicle length. Topcrosses appeared to be useful for preliminary screening of sorghum inbred lines. They effectively identified superior A-lines. High heritability estimates were obtained by variance components and parent-offspring regression for all the traits studied,

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except for growth rate. Positive and very high correlations were found between grain yield, harvest index, and test weight. Negative relationships were detected for grain yield with heading date. Specific parental combinations and heterotic effects were observed.

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استفاده از تاپ کراس های سورگوم در گزینش لاین های نرعیتم

مطلوب

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چکیده

تاپ کراس های حاصل از تلاقی ۴۰ لاین تصادفی نرعیتم سورگوم (لاین A) با یک والد نر دارای زمینه ژنتیکی گسترده (مخلوطی از ۱۰۰ لاین برگرداننده باروری، لاین R)، از نظر قابلیت ترکیب پذیری عمومی، برآوردهای وراثت پذیری، و همبستگی های فنوتیپی و ژنوتیپی در دو محیط

تولید متفاوت ارزیابی شدند. عملکرد دانه، شاخص برداشت، سرعت رشد، وزن حجمی، ارتفاع بوته، تعداد روز تا ۵۰ درصد گلدهی و طول خوشه اندازه گیری شدند. تفاوت های آماری معنی داری بین دو محیط، بین تاپ کراس ها، بین لاین های نر بارور مادری (لاین B) و بین تاپ کراس ها و لاین های B برای تمام صفات مورد مطالعه، مشاهده شد. تاپ کراس ها در گزینش مقدماتی در ژرم پلاسما سورگوم مفید واقع شدند. تاپ کراس ها، لاین های مطلوب A را مشخص ساختند. برآوردهای وراثت پذیری با توجه به اجزاء متشکله واریانس و رگرسیون والد-تایچ محاسبه شد. تمام صفات مورد بررسی، به جز سرعت رشد، وراثت پذیری بالائی داشتند. همبستگی های مثبت و معنی داری بین عملکرد دانه، شاخص برداشت وزن حجمی مشاهده شد. همبستگی عملکرد دانه با تعداد روز تا ۵۰ درصد گلدهی منفی بود. ترکیب های مناسب والدینی و اثرات هتروزیس مشاهده شد.

INTRODUCTION

The utilization of hybrid vigor in plant breeding requires the evaluation of inbred or pure lines in hybrid combinations (6). Individual lines can be tested in different single cross combinations, but this limits the number of lines which may be evaluated. The topcross method is widely used in the first evaluation of new lines to evaluate their general combining abilities (18, 27). For this purpose, the lines under evaluation are crossed with a common pollen parent with a broad genetic base. Sprague and Tatum (27) have defined the concepts of general and specific combining abilities in term of topcross data. Usually, a random mating or a mixture of lines is used as the

topcross tester (6). The selected lines are then subjected to more detailed testing in single or double crosses to determine the specific combining ability.

Following the recovery of cytoplasmic-genetic male sterility (28), F1 hybrid production became a practical and new breeding approach in sorghum (9, 20). Topcrosses in sorghum have not been explored extensively as in corn (2, 18, 22). However, after the development of a large number of male-sterile (A), restorer (R) and maintainer of male-sterility (B) lines for hybrid seed production, results regarding the most efficient breeding method and type of tester to identify superior lines have been published (1, 2, 5, 18, 20, 22, 23, 26).

Significant general combining ability (GCA) effects have been reported for grain yield and some other related characters such as days to 50% flowering, harvest index, 1000-seed weight, and plant height (8, 9, 16, 29). Laosuwan and Atkins (10) found that GCA effects of R-lines accounted for the largest portion of the variation for grain yield, heads per plant, 100-seed weight and seeds per head. They reported that heterosis for grain yield was greater than for any of the yield components.

Ross and Kofoed (24) evaluated A-line x random mating population topcrosses and found significant differences in combining ability of A-lines for grain yield. Also, Hookstra *et al.* (7) obtained similar results for grain yield, plant height, days to flowering and tillering. They conclude that topcrosses appear to be suitable for preliminary evaluation of grain sorghum inbred lines and selection of superior A-lines.

The present study was undertaken in an attempt to determine: (a) the general combining ability effects of a group of male-sterile lines, (b) the magnitude of heritability and heterosis estimates, and (c) genotypic and phenotypic correlation coefficients between different quantitative traits in grain sorghum.

MATERIALS AND METHODS

Genotypes were obtained from International Crop Research Institute for Semi-Arid Tropics (ICRISAT). Forty randomly selected cytoplasmic-genetic male sterile A-lines of grain sorghum were topcrossed to a genetically broad-based pollen parent which was a mixture of equal amount of seed from 100 restorer R-Lines. Topcrosses were made in an isolated crossing block in which the female parents were planted in 3 randomly placed single rows 5 m long and 0.75 m apart. One row of the pollen parent was planted between 2 female rows. Equal amounts of seed obtained from each female line in different replications were bulked to provide the topcross progeny of that line for evaluation purposes.

In 1991, the 40 topcross progenies, 40 maintainer B-lines, which were genetically the same as A-lines but male fertile, and the male parent were evaluated in an 8×8 triple lattice design in 2 different production environments. The soil at the experimental sites was medium-textured predominantly Aridisols series. In the favorable environment standard tillage, fertilizer (200 kg ha⁻¹ ammonium phosphate, 48% P₂O₅ and 18% N, before planting and 100 kg ha⁻¹ urea, 46% N, as topdressing), herbicide, insecticide, and irrigation practices were used. In the unfavorable environment the cultivation practices (amount of fertilizer and irrigation water, and number of herbicide and insecticide applications) were reduced by one half. Planting dates in the favorable and unfavorable environments were May 1st and 15th, respectively. A 4-row plot, 5×2.4 m in size was used in both environments. Plots were over-planted and thinned to a within row plant spacing of 15 cm, giving a final stand of about 111000 plants ha⁻¹.

Data were obtained for each plot for heading date (number of days from planting to the date when 50% of plants showed a completely emerged head), plant height (cm) at harvest (ground level to the top of the panicles), and panicle length (cm) (average length of 20 randomly selected heads from each plot). From each experimental unit a 3-m portion of the central two rows (2.25 m) was hand-harvested, dried to a uniform moisture level, threshed and weighed for grain yield and biomass determinations. Plot grain yields (kg plot^{-1}) were adjusted to 12 % moisture prior to analysis. A random sample of 500 ml threshed grain was weighed for test weight determinations ($\text{g } 500^{-1} \text{ ml}$). Finally, harvest index (percentage) was calculated as the ratio of grain yield to biomass (both at 0 % moisture levels) and growth rate (g day^{-1}) was obtained as biomass divided by the number of days from emergence to 50% flowering.

Data from each environment were analyzed as a triple lattices, but because the lattice design did not produce an increase in efficiency over the randomized complete block, the experiments were subsequently analysed as a randomized complete-block design. A Combined analysis of the two environments was calculated to provide estimates of the variances among the topcrosses, B-lines, topcrosses vs. B-lines (an estimate of average heterosis), their interactions with environment and pooled error. A combined analysis of covariance over environments was also done. After statistical analysis the yields and test weights were converted to t ha^{-1} and hectoliter, respectively.

The effects of environment were considered fixed while genotypic effects were assumed to be random. Tests of significance were made by using the error terms in calculating the F ratios. The GCA effects were calculated as mean deviations of each topcross performance from their overall mean. The GCA estimates were tested for significance from zero using a two-tailed t-test (25). The mean squares and mean cross products were equated with their expectations to obtain genotypic and phenotypic variance and covariance components, respectively (16). These components were used to

calculate heritability estimates as well as phenotypic and genotypic correlations. The phenotypic variance (σ^2_{ph}) among the means of topcrosses tested in r replicates and e environments may be expressed as:

$$\sigma^2_{ph} = \sigma^2_g + \sigma^2_{ge/e} + \sigma^2_{re/re}$$

where, σ^2_g is the genotypic variance among topcrosses, σ^2_{ge} is the topcrosses \times environment interaction variance, and σ^2 is the error variance. Covariances were calculated in a similar manner. Heritabilities (h^2) were estimated as $\sigma^2_g / \sigma^2_{ph}$ with standard error:

$$SE(h^2) = SE(\sigma^2_g) / [\sigma^2_{re} + \sigma^2_{ge/e} + \sigma^2_g]$$

$$SE(\sigma^2_g) = \{2/c[\sum MS_i / (df_i + 2)]\}^{1/2}$$

where, C is the coefficient of variance component in the expected values of mean square formula. MS_i and df_i are the mean square and degrees of freedom of the i_{th} component of variance (6).

Heritability estimates were also calculated as twice the regression coefficient of topcross performances on B-lines (4). Genotypic correlations were calculated as:

$$r_{gxy} = \sigma_{gxy} / (\sigma^2_{gx} \sigma^2_{gy})^{1/2}$$

where, σ_{gxy} is the genotypic component of the covariance between traits x and y , and σ^2_{gx} and σ^2_{gy} are the genetic component of variance for traits x and y , respectively. Phenotypic correlations were calculated as:

$$r_{pxy} = Mc_{pxy} / (MS_x MS_y)^{1/2}$$

where, MC_{pxy} is the mean cross product of traits x and y, and MS_x and MS_y are the squares among topcrosses for the traits x and y, respectively.

The additive genetic variance was estimated on the assumption that topcross progenies are half-sib families (4, 6). Therefore, the variance among topcrosses is equal to the covariance of half-sibs, which is $[(1 + F) 4] \sigma_A^2$, where σ_A^2 is the additive genetic variance and F is the coefficient of inbreeding, which is equal to one for inbred parents.

RESULTS AND DISCUSSION

The genotype \times environment interactions obtained for yield and other characteristics were not statistically significant (Table 1). Therefore, only the results of the combined analysis of variance will be discussed.

Means and ranges of the traits measured for 40 topcrosses and 40 B-lines evaluated in the favorable and unfavorable environments are presented in Table 2. Grain yield is of primary importance, but other characters also determine the agronomic value of a genotype. Within the topcrosses, wide ranges of values existed for all traits measured (Table 2). This result validates the estimation of genetic parameters. For all traits, the mean of topcrosses exceeded those of the respective B-lines. Interestingly, the mean performances of the topcrosses in the unfavorable environment were generally greater than those of the B-lines grown under favorable environmental conditions. Combined analyses of variance for the two environments showed that the mean squares for environments, among topcrosses, among B-lines, and topcrosses vs. B-lines were highly significant ($P < 0.01$) for all traits.

Table 1. Means, estimates of variance components, and heritabilities from the analyses of variance of topcross lines in two environments.

Trait	Mean	Additive variance	Mean square		Heritability [†]	
			Genotype × environment	Average heterosis [‡]	Reg Op	Var. Comp.
Yield (t ha ⁻¹)	5.1 ± 0.26	2.10 ± 0.33	2.41	473.4**	0.84 ± 0.16	0.72 ± 0.22
Harvest index(%)	34.2 ± 1.35	52.00 ± 9.06	54.00	1/29.8**	0.58 ± 0.18	0.66 ± 0.23
Growth rate (g day) ⁻¹	43.5 ± 1.73	30.34 ± 1.57	103.50	12836.5**	0.28 ± 0.18	0.37 ± 0.27
Plant height (cm)	199.2 ± 3.31	538.04 ± 5.88	179.70	304299.0**	0.30 ± 0.26	0.90 ± 0.22
Days to 50 % heading	93.3 ± 0.78	37.35 ± 4.37	6.40	2669.3**	0.86 ± 0.27	0.94 ± 0.22
Panicle length (cm)	26.6 ± 0.30	2.86 ± 0.42	2.40	722.5**	0.42 ± 0.25	0.78 ± 0.23
Test weight Kg ha ⁻¹	73.6 ± 0.63	5.94 ± 1.39	17.14	320.6**	0.70 ± 0.30	0.84 ± 0.08

†- Based on parent-offspring regression (Reg Op) and variance components (Var. Comp).

§- Topcrosses vs B-lines.

** Significant at the 1% probability level.

Table 2. Mean Values (above) and ranges (below) for seven characteristics of 40 topcrosses and B-lines of sorghum grown in favorable and unfavorable environments.

Trait	Topcrosses		B-lines	
	Favorable environment	Unfavorable environment	Favorable environment	Unfavorable environment
Yield (t ha ⁻¹)	5.73±0.15 3.05-8.71	4.48±0.151 18-8.03	3.67±0.13 0.81-5.23	2.56±0.17 0.78±5.11
Harvest index(%)	34.7 ± 0.73 16-50	33.8±0.82 19-44	30.8±0.82 13-42	28.3±0.81 11-39
Growth rate (g day ⁻¹)	46.0±0.95 17-67	41.0±1.03 21-61	33.0±0.97 18-57	28.0±0.94 13-47
Plant height (cm)	207.8±2.03 142.2-241.9	190.6±1.77 130.9-219.2	154.8±2.69 103.8-229.7	142.8±2.42 102.1-221.8
Days to 50% heading	98.0±0.46 82.1-110.0	88.6±0.43 80.1-99.2	102.7±0.84 90.1-142.5	93.2±0.79 79.6-126-8
Panicle length (cm)	27.3±0.17 24.9-30.3	26.0±0.18 22.1-29.1	24.6±0.30 19.1-33.5	23.5±0.27 18.6-30.0
Test weight kg ha ⁻¹	75.5±0.35 70.4-80.6	71.7±0.44 61.2-78.1	73.3±0.35 63.0-78.3	67.2±0.53 54.8-75.7

Estimates of variance components from analysis of variance for each trait over environments are given in Table 1. In theory, the variation among a random sample of topcrosses is equal to one half the additive genetic variance. As it is revealed from the estimates of additive genetic variance and heritability estimates based on regression of topcrosses on B-lines (Table 1), a large proportion of phenotypic variance of yield and other traits, except growth rate, is attributable to additive genetic variance among topcrosses.

Preponderance of additive variance for harvest index (7, 16, 17, 29), days to 50% heading (7, 16), and grain yield (7, 10, 16, 24) has been reported earlier. Since the estimates of additive genetic variance were relatively high for grain yield and most of the traits studied, preliminary screening of the relative potential of these female parents (A-lines) for use in hybrid combination could be accomplished effectively by topcrossing them to a genetically broad-based tester and comparing the performance of the hybrids. The mean squares for topcrosses vs. B-lines, which indicate average heterosis effects, were statistically significant for all traits measured (Table 1). Therefore, specific combinations of A- and R-line crosses with higher yield and other traits relative to parental lines are expected. The presence of specific parental combination and heterotic effects for agronomic characteristics of sorghum have been reported previously (1, 3, 12, 19, 21, 26).

Estimates of GCA effects for the 10 highest yielding of the 40 topcrosses based on the two environments are presented in Table 3. Within the set of these 10 highest yielding topcrosses, a wide range of values existed for other traits. A-line number 6 produced the highest yielding topcross which reflects the largest significant GCA effects for harvest index and the second highest values for plant height and panicle length. Low and negative GCA effects for days to flowering and plant height are necessary for ideal genotype. In general, A6 would be a useful parental line for hybrid sorghum. A30 and A10 also produced high yielding topcrosses with large GCA effects. A30 had the highest mean and GCA effect for growth rate and a positive GCA effect for days to flowering. A10 had a negative and relatively high GCA effect for plant height, and the lowest mean and the highest negative GCA effects for days to flowering, as well as the largest mean and GCA effects for harvest index and test weight. These two characters showed positive and significant correlations with grain yield (Table 4), and had high heritability estimates. Therefore, it would be possible to select specific cross combinations between A-lines and some R-lines, for high grain

yield based on harvest index and test weight. Previously published results have also clearly indicated differences in combining ability of A-lines for grain yield and other traits studied. In these studies populations have been found to be satisfactory testers (7, 11, 14, 15, 18).

Table 3. Estimate of GCA effects for the 10 highest yielding of 40 topcrosses in two environments.

Topcross	Traits						
	Yield (t ha ⁻¹)	Harvest index (%)	Growth rate (g day ⁻¹)	Plant height (cm)	Days to 50 % heading	Panicle length (cm)	Test weight (kg ha ⁻¹)
6	3.27**	11.3**	1.9	-18.4**	-2.7**	1.57*	2.06
30	1.45**	5.8*	8.0**	-8.5*	6.6**	-0.75	1.64
10	1.41**	11.3**	-1.4	-7.6*	-8.8**	-0.23	4.42**
31	1.39**	6.8*	2.8	0.2	-4.8**	0.98	3.86**
82	1.26**	1.8	5.1*	-19.1**	-7.4**	0.01	1.81
20	1.24**	6.8*	-2.3	3.3	-2.9**	-0.60	0.62
39	1.21**	0.8	7.7*	6.3	-1.6	1.50*	0.08
18	0.93**	1.8	6.3*	1.1	-0.6	-1.20*	0.90
38	0.78*	-3.2	5.1*	-4.8	2.8*	2.15*	1.00
2	0.66*	5.8*	-4.3	-10.0*	2.2*	0.30	3.75**

† Individual A-line mean = experiment mean (Table 1) + GCA effect

* and ** Significantly different from zero at 5 and 1% probability levels, respectively.

Heritability estimates are presented in Table 2. The heritability estimate for grain yield was greater than would normally be expected. This was probably due to application of a genetically diverse population used as pollen parent in this study. Heritability estimates for grain yield of sorghum have

ranged from 6 to 76%, depending on the population and method of calculation (3, 8, 13). The estimates of heritability for other traits, except growth rate, revealed that these characters can be altered through selection. Knowledge concerning the inheritance of quantitative traits and their relationships should increase the effectiveness of selection.

Table 4. Genotypic (above) and phenotypic (below) correlations between seven characters of sorghum topcrosses over 2 environments.

Traits	Y	HI	GR	HD	PL	TW
Yield(Y)						
Harvest index	0.80					
(HI)	0.86					
Growth	0.36	0.28				
rate(GR)	0.36	-0.14				
Plant height	0.46	0.63	0.22			
(PH)	0.31	0.44	0.21			
Days to 50% heading (HD)	-0.71	-0.79	-0.38	-0.35		
	-0.64	-0.66	-0.29	-0.29		
Panicle lenght	0.31	0.18	0.11	0.12	0.04	
(PL)	0.28	0.13	0.12	0.12	0.03	
Test weitht	0.51	0.57	0.11	-0.16	-0.80	0.04
(TW)	0.44	0.39	0.25	-0.10	-0.56	0.11

† The correlation coefficients greater than 0.30 and 0.39 are significantly different from zero at the 5% and 1% probablity levels, respectively.

The estimates of genotypic and phenotypic correlations are shown in Table 4. The genotypic correlations were generally greater than the phenotypic correlations. Grain yield was very closely and positively correlated with harvest index, plant height, and test weight. Harvest index and test weight were also positively correlated. Plant height and days to 50%

heading were negatively correlated. All other correlations were low and of no predictive value. Days to 50% heading was negatively correlated with grain yield. similar result has been noted by Torchi (30) who worked on the same material. The presence of some late maturing genotypes among the material studied, and early fall cold weather which caused low yields, may be the reason for this negative correlation. High and positive relationships have been reported between yield, and plant height, and between harvest index and days to flowering (8, 14, 16, 22).

Test weight is an important component of yield, and harvest index is the proportion of biological yield that measures the physiological efficiency of the plant. Since both of these characters are highly heritable (Table 3) and positively correlated to grain yield, it is possible to use them as selection criteria in breeding programs. According to the results obtained, early maturing and tall genotypes would have higher yields. If negative association between traits is due to pleiotropic effects, it would be very difficult to obtain the desired combination, but if linkage is involved, special breeding programs such as recurrent selection based on recombination between selected genotypes or mutation breeding are needed to break these linkages (4, 6). Imposing selection based on indices is also another efficient alternative method to overcome the undesirable effects of negative correlations (6).

In general, the results obtained supported the use of topcrosses for identifying sorghum lines with superior GCA for use in producing hybrids. The germplasm studied included a range of A-lines, and a genetically broad based population used as the topcross tester effectively identified the superior A-lines that are immediately usable. Depending upon the objective, superior R-lines should have improved GCA or specific combining ability (SCA) and have to be extracted through inbreeding or conventional pedigree method. However, the only true test of the value of a line that will ultimately be used in hybrid seed production is to test that line in hybrid combination.

In an attempt to do this, a separate experiment was conducted to select the best hybrid combinations of A×R lines and the results will be published in a separate article.

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