

## Seed production potential of ICRISAT-bred parental lines of two sorghum hybrids in the central Rift-valley of Ethiopia

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### Abstract

An experiment was carried out to study the seed production potential of the parental lines of two sorghum hybrids in the central Rift-valley of Ethiopia. The two important seed production attributes considered for the study were synchronization of flowering and row ratio of the parental lines and their interaction with the test environments. A total of 12 treatment combinations related to these attributes were organized. The ANOVA revealed significant differences among the treatments and among the environments for outcrossing percentage and seed yield. Although a 4:4 row ratio showed maximum outcrossing, a 2:6 row ratio gave maximum hybrid seed per hectare and thus is more profitable. Sowing date brought about variation in outcrossing percentage and yield in the parental lines of the two hybrids studied. In ICSA 15 × 3443-2-OP, regardless of the row ratio, yield decreased by 36.1% when the pollinator parent was sown 10 days later than the seed parent. The row orientation against the prevailing wind direction could have also contributed to variation in seed production of the parental lines. Therefore, when hybrid sorghum seed production is planned, synchronization of flowering and row ratio of the parental lines and the row orientation should be considered.

### Introduction

Sorghum (*Sorghum bicolor*) is the third important food grain in Ethiopia. Research on the development of varieties of the crop for about a century resulted in various high-yielding varieties. However, no single hybrid has been released so far in the country for commercial production. Although currently there are some hybrids in the pipeline, there is a paucity of information in the country regarding their seed production potential under natural environment.

Seed production is a vital part of any hybrid program with percent seed set on male sterile plants being an important economic factor in seed production (Imre 1966).

For a given hybrid to be released for commercial production, it should pass two distinct stages. First, the hybrid is evaluated for per se performance. Second, the parents of the selected hybrid are evaluated for various seed production attributes. The hybrid seed production attributes are synchronization of flowering of the parental lines (A- and R-lines), cross-pollination potential under natural environment and the row ratio of the seed parent to the pollinator parent.

One of the many problems breeders and producers of hybrid grain sorghum seed have encountered while trying to develop new hybrids is the crossing of lines differing in flowering time (Trybom et al. 1978). Sorghum pollen grains remain viable only for a very short time (in hours) whereas the stigma remains viable for about ten days. However, stigma receptivity increases and reaches its maximum on the fifth day after flowering (Puttarudrappa and Goud 1970, Patil and Goud 1980) and decreases gradually thereafter. Therefore, unless the pollen parent flowers within the range of maximum stigma receptivity of the seed parent, seed production is mostly limited.

Several methods have been used to delay flowering of one of the early parent lines. Delayed planting of the earlier maturing line is the method most often used. Other methods reviewed by Trybom et al. (1978) include clipping the earlier maturing line in its early stages of growth, differential irrigation, differential fertilization, use of contact herbicides and flaming. Although the latter approaches can be used to delay flowering, they may have some limitations associated with them. Most of the male parents in our hybrid development program flower earlier than their seed parents. Therefore, flowering should be delayed in these male parents.

Planting ratios of male and female lines should be worked out for each hybrid so as to maximize the seed yield of the hybrid. The ideal planting ratio in sorghum is two male rows alternated by four female rows where the male line has smaller heads and a shorter flowering span; also two male rows alternated by six female rows where the male line has larger panicles and a longer flowering span (Murty 1999). Therefore, this experiment was carried

out to investigate the seed production potential of the parental lines of two sorghum hybrids via various seed production attributes under natural wind pollination.

### Materials and methods

**The experimental sites.** The experiment was carried out at Melkassa Agricultural Research Center (39°21' E, 8°24' N) during the 2004 rainy season (June–October) and at Werer irrigated nursery during 2004 and 2005 off-seasons (December–April) giving a total of three environments. Table 1 shows the data recorded on various variables at the experimental site at Werer.

**Treatment combinations.** The trial was a three-factor factorial experiment in randomized complete block design (RCBD) with three replications, which gave a total of 12 treatment combinations. The factors, their levels and description are given in Table 2. The plot size was 5 m × 0.75 m × 8 rows. Fertilization and management practices other than sowing date and row ratio were as per the recommendation of sorghum production in the study area.

**Table 1. Data for some variables recorded at Werer experimental site in Ethiopia.**

Variable <sup>2</sup>	Environment <sup>1</sup>	
	Werer-2004	Werer-2005
Temperature (during flowering)		
Minimum (°C)	16.2	16.8
Maximum (°C)	32.7	35.4
Average relative humidity (%)	49	45
Prevailing wind direction	N→S	N→S
Wind speed (average at 2 m) (m s <sup>-1</sup> )	0.15	1.67
Row orientation	N→S	E→W

1. N = North, S = South, E = East, W = West.
2. Data on relative humidity, wind direction and wind speed were for five months from December to April.

The two hybrids under study were: (1) H<sub>1</sub> – ICSA 15 × ICSR 161; and (2) H<sub>2</sub> – ICSA 15 × 3443-2-OP, which have a common seed parent ICSA 15. Moreover, ICSB 15 was included in the experiment as control for the purpose of calculating outcrossing percentage (OC%). The parental lines (ICSA 15, ICSB 15 and ICSR 161) were selections from a large pool of A- and R-lines received from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). 3443-2-OP is a variety released as *Teshale* for production in the lowlands of Ethiopia.

**Data recording and analysis.** Data were recorded on time to 50% flowering, plant height and panicle length in both parents and seed yield and number of seeds per panicle on the seed parent. Previous results have been reported that when the night temperature falls below 11°C, breakdown of male sterility is noticed in sorghum. As this may lead to a faulty interpretation of the results, attempts have been made to avoid it through calculating selfing and later sterility percentage.

For calculating selfing percentage, 5 heads from the female rows were randomly bagged just before flowering. Later the bagged heads were harvested and the selfed seeds in each of the bagged heads were hand picked and counted using Seed Buro seed counter (Seedburo Inc) and their mean was calculated. Selfing percentage was estimated by dividing the number of seeds on the bagged heads by the number of seeds on the control (ICSB 15). Further, sterility percentage was estimated by deducting selfing percentage from 100.

For the purpose of estimating the OC%, 5 heads were randomly tagged from the female rows during flowering. The tagged heads were harvested and threshed and the seeds were counted and divided by 5 to get the average. The OC% was calculated using the following formula:

$$OC\% = \frac{(OCS - SS)}{CS} \times 100$$

where OCS = mean number of outcrossed seeds; SS = mean number of selfed seeds; and CS = mean number of seeds on control.

**Table 2. The factors and their levels used in the experiment on sorghum hybrids and parents.**

Factor	Factor levels	Description
Sowing date	S <sub>0</sub>	Male and female parents sown on the same date
	S <sub>1</sub>	Male parent planted 5 days after the female parent
	S <sub>2</sub>	Male parent planted 10 days after the female parent
Row ratio	R <sub>1</sub>	4 rows male parent : 4 rows female parent
	R <sub>2</sub>	2 rows male parent : 6 rows female parent
Hybrid	H <sub>1</sub>	ICSA 15 × ICSR 161
	H <sub>2</sub>	ICSA 15 × 3443-2-OP

The data were subjected to analysis of variance and mean separation using SAS system for windows version 8.0.

## Results and discussion

**Seed yield on the male sterile line.** The analysis of variance revealed significant difference among the treatments for seed yield. There was also significant difference among the environments indicating that the environments were diverse. However, treatment  $\times$  environment interaction was not significant (Table 3). There was no main factor, which was found to be significant except row ratio. In general more seed was harvested per hectare from 2:6 row ratio ( $R_2$ ) than 4:4 ( $R_1$ ). Sowing date  $\times$  hybrid  $\times$  row ratio interaction was significant indicating the variation in seed production of the pollinator lines of the two hybrids with changed sowing date and row ratio. In  $H_1$ , the highest yield was obtained when both the parental lines were sown on the same day and showed a decreasing trend thereafter. Thus, in this hybrid, delayed sowing of the pollinator parent 10 days after the seed parent resulted in yield reduction of 36.1% regardless of the row ratio. However,  $H_2$  gave variable yield when the sowing date was changed with row ratio. Therefore, in this hybrid, using  $R_1$  maximum yield (949 kg ha<sup>-1</sup>) was obtained when the pollinator was

sown 10 days later than the seed parent. But using  $R_2$  the highest yield (1096 kg ha<sup>-1</sup>) was obtained when the pollinator was sown 5 days later than the seed parent (Table 4). The mean seed yield of  $H_2$  was better than that of  $H_1$  though differences were not significant. Since the seed parent was common to both hybrids, if there were any significant yield differences between the two hybrids, it would have been attributed to the differences associated with the pollen producing ability of the pollinator parents. In general, maximum yield (1096 kg ha<sup>-1</sup>) was obtained when the pollinator parent of  $H_2$  was sown 5 days after the seed parent at a row ratio of 2:6 (Table 4).

**Selfing percentage.** Selfing percentage was estimated in the male sterile female parent to declare whether the harvested seed was actually outcrossed (hybrid). For this, selfing in the bagged heads was compared with that in the control plot (which was 100%). The experimental result revealed that selfing percentage in all the bagged heads was close to zero indicating the harvested seed was truly outcrossed. In fact, ICSA 15 is among the best performing and stable A-lines under Ethiopian condition for more than a decade.

**Outcrossing percentage.** Outcrossing percentage or the proportion of florets that got fertilized by foreign pollen is determined by a number of environmental and plant factors. While temperature, wind direction and intensity, and relative humidity are the main environmental variables, the length of pollen viability and stigma receptivity, height differences of the parents [a shorter A-line might be more favorable (de Vries 1971)] and panicle size which can carry maximum number of extruded anthers are the main plant factors that influence the amount of outcrossing.

In the present experiment, the ANOVA showed significant differences among the treatments, environments and treatment  $\times$  environment interaction (Table 5) for OC%. The mean outcrossing in 2005 (47.8%) was better than that in 2004 (35.9%) (Table 6). This can be justified in various ways. Firstly, the wind speed, which is among the necessary factors helping pollen dispersion was higher in 2005 (1.67 m s<sup>-1</sup> in 2 m distance) than in 2004 (0.15 m s<sup>-1</sup>). Secondly, in 2005 the row orientation was E $\rightarrow$ W direction while the prevailing wind direction was perpendicular to it (ie, N $\rightarrow$ S), which is more preferred for better seed set by dispersing more pollen to the seed parent.

Sowing date and the first order interaction, sowing date  $\times$  hybrid were significant for OC%. Like the seed yield, the second order interaction, sowing date  $\times$  row ratio  $\times$  hybrid was also significant indicating that the three factors were not working independently of one

**Table 3. Mean squares from the analysis of variance of the various treatments for seed yield at Werer, Ethiopia in 2004 and 2005.**

Source	df	Mean square <sup>1</sup>
Replication	2	588998
Treatment (T)	11	185742**
Sowing date (S)	2	121278
Hybrid (H)	1	158578
Row ratio (R)	1	298121*
S $\times$ H	2	289156*
S $\times$ R	2	61972
H $\times$ R	1	199607
S $\times$ H $\times$ R	2	221024*
Environment (E)	2	2093399**
T $\times$ E interaction	11	46696
S $\times$ E	2	78294
H $\times$ E	1	55389
R $\times$ E	1	36136
S $\times$ H $\times$ E	2	51691
S $\times$ R $\times$ E	2	21200
H $\times$ R $\times$ E	1	1066
S $\times$ H $\times$ R $\times$ E	2	59346
Error	46	58013

1. \* = Significant at  $P < 0.05$ ; \*\* = Significant at  $P < 0.01$ .

**Table 4. A three-way table of combined totals showing the effect of the three factors on seed yield in 2004 and 2005 at Werer, Ethiopia.**

Hybrid	Sowing date	Seed yield (kg ha <sup>-1</sup> )			
		Row ratio R <sub>1</sub>	Row ratio R <sub>2</sub>	Sum	Mean
H <sub>1</sub>	S <sub>0</sub>	927	941	1868	934
	S <sub>1</sub>	872	805	1678	839
	S <sub>2</sub>	535	659	1194	597
	Sum	2335	2405	4740	
	Mean	778	802	1580	790
H <sub>2</sub>	S <sub>0</sub>	812	967	1780	890
	S <sub>1</sub>	539	1096	1635	818
	S <sub>2</sub>	949	939	1888	944
	Sum	2300	3002	5303	
	Mean	767	1001	1768	884
	Sum	1545	1802		
	Mean	773	901		

another. The three-way table (Table 7) shows the relative outcrossing potential of the three main factors when combined. With R<sub>2</sub>, there was a consistently decreasing trend in outcrossing from sowing date S<sub>0</sub> to S<sub>2</sub> in both the hybrids. However, when R<sub>1</sub> was considered, there was an inconsistent trend of OC% in relation to sowing date. Accordingly in H<sub>1</sub>, the pollinator parent when sown 5 days later than the seed parent at a row ratio of 4:4 gave the highest OC% (54.17%). However, in similar sowing date treatment, H<sub>2</sub> gave the lowest OC%. Regardless of the row ratio, sowing date S<sub>0</sub> gave the maximum outcrossing in both hybrids. The hybrid H<sub>2</sub> gave better outcrossing and hence gave better yield than H<sub>1</sub>.

**The effect of height and panicle length differences between the parents on outcrossing.** If the parents are of similar height or if the pollen parent is taller than the seed parent, no difficulty is usually experienced for good seed set; but if the pollen parent is considerably shorter than the seed parent, obtaining good seed set even when the nicking is done properly, is rather difficult (Murty 1999). In the present experiment, 3443-2-OP was very much taller than ICSA 15 (by about 50–80 cm) but ICSR 161 was only 10–15 cm taller. No matter how much, both of the pollinator parents were taller than the seed parent to bring about adequate level of outcrossing. The density of pollen in the air is further influenced by anther extrusion ability and the panicle size of the pollen parent.

**Table 5. Mean squares from the analysis of variance of the various treatments for outcrossing percentage at Werer, Ethiopia in 2004 and 2005.**

Source	df	Mean square <sup>1</sup>
Replication	1	146.01**
Treatment (T)	11	565.77**
Sowing date (S)	2	1412.35**
Hybrid (H)	1	0.01
Row ratio (R)	1	86.68
S × H	2	988.76**
S × R	2	86.68
H × R	1	42.01
S × H × R	2	559.60**
Environment (E)	1	3294.01**
T × E interaction	11	70.14*
E × R	1	196.68*
E × H	1	78.13
E × S	2	90.26
E × H × R	1	1.68
E × H × S	2	6.13
E × R × S	2	68.43
E × S × H × R	2	82.68
Error	46	82.68

1. \* = Significant at  $P < 0.05$ ; \*\* = Significant at  $P < 0.01$ .

In spite of differences in the panicle size of the male lines (data not shown), it was not adequate to bring about any significant variation in cross pollination.

**Row ratio vs profitability of hybrid seed production.**

The mean seed yield from a row ratio of 4:4 ( $R_1$ ) was 773 kg ha<sup>-1</sup> whereas from a 2:6 ratio ( $R_2$ ), it was 901 kg ha<sup>-1</sup> regardless of the hybrids used in the study (Table 4). The experimental result revealed that this yield difference was not attributed to seed set but to the differences in size of plots allotted to the seed parent. The actual plot size covered by the seed parent was 50% of a hectare for the former and 75% of a hectare for the latter. In terms of

seed set potential, one can see that a 4:4 row ratio is better (outcrossing 42.08%) than a 2:6 row ratio (outcrossing 39.89%) though differences were not significant (Table 7). However, this result clearly shows that the later row ratio is more profitable than the former in terms of the hybrid seed produced. On the contrary, more seed of the restorer parent can be harvested in a hectare using the 4:4 row ratio. This calls for a comparison of the marginal return from the sale of the seed of the hybrids and production of the seed of the pollinator line for the next round of hybrid seed production.

Previous reports showed that sorghum hybrids perform better than the open-pollinated varieties in terms

**Table 6. Mean seed yield and outcrossing percentage (OC%) on sorghum male sterile line as affected by the various treatment combinations in two off-seasons at Werer, Ethiopia.**

Treatment combinations	Seed yield (kg ha <sup>-1</sup> )			OC%		
	2004	2005	Mean	2004	2005	Mean
$S_0R_1H_1$	618	1237	927	37.0	62.0	49.5
$S_0R_2H_1$	616	1266	941	52.2	62.0	55.5
$S_0R_1H_2$	625	999	812	39.9	58.0	47.5
$S_0R_2H_2$	843	1091	967	34.2	47.0	41.5
$S_1R_1H_1$	713	1032	872	48.4	62.3	54.2
$S_1R_2H_1$	673	937	805	31.4	40.0	35.2
$S_1R_1H_2$	498	580	539	29.9	36.0	35.0
$S_1R_2H_2$	875	1317	1096	37.2	46.3	40.8
$S_2R_1H_1$	488	582	535	18.9	33.0	24.8
$S_2R_2H_1$	442	876	659	22.2	33.3	26.7
$S_2R_1H_2$	805	1093	949	34.7	51.7	41.5
$S_2R_2H_2$	800	1078	939	35.9	41.3	39.7
Mean	666	1007	837	35.9	47.8	41.0
LSD (0.05)	356	395	280	9.3	11.7	7.1
CV (%)	31.6	23.2	29.8	17.8	14.4	14.9

**Table 7. A three-way table showing the integration of sorghum hybrids, sowing date and row ratio in outcrossing percentage (OC%).**

Hybrid	Sowing date	OC%			
		Row ratio $R_1$	Row ratio $R_2$	Sum	Mean
$H_1$	$S_0$	49.5	55.5	105	52.5
	$S_1$	54.17	35.17	89.34	44.67
	$S_2$	24.83	26.67	51.5	25.75
	Sum	128.5	117.34	245.84	40.97
	Mean	42.83	39.11		
$H_2$	$S_0$	47.5	41.5	89	44.5
	$S_1$	35	40.83	75.83	37.92
	$S_2$	41.5	39.67	81.17	40.59
	Sum	124	122	246	41.17
	Mean	41.33	40.67		
	Sum	252.5	239.34		
	Mean	42.08	39.89		

of yield and other agronomic characteristics. However, hybrid seed production in this crop is a low profit business due to its reduced yield coupled with low seed rate. The process of hybrid seed production requires studying the variation in the parental lines for flowering time and the determination of the more profitable row ratio under natural wind pollination. During the process of hybrid development, the characteristics of the parental lines in relation to seed production should be determined in order to save time and cost involved.

## Conclusion

In the present experiment, attempts were made to evaluate the synchronization of flowering and the row ratio between the parental lines of two sorghum hybrids. The results have shown that the parental lines had good level of synchronization. In general, 2:6 row ratio gave better yield than that of 4:4 row ratio regardless of the hybrids used in the study. However, row ratio was associated with sowing date of the two hybrids. Using a 2:6 row ratio, both hybrids gave the highest yield when the parents were sown the same day and showed a reduced trend there onwards. However, using a 4:4 row ratio, sowing date was variable in the two hybrids.

The hybrid seed yield was highly affected by the environment. In general, the mean yield of all the treatments in 2005 was higher than that in 2004. This was due to the variation in the climatic variables and row orientation, which were vital for seed set. Therefore, for better hybrid seed yield, a relatively higher wind speed and a row orientation perpendicular to the direction of the prevailing wind direction should be considered.

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