Journal of the Iowa Academy of Science: JIAS

Volume 95 | Number Article 5

1988

Agronomic Performance of Sorghum Hybrids Produced by using Different Male-sterility-inducing Cytoplasms

A. J. Maves
Iowa State University

R. E. Atkins
Iowa State University

Jour. Iowa Acad. Sci. 95(2):43-46, 1988

Agronomic Performance of Sorghum Hybrids Produced by using Different Male-sterility-inducing Cytoplasms[1]

A. J. MAVES and R. E. ATKINS

Department of Agronomy, Iowa State University, Ames, Iowa 50011

Experiments were conducted in three environments in Iowa to obtain information on the effects of different cytoplasms on agronomic characters in grain sorghum (*Sorghum bicolor* L. Moench) hybrids. Compared with A1 cytoplasm, A2 and A3 cytoplasms delayed flowering; reduced the percentage of fertile pollen, the number of seeds per panicle, and grain yield; and increased 100-seed weight of the hybrids. Hybrids with A2 or A3 cytoplasm did not differ ($p \leq 0.05$) from those in A1 cytoplasm for plant height, panicles per plant, or for length, width, and area of the third and fourth leaves from the top of the plant. A2-cytoplasm hybrids did not differ ($p \geq 0.05$) from those in A3 cytoplasm for any of the traits measure. The results are discussed relative to the performance of hybrids with pollen fertility restored and not restored.

INDEX DESCRIPTORS: *Sorghum bicolor* L. Moench, male sterility, cytoplasm, hybrid, agronomic traits, pollen fertility

Commercial production of hybrid seed in sorghum (*Sorghum bicolor* L. Moench) became feasible economically after the discovery of a cytoplasmic-genetic male-sterility system (Stephens and Holland, 1954). The combination of cytoplasm from 'milo' sorghum and recessive nuclear factors for fertility restoration from 'kafir' types produced plants that were pollen sterile. Hybrid seed of sorghums has been produced for more than three decades by using seed parents with the milo cytoplasm (designated A1) and male parents that possessed dominant nuclear factors for pollen fertility restoration (R-lines) in that cytoplasm.

The use of a singular cytoplasm source for seed production resulted in cytoplasmic uniformity among sorghum hybrids. Uniformity of cytoplasm is undesirable because a cytoplasm-specific hazard, such as the association of susceptibility to *Helminthosporium maydis* and T cytoplasm in maize (*Zea mays* L.) could be devastating to hybrid sorghum production, and genetic diversity among hybrids is limited by restrictions placed on parental combinations. The need for greater diversity of parents among sorghum hybrids has been emphasized by several scientists (Harvey, 1977; National Research Council, 1972).

New sources of male-sterility-inducing cytoplasm were discovered by examining patterns of pollen fertility restoration in crosses among an array of sorghum breeding lines (Schertz and Ritchey, 1978; Quinby, 1980; Worstell *et al.*, 1984). The more diverse and potentially useful of these cytoplasms have been designated A2, A3, and A4 to delineate them from the A1 milo source. A2 and A3 cytoplasm inbred lines have been registered (Schertz, 1977, 1984). Mitochondria and chloroplast DNA restriction fragment analyses have verified that organelle genomes differ among cytoplasms (Pring *et al.*, 1982; Conde *et al.*, 1982; Dixon and Leaver, 1982).

Chisi and Miller (1987) compared the combining ability and heterosis of hybrids in A1 and A2 cytoplasms. Thirty-six hybrids were produced by crossing six fertility-restorer lines onto three A1- and three A2-cytoplasm seed parents. A1 hybrids consistently yielded more than the A2 hybrids except for two parental combinations. Most A2 hybrids had low pollen-fertility scores and were earlier than A1 hybrids. Comparisons that involve A3 hybrids have not been published.

The objective of our study was to evaluate the agronomic performance of 30 F_1 hybrids in A1, A2, and A3 cytoplasms. To be effective substitutes for the widely used A1 cytoplasm, A2 and A3 cytoplasms should exhibit no adverse effects that would lower the value of sorghum hybrids or decrease the efficiency of producing hybrids.

MATERIALS AND METHODS

Thirty hybrids were produced by crossing 10 inbred lines used as males to each of three inbred lines used as females. The male parents were known pollen-fertility restorers when crossed to male-sterile lines that possessed A1 cytoplasm. Their fertility restoration capability in A2 and A3 cytoplasm was not known. Male parents were Tx 423, Tx 428, Tx 430, Tx 2536, Tx 2567, Tx 7078, NB 9040, IA 28, OKY 33, and KS 55. The female parents contained the same nuclear factors (i.e., from Combine Kafir 60, Tx 3197), but they differed in cytoplasm source. Designations for the cytoplasm sources were A1 (Tx 3197), A2 (IS12662C), and A3 (IS1112C). The three cytoplasms interacted with the CK 60 nuclear factors in a way that inhibited the production of functional pollen. Controlled hand-pollinations were made at Ames, Iowa, in 1984 to produce seed of each hybrid.

Hybrids were replicated three times and arranged in a randomized-complete-block design at each of three locations in Iowa in 1985. Planting dates at Beaconsfield, Castana, and Ames were May 17, May 22, and June 4, respectively. Individual plots were single rows 4.3 m long, with 102 cm between rows. At the three- to five-leaf stage, plots were thinned to one plant every 10 cm (96,900 plants/ha). After thinning, a center 3-m section of uniformly spaced plants in each plot was marked with garden stakes and used as the experimental unit for grain yield and the components of yield.

Data were recorded for grain yield, seeds/panicle, 100-seed weight, panicles/plant, and plant height in each environment. Days to midbloom, percentage pollen fertility, and length, width, and area of the third and fourth leaves from the top of the plant were recorded only at Ames. The area of these leaves is correlated closely with total-plant leaf area in sorghum (Atkins and Bueno, 1981). Leaf measurements were made on three plants in each plot.

The pollen-fertility estimates were obtained by sampling three plants outside the staked section in each plot. One branch was removed from each selected panicle a point just below anther extrusion. These branches were dried naturally and stored at room temperature. Pollen fertility was determined by staining the pollen grains with a solution of 2 g KI + 0.2 g I in 100 ml water (Brooks and Brooks, 1967). Three microscope fields were examined for each slide, and pollen grains that were plump and circular, with dark purple coloration, were classed as fertile.

Combined analyses of variance were computed for data recorded at all locations. Locations were considered random, and male and female parents were considered fixed effects. The means squares attributable to male and female parent sources of variation reflect differences among the hybrids that are due primarily to genetic and cytoplasmic

[1]Journal Paper No. J-12752 of the Iowa Agric. and Home Econ. Expt. Stn., Ames, IA 50011. Project No. 2573.

influences, respectively. Differences in female parent effects were of primary interest in this study. The sums of squares due to female parents were partitioned into two orthogonal single-degree-of freedom contrasts to assess the variation attributable to A1 vs. A2 + A3 and A2 vs. A3 cytoplasm effects. Means for hybrids grouped by female parents were compared by using Duncan's multiple range test at the 5% level of probability and LSD (0.05) values were determined for the means of each character (Steel and Torrie, 1960).

RESULTS

Environmental conditions during 1985 in Iowa generally were favorable for sorghum growth and development. Limited soil moisture seemed the major constraint to plant development. Average grain yields at Beaconsfield, Ames, and Castana were 7.16, 5.97, and 4.19 Mg/ha, respectively.

Combined analyses of variance indicated that variation attributable to locations was highly significant ($p \leq 0.01$) for grain yield, seeds/panicle, 100-seed weight, panicles/plant, and plant height. Location mean squares, in most instances, were larger than those for other sources of variation. Variation ascribable to male and female parent influences was highly significant for grain yield, seeds/panicle, and 100-seed weight, but not for panicles/plant. The male parent source of variation was highly significant for plant height. The male x female interaction mean square was not significant for grain yield, panicles/plant, and plant height, but it exceeded the 0.01 probability level for seeds/panicle and 100-seed weight.

Mean squares for the comparison of A1 vs. A2 + A3 cytoplasms were highly significant for grain yield, seeds/panicle, and 100-seed weight. In contrast, the A2 vs. A3 source of variation was not significant for any of the traits measured.

Comparisons of the relative performance of hybrids can be made from the means grouped according to female parents (Table 1). Hybrids that possessed A1 cytoplasm were significantly higher in mean grain yield and seeds/panicle and significantly lower in 100-seed weight than hybrids that possessed A2 or A3 cytoplasm, but they did not differ ($p \leq 0.05$) for panicles/plant and plant height.

The analysis of variance for percentage fertile pollen indicated that the mean squares for males, females, and the male x female interaction were highly significant. Partitioning of the sums of squares for females indicated that hybrids with A1 cytoplasm were markedly different ($p \leq 0.01$) in pollen fertility from hybrids that possessed A2 or A3 cytoplasm. The comparison for hybrids with A2 vs. A3 cytoplasm was not significant.

Pollen fertility was retored in all hybrids with A1 cytoplasm. Mean percentage fertile pollen was 70.0 (Table 2), with individual hybrids ranging from 38.2 to 93.7%. When the hybrids were produced by using A2 or A3 cytoplasm, only those that had the NB 9040 male parent were fertile (76.8 and 81.0%, respectively). Means for pollen fertility were 7.7% for all A2 hybrids and 8.1% for all A3 hybrids. Potential effects of the lack of pollen dispersal by part of the hybrids are addressed in the discussion section.

Table 1. Means for hybrids grouped by female parents for grain yield, yield components, and plant height for combined experiments at Ames, Beaconsfield, and Castana, Iowa in 1985.

Female	Grain yield[a] (Mg/ha)	Seeds/ panicle	100-seed weight (g)	Panicles/ plant	Plant height (cm)
A1	6.15a	1806a	2.73a	1.36a	128.7a
A2	5.57b	1512b	3.07b	1.33a	128.3a
A3	5.73b	1501b	3.12b	1.35a	128.2a
LSD (0.05)	0.18	56	0.06	0.05	1.4
C.V. (%)	10.37	11.81	7.06	12.67	3.79

[a]Means within a column followed by the same letter do not differ at the 0.05 probability level by use of Duncan's Multiple Range Test.

Table 2. Means for hybrids grouped by female parents for percentage fertile pollen, days to midbloom, and leaf area for experiment at Ames, Iowa in 1985.

Female	Fertile pollen[a] (%)	Days to midbloom	Leaf area (cm^2) Third from top	Leaf area (cm^2) Fourth from top
A1	70.0a	70.5a	321.6a	384.2a
A2	7.7b	71.9b	324.2a	386.4a
A3	8.1b	71.8b	314.8a	383.3a
LSD (0.05)	5.9	0.6	30.2	26.8
C.V. (%)	30.05	1.60	14.78	11.82

[a]Means within a column followed by the same letter do not differ at the 0.05 probability level by use of Duncan's Multiple Range Test.

Analyses of the midbloom data showed that mean squares for males, females, and the male x female interaction were significant ($p \leq 0.01$). A1 hybrids were significantly earlier than those in A2 or A3 cytoplasm (Table 2). Significant variation for days to midbloom between hybrids with A2 vs. A3 cytoplasm was not detected.

Variance analyses for area of the third and fourth leaves from the top of the plant were significant ($p \leq 0.01$) only for the male parent source of variation. Means for the three cytoplasm sources (Table 2) were nearly alike. Measurements of the length and width of these leaves (data not presented) likewise did not show significant differences attributable to sources of variation other than male parents.

DISCUSSION

Diversification of the cytoplasmic and genetic germplasm base in sorghums may lead to improved hybrids and serve as a safeguard against potential hazards to production. Our experiments with hybrids in two alternative cytoplasm sources indicated that their performance for a number of agronomic characters was appreciably different from that of hybrids with A1 milo cytoplasm. The A2 and A3 cytoplasm hybrids yielded less, had fewer seeds/panicle, larger seed, and were later for midbloom date. The delay in midbloom was only 1.5 days and likely of little consequence. Atkins and Kern (1972) also found differences in midbloom between cytoplasm sources as small as 1.3 days that were significant statistically, but they considered them of minor importance practically.

Reductions in grain yield and seeds/panicle for A2 and A3 cytoplasm hybrids in comparison with A1 hybrids averaged 8 and 17%, respectively. Losses of that magnitude raise questions about desirability of the alternative cytoplasms. Larger seed of the A2 and A3 hybrids should provide some compensation in the use of these cytoplasm sources. But, overall, they seem inferior to the A1 cytoplasm for hybrid seed production. If attention is focused on relationships between grain yield and pollen fertility in our study, however, there is reason to temper that conclusion.

Hybrids with A1 cytoplasm averaged markedly higher in percentage fertile pollen than those with A2 and A3 cytoplasm. All but two of the hybrids with A2 and A3 cytoplasm were completely male sterile. Those hybrids, therefore, were dependent on pollen dispersed from fertile entries and from hybrids in adjacent experiments, breeding nurseries, and border rows for fertilization and seed set.

Plantings of fertile sorghum genotypes around each experiment were extensive. At Ames, the experiment was placed centrally within

1.8 ha of yield trials and breeding nurseries. Surrounding experiments and border rows at Beaconsfield and Castana were fewer, but they occupied sizable adjacent areas. Sparse seed set was not prominent visually in any of the tests. Even so, male-sterile entries may have been at a disadvantage in terms of fertile pollen at critical times. Hybrids with A1 cytoplasm may have had the advantage of a greater pollen load through self-pollination as well.

Evidence that bears on the contention that lower grain yields of the A2 and A3 cytoplasm hybrids may have been related to the mode and efficiency of pollination as well as influences of the cytoplasms was obtained by partitioning the males x females interaction into single-degree-of-freedom contrasts. The comparison, A1 vs. A2 + A3 was divided further to compare hybrids with NB 9040 male parent and those with the other nine male parents. NB 9040 produced fertile hybrids with all cytoplasms; the other males produced fertile hybrids only in A1 cytoplasm.

Means in Table 3 for hybrids with parental combinations of NB 9040 and A1 cytoplasm, NB 9040 with A2 or A3 cytoplasms, and the remaining nine male parents combined with A1 cytoplasm are similar for all characters. Hybrids with the other nine male parents and A2 or A3 cytoplasm showed significantly different values ($p \leq 0.01$ or $p \leq 0.05$) for these traits compared with the other groupings. The other groups all displayed high percentages of fertile pollen, but hybrids with the nine male parents in A2 and A3 cytoplasm were pollen sterile. The male-sterile group had lower grain yield, fewer seeds/panicle, and larger seed compared with the fertile hybrids.

Table 3. Means for hybrids for grain yield, seeds/panicle, 100-seed weight, and percentage fertile pollen grouped by female parents and fertility restoration performance of male parents.[a]

Female	Male		Female	Male	
	NB 9040	Other 9		NB 9040	Other 9
	Fertile pollen (%)			100-seed weight (g)	
A1	93.7	67.7	A1	2.8	2.7
A2 + A3	78.9	0.0	A2 + A3	2.8	3.1
	Seeds/panicle (no.)			Grain yield (Mg/ha)	
A1	1747	1816	A1	6.21	6.15
A2 + A3	1817	1474	A2 + A3	6.13	5.60

[a]Means for percentage fertile pollen are from the 1985 Ames experiment. Means for the other characters are from combined analyses of data from Ames, Beaconsfield, and Castana.

Grain yields of corn hybrids that were male sterile (i.e., hybrids with T cytoplasm and no restorer genes) and male fertile (i.e., counterpart hybrids with normal cytoplasm) were reported equal in some environments, but male-sterile hybrids had significantly higher yields in others (Rogers and Edwardson, 1952; Duvick, 1958). Duvick concluded that the male-sterility-inducing cytoplasm had a depressing effect on grain yield, but physiological effects of the male-sterile condition not diverting metabolites into pollen development actually increased yield.

Comparisons between male-sterile (A-lines) and male-fertile genotypes (B-lines and R-lines) in wheat (*Triticum aestivum* L.) were made with data obtained from hybrid seed production blocks by Johnson and Lucken (1986). The male steriles had reduced seed set and grain yield, but larger seed than their counterpart B-lines and R-lines. These results agree with our results with sorghum, but they conflict with results cited for corn. Differences in yield response relative to male sterility likely are associated with natural mode of pollination for the species. Corn is naturally cross pollinated, but wheat and sorghum are predominantly self pollinated. Reduced efficiency of cross pollination may have contibuted to the decreased seed set and grain yield in wheat and sorghum.

Studies that utilize only male parents that restore fertility to sorghum hybrids with all three cytoplasms (A1, A2, A3) would eliminate the confounding of male sterility in some hybrids and provide a clear-cut evaluation of effects of the different cytoplasms on agronomic performance. Implementation of experiments comparing an appreciable number of male parents that restore pollen fertility in A1, A2, and A3 cytoplasm would be difficult presently. Surveys of fertility restoration among sorghum inbreds to A2 and A3 cytoplasm are under way, but published results are few and lacking in detail. The limited information available shows a dearth of good restorers to A2 cytoplasm. Information on fertility restoration in A3 cytoplasm is still more sparse, but results indicate that hybrids with that cytoplasm are distinct from those in other cytoplasms with most male parents (Worstell *et al.*, 1984).

Sorghum breeders need to pursue the diversification of male-sterility-inducing cytoplasms available for hybrid seed production. Incorporation of A2 and A3 cytoplasms into the B-lines of existing breeding programs would increase cytoplasmic and genetic variability. Although significant differences for some agronomic traits were attributed to cytoplasmic influences, questions remain about the cause of these differences.

The reductions in grain yield attributed to A2 and A3 cytoplasms, although significant statistically, were not large. A production hazard specific to A1 cytoplasm could lower yields much more than the reductions attributed to A2 and A3 cytoplasms in our experiments. New parental combinations, not possible with A1 cytoplasm, may produce hybrids that outperform existing hybrids. The greater genetic variability that can be utilized with different cytoplasms may offset potential adverse influences that might accompany these cytoplasms. Evaluations of hybrids made from these combinations may show that A2 and A3 cytoplasms are suitable alternatives to the A1 cytoplasm even if no A1-specific hazard occurs.

REFERENCES

ATKINS, R. E., and A. BUENO. 1981. Estimation of total-plant leaf area in grain sorghum. Iowa State J. Res. 56:193-204.

———, and J. J. KERN. 1972. Cytoplasm effects in relation to agronomic performance of grain sorghum, *Sorghum bicolor*, L. Moench. Crop Sci. 12:777-780.

BROOKS, J. S., and M. H. BROOKS. 1967. Pollen abortion in relation to cytoplasmic-genetic male sterility in sorghum. Crop Sci. 7:47-50.

CHISI, M., and F. R. MILLER. 1987. Combining ability and heterosis in A1 and A2 cytoplasms. p. 85-86. *In*. Proc. 15th Biennial Grain Sorghum Research and Utilization Conf., Grain Sorghum Producers Assoc., Lubbock, Texas.

CONDE, M. F., D. R. PRING, K. F. SCHERTZ, and W. M. ROSS. 1982. Correlations of mitochondrial DNA restriction endonuclease patterns with sterility expression in six male-sterile sorghum cytoplasms. Crop Sci. 22:536-539.

DIXON, L. K., and C. J. LEAVER. 1982. Mitochondrial gene expression and cytoplasmic male sterility in sorghum. Plant Mol. Biol. 1:89-102.

DUVICK, D. N. 1958. Yields and other agronomic characteristics of cytoplasmically pollen sterile corn hybrids compared to their normal counterparts. Agron. J. 50:121-125.

HARVEY, P. H. 1977. Sorghum germplasm base in the United States. p. 168-198. *In*. Proc. 32nd Annu. Corn Sorghum Res. Conf., 6-8 Dec. Chicago, Illinois. Am. Seed Trade Assoc., Washington, DC.

JOHNSON, K. M., and K. A. LUCKEN. 1986. Characteristics and performance of male-sterile and hybrid seed produced by cross-pollination in hard red spring wheat. Crop Sci. 26:55-57.

NATIONAL RESEARCH COUNCIL. 1972. Genetic vulnerability of major

crops. NAS-NRC, Washington, D.C.
PRING, D., M.F. CONDE, and K. F. SCHERTZ. 1982. Organelle genome diversity in sorghum: Male sterile cytoplasms. Crop Sci. 22:414-421.
QUINBY, J. R. 1980. Interaction of genes and cytoplasms in male sterility in sorghums. p. 175-184. *In.* Proc. Annu. Corn Sorghum Res. Conf., 9-11 Dec., Chicago, Illinois. Am. Seed Trade Assoc., Washington, DC.
ROGERS, J. S., and J. R. EDWARDSON. 1952. The utilization of cytoplasmic male-sterile inbreds in the production of corn hybrids. Agron. J. 44:8-13.
SCHERTZ, K. F. 1977. Registration of A2 Tx 2753 and B Tx 2753 sorghum germplasm. Crop Sci. 17:983.
———. 1984. Registration of A3 Tx 398 sorghum. Crop Sci. 24:833.
———, and J. M. RITCHEY. 1978. Cytoplasmic-genic male-sterility systems in sorghum. Crop Sci. 18:890-893.
STEEL, R. G. D., and J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York.
STEPHENS, J. C., and R. F. HOLLAND. 1954. Cytoplasmic male-sterility for hybrid sorghum seed production. Agron. J. 46:20-23.
WORSTELL, J. V., H. J. KIDD, and K. F. SCHERTZ. 1984. Relationships among male-sterility inducing cytoplasms of sorghum. Crop Sci. 24:186-189.