Natural Outcrossing of Sorghum and Sudangrass in the Central Great Plains

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ABSTRACT

Amount of natural outcrossing in a species is one of the primary determining factors in selection of breeding methods. Existing literature on natural outcrossing of sorghum is dated and limited in geographical reference. Experiments were designed to investigate natural outcrossing in contemporary sorghum, *Sorghum bicolor* (L.) Moench, B- and R-lines, and in sudangrass, *Sorghum bicolor* (L.) Moench. Four R-lines and four B-lines were seeded in rows in isolation at Mead, NE, and allowed to intercross in each of 2 yr. Bulked seed from each row was planted and proportion of offtypes recorded. Outcrossing ranged from 0.1 to 13% in R-lines, and from 0.5 to 9% in B-lines. Outcrossing among sudangrass plants with white or green midribs transplanted in isolation in each of 2 yr was estimated by utilizing the dominant gene for white midrib as a marker. Panicles from green midrib plants were tagged to indicate approximate date of pollination. At maturity, the panicles were divided into top, middle, and bottom thirds and threshed. Seed from each panicle section was planted and proportion of white midrib plants recorded. Outcrossing ranged from 0 to near 100% on individual sudangrass plants and was highly variable. Harvest of panicles pollinated during the middle of the pollination period should maximize outcrossing in sudangrass, but the use of nuclear male-sterility genes is still recommended for improvement of sudangrass through recurrent selection. For sorghum breeding procedures requiring a high degree of self-pollination, selfing under pollination bags is recommended.

Amount of natural outcrossing in a species is one of the primary determining factors in selection of breeding methods. Recurrent selection methods are most easily applied to highly outcrossing species, but are utilized with species such as sorghum by forcing outcrossing via the use of nuclear male-sterility genes (Ross, 1973). Conversely, breeding programs utilizing the pedigree approach are based on genetic segregation during selfing generations subsequent to the crossing of selected individuals. Selfing is assured in sorghum by isolating individual inflorescences under pollinating bags. Although hybrid sorghum is routinely produced using cytoplasmic male-sterility (Harvey, 1977), genetic improvement of parental lines is based on breeding techniques that rely largely on self fertilization.

Existing literature on natural outcrossing of sorghum is dated and limited in geographical reference. Varietal sorghums used by Karper and Conner (1919) and Sieglinger (1921) are no longer representative of genetic resources available to the sorghum industry today. Some evidence of natural outcrossing in sorghum is anecdotal (Quinby et al., 1958) rather than experimentally based. Karper and Conner (1919) reported an average of 6% cross-fertilization in 'White Milo' in Texas, and noted differing percentages of cross-fertilization among heads. Sieglinger (1921) reported an average of 5% cross-fertilization between white and yellow milo in Oklahoma.

Fewer experiment results are published on natural outcrossing of sudangrass. Hogg and Ahlgren (1943) showed an average of 7% natural cross-fertilization of 'Piper' sudangrass with 'Leoti' sorghum in Wisconsin. Garber and Atwood (1945) reported 18 to 77% outcrossing in an experimental line of sudangrass using seed produced in Pennsylvania, with an increase in cross-fertilization between 6 and 26 August.

Maunder and Sharp (1963) showed outcrossing percentages of sorghum in Texas to be affected by genotype and floret position on the panicle. 'Combine Kafir 60-B' averaged 3% outcrossing on the upper one-fourth and 1% outcrossing on the lower three-fourths of the panicle. 'Double Dwarf White Feterita' averaged 10% outcrossing on the upper one-fourth and 4% outcrossing on the lower three-fourths of the panicle. The authors concluded that "for increased purity (selfing), it would seem justifiable to discard the upper portion of open-pollinated panicles at the time of threshing." One might also conclude that percentage of natural outcrossing could be maximized by saving only the upper portion of the panicle.

Two separate experiments were conducted to investigate natural outcrossing in sorghum (grain-type) and sudangrass in the temperate climate of Nebraska. Objectives of the sorghum experiment were to estimate outcrossing in contemporary sorghum lines and to compare outcrossing percentages among individual sorghum B- and R- lines. The objective of the sudangrass experiment was to determine if anthesis date and location of florets on the panicle affect natural outcrossing.

MATERIALS AND METHODS

Both experiments were conducted at the University of Nebraska Agricultural Research and Development Center, Mead, NE. This facility is located at 41°10′ N, 96°28′ W, 334 m above sea level. Prevailing southerly winds average 4.3 m s^{-1} during the month of August when sorghum and sudangrass pollination occurs.

Sorghum Experiment

Four R-lines (RTx430, RTx2741, RTx2737, and RTx2783) and four B-lines (BTx399, BTx623, BTx3042, BKS57) were seeded in 7.6-m long rows spaced 76 cm apart in spatial isolation (minimum of 400 m) from other sorghum pollen sources in a randomized complete block design with four replications per isolation. Two separate isolations of R-lines and two separate isolations of B-lines were established in each of 2 yr. Rows were oriented perpendicular (east-west) to prevailing southerly winds. Planting dates were 26 May 1988, and 18 May (R-lines) and 31 May (B-lines) 1990. The isolations were fertilized with 11.2 kg ha^{-1} N prior to planting. Propachlor [2-chloro-*N*-(1-methylethyl)-*N*-phenylacetamide] and atrazine [6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5,-triazine-2,4-

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diamine] were applied at 3.36 and 1.12 kg ha^{-1}, respectively, immediately after planting for weed control. No supplemental irrigation was applied. Ten open-pollinated panicles were harvested from each row, and bulked by row.

Each bulked-row sample was planted in a single row of 90-m length on 27 May 1992 to determine frequency of outcrossing. Agronomic practices in 1992 were the same as those used to produce the seed in 1988 and 1990. Offtypes (outcrosses) were defined as plants that differed (e.g., height, panicle shape, seed color) from the pure line phenotype. Number of offtypes and number of total plants were recorded for each row, and percentage of outcrosses was calculated as (number offtypes/number total plants) × 100. Frequency of indigenous variation within the original pure lines was assumed to be zero for this experiment.

Data from each year were analyzed independently by the General Linear Model procedure of SAS (SAS Institute, 1990). Isolations and lines were considered random effects. Data pooled across isolations were analyzed with the SAS model, outcross = isolation replicate(isolation) line isolation × line. Data from individual isolations were analyzed using the SAS model, outcross = replicate line, and residual error was used to test significance of line effects.

Sudangrass Experiment

Two closely related sudangrass B-lines of similar height were used in this experiment. BN118 is homozygous for the dominant *D* allele producing the phenotype "white midrib". BN119 is homozygous for the recessive *d* allele producing the phenotype "green midrib" (Gorz et al., 1990). Individual plants of each genotype were transplanted into isolations on 31 May 1990 and 29 May 1991. Genotypes were arranged in a checkerboard pattern, alternating green and white midrib plants 76 cm apart in each direction. Isolations were square in shape (10 by 10 plants). Cultural treatment was as above for sorghum. At 9, 11, and 13 wk after transplanting, two panicles (at 50% anthesis) from each green plant were tagged to indicate approximate date of pollination.

Individual tagged panicles from 32 green midrib plants were harvested at maturity (plants from outside rows were excluded to eliminate border effects). Each panicle was then divided into three fractions: top one-third, middle one-third, and bottom one-third. For each green midrib plant, 18 seedlots were generated: two panicles from each of three pollination dates, with each panicle divided into three fractions.

Each seedlot was planted in a 7.6-m long row on 10 June 1993. White midrib plants and total plants were counted in each row. The following assumptions were made to determine outcrossing percent. (i) BN118 and BN119 produced equal quantities of pollen. (ii) Pollen from the two genotypes was randomly distributed within the isolations. (iii) Pollen from the two genotypes was equal in prepotency. Percent outcrosses (individual-plant basis) would then equal ([number white midrib plants × 2]/total number of plants) since one-half of actual outcrosses would be green midrib and not distinguishable from selfs. Frequency of spontaneous mutation from green to white midrib, or white to green midrib, was assumed be zero for this experiment.

Data were analyzed by the General Linear Model procedure of SAS (SAS Institute, 1990). Data from each year were analyzed independently, and data from individual green midrib plants were considered repeated measures. Pollination date and panicle section were considered to be fixed effects in the SAS model: outcross = date section(date), and residual error was used to test significance of date and panicle section effects.

Table 1. Effect of genotype on outcrossing percentage of four grain-type sorghum R-lines and four B-lines grown in separate isolations in Mean, NE, in 1988 and 1990.

	1988		1990	
Line	Isolation 1	Isolation 2	Isolation 3	Isolation 4
	% Outcrossing			
RTx2783	13.0	1.9	11.9	9.5
RTx2741	4.1	1.9	5.4	6.2
RTx430	0.2	1.8	0.1	0.2
RTx2737	0.1	1.9	0.4	0.1
Mean	4.4	1.9	4.5	4.0
LSD (P = 0.05)	3.6	2.2	4.1	4.6
	Isolation 5	Isolation 6	Isolation 7	Isolation 8
BTx399	9.0	3.4	2.0	1.9
BTx3042	4.1	1.9	2.4	0.5
BKS57	3.8	3.7	3.8	3.6
BTx623	3.5	1.3	0.7	0.4
Mean	5.1	2.6	2.2	1.6
LSD (P = 0.05)	10.9	2.2	2.2	1.8

RESULTS AND DISCUSSION

Sorghum Experiment

Isolation effects and their interactions were significant for R-lines in 1988. For consistency and ease of interpretation, all data are therefore presented for each isolation in each year (Table 1).

RTx430 and RTx2737 exhibited very low frequency of outcrossing in both years, with offtype percentages of <2% in all isolations (Table 1). RTx2783 exhibited high outcrossing in both years, with nearly 10% or higher offtypes in three of the four year × isolation combinations. Line effects on outcrossing among B-lines were significant for only one isolation in 1988 and both isolations in 1990 (Table 1). In all three instances, BKS57 exhibited slightly higher outcrossing than BTx623.

Based on this limited number of B- and R-lines, it can be concluded that differences in outcrossing exist among individual sorghum lines with outcrossing percentages ranging from nearly zero to over 10%. These values are in a similar range, or are only slightly higher than outcrossing frequencies reported much earlier for sorghum (Karper and Conner, 1919; Sieglinger, 1921) in lower latitudes in the USA. The significant isolation effect for R-lines in 1988 was caused by the unexplained relatively low frequency of outcrossing in RTx2783 in Isolation 2, and is an indication that at least for some lines, outcrossing frequency can be variable and unpredictable, even in similar environments.

Outcrossing frequencies for these experiments were based on bulked progeny of single rows of fertile inbred lines (as previously reported in the literature), not for outcrossing of individual plants. If the assumption is made that cross-pollination by plants within a pure-line row was at least equally probable as pollination by plants from neighboring but different pure-line rows, actual outcrossing of individual plants would be expected to be at least double the values reported (0–26%) because pollen from within the row would not produce offtype plants. Therefore, for sorghum breeding procedures requiring a high degree of self-pollination, selfing under pollination bags is recommended.

Table 2. Effect of pollination date and panicle section on outcrossing of sudangrass at Mean, Nebraska, in 1991 and 1992.

1991			1992		
Pollination date	Panicle section	Outcross (%)	Pollination date	Panicle section	Outcross (%)
01 Aug.	Bottom	34.9	28 July	Bottom	12.8
	Middle	34.6		Middle	19.7
	Top	38.2		Top	29.1
	LSD (P = 0.05)	15.3		LSD (P = 0.05)	12.4
Overall date mean		36.0	Overall date mean		20.6
15 Aug.	Bottom	49.2	15 Aug.	Bottom	30.3
	Middle	61.3		Middle	37.2
	Top	60.1		Top	48.7
	LSD (P = 0.05)	15.0		LSD (P = 0.05)	11.2
Overall date mean		57.1	Overall date mean		38.9
30 Aug.	Bottom	53.7	30 Aug.	Bottom	22.2
	Middle	68.3		Middle	18.4
	Top	60.6		Top	27.4
	LSD (P = 0.05)	15.4		LSD (P = 0.05)	11.3
Overall date mean		61.2	Overall date mean		22.6
Among dates	LSD (P = 0.05)	8.8	Among dates	LSD (P = 0.05)	6.8

Sudangrass Experiment

Pollination date affected outcrossing in both years (Table 2). High outcrossing occurred at the middle pollination date in both years, averaging 57% in 1991 and 39% in 1992. The middle pollination date coincides with the period in which most tillers were observed to be entering anthesis, and overall pollen density would have been expected to be highest. A low amount of outcrossing occurred at the earliest pollination date in both years. The number of tillers observed entering anthesis was lowest at that date. In 1991, the latest pollination date had relatively high outcrossing, but in 1992, the latest pollination date had relatively low outcrossing. Harvest of panicles pollinated during the middle of the pollination period should maximize outcrossing in sudangrass.

The panicle section from which seed was obtained did not affect outcrossing at any harvest date in 1991. Panicle section did affect outcrossing in 1992 at the early and middle harvest dates (Table 2). In these two instances, outcrossing was higher in the upper one-third of the panicle than in the lower one-third of the panicle. Although these sudangrass outcrossing values are considerably higher, this observation of differential outcrossing within the panicle agrees with the observations of Maunder and Sharp (1963) in grain-type sorghum.

Although this experiment demonstrates that outcrossing of sudangrass can be enhanced by harvesting seed from the top portion of panicles that enter anthesis during the middle of the reproductive period, this process falls short of ensuring adequate natural outcrossing to encourage application of recurrent selection to sudangrass without the use of nuclear male-sterility. Previous literature reports sudangrass outcrossing ranging from 7 (Hogg and Ahlgren, 1943) to 77% (Garber and Atwood, 1945), not accounting for the probability of pollination by other plants from the same line or variety. In this experiment, sudangrass outcrossing frequencies for individual plants (data not shown), regardless of treatment, were highly variable and ranged from 0 to 100%. Because of the highly variable amounts of natural outcrossing in sudangrass, the continued use of male-sterility genes to effectively recombine sudangrass populations is recommended.

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