HETEROFERTILIZATION IN GRAIN SORGHUM

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CHAPTER I

INTRODUCTION

Plant breeders have long made use of hybridization as a method of plant improvement, and with the application of the principles of genetics, the inheritance of certain characteristics of various crops has been established. Thus when a cross is made between two plants of the same species which have a known genetic "marker", a certain phenotypic expression of the trait is expected. Such genetic "markers" have been used to indicate that a successful cross has been obtained.

Grain sorghum is one of many plants in which double fertilization occurs resulting in the formation of an embryo and endosperm in the mature kernel. The normal expectation is that the embryo and endosperm will be fertilized by gametes of the same genetic constitution. Some observations of genetic markers for both the endosperm and seedlings indicated that this might not always be true. Such cases have been described as being the result of heterofertilization.

The purpose of this study was to investigate the occurrence of heterofertilization in certain lines of grain sorghum. Data were obtained on the occurrence of heterofertilization under open pollination conditions and from controlled crosses. Also, estimates of preferential fertilization or pollen vigor were made.

CHAPTER II

LITERATURE REVIEW

The term heterofertilization has been applied by Sprague (1932) to the process resulting in those exceptional cases in which the embryo and endosperm differ genetically. Sprague suggested that this could be due to the egg and polar nuclei differing genetically and fusing with identical sperms, or the egg and polar nuclei being the same genetically and fusing with sperms having different genotypes. Four ways in which heterofertilization might occur are (1) the persistence and functioning of more than one magaspore, (2) mutation, (3) nondisjunction of one or more chromosome pairs when the generative nucleus divides to form the sperm, and (4) the functioning of genetically unlike sperms from two pollen grains.

Sprague (1932) made use of an endosperm and seedling marker in his investigations on corn. Aleurone color (dominant to colorless) was the endosperm marker, and scutellum color (dominant to colorless) was the seedling marker. Female lines were used that were colorless for both markers. Pollen was made available from pollinators which were colored for both markers. Aleurone color, exhibiting xenia, showed expression on the female kernels when a cross had occurred. Likewise if no cross had occurred the aleurone was colorless and the seedling was expected to have a colorless scutellum. Instances were found where seeds with colorless aleurone produced seedlings with colored scutellum. Such

occurrences were reported to be the result of heterofertilization.

Sprague (1932) indicated that heterofertilization was due to the fusion of the egg and polar nuclei with sperms of unlike genotypes. His results from pollinations with mixed pollen indicated that the genotype differences were usually due to the participation in fertilization of sperms from more than one pollen grain. The other explanations of heterofertilization, nondisjunction and mutation, played only minor roles in the occurrence of heterofertilization.

Roman and Ullstrup (1951) attributed some results which they obtained in work with corn to heterofertilization. Other workers have obtained results which appeared very abnormal and could be due to heterofertilization, but they lacked conclusive evidence.

Rhoades (1936) confirmed Sprague's assumption that more than one pollen tube could penetrate the embryo sac. Cytological observations in corn showed 11.0% of the embryo sacs examined contained more than one pollen tube.

Observations similar to those of Rhoades were made by Artschwager and McGuire (1949) on sorghum. They noted that many pollen tubes grew down the stylar canal into the cavity of the ovary. In this process they noted that the pollen tube grew through the stigmatic papillae into a lateral branch of the stigma and continued downward into the conductive tissue of the style. The pollen tube grew between the integument and the ovary wall and entered the embryo sac by pressing apart the nucellus cells. It was at this point that functioning of additional pollen tubes was limited. Apparently only one or two entered the micropyle and discharged their sperms.

Most pollen tubes discharged two sperms one of which normally fused

with the egg nucleus to produce the embryo and the other sperm fused with the polar nuclei to produce the endosperm. Artschwager and McGuire (1949) frequently observed sperms in the vicinity of the egg and even inside the egg membrane, but no actual fusion between sperm and egg nuclei.

Pollen falling on a receptive stigma of sorghum apparently germinated immediately if conditions were favorable (Artschwager and McGuire, 1949). Stephens and Quinby (1934) reported fresh sorghum pollen germinated in 20 minutes to 1 hour. They also studied the time interval from pollination to fertilization on Spur Feterita flowers. No evidence of fertilization was found 6 hours after pollination, but 12 hours after pollination such evidence was noted. They concluded that fertilization in sorghum commonly took place within 8 to 10 hours after pollination. Later Artschwager and McGuire (1949) found this interval to be as short as 100 to 120 minutes for some sorghums. Stephens and Quinby (1934) also showed stigmas to be receptive from 48 hours before blooming to as much as 8 days after blooming (possibly 16 days in cooler weather).

Reed (1930) described the sorghum plant as having a panicle type of inflorescence with spikelets arranged in pairs along the rachis. The basal spikelet of each pair was perfect, composed of two outer glumes and a lemma and palea enclosing the ovary with the two branched stigmas and the three stamens. The upper spikelet was described as sterile, or sometimes staminate. Similar descriptions were noted by Cowgill (1926) and Artschwager and McGuire (1949).

Sorghum, unlike corn, is generally considered to be a self-pollinated crop. Reed (1930) described flowering in sorghum as starting at the apex and progressing down the head over a period of several days. He

noted that the stigmas of the flowers often matured and pushed out first, followed later by the anthers. He thus concluded that sorghum was highly self-pollinated by the flowers above on the same head. Such flowering seemed to offer ample opportunity for a substantial amount of outcrossing to occur. Sieglinger (1921, 1951) found outcrossing to occur as high as 29%, depending on the lines used and environmental conditions, with 5% or more as a reasonable expectation. Reed (1930) also noted that natural crossing depended on the variety, winds, and sometimes insects.

To conduct a study on heterofertilization in sorghum genetic markers were needed. Reed (1930) found red coleoptile color to be dominant to green in his studies with several varieties of sorghum. He noted that a 3:1 (red to green) segregation resulted in the F_2 generation. This indicated monogenic inheritance. Coleoptile color was thus comparable to scutellum color of corn.

Three mutant lines of sorghum with endosperm markers were identified. One of these lines was noted as a defective endosperm type and called "defective". This line was found in an F_2 field hybrid progeny of Durra C.I. No. 696 in 1945 by Sieglinger (1951). This character showed xenia in the F_0 generation and a simple recessive inheritance. The grain had a very collapsed or degenerate endosperm. This line also conveniently had green coleoptile color.

The second mutant line was noted as a shrunken or sugary endosperm type. Karper and Stephens (1936) found it to be similar to sugary in corn and to have a simple recessive (3:1) inheritance. Similar descriptions and inheritance of this character were also noted by Ayyangar (1936) who called it "dimpled". Sugary sorghum seeds wrinkled as they

matured and varieties with corneous seeds and juicy stems wrinkled the most. The seed was about one fourth smaller than normal seed and contained at least twice as much sugar (Karper and Stephens, 1936). Xenia occurs producing plump kernels when pollen from normal grain sorghum fertilizes the polar nuclei. Lines with shrunken endosperm and green coleoptile were located also.

The third mutant line used in this study was noted as "dent" or "hollow". This line also was from a Durra type sorghum from India. The grain was characteristically dented or dimpled on the end opposite the germ, or the kernel appeared plump but was hollow where the starch deposit in the endosperm would normally be. The inheritance of this endosperm character had not been determined but was believed to be recessive to the normal plump kernels. Lines of this endosperm type which had green coleoptile were located also.

CHAPTER III

MATERIALS AND METHODS

The initial selection of the material began in the fall of 1965 at the Agronomy Research Station, Perkins, Oklahoma. Self- and open-pollinated heads of the defective, shrunken, and dent mutant pure lines were selected and harvested by hand. Heads which were segregating for defective, shrunken, and dent endosperm were selected and harvested, also. These segregating heads were both self- and open-pollinated, and originated from controlled crosses or from field crosses.

Heads were threshed separately and the seeds from heads which were segregating for defective endosperm were separated into plump and defective types of kernels. The same procedure was followed with heads segregating for shrunken endosperm, but difficulty in classification of the seeds from heads of the dent line prevented their separation. The open heads of the homozygous defective and the homozygous shrunken endosperm lines were examined and the plump kernels were separated out.

Seed from segregating heads was sown on June 14, 1966 at the Agronomy Research Station, Perkins, Oklahoma, in 40-inch rows, 50-feet long. The seed was sown in paired rows with the plump seed planted in one row and the defective (or shrunken) seed planted in the adjoining row. The defective or shrunken seed from homozygous open-pollinated heads of these types was planted at this time, and the plump seed obtained from these heads was planted June 26, 1966 by hand in short rows since very

few seeds were available.

The resulting plants were classified for the mutant endosperm characters. Plump kernels were expected to produce plump or segregating progeny. Any homozygous mutant-type plants produced from plump kernels were considered to be the result of heterofertilization. Mutant type endosperm kernels were expected to produce mutant type progeny. Any segregating plants were to be considered the result of heterofertilization. An outline of the methods for this portion of the study is given in Appendix A.

Preliminary controlled crosses were made in the greenhouse during the winter of 1965-66. Homozygous defective, shrunken, and dent endosperm lines, each having green coleoptile color, were established to be used as female parents. At the same time plants of the following varieties having red coleoptile color were established to be used as male parents: Dex broomcorn, Lahoma sudangrass, Piper sudangrass, R OK Y10 (red seeded, yellow endosperm pollinator released by the Oklahoma Agricultural Experiment Station), Early Hegari, B Wheatland, B OK 8 (red seeded maintainer released by the Oklahoma Agricultural Experiment Station), Combine 7078, and Black Spanish broomcorn.

Both emasculated and nonemasculated crosses were made. In the case of the emasculated crosses individual florets of a branch of a head of one of the female lines (defective, shrunken, or dent) were emasculated by hand and a combination of pollen was applied several times. The combination of pollen was made up of approximately equal amounts of the female plants own pollen and pollen from one or two of the male parents (plump kernels). Nonemasculated crosses were attempted by applying pollen from a single male or from a combination of male parents to the

heads of the female lines (defectiven, shrunken, and dent) each day they were in bloom. Since the female lines own pollen was shed daily, florets could be self- or cross-pollinated. Seed from the emasculated crosses were planted, and plants classified for coleoptile color and endosperm type. Nonemasculated pollinations were threshed separately, and the seed was separated into plump and mutant type kernels. This material was sown at Perkins, July 13, 1966, and later classified according to plant characters and seed type.

Additional controlled crosses were made in the field in 1966. Defective, shrunken, and dent lines were used as female parents, and five varieties with red coleoptile and normal grain were used as male parents. They were Dex broomcorn, Early Hegari, Lahoma sudangrass, B Wheatland (2 sources) and B OK 8. These male and female parent lines were sown in 2-row, 50-foot-long plots in an arrangement that permitted two adjacent rows of the defective line to be windward of two adjacent rows of each male parent. Then the planting was duplicated. One block of the shrunken line and of the same pollen parents was sown in a similar manner. Also, one block of the dent (female) line was interplanted with single rows of the pollen parents. B Wheatland and Early Hegari, however, were sown in 2-row plots. The female lines were planted June 27, 1966, but several of the pollen parents were sown later in an attempt to match bloom dates.

Emasculated crosses and nonemasculated crosses among these rows were made as in the greenhouse, and the resulting seeds were threshed, classified, and sown in the greenhouse in flats for coleoptile color classification. Open-pollinated heads of the mutant lines also were selected at random among those blooming at the same time as the adjacent

pollen parents. These heads were bagged after anthesis for identification and protection. After harvest the heads were threshed individually and the seeds were treated as the controlled crosses. An outline of the procedure for this portion of the study is given in Appendix A.

All seedling tests were conducted in the greenhouse at the Agronomy Research Station, Stillwater, Oklahoma. Seeds were treated with captan, sown in sandy loam soil in flats, 22 x 15 x 3.75 inches, in 13 rows per flat. Vermiculite was used to cover the seeds to get maximum emergence. The material for the tests was divided so as to grow a portion of the different groups of seeds in each of six greenhouse runs, since not all of it could be grown at once. A completely random arrangement of the entries was used in the flats. The tests were sown May 13 and 31, June 14 and 28, and July 8 and 19, 1967. Coleoptile colors of the seedlings were classified on the fifth, sixth, and seventh day after planting for each run. To check the classification a sample of five seedings from each category of endosperm and coleoptile color was transplanted to the field at Perkins, Oklahoma.

A few plump kernels appeared on the bagged check heads of the homozygous mutant endosperm types in the 1966 field planting. These were sown for identification of the resulting plants in 1967.

The seeds from emasculated crosses made in the 1966 field planting were germinated in the germinator, transplanted to cans in the greenhouse and grown to maturity for endosperm classification.

CHAPTER IV

EXPERIMENTAL RESULTS

Field Study

The results of the 1966 field classification of endosperm types of plants are given in Tables I and II. Table I includes results from seed planted from the homozygous and segregating heads harvested in 1965. The categories of plants which should be the result of heterofertilization are marked. The percent heterofertilization was calculated for each category except two. In these two categories there were far more plants than expected in the heterofertilization classification. The categories with percent heterofertilization calculated gave a range of values from 0.0 to 13.2 which seemed reasonable estimates of heterofertilization by comparison with Sprague's (1932) results.

Table II gives the endosperm classification of the plants grown from the preliminary controlled crosses made in the 1965-66 greenhouse experiment. No heterofertilization plants were recorded from plump seeds from either mutant. The heterofertilization plants from shrunken seeds made up 2.2 percent of the resulting plants. The results of defective seeds are again contrary to expected with far too many individuals in the heterofertilization group. This and the other field results indicated that the classification of the defective endosperm marker should be considered more carefully.

TABLE I

HETEROFERTILIZATION BASED ON THE SUMMARY OF FIELD CLASSIFICATION OF ENDOSPERM TYPES ON PLANTS GROWN FROM SEED FROM HOMOZYGOUS DEFECTIVE AND SHRUNKEN LINES AND FROM SEGREGATING HEADS, PERKINS, 1966

Endosperm type of Source plant	Seed planted	No. of plants in endosperm classes[a]			Hetero-fertilization[b] (%)
Defective:		Def.	Seg.	Plp.	
Homo.[c] (open)	Def.	510	26[d]	0	4.9
	Plp.	2[d]	33	1	5.7
Seg. (bagged)	Def.	633	1087[e]	7	-
	Plp.	25[d]	2427	1840	0.6
	Totals	688	3514	1847	
Seg. (open)	Def.	134	215[e]	0	-
	Plp.	1[d]	324	289	0.2
	Totals	135	539	289	
Shrunken:		Shr.	Seg.	Plp.	
Homo. (open)	Shr.	276	4[d]	0	1.4
	Plp.	0	0	0	-
Seg. (open)	Shr.	46	7[d]	0	13.2
	Plp.	0[d]	34	29	-
	Totals	46	41	29	

[a]Def. denotes defective, Seg. denotes segregating, Plp. denotes plump, Shr. denotes shrunken.

[b]Percent heterofertilization figured on number of heterofertilization individuals from seed type divided by total number of plants from seed type planted.

[c]Homo. denotes homozygous.

[d]Plants resulting from heterofertilization.

[e]Plants expected to be the result of heterofertilization, but too many to be realistic.

TABLE II

HETEROFERTILIZATION BASED ON THE SUMMARY OF 1966 FIELD CLASSIFICATION OF ENDOSPERM TYPES ON PLANTS GROWN FROM SEED FROM POLLINATIONS MADE IN 1965-66 GREENHOUSE

Endosperm type of Female plant	Seed planted	No. of plants in endosperm classes[a]			Hetero-fertilization[b] (%)
		Def.	Seg.	Plp.	
Defective	Def.	16	106[c]	0	-
	Plp.	0[d]	23	5	-
		Shr.	Seg.	Plp.	
Shrunken	Shr.	396	9[d]	0	2.2
	Plp.	0[d]	44	0	-

[a]See footnote a, Table I.
[b]See footnote b, Table I.
[c]See footnote e, Table I.
[d]See footnote d, Table I.

Seeding Tests

The results of the seedling tests are given in Tables III through VI. Coleoptile color was used as a marker gene in the seedling tests to detect heterofertilization. Table III contains the overall results on heterofertilization and germination for all endosperm types of seed planted and the averages for the defective and shrunken types. The seedlings resulting from heterofertilization are marked in the table. It should be noted that in these results defective and shrunken seed normally give rise to seedlings with green coleoptile color while plump seed from either type give rise to seedlings with red coleoptile. When these patterns of markers are reversed the seedlings are believed to be the result of heterofertilization. The 1966 field results indicated a

TABLE III

HETEROFERTILIZATION AND GERMINATION VALUES FOR ALL POLLINATIONS MADE IN 1966 FIELD ON DEFECTIVE AND SHRUNKEN ENDOSPERM TYPES AS DETERMINED FROM COLEOPTILE CLASSIFICATION IN SEEDLING TESTS

Endosperm type	Number seed tested	Number of seedlings with coleoptile color indicated			Germination[a] (%)	Heterofertilization[b] (%)
		Red	Green	Unclassified		
From pollination on defective endosperm type:						
Defective	18900	101[c]	2699	155	15.6	3.6
Intermediate	8683	1211	858	343	27.8	-
Plump	5581	1725	613[c]	416	49.3	26.2
			Weighted average		24.5	13.9
From pollinations on shrunken endosperm type:						
Shrunken	11800	25[c]	6220	285	55.3	0.4
Intermediate	1532	22	581	62	43.4	-
Plump	1314	770	170[c]	131	81.5	18.1
			Weighted average		56.4	2.7
			Overall arithmetic average		40.5	8.3

[a]Calculated on basis of total seedlings emerging divided by total seeds planted (calculated in same manner for all tables).

[b]Calculated on basis of number of heterofertilization seedlings divided by the total seedlings, omitting unclassified category.

[c]Seedlings resulting from heterofertilization.

need for a more careful classification of endosperm type, so a category of "intermediate" was used to include seed for which difficulty was experienced in the endosperm classification. A similar category was found to be necessary for coleoptile color. Heterofertilization was not calculated on the data from the intermediate classification of endosperm.

Germination percentages were very low in the seedling tests for some categories, ranging from 15.6 for defective to 81.5 for plump seed from shrunken female heads. Estimates of heterofertilization ranged from 0.4 to 26.2 percent with the values obtained from the plump categories being considerably higher than those from the defective or shrunken groups.

A suitable statistical analysis could not be devised for these data. Therefore, it was decided to calculate percent germination and heterofertilization in as many different categories and classifications as possible.

Table IV gives a subdivision of the various endosperm types into controlled and open type crosses. The categories and classifications for heterofertilization are the same. The calculations were made by the same methods as in Table III. It was evident in this table, as in Table III, that plump seed from either line produced the greater amount of heterofertilization. There was a difference in the amount of heterofertilization between the open (30.7%) and controlled crosses (15.7%) of the plump from defective category, but less difference in the defective groups and probably no difference in either category from shrunken types. It is evident from the average germination percentages that the seed from open pollinations (33.8%) of the defective mutant

TABLE IV

HETEROFERTILIZATION AND GERMINATION VALUES FOR CONTROLLED VS. OPEN POLLINATIONS AS DETERMINED FROM COLEOPTILE CLASSIFICATION IN SEEDLING TESTS

Endosperm type	Type cross	Number seed tested	Number of seedlings with coleoptile color indicated			Germination[a] (%)	Heterofertilization[b] (%)
			Red	Green	Unclassified		
From pollinations on defective endosperm type:							
Defective	Control	10500	57[c]	876	101	9.9	6.1
	Open	8400	44[c]	1823	49	22.8	2.4
Intermediate	Control	3933	318	208	108	16.0	-
	Open	4750	893	650	240	37.5	-
Plump	Control	2132	585	109[c]	149	39.5	15.7
	Open	3449	1140	504[c]	267	55.4	30.7
			Weighted average controlled			15.2	10.2
			Weighted average open			33.8	15.6
From pollinations on shrunken endosperm type:							
Shrunken	Control	6900	11[c]	4127	79	61.1	0.3
	Open	4900	14[c]	2093	206	47.2	0.7
Intermediate	Control	971	21	462	28	52.6	-
	Open	561	1	119	34	27.5	-
Plump	Control	1059	608	138[c]	110	80.8	18.5
	Open	255	162	32[c]	21	84.3	16.5
			Weighted average controlled			62.5	3.1
			Weighted average open			46.9	2.0

[a]See footnote a, Table III.
[b]See footnote b, Table III.
[c]See footnote c, Table III.

germinated better than those of the controlled crosses (15.2%), but this pattern was reversed in the shrunken mutant (46.9% for open to 62.5% for controlled crosses).

Heterofertilization percentages for pollinators are given in Table V. Results of the controlled pollinations using only one pollinator are given in the first column. The second column gives the results of the controlled crosses using two pollinators in combination. Some combinations of pollinators were not used. Those used represented contrasting types. Combine 7078 gave the greatest amount of heterofertilization (12.4%) with B Wheatland (9.8%) and Dex-Lahoma (9.4%) next in rank.

TABLE V

HETEROFERTILIZATION VALUES FOR POLLINATORS FROM CONTROLLED CROSSES AS DETERMINED FROM COLEOPTILE CLASSIFICATION IN SEEDLING TESTS

One pollinator	Heterofertilization (%)	Two pollinators[a]	Heterofertilization (%)
Dex	2.1	Dex-Lahoma	9.4
Lahoma	3.7	Dex-B Wheatland	2.1
B Wheatland	9.8	Dex-B OK 8	4.5
B OK 8	1.7	Dex-Early Hegari	2.4
Early Hegari	4.5	Lahoma-B Wheatland	1.6
Combine 7078	12.4	Lahoma-B OK 8	2.8
B Wheatland ASA	6.5	Lahoma-Early Hegari	4.7
Average	5.8		3.9

[a]Pollen from two pollinators mixed in approximately equal amounts.

Table VI gives the heterofertilization and germination percentages for different pedigrees within the defective and shrunken endosperm types. The heterofertilization values ranged from 2.0 to 71.4 percent for the defective types. The number of seeds planted from the various pedigrees varied greatly. The high figure of heterofertilization for the Defective x Ryer line is based only on a few seeds coupled with poor germination. Heterofertilization percentages for the totals were 10.4, 15.8, and 21.2 for Ryer x Defective, Defective, and Defective x Ryer, respectively. Excluding the estimate for Defective x Ryer, which was based on a limited number of observations, the values did not seem to indicate a difference in frequency of heterofertilization due to the pedigree source of the defective mutant line.

For the shrunken types heterofertilization estimates ranged from 0.1 to 22.5%. The average heterofertilization percentages were 1.9, 7.2, and 0.6 for Kashakasha x 10-2, White shrunken, and Forage shrunken, respectively, indicating a possible difference in the rate due to pedigree source.

Emasculated Crosses

Heterofertilization and germination results of the emasculated crosses are given in Table VII. These emasculations and pollinations were made in the field in 1966 for both the defective and shrunken types. All pollinators were not used and poor seed set was obtained for those made. The result was that few seeds were available for testing, and low germination was obtained, especially for the seed from the shrunken line.

Two plants out of 16 were identified as being the result of heterofertilization among the crosses on the defective mutant, and none from

TABLE VI

HETEROFERTILIZATION AND GERMINATION VALUES FOR DIFFERENT PEDIGREES WITHIN ENDOSPERM TYPES OVER ALL TYPE POLLINATIONS AS DETERMINED BY COLEOPTILE CLASSIFICATION IN SEEDLING TESTS

Endosperm type	Female pedigree	Number seed tested	Number of seedlings with coleoptile color indicated			Germination[a] (%)	Heterofertilization[b] (%)
			Red	Green	Unclassified		
		From pollinations on defective endosperm type					
Defective	Def. x Ryer	300	5[c]	2	1	2.7	71.4
	Defective	12700	36[c]	1762	32	14.4	2.0
	Ryer x Def.	5900	60[c]	935	122	18.9	6.0
Intermediate	Def. x Ryer	204	49	17	2	33.3	-
	Defective	6508	784	678	254	26.4	-
	Ryer x Def.	1971	378	163	87	31.9	-
Plump	Def. x Ryer	52	24	2[c]	1	51.9	7.7
	Defective	4072	981	478[c]	310	43.4	32.8
	Ryer x Def.	1457	720	133[c]	105	65.8	15.6
						Averages	
Totals	Def. x Ryer	556	78	21	4	18.5	21.2
	Defective	23280	1801	2918	596	22.8	15.8
	Ryer x Def.	9328	1158	1231	314	28.9	10.4

TABLE VI (Continued)

Endosperm type	Female pedigree	Number seed tested	Number of seedlings with coleoptile color indicated			Germination[a] (%)	Heterofertilization[b] (%)
			Red	Green	Unclassified		
	From pollinations on shrunken endosperm type:						
Shrunken	Kash. x 10-2	5500	17[c]	1443	269	31.4	1.2
	White shrunken	1900	1[c]	1368	6	72.4	0.1
	Forage shrunken	4400	7[c]	3409	10	77.9	0.2
Intermediate	Kash. x 10-2	894	7	84	58	16.7	-
	White shrunken	610	12	483	4	81.8	-
	Forage shrunken	28	3	14	0	60.7	-
Plump	Kash.x 10-2	140	77	13[c]	8	70.0	14.4
	White shrunken	908	493	143[c]	111	82.3	22.5
	Forage shrunken	266	200	14[c]	12	85.0	6.5
						Averages	
Totals	Kash.x 10-2	6534	101	1540	335	30.2	1.9
	White shrunken	3418	506	1994	121	76.7	7.2
	Forage shrunken	4694	210	3437	22	78.2	0.6

[a]See footnote a, Table III.
[b]See footnote b, Table III.
[c]Seedlings resulting from heterofertilization.

TABLE VII

HETEROFERTILIZATION AS DETERMINED FROM ENDOSPERM CLASSIFICATION OF F_1 PLANTS FROM EMASCULATED CROSSES MADE IN 1966 FIELD ON DEFECTIVE AND SHRUNKEN ENDOSPERM TYPES

Pollen parents	Resulting seed	No. seed planted	Classification of endosperm of seed on F_1 plant	
Emasculated defective:			Defective	Segregating
B OK 8 + Defective	Defective	5	4	1[a]
	Plump	1	0	1
Dex + Defective	Defective	5	no plants	
	Intermediate	1	1	0
	Plump	1	0	1
Early Hegari + Defective	Defective	4	0	1[a]
	Plump	1	0	1
B Wheatland + Defective	Defective	3	3	0
	Intermediate	1	no plants	
	Plump	1	no plants	
Average germination= 56.5%				
Average heterofertilization=16.5%				
Emasculated shrunken:			Shrunken	Segregating
Dex + Shrunken	Shrunken	7	2	0
Early Hegari + Shrunken	Shrunken	5	2	0
B Wheatland + Shrunken	Shrunken	2	no plants	
Average germination= 28.6%				
Average heterofertilization= 0.0%				

[a]Seedlings resulting from heterofertilization

the shrunken mutant. Intermediate types were not included in the calculations.

Pollen Vigor

The information in Tables VIII and IX is related to the pollen vigor study. The data are based on the results of the controlled pollinations only.

Table VIII gives the number of plump endosperm seed resulting when the indicated pollinator or pollen combination was applied to the mutant female, defective or shrunken endosperm. This information should indicate the relative vigor or competitive ability of the pollen applied with the mutant pollen. Pollinators produced more plump seed per head on the defective endosperm type than on the shrunken type in nine of fourteen comparisons. However, the highest three plump seed frequencies were on the shrunken endosperm type.

The pollen combination of Lahoma-Early Hegari produced the greatest number of plump seed per head on the defective mutant (48.2). Lahoma (41.3) and Early Hegari (44.0) singly gave the next highest plump seed per head on the defective mutant. Dex (62.2), B Wheatland (59.2), and Combine 7078 (57.8), singly gave the greatest number of plump seed per head on the shrunken female. B Wheatland and Combine 7078 also gave the greatest percent heterofertilization. The combination of pollinators giving the most plump seed on shrunken was Dex-B Wheatland. Early Hegari gave the most plump seed per head (29.7) when averaged over both females. It was also observed that the open pollinations produced more plump seed per head on the defective type and on the average than any of the controlled pollinations.

TABLE VIII

NUMBER OF PLUMP SEED OBTAINED WHEN DIFFERENT POLLINATORS WERE USED ON THE DEFECTIVE AND SHRUNKEN ENDOSPERM TYPES AS AN INDICATION OF POLLEN VIGOR

Pollinator or pollen combination	Defective female			Shrunken female			Average plump seed/ head
	No. of heads	Total plump seed	Plump seed/ head	No. of heads	Total plump seed	Plump seed/ head	
Dex	10	54	5.4	5	311	62.2	24.3
Dex-Lahoma	5	149	29.8	5	5	1.0	15.4
Dex-Wheatland	5	99	19.8	5	144	28.8	24.3
Dex-B OK 8	5	46	9.2	5	8	1.6	5.4
Dex-Early Hegari	5	168	33.6	5	13	2.6	18.1
Lahoma	10	413	41.3	5	10	2.0	28.2
Lahoma-B Wheatland	5	100	20.0	5	7	1.4	10.7
Lahoma-B OK 8	5	36	7.2	5	65	13.0	10.1
Lahoma-Early Hegari	5	241	48.2	5	32	6.4	27.3
B Wheatland	10	110	11.0	5	296	59.2	27.1
B OK 8	10	295	29.5	5	2	0.4	19.8
Early Hegari	10	440	44.0	5	5	1.0	29.7
Combine 7078	10	100	10.0	5	289	57.8	25.9
B Wheatland ASA	10	74	7.4	5	0	0	4.9
Open	84	4240	55.0	49	255	5.2	33.8

A sample of seedlings with red coleoptile color was transplanted from the greenhouse tests to 1967 field and the adult plant type was classified to obtain the data in Table IX. These seedlings were from seed where the indicated pollen combination was used on the defective and shrunken endosperm types. The biggest difference was noted in the Dex-B Wheatland combination, where Dex produced 35 hybrid to 9 for B Wheatland. Dex and Lahoma appeared to have some pollen vigor advantage over Early Hegari where in combination with it. More data are needed for several or all combinations, especially on the shrunken line.

Seedling Check

Table X gives the results of a check on the classification of the seedling test. Adult plant endosperm classifications were taken in 1967 field on a sample of the seedlings from all categories and all runs of the seedling tests. This gave a further check on the effectiveness of the markers used and the ability to classify them correctly. It might be noted that all parental combinations of the markers (defective-green, plump-red, and shrunken-green) proved to be essentially correct. Also, all of the seedlings from the defective line that had red coleoptile proved to be crosses (except one). Although this heterofertilization category of defective-red proved to be essentially correct, the other three heterofertilization categories (plump-green, shrunken-red, and plump-green) were not nearly as correct as they should have been. Furthermore, the majority of the unclassified seedlings from defective and shrunken categories should have been classified as green. Most of the seedlings from defective intermediate unclassified should have been red and from shrunken intermediate unclassified green, but the

TABLE IX

RELATIVE POLLEN VIGOR OF INDICATED POLLINATOR IN COMBINATION AS DETERMINED BY SEEDLING AND ADULT PLANT CLASSIFICATION FROM CONTROLLED CROSSES

Pollen combination	No. of hybrids resembling pollinator used on:		
	Defective	Shrunken	Totals
Dex	7	8	15
Early Hegari	7	2	9
Dex	11	0	11
Lahoma	11	1	12
Dex	5	4	9
B OK 8	7	1	8
Dex	30	5	35
B Wheatland	9	0	9
Lahoma	16	0	16
B Wheatland	6	1	7
Lahoma	1	0	1
B OK 8	2	0	2
Lahoma	11	11	22
Early Hegari	8	1	9

TABLE X

ADULT PLANT ENDOSPERM CLASSIFICATION OF SEEDLING TRANSPLANTS IN 1967 FIELD AS A CHECK ON HETEROFERTILIZATION AND COLOEPTILE CLASSIFICATION OF SEEDLINGS

Endosperm type of seed planted	Coleoptile color of resulting seedling	No. of adult plants with indicated endosperm classification		
		Defective	Segregating	Shrunken
Defective	Green	22	0	
	Unclassified	14	9	
	Red[a]	1	29	
Intermediate	Green	20	8	
	Unclassified	2	19	
	Red	0	39	
Plump	Green[a]	13	15	
	Unclassified	3	25	
	Red	0	88	
Shrunken	Green		1	30
	Unclassified		2	23
	Red[a]		9	12
Intermediate	Green		0	24
	Unclassified		2	16
	Red		14	5
Plump	Green[a]		10	15
	Unclassified		16	4
	Red		48	0

[a]Heterofertilization categories.

majority of the plants in both plump unclassified categories should have been red.

The bagged checks of the mutants produced some intermediate to plump seeds which were planted in the 1967 field and classified for plant and endosperm type. A mix-up occurred in the identification and handling of the heads and grain from the defective mutant. The plump seed from the bagged defective heads produced hybrid types from all the pollinators available. This information was thus of no use as a check for mutation or any other cause of plumpness of seed for the defective mutant. But the checks on shrunken mutant produced no definite plump kernels and all plants resulting from these intermediate endosperm seed were homozygous shrunken types.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Grain sorghum is one of many plants which has double fertilization resulting in the formation of the embryo and endosperm in the mature kernel. Indications are that gametes from two different pollen grains can be involved in fertilization, one for embryo formation and another for endosperm formation. These can be of different genetic constitution as a result of heterofertilization.

Field Study

In line one of Table I the homozygous defective endosperm source plants were not bagged and therefore were subject to foreign pollen. The defective type seed sown from these plants gave 510 homozygous defective type plants and 26 plants which were segregating for endosperm type. Since all the seeds planted were defective, this indicated that no cross had occurred, at least not for the endosperm. However, the 26 segregating plants indicated that a cross had occurred to form the embryo, thus heterofertilization must have been responsible for this group. The two homozygous defective endosperm plants in line two of Table I which developed from plump seed, appeared to be the result of a cross on the endosperm and not the embryo. The percentages of heterofertilization for these two groups (4.9 and 5.7) were reasonable when compared with Sprague's (1932) results in corn.

The data in lines three and six of Table I from the segregating defective endosperm plants (bagged and open) were not expected. The endosperm classification of plants from defective endosperm seed was expected to give results similar to those obtained for the defective seed from the homozygous defective types. Instead, in both the bagged and open heads there were more segregating than defective endosperm types, and far more than could normally be attributed to heterofertilization.

Several factors may have contributed to these excessive numbers of plants in the segregating class. One, homozygous defective endosperm seeds germinated very poorly in most of the rows, and it could have been that the defective seeds with crossed embryos (heterofertilization) germinated much better, relatively, thus resulting in more segregating plants than expected. Two, pollen grains on a segregating head carrying the gene for plump (normal) endosperm could have a selective advantage over pollen grains carrying the gene for defective endosperm. Three, misclassification could have resulted in some crossed seeds being classified as defective rather than plump. Four, misclassification could have resulted in potentially plump seeds being classified as defective due to poor seed development for any one of several reasons such as disease, insects, cultural deficiencies or other environmental factors. And five, misclassification could have resulted from a lack of complete expression of xenia in the endosperm having the genotype, Aaa, which was expected to be plump. This last explanation may appear more valid when the numbers of segregating and plump plants from plump seed are considered. A ratio of two segregating to one plump was expected but the observations show too few segregating. The totals for the segregating defective (bagged and open) sources show that, allowing for poor

germination in the defective class, the ratio of defective to segregating to plump approaches a 1:2:1 ratio of a simply inherited character in F_2. But the large number of segregating endosperm plants obtained from the defective endosperm seed casts considerable doubt on this. It appeared that the most reliable information on heterofertilization utilizing the defective endosperm character was obtained from the homozygous defective type exposed to foreign pollen (line 1 and 2, Table I) and from the plump endosperm types from segregating heads (lines 4 and 7, Table I).

Data on heterofertilization from shrunken endosperm types in Table I were based on smaller numbers of observations than for the defective. No obvious bias in the data was apparent, however.

In Table II defective seed planted from the defective female plants gave 16 defective and 106 segregating plants. The same possible explanations for the large number of segregating plants that were proposed for the same situation in Table I apply. The majority of these plants probably resulted from normal fertilization, but the plump factor failed to express complete dominance, or the environment retared its expression. Many of the mutant plants used as females in the greenhouse in this portion of the study were unthrifty. This condition plus greenhouse environment may have hindered endosperm development of the crossed seed.

Seedling Tests

There was some difficulty in classifying the coleoptile color, and a questionable or unclassified category was created.

The germination percentages were rather low, especially for the defective endosperm type. The defective endosperm seed had 3.6%

heterofertilization which was similar to the 4.9% obtained in the 1966 field study. The unclassified coleoptile category was not used in figuring percent heterofertilization.

It may be noted that the intermediate category of seeds produced many red seedlings indicating that they were crosses in the ebmryo. On the other hand two-thirds as many green seedlings indicated no cross and together with the unclassified seedlings equalled the number of red seedlings. This indicated that the intermediate defective seeds consisted of half potentially plump seeds, and it also excludes the possibility of including the intermediate defective with either the defective or the plump group.

The plump endosperm seed would normally be expected to produce all red coleoptile seedlings. The heterofertilization seedlings, in this case, had green coleoptile color and represented 26.2% of the seedlings classified. This figure was considerably higher than any obtained previously. A possible explanation of this was that the endosperm of the seeds was plump which indicated that plump pollen was involved in the fertilization. This made the occurrence of heterofertilization more likely, especially since these plants produced their own defective pollen, some of which could land on the stigmas. In contrast the seeds with defective endosperm might not have had plump pollen on their stigmas at all. The average heterofertilization for the defective line was raised considerably by the heterofertilization percentage of the plump seed. The 13.0% average heterofertilization for the defective type is somewhat larger than expected but comparable to some of Sprague's (1932) results.

The seed from the shrunken females gave better germination but

lower heterofertilization values. Again the intermediate category was not used for heterofertilization values, but the seed of this type did produce substantially more green coleoptile seedlings than red indicating that the intermediates might be combined with the shrunken. The average heterofertilization was more than 10% lower than that of the defective type.

The overall average was an arithmetic mean of the weighted averages of the two endosperm types. This would mean that the frequency of heterofertilization for the populations studied was around 8.3%, and was within the range of heterofertilization given by Sprague (1932) for his studies with corn.

Table IV presents the same data as Table III except that the information is subdivided into the type of cross made. Germination of the seed from controlled crosses on the defective line was lower in all cases, probably due to more fungal growth. The reverse was true for the shrunken line, however.

There was no consistent pattern for the percent heterofertilization values. From the defective endosperm type the defective seed from controlled crosses gave the greatest frequency of heterofertilization, but the opposite was true for the plump endosperm seed. The division of the intermediate categories for both defective and shrunken endosperm types into controlled and open types of crosses resulted in ratios of red, green, and unclassified seedlings very similar to those from the combined data in Table III. Thus no additional insite was gained on the genotypes of this intermediate class. On the average the open pollinations on defective gave the greatest percent heterofertilization. In contrast seed from the shrunken endosperm type showed similar

heterofertilization values for controlled and open types of crosses.

Before the study was started a greater amount of heterofertilization was expected from the controlled crosses, since this type of pollination insured the presence of plump type pollen on the mutant flowers (defective or shrunken). Stephens and Quinby (1934), however, found maximum blooming of some sorghums at 1-2 a.m. This being the case, the open pollinations would have had the best opportunity for crossing since the hand pollinations for the controlled crosses were done at 8:30 a.m. to 12:00 noon. Probably a high percentage of the florets were already pollinated and fertilized by this time, depending on the weather conditions. The difference in percentage of heterofertilization for the plump from defective endosperm type could be explained on this basis.

Table V presents percentages of heterofertilization attributed to or associated with the various pollinators or pollen combinations used. Dex broomcorn and Lahoma sudangrass which ordinarily produce an abundance of outcrosses when grown near grain sorghums failed to produce an increase in frequency of heterofertilization. When one compares percent heterofertilization for the individual pollinators with the average of combinations in which they were involved, the individual pollinators Dex, Lahoma, and B OK 8 produced a lower percentage of heterofertilization than did the combinations. However, B Wheatland pollinator produced several times the percentage of the average of its combinations. It would appear that the combinations of pollen produced higher percentages of heterofertilization in the majority of the comparisons. This could be merely a mathematical advantage where more kinds of pollen have a better chance of producing heterofertilization.

Table VI gives germination and heterofertilization percentages for

the various pedigrees of the respective endosperm types used as females. The three lines differed from one another in some phenotypic traits but not in the endosperm marker. Further explanations and descriptions of these lines may be found in Appendix B. Also, pictoral description of the endosperm classification of seeds planted for the seedling tests are given in Figure 1 of Appendix C.

A fair comparison among the lines with defective endosperm can not be made since the sampling was unequal. On the average the Defective x Ryer line gave the lowest percent germination and the highest percent heterofertilization, but these figures were based on the smallest sample taken. The regular defective endosperm line was intermediate for both germination and heterofertilization percentages.

The average values for heterofertilization were smaller for all three of the lines with shrunken endosperm as compared to the defective lines. The White shrunken line had a substantially higher percent heterofertilization than the other two shrunken lines. The Kashakasha x 10-2 line was considerably lower in germination. No definite reason for the differences in the relative amount of heterofertilization for these lines can be offered. The earliest blooming line of the defective type was the Ryer x Defective line, and it gave the lowest percent heterofertilization of the three defective lines. The opposite was true for the shrunken lines. The White shrunken line was the earliest shrunken endosperm line to bloom, and it gave the greatest amount of heterofertilization. This shrunken line bloomed two days before the regular defective line did.

The White shrunken line could be the highest of the shrunken types because all of the pollinations with B Wheatland and Combine 7078, which

were the highest heterofertilization pollinators, were made on it. But all of the B Wheatland and Combine 7078 pollinations on the defective type were made on the Ryer x Defective line, which gave the lowest percent heterofertilization of the defective type. All pollinations with B Wheatland and Combine 7078 were done at about the same time or within a week of each other.

Further reference to the seedling test is given in Table XI of Appendix C where heterofertilization and germination values obtained for each endosperm type in each greenhouse test is given.

Emasculated Crosses

From data in Table VII from emasculated crosses it is evident that the mutant pollen was most frequently involved in the fertilization. Although few seed were obtained in this portion of the study the results were similar to that of the seedling tests. The average amount of heterofertilization for the seed from the defective endosperm type was 16.5%. This compares favorably to the 13.9% obtained in the seedling test. No heterofertilization was recorded for the shrunken endosperm seed, but only four plants were obtained.

Pollen Vigor

The results in Table VIII on pollen vigor indicated that there might be an interaction between pollinators and the mutants used as females. Pollinators producing many plump seeds on defective, failed to do so on the shrunken.

Environmental factors could have had a profound effect on these data also. The defective and shrunken lines bloomed during slightly

different periods and the temperature, moisture, humidity, and light could have been different. Also, pollinations for one set of data could have been made early in the day when the pollen was more viable compared to another set of pollinations. Plant height differences could have had an influence, also.

Data in Table IX could give better estimates of relative pollen vigor than data in Table VIII. The data in Table IX was based on adult plant classifications of F_1 plants from the indicated pollen combinations used. It appeared that pollen from Dex and Lahoma were more competitive than pollen from the grain type pollinators, in most cases. They also appeared to be equally competitive with each other in combination.

The studies conducted do not represent all combinations of pollinators used but only combinations of contrasting phenotypes. The information given is only a sample and not sufficient, especially on the shrunken type, to draw definite conclusions.

Seedling Check

In Table X it is difficult to explain why so many of the plants whose seedlings were classified as green (and expected to be selfs) could produce adult plants that segregated for endosperm indicating that the embryo was a hybrid (lines 4, 7, and 16). Similarly it is difficult to explain why plants whose seedlings were classified as red (and expected to be hybrids) could produce adult plants homozygous mutant, indicating that the embryo was a self (lines 12 and 15).

The intermediate endosperm classification of seed did not produce the same proportion of F_1 plants in the defective and shrunken mutants.

The intermediate class from the defective mutant gave 22 selfs or non-crosses and 66 crosses or F_1 plants. This would indicate that most of this seed resulted from crossing or hybridization, irrespective of coleoptile color. The intermediate category from the shrunken mutant produced 16 F_1 plants and 45 selfs. Thus the intermediate group from the shrunken source was mainly composed of mutant types. There was not enough consistency to allow combining the intermediate group with either mutant or plump categories for study.

On the basis of these results the values of heterofertilization are probably overestimated. It would be very difficult to locate the main source of error, if there was one. Many factors could be involved in the expression of these markers. Study of the results failed to associate a large portion of the error with any particular mutant line or pollinator. One line of the shrunken types, Kashakasha x 10-2, had more seedling color than desired which interfered with the coloeptile color classification of its seedlings. The Lahoma pollinator did not have a good red coleoptile color and was a source of some of the error. It would not be fair to attribute a large amount of the error to these two lines though, since errors in classification were found for nearly all combinations.

Coleoptile color expression is subject to environmental influence and a controlled environment should be used. Coleoptile color was classified for three consecutive days on the seedlings, and the original classification on the first day was sometimes changed by the third day. Some seedlings classified as red tended to loose color by the third day, and some classified as green gained color by the third day. Classifications were changed occasionally by comparison to other seedlings in the

row and seedlings of the parental type.

Several other sources of error were possible. One was the original endosperm classification of the seed planted. The intermediate classification and the 1966 field results indicated this. It was also possible for a plump endosperm seed to arise from fertilization by a green coleoptile pollinator which was not in the immediate area. This could occur on the open pollinated heads.

An intensive study should be given the markers and their expression before undertaking future studies of this nature. The inheritance and environmental influence should be checked, especially for the defective endosperm type. A chi-square for one gene and lack of dominance on the 1966 field data for the defective type, even after germination adjustments, failed to give a good fit. The method of classification limited checking any multiple gene hypothesis. The seedling marker introduced an additional source of error. Further use of this marker should only follow consideration of environmental influences on its expression. Better control of environmental factors should be given in such a study.

Even with consideration for various points of error in this study the data indicate that heterofertilization does occur in sorghum. The indicated error casts doubt on the estimates of the frequency of heterofertilization. Also, no consideration is given the case when two pollen grains of the same genetic constitution are involved in fertilization. In the strict sense of the definition this is not heterofertilization, but it is not normal fertilization either. There is no genetic test for this situation.

CHAPTER VI

SUMMARY

A study was conducted to determine the occurrence of heterofertilization in sorghum. Two mutant endosperm types with a recessive seedling marker were selected to use in this study. Seven pollinators with dominant plump endosperm and seedling markers were also used.

The study was divided into a field study and a seedling study which was conducted in the greenhouse. The field study was concerned with classification of endosperm of adult plants in the 1966 field planting at the Agronomy Research Station, Perkins, Oklahoma. The seedling study was done with seed obtained from the two mutant endosperm types which were subjected to pollen from the various pollinators used in the 1966 field planting at Perkins. The seedling marker, coleoptile color, was used in classification of these seedlings.

Estimates were made on the frequency of heterofertilization associated with each mutant endosperm type, each line of mutant type, each pollinator, and each type of cross made. Estimates of preferential fertilization or pollen vigor were also made for the various pollinators used.

Estimates of heterofertilization from the field study were 1.0% for the defective type and 2.3% for the shrunken type. From the seedling tests estimates of heterofertilization obtained by averaging over pedigrees, pollinators, and type of crosses, were 13.9% for the defective

type and 2.7% for the shrunken type. The overall average for all data was 4.9% heterofertilization. It appeared that the type of cross influenced the frequency of heterofertilization in the defective type since values of 10% and 15% for controlled and open crosses, respectively, were obtained. For shrunken no difference was apparent. The amount of heterofertilization associated with the various pollinators ranged from 1.7% for B OK 8 to 12.4% for Combine 7078, among the single pollinators. Among the combination pollinators the range was from 1.6% for Lahoma-B Wheatland to 9.4% for Dex-Lahoma. Averages of single and combination pollinators were greatly different. The amount of heterofertilization associated with pedigrees ranged from 2.0% to 71.4% (small sample), but it was difficult to ascribe any real difference to the pedigree source (unequal sampling). The seedling transplant check on these estimates of heterofertilization indicated they all may be somewhat high.

The two tests for pollen vigor or preferential fertilization did not produce the same results. The pollen vigor of a particular pollinator seemed to depend on the female or mutant line with which it was considered. Dex seemed to be the most vigorous on the shrunken female and Lahoma and Early Hegari on the defective endosperm type, for one of the tests. Overall Dex and Lahoma pollen seemed to be somewhat more vigorous than the grain type pollinators.

Further study would be needed to make better estimates of the frequency of heterofertilization in sorghum.

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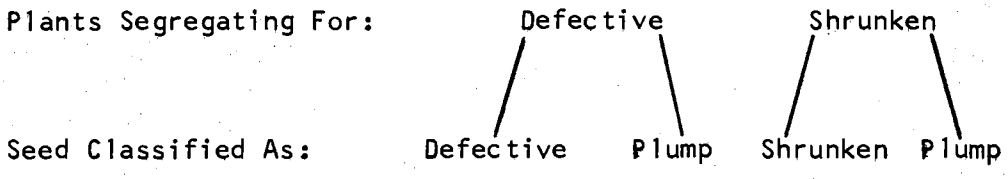
APPENDIXES

APPENDIX A

Methods Outline

Field Study

1965 Field: Segregating mutant type heads (bagged and open) were harvested and the seed classified.



1966 Field: A sample of seed of each of the above endosperm types from each head was planted and the resulting plants classified for endosperm type as follows:

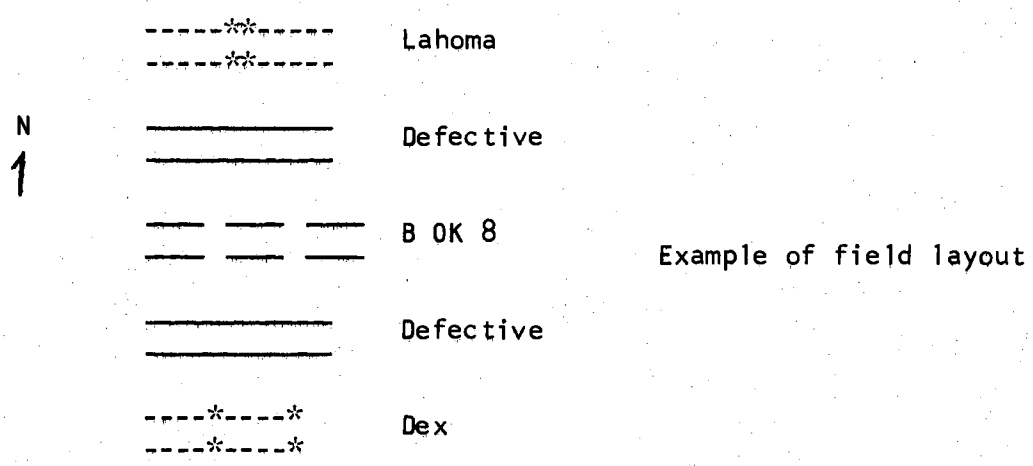
Seed planted:		Defective	Plump	Shrunken	Plump
Resulting plants classified as:	1)	Defective	Defective	Shrunken	Shrunken
	2)	Segregating	Segregating	Segregating	Segregating
	3)	Plump	Plump	Plump	Plump

Heads which were homozygous defective and homozygous shrunken, but were open and subject to outcrossing in the 1965 field, were handled in the same manner. The main difference was that they had very few plump kernels (outcrosses).

Seedlings Tests (Pollinations)

1965 Field: Homozygous bagged heads of the mutants (defective, shrunken, dent) were harvested for planting in 1966 field.

1966 Field: Two rows of mutant type seed from the homozygous plants were sown between two rows of pollinator in the field and both open and controlled pollinations made on the mutants.



Example of field layout

Two such blocks were made using the defective mutant, one for the shrunken, and one for the dent (single rows).

1967 Greenhouse:

Heads of the mutants (on which pollinations were made) were threshed, the seed classified for endosperm type, and a sample of each endosperm type from each head planted in a flat and the resulting seedlings classified for coleoptile color.

APPENDIX B

DESCRIPTION OF FEMALE LINES

I. Defective endosperm type: This mutant type was first found in an F_2 field hybrid progeny of Durra C. I. No. 696 in 1945 by Sieglinger (1951). The grain had a very collapsed or degenerate endosperm. The original line resulting from this selection plus two additional lines obtained from crosses of the defective endosperm line with Ryer Milo were used.

   A. Defective Endosperm 1-2-1-1 was the original line selected. It had red grain, awns, and was a grain type about 40 inches tall. It began blooming 52 days after planting.

   B. Ryer x Defective Endosperm 1-2-1-1 was another line with defective endosperm. This line was homozygous for defective endosperm but segregating for other characters, such as height and bloom date. The plants ranged from 29 to 61 inches in height.

   C. Defective Endosperm 1-2-1-1 x Ryer was a pure line that looked essentially like the Defective Endosperm 1-2-1-1 parent.

II. Shrunken endosperm type: This shrunken, sugary, or dimpled endosperm marker had been noted by several workers. The seeds were usually about 25% smaller than normal seeds and contained at least twice as much sugar. Three lines were used of this type also.

A. Kashakasha x 10-2-2-1-1-1 was a line with red grain and about 54 inches tall. The heads were oval and compact and bloomed two days later than the Defective Endosperm 1-2-1-1.

B. White shrunken (Sterile Tan Redlan x Large Shrunken Seed) was short (33 inches) and had tan plant and white grain. It took about 50 days to bloom.

C. Forage shrunken, Kashakasha type (Shrunken Sorgo x Tan Sumac), was the third line. It was a tall forage type (70 inches) with small wrinkled brown grain and a tan juicy stalk.

APPENDIX C

SEEDLING TESTS DATA

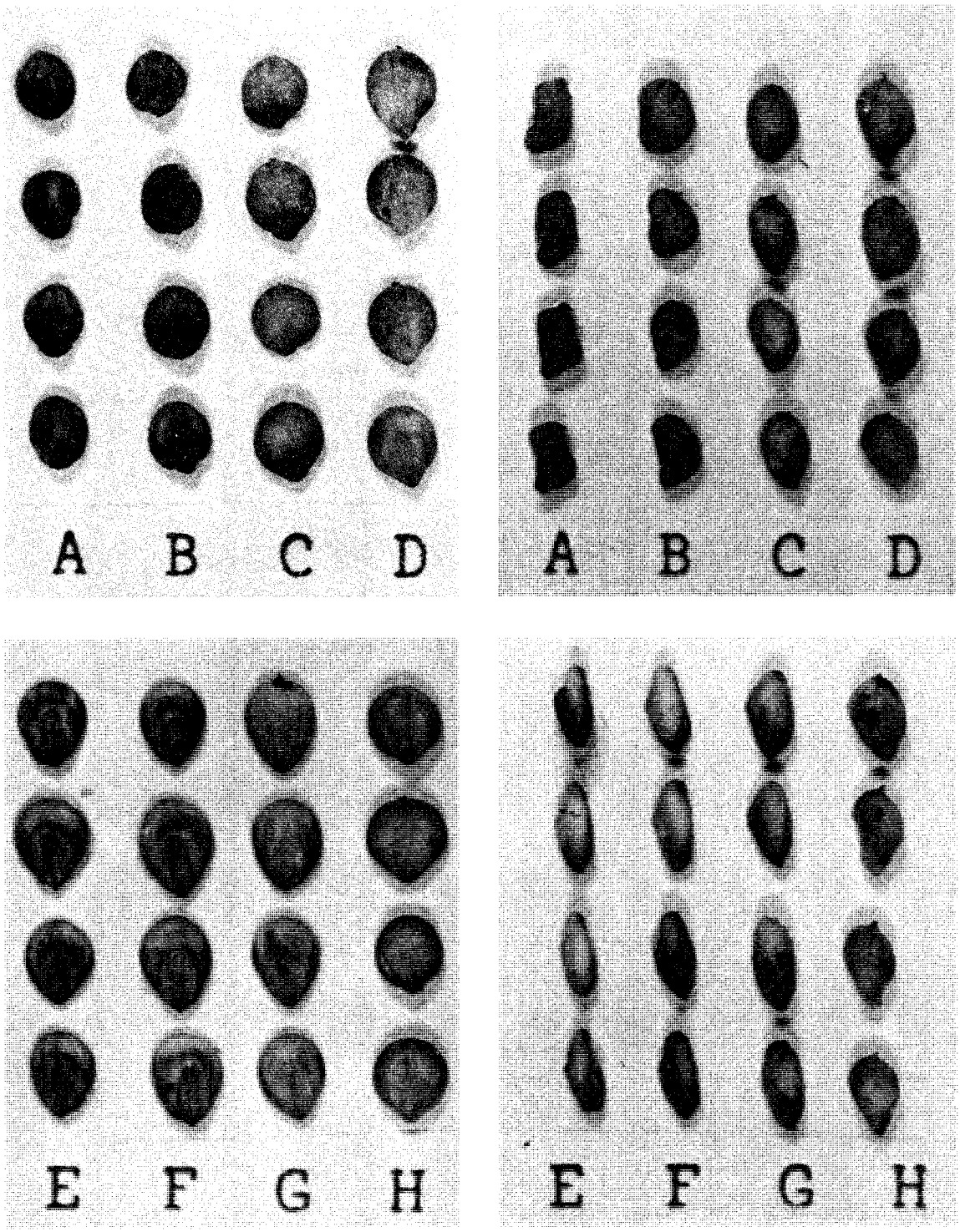


Figure 1. Typical kernels of endosperm types studied. Upper left (germ view) and upper right (lateral view) show A) shrunken endosperm, B) intermediate-shrunken, C) plump from shrunken, and D) plump (Combine 7078). Lower two show same respective views of E) defective endosperm F) intermediate-defective, G) plump from defective, and H) plump (Combine 7078).

TABLE XI

HETEROFERTILIZATION AND GERMINATION VALUES FOR EACH GREENHOUSE TEST OVER ALL POLLINATIONS, AS DETERMINED BY COLEOPTILE CLASSIFICATION IN SEEDLING TESTS, GREENHOUSE, 1967

Endosperm type	No. seed tested	Coleoptile Color			Percent	
		Red	Green	Unclassified	Germination	Heterofertilization
Test number one:						
Defective	3200	29[a]	767	18	25.4	3.6
Intermediate	1681	353	201	31	34.8	-
Plump	1026	422	99[a]	43	55.0	19.0
Shrunken	2400	11[a]	1168	94	53.0	0.9
Intermediate	342	7	107	7	35.4	-
Plump	262	203	20[a]	3	86.3	9.0
				Average	40.2	5.8
Test number two:						
Defective	3200	26[a]	651	31	22.1	3.8
Intermediate	1640	270	186	61	31.5	-
Plump	1256	405	182[a]	73	52.5	31.0
Shrunken	2400	5[a]	1307	68	57.5	0.4
Intermediate	237	1	49	8	24.5	-
Plump	235	117	41[a]	13	72.8	25.9
				Average	38.9	9.3
Test number three:						
Defective	3100	11[a]	438	50	16.1	2.4
Intermediate	1423	183	145	63	27.5	-
Plump	1173	300	141[a]	117	47.6	32.0
Shrunken	2400	3[a]	1211	19	51.4	0.2
Intermediate	386	3	194	20	56.2	-
Plump	215	73	67[a]	41	84.2	47.8
				Average	35.4	9.9
Test number four:						
Defective	3100	19[a]	319	25	11.7	5.6
Intermediate	1487	188	168	62	28.1	-
Plump	878	239	95[a]	77	46.8	28.4
Shrunken	2400	3[a]	1321	42	56.9	0.2
Intermediate	314	3	192	2	62.7	-
Plump	225	149	23[a]	24	87.1	13.4
				Average	35.1	6.5

TABLE XI (Continued)

Endosperm type	No. seed tested	Coleoptile Color			Percent	
		Red	Green	Unclassified	Germination	Heterofertilization
Test number five:						
Defective	2800	5[a]	364	15	13.7	1.4
Intermediate	1301	170	105	104	29.1	-
Plump	737	232	68[a]	80	51.6	22.7
Shrunken	2200	3[a]	1213	62	58.1	0.2
Intermediate	253	8	39	25	28.1	-
Plump	377	228	19[a]	50	78.8	7.7
				Average	36.4	4.5
Test number six:						
Defective	3500	11[a]	160	16	5.3	6.4
Intermediate	1148	47	53	22	10.6	-
Plump	511	127	28[a]	26	35.4	18.0
				Average	9.5	12.0

[a]Seedlings resulting from heterofertilization.

VITA

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