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Effect of the Waxy Gene On Grain Yields Of Sorghum

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Waxy endosperm of sorghum has been used for many years by the Chinese in preparation of foods for special occasions (10).**

Tapioca starch, produced from the roots of the cassava plant, *Manihot esculenta* Crantz, is used in the United States as food, and also for the manufacture of adhesives for postage stamps, envelopes, boxes and packages, and for sizings used in the textile and paper industries.

Tapioca starch has been imported for many years, the chief source of supply formerly being the Dutch East Indies. World War II resulted in the loss of this source, and users began searching for a domestically-produced substitute.

The endosperm starch from waxy (glutinous) cereals differs from that of the non-waxy ones in both chemical structure and physical characteristics. It has been named waxy because the cut surface of kernels which have this character have a uniform waxy, opaque appearance, while the cut surface of kernels with the usual type of endosperm either is more lustrous and hard or else white and starchy in appearance. Grasses known to have strains with waxy endosperm include corn, *Zea mays* L. (7); sorghum, *Sorghum vulgare* Pers. (15); rice, *Oryza sativa* L. (19); barley, *Hordeum vulgare* L.; proso millet, *Panicum miliaceum* L. (10); and coix, *Coix lachryma-jobi* L. (15). Of these, corn and sorghum have been investigated most thoroughly.

The starch of the waxy cereals has many properties similar to those of tapicoa starch. Domestically-produced starch from waxy cereals can be used as a substitute for tapioca starch.

Data with regard to the genetic constitution and segregation of the starchy and waxy genes have been obtained with various crops. Informa-

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** Figures in parentheses refer to "Literature Cited," p. 14.

tion is limited relative to the effects of starchy, heterozygous and waxy types on yielding ability in grain sorghums. Such information would be of considerable value to the plant breeder and could facilitate the development of high-yielding waxy hybrids of probable commercial importance as a source of starch.

The primary objective of the investigation reported in this bulletin was to determine the relative yields of the different genotypes. However, the conditions of the experiment made it possible to collect considerable information relative to the segregation of the waxy and starchy alleles; therefore that information is also reported and discussed.

Review of Literature

Investigations of the heredity of the waxy gene in sorghum show that when waxy is crossed with homozygous starchy the crossed seeds have a starchy endosperm. The starchy endosperm of the hybrid seed cannot be distinguished from that of homozygous starchy varieties. However, F_1 generation plants produce both waxy and starchy seeds in a typical segregating manner. Karper (12) was the first to report that the waxy gene in sorghum is inherited as a simple Mendelian recessive. This agreed with earlier reports of the behavior of the waxy gene in maize and rice (2,5,6,11,17,18,19). The proportion of starchy and waxy seeds produced on segregating plants fits the monohybrid 3:1 ratio.

The iodine test has been used in sorghum as a rapid means of distinguishing the waxy from the starchy phenotype. Karper (12) reports that the cross-sectioned surface of the waxy seed stains reddish-brown, whereas the starchy seeds give a typical blue starch reaction when stained with an aqueous solution of potassium iodide and iodine. He also reported that the reddish staining of the waxy seed is confined to the endosperm starch, the pericarp and embryo staining blue. The waxy pollen of rice, corn and sorghum likewise stains a reddish-brown color and the starchy pollen stains blue when treated with a potassium iodide solution (2,5,6,11,17,18,19).

Demerec (11), Brink and MacGillivray (5), Kiesselbach and Peterson (17), Longley (18), and Brink (2) showed the presence of the waxy and starchy allelomorphs in the male gametophyte of segregating plants in which the waxy and starchy pollen grains are produced in equal proportion.

Experimental results with corn show a deficiency of waxy kernels in the F_2 generation (1,2,8,14,17). Kiesselbach and Peterson (17) summarized all the available corn data which show a waxy kernel deficiency of 1.1% from the expected 25% when the heterozygote is selfed and 0.7% deficiency from the expected 50% when the heterozygote is back-crossed to the recessive. They conclude, however, that the evidence at hand seems insufficient to definitely establish the causes or significance of these deviations. In a study of 6,879 rice kernels, Parnell (19) reports a waxy kernel deficiency of 1.9%.

Brink and Burnham (4), Brink and MacGillivray (5), Kiesselbach and Peterson (17), and Kempton (14) have offered several explanations to account for the deficiency of waxy kernels. The following causes have been proposed: (a) Lack of vigor of the pollen carrying the waxy gene, causing slower growth of the pollen tube; (b) differential selection of sperm by female gametes; (c) greater death rate of zygotes homozygous for waxy; (d) differential death rate of the gametes during their formation so that equal numbers do not function.

Experimental data are sparse regarding the effect of the waxy gene on the yield of grain of sorghum and other grass crops such as corn, rice, barley, and proso millet when the parentage of the waxy and starchy genotypes is identical.

The second generation hybrids from a waxy crossed with a sweet type corn produced starchy, waxy, and sweet seeds on the same self-fertilized plant, according to Collins and Kempton (9). Separate weights of the three classes of seed showed that in nearly every ear the starchy seeds were heavier than the waxy, which in turn were heavier than the sweet. They concluded the differences in weight were due to differences in size, since the specific-gravity determinations showed no significant differences.

Kiesselbach (16) reports that three standard waxy hybrids averaged 5.6% less grain than three starchy hybrids that were essentially isogenic except for the waxy gene. He suggests that a difference in gene-controlled enzyme relationships within the developing grain are destined to yield approximately 5% less than their starchy counterparts.

A similar study with sorghum was made by Karper and Quinby (13) in 1937. To determine to what extent the waxy gene normally reduces the weight of the seed, the starchy and waxy seeds from three segregating heads of sorghum were separated and weighed. The starchy seeds were found to have a relative weight of 104.4% as compared to 100% for the waxy seeds. However, increases four times this large were obtained when hegari or milo was used as one of the parents.

Materials and Methods

The major portion of this study was conducted with five strains of grain sorghum during the years 1946, 1947, and 1949. A preliminary study had been made in 1944, using 13 strains, including the five later used for the major study.

The waxy parent for four of the five strains was a fixed selection of Standard Blackhull kafir X Leoti (C. I. No. 71 X F. C. I. No. 6610-2-7), and for the fifth strain it was Cody (H. C. No. 39-142). The strains were:

Strain A—(71 X Leoti-2-7) X Bonar durra.
Strain B—(71 X Leoti-2-7) X Yellow darso.
Strain C—(71 X Leoti-2-7) X Shallu C. I. No. 85.
Strain D—(71 X Leoti-2-7) X Corneous durra C. I. No. 695.
Strain E—Cody X Yellow darso.

Seed of these strains was in the F_3 generation in 1944, and corresponding successive generations in succeeding years. The plants were grown on the Oklahoma Station's Agronomy Farm near Stillwater.

Yields of seed were determined in grams per plant in 1946 and 1947. The variation among the yields of individual plants within a genotype made it difficult to accurately measure small differences between genotypes, therefore a plot technique was adopted in 1948 and continued through 1950. However, usable data were obtained only in 1949, since the seed crop was partially destroyed by birds in 1948 and by sorghum webworm in 1950.

For the individual plant studies in 1946 and 1947, seed from heterozygous self-fertilized plants of each strain was planted in rows 42 inches apart, with plants spaced uniformly 1 foot in the row. The heads were bagged previous to pollen shedding, and genotypic determinations were made by staining a small amount of the pollen with a solution of potassium iodide. Pollen from waxy plants stained reddish-brown, from starchy plants blue, and from heterozygous plants approximately 50% reddish-brown and 50% blue. Chi square was used to test goodness of fit to expected ratios.

In the 1944 work, mature seed was used to determine the segregation and percentage of waxy. Seed staining reddish-brown when treated with the potassium iodide reagent was classified as waxy, and that staining blue was recorded as starchy. The strains used are listed in reporting the results (Table 5, page 12).

For the plot studies, a composite sample of waxy seed from each strain and starchy seed from each strain was planted in 2-row plots 62.5 feet long. A 5 x 5 Latin square design was used, with the waxy and starchy genotypes randomized in a split plot on each cell of the square.

Data from both individual plant and plot studies were evaluated by analysis of variance.

Results

YIELDS AND SEED WEIGHT

Individual Plant Studies

YIELD

Table 1 shows yields obtained from 1,131 individual bagged plants during 1946 and 1947. Analyses of variance were run both years on the data from each of the five strains. Significant differences were found between genotypes at the 5% level in 1946 for strains B and E, and at the 1% level for strain D. In 1947, no significant differences between genotypes were found in any of the strains. Even though the plants were in the F_5 and F_6 generations of selfing, were uniformly spaced in the row, and stands were good, there was tremendous variation between plants. This resulted in large experimental errors and made it difficult to measure differences between genotypes by anaylsis of variance.

The starchy plants consistently yielded more than the waxy plants from these isogenic strains. In only one of the 10 strain years did waxy yield better than starchy. This occurred in 1947 in strain C, which is late in maturity. All genotypes in strain C were injured by drouth in 1947. However, it is doubtful whether drouth was responsible for the

TABLE 1.—AVERAGE YIELDS OF WAXY, HETEROZYGOUS, AND STARCHY GENOTYPES FOR 1,131 PLANTS WITHIN FIVE STRAINS OF SORGHUM; 1946 AND 1947.

			Average yield per plant			
Strain	Pedigree	No. of plants	Genotype	1946 Gms.	1947 Gms.	2-Year average percent*
A.	(71 X Leoti-2-7)	49	Waxy	61.3	60.6	100.0
	X Bonar durra	112	Heterozygous	65.0	68.0	109.1
		55	Starchy	67.1	75.9	117.3
B.	(71 X Leoti-2-7)	51	Waxy	63.7	66.5	100.0
	X Yellow darso	105	Heterozygous	64.8	71.9	104.9
		60	Starchy	72.6	67.7	107.9
C.	(71 X Leoti-2-7)	58	Waxy	54.9	34.3	100.0
	X Shallu	119	Heterozygous	53.0	34.2	98.0
		59	Starchy	59.1	33.9	103.3
D.	(71 X Leoti-2-7)	54	Waxy	49.8	52.2	100.0
	X Corneous durra	118	Heterozygous	51.8	54.8	104.5
		70	Starchy	57.7	54.2	109.8
E.	Cody X Yellow darso	49	Waxy	51.7	62.5	100.0
		116	Heterozygous	58.6	60.4	105.0
		56	Starchy	60.7	71.8	116.2
	total	1,131				

* Percent based on waxy as 100.

waxy yielding more than the starchy. In the other four strains, starchy proved to be superior in yield.

The two-year average yield of all waxy, heterozygous and starchy plants shows a superiority for starchy over waxy ranging from 3.3% in strain C, to 17.3% in strain A, with an average of 10.9%. The heterozygous genotype was intermediate between the waxy and starchy genotypes in all strains except C, in which it produced 98% as much as the waxy. Considering waxy as 100%, the heterozygous genotype yielded 104.3% and the starchy 110.9%, as an average for all strains.

Weight per 1,000 Seed

A comparison of the weight per 1,000 seed of the waxy, heterozygous and starchy genotypes in all strains was made in 1946 and of the waxy and starchy genotypes in 1947 (Table 2). Samples of starchy seed weighed more in four of the five strains in both 1946 and in 1947. The heterozygous genotype, which was checked in only 1946, was intermediate. As an average for two years, 1,000 starchy seed weighed 0.77 gram more than 1,000 waxy.

Webster* in Nebraska made weight determination in 1948 on samples of 100 seed from nine strains developed from one cross. He found the waxy weighed 0.08 gram less than starchy per 100 seed, or 0.8 gram less on a 1,000-seed basis.

Plot Studies

Yield

Table 3 shows yields from composite samples of waxy seed from each strain and starchy seed from each strain planted in field plots in 1949. They indicate a variable but significant superiority of starchy types over waxy. Considering the waxy genotype as 100%, starchy yielded 103.7% in strain B, 104.5% in strain D, 106.2% in strain A, 115.5% in strain E, and 120.1% in strain C. The average superiority of starchy over waxy was 9.2%. The interaction of variety times genotypes was not significant, indicating a consistent superiority of the starchy type.

Test Weight

Test weights for the waxy and starchy genotypes in each strain in 1949 are reported in Table 2. The starchy seed were heavier in all

* Courtesy of Mr. O. J. Webster, Assistant Agronomist, Nebraska Agr. Exp. Sta., Lincoln, Nebraska.

strains, varying from 0.2 of a pound in strain B to 2.9 in strain C. The average weight-per-bushel advantage of the starchy genotypes was 1.2 pounds.

GENE SEGREGATION

Table 4 gives the observed numbers for each genotype among the 1,131 individual plants studied in 1946 and 1947, and also the expected numbers based on a 1-2-1 segregation. The combined Chi Square value of 4.85 with a probability of 0.87 for the five progenies indicates a good fit of the data to a 1-2-1 hypothesis.

TABLE 2.—WEIGHT IN GRAMS PER 1,000 SEED (1946 AND 1947) AND IN POUNDS PER BUSHEL OF SEED (1949) FROM FIVE SORGHUM LINES ISOGENIC EXCEPT FOR THE GENE FOR WAXY ENDOSPERM.

		Grams per 1,000 seed			Pounds per bushel
Strain	Genotype	1946	1947	Av.	1949
A	Waxy	29.45	28.20	28.84	54.3
	Heterozygous	29.57	----	----	----
	Starchy	30.12	29.25	29.69	55.2
B	Waxy	28.21	23.94	26.08	54.4
	Heterozygous	28.85	----	----	----
	Starchy	28.68	25.85	27.27	54.6
C	Waxy	19.87	20.97	20.42	54.4
	Heterozygous	20.88	----	----	----
	Starchy	21.01	21.82	21.42	57.3
D	Waxy	23.69	20.34	22.02	54.0
	Heterozygous	23.22	----	----	----
	Starchy	23.00	21.99	22.49	55.0
E	Waxy	25.96	22.01	23.99	53.8
	Heterozygous	25.22	----	----	----
	Starchy	27.56	21.14	24.35	54.9
Average	Waxy	25.43	23.09	24.27	54.2
	Heterozygous	25.75	----	----	----
	Starchy	26.07	24.01	25.04	55.4

TABLE 3.—YIELDS FROM PAIRS OF SORGHUM LINES ISOGENIC EXCEPT FOR THE GENE FOR WAXY ENDOSPERM; 1949.

Strain	Genotype	Yield* (pounds per acre)	Percent of waxy
A	Waxy	1,060	100.0
	Starchy	1,126	106.2
B	Waxy	1,040	100.0
	Starchy	1,072	103.1
C	Waxy	890	100.0
	Starchy	1,067	119.9
D	Waxy	641	100.0
	Starchy	670	104.5
E	Waxy	493	100.0
	Starchy	570	115.5
Average	Waxy	825	100.0
	Starchy	901	109.2

TABLE 4.—OBSERVED AND EXPECTED NUMBERS OF WAXY, HETEROZYGOUS, AND STARCHY PLANTS IN FIVE SELF-FERTILIZED, HETEROZYGOUS STRAINS OF SORGHUM; AND VALUES OF CHI SQUARE UNDER THE 1:2:1 HYPOTHESIS; 1946 AND 1947.

Strain	Pedigree	Total plants	Observed			Expected			Chi Square
			Waxy	Hetero-zygous	Starchy	Waxy	Hetero-zygous	Starchy	
A	(71 X Leoti-2-7) X Bonar durra	216	49	112	55	54.00	108.00	54.00	0.63
B	(71 X Leoti-2-7) X Yellow darso	216	51	105	60	54.00	108.00	54.00	0.95
C	(71 X Leoti-2-7) X Shallu	236	58	119	59	59.00	118.00	59.00	0.02
D	(71 X Leoti-2-7) X Corneous durra	242	54	118	70	60.50	121.00	60.50	2.26
E	Cody X Yellow darso	221	49	116	56	55.25	110.50	55.25	0.99
	Total	1131	261	570	300				4.85

D. F.=10. P=0.87.

TABLE 5.—OBSERVED NUMBERS OF WAXY AND STARCHY SEED FROM 13 SELF-FERTILIZED, HETEROZYGOUS SORGHUM STRAINS; 1944.

Strain pedigree	Waxy	Starchy	Percent waxy
71 X Leoti-2-7 X Dwarf White feterita	1280	4608	21.74
71 X Leoti-2-7 X Corneous durra	1841	5875	23.86
71 X Leoti-2-7 X Shallu	1141	3677	23.68
71 X Leoti-2-7 X Yellow darso	1314	4713	21.80
71 X Leoti-2-7 X Tift Sudan	1436	6348	18.45
71 X Leoti-2-7 X Bonar durra	990	3576	21.68
71 X Leoti-2-7 X Dwarf kafir	1059	3625	22.61
71 X Leoti-2-9 X Dwarf kafir	413	1299	24.12
Cody X Dwarf White feterita	1109	3594	23.58
Cody X Dwarf kafir	1689	5388	23.87
Cody X Yellow darso	1243	4078	23.36
((White Sumac X Collier) X Leoti) X Tall Red Kafir No. 7	1248	3582	25.84
Sedan Red kafir X ((White Sumac X Collier) X Leoti)	783	2380	24.75

The waxy genotype in Table 1 contains fewer plants in each strain than the starchy genotype. Based on an expected 25 percent, strain A had 22.7 percent waxy, strain B, 23.6, strain C, 24.6, strain D, 22.3, and strain E, 22.2. This shows an average deficiency for waxy in the five strains of 1.90%.

The exploratory study made in 1944 on the seed of 13 hybrid sorghum strains resulted in an average deficiency of 1.97% waxy. The percentage of waxy plants produced within the 13 strains ranged from 18.45 to 25.84 (Table 5). In only one of the 13 strains did the percent waxy exceed the expected 25%.

Discussion

YIELD AND SEED WEIGHT

In both the individual plant study and the plot study, the starchy genotype was superior in yield by about 10%; and the weight per 1,000 seed and test weight per bushel were both higher in the starchy genotype than in the waxy. During the three years of testing, all these strains were in the F_5, F_6, and F_8, which should indicate a high degree of homozygosity. The waxy and starchy lines in each strain are known to be different for the waxy gene, and probably these lines are different for other genes. It is possible that the waxy gene alone is responsible for the deleterious effect on yield and test weight. However, it seems more probable that these depressive effects may be associated with a gene or group of genes tightly linked to the waxy gene in chromosome 3. The fact that some high yielding waxy strains have been produced indicates that the waxy is not solely responsible for the depression in yield and test weight; and, if this should prove to be the case, high yielding waxy combination would be possible by breaking this linkage.

There is evidence that the chemical development of the waxy endosperm is arrested just short of reaching its full expression which would result in starchy endosperm. This might be responsible for the lower yield and smaller seeds.

GENE SEGREGATION

The deficiency of waxy plants below the number to be expected on a 1-2-1 hypothesis has also been reported in corn (1,2,8,14,17) and in rice (5,18). Several hypotheses have been formulated to account for this deficiency (4,5,6,14,17). The most logical explanation seems to be a differential rate of pollen tube growth (6). That is, a pollen carrying the waxy gene would produce a pollen tube that grows more slowly than pollen carrying the starchy gene. This would result in a smaller percentage of ovules being fertilized by waxy pollen.

The fact that counts of the numbers of waxy and starchy pollen grains from segregating plants in five strains did not differ significantly from the expected 50-50 ratio would indicate that sporogenesis was normal. Similar results were reported in sorghum by Karper (12), in rice by Chao (6), and in corn by Brink (3).

Summary

Experiments were conducted for three years at the Oklahoma Agricultural Experiment Station to determine the type of segregation and the relative yields of the waxy and starchy endosperm in sorghum. The pollen of five strains segregating for waxy were studied by use of the potassium iodide test. The waxy and starchy genes segregated on 1-2-1 monohybrid basis with a Chi Square probability of 0.87. Even though an acceptable fit to a 1-2-1 segregation was found, the expected percentage of waxy plants was consistently low. On the basis of an expected 25% waxy, the average deficiency was 1.90%. Similar studies conducted on the seed of 13 other segregating hybrid strains showed a deficiency of 1.97% from the expected 25% waxy. This deficiency of waxy plants is thought to be due to slower pollen tube growth of waxy pollen, which results in a smaller percentage of ovules being fertilized by waxy than by starchy pollen.

The yield of individuality spaced plants showed a 10 percent superority of starchy types over waxy. The heterozygous plants were intermediate in yield. When tested in large field plots the starchy plants were also superior to the waxy by 10 percent.

The weight per 1,000 seed was 0.77 gram in favor of starchy. Likewise starchy seeds outweighed waxy seeds by an average 1.2 pounds per bushel.

The depressive effects on yield and weight of grain may be due to a group of undesirable genes linked to waxy, rather than to the waxy gene alone.

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