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1. Genetic control of carbohydrate type and quantity in maize kernels.

Culpepper and Magoon (1924, J. Agr. Res. 28:403-443), in a test of sweet corn (su₁) and dent corn (Su₁) varieties at 5-day intervals beginning at 5 days after pollination and ending at 30 days, observed that: (1) dry matter increased in both types with age; (2) total sugar increased up to the 15th day, followed by a decrease (sweet corn had about twice as much sugar as dent); (3) reducing sugars showed larger increases in su, than in Su₁ genotypes; (4) sucrose increased to the 15th day, followed by a decrease (su₁ had about twice as much sucrose as Su₁); (5) water-soluble polysaccharides (VSP) increased rapidly in su₁ but remained very low in Su₁ (at 30 days su₁ had about 10 times as much VSP as dent); and (6) starch increased rapidly in both types, Su₁ having more starch than su₁.

During recent years several other genes have been shown to alter carbohydrate type and quantity at maturity or during kernel development. The gene wx (Andrew et al, 1944 J. Agr. Res. 69:355-371) increases sugars and WSP on a percentage basis in a sul background and alone. The effects of bt, and bt, were reported by Cameron and Teas (1954, Amer. Journ. Bot. 41:50-55). Each brittle gene increased sugar and reduced starch content at mid-development and beyond. No increase in WSP was observed. Laughnan (1953, Genetics 38:485-499)reported similar effects at kernel maturity for the gene sh. Sul she kernels contained a high percentage of sucrose and less starch than sul, but had very little WSP. The double recessive sul she had even higher sugar and less starch than sul. The WSP content was about the same as she alone (about 2%).

Mangelsdorf (1947, Genetics 32:448-458) and Cameron (1947, Genetics 32:80-81) reported that in mature kernels the gene du, when homozygous with su, produced less starch, more WSP, and perhaps more sugars than su, alone. Horovitz et al (1941, Annal. Inst. Fitotec. Sta. Catalina 3:37-44) reported that su2 su, produced about 14% sugar in mature kernels in contrast to about 5% for su, alone. Additional data by Dwonch et al (1951, Cereal Chem. 28:270-280) and Dunn et al (1953, Agron. Jour. 45:101-104) indicate that in mature kernels starch is lower and WSP is sometimes higher in su, du and su, su, than in su.

The genes ae, su2, and du have been reported to alter the proportion of the two starch fractions, amylose and amylopectin (Kramer et al, 1949, Agron. Jour. 41:409-411, 1958, Agron. Jour. 50:207-210; Vineyard and Pear, 1952, Maize Genetics Coop. News Letter 26:5; Zuber et al, 1958 Agron. Jour. 50:9-12; and others). Amylose is a straight

chain molecule having 200-300 glucose units and stains blue with iodine. Amylopectin, which has a higher molecular weight than amylose, consists of branched chains with about 25 glucose units in each branch and stains red with iodine. Weatherwax (1922, Genetics 7:568-572) was the first to show that waxy (wx) corn starch stained red with iodine. Sprague et al (1943, Agron. Jour. 55:817-822) showed that the standard waxy endosperm contains no amylose but all amylopectin starch.

According to Zimmerman (1960, Ann. Rev. Plant Physiol. 11:167) and Porter (1962, Ann. Rev. Plant Physiol. 13:303-328) the main transport material in higher plants is sucrose, the first free sugar after photosynthesis. There is evidence that sucrose is the main glucose donor in the formation of polysaccharides which may become increasingly larger until starch is formed. It is thought by some that amylopectin is formed by the rearrangement of preformed amylose chains (Porter, 1962; Whistler and Young, 1960, Cereal Chem. 37:204). However, Erlander (1961, Cereal Chem. 37:81) claims that amylose arises from debranching of amylopectin and that the absence of amylose in waxy starch can be accounted for by the absence of the debranching enzyme. There seems to be insufficient evidence to conclude that either amylose or amylopectin is formed first (Porter, 1962).

Research at The Pennsylvania State University with genetic mutants affecting the properties of maize endosperm is being carried out with the following objectives in mind: (1) to determine the effects of specific genes and gene combinations on carbohydrate type and quantity at varying endosperm maturities; (2) to elucidate the pathway (or pathways) of carbohydrate snythesis and the "gene-enzyme" or "gene-enzyme component" relationships; and (3) the application of this knowledge, where feasible, in improving the quality of sweet corn or in the breeding of types for particular industrial purposes.

In 1961 thirty genetic lines were obtained from Dr. H. H. Kramer. The genotypes of these lines are shown in Table 1. They are single, double and triple recessives of ae, du, sh2, su1, su2, and wx. All these mutants were studied in a background related to the single cross, W23/L317, except Golden Cross Bantam sweet corn (su1). The backgrounds are not isogenic; therefore, possible background effects must be kept in mind. These lines were grown in a replicated trial in 1961 and fresh kernels samples of 50 grams were taken from 2 - 3 ears at 16, 20, 24, and 28 days after pollination and stored in 95% ethanol within 2 hours after removal from the plant.

Dry matter content was determined by weight of the alcohol insolubles plus the alcohol solubles. Chemical analyses were made for reducing sugars, sucrose (after Hassid, 1936, Ind. and Eng. Chem. 8: 138-140), water-soluble polysaccharides (after Cameron, 1959, Agron. Jour. 51:424-427), and starch (after Hixon, 1944, Iowa A.E.S. Report, Part II). Total carbohydrate content was calculated by summing the weights of the individual carbohydrates analyzed.

Very significant differences were noted for all carbohydrate and dry matter analyses between genotypes at all kernel maturities. Very significant differences were observed between maturities within genotypes for all characteristics measured. These data are presented in Table 1.

Dry Matter content. The dry matter content of normal increased from 15.7% at 16 days to 43.8% at 28 days after pollination. All the recessive genotypes, with the exception of su, and su, wx, tended to be significantly less than normal. The genotypes that were exceptionally low in dry matter were sh, ae wx, du sh, sh, sn, ae du su, ae du wx, ae su, wx, and ae su, wx. The other genotypes were intermediate between these and normal. It is important to keep these dry matter differences between genotypes in mind when comparing the quantitites of particular carbohydrates reported here as percentages of dry matter. Actual weights and percentages of fresh weights have been omitted here in interest of space.

Reducing sugars. The reducing sugars content of normal decreased from 9.4% at 16 days to 0.8% at 28 days after pollination. All other genotypes possessed approximately the same amount of reducing sugars as normal except sh, su, ae wx, du wx, sh su, ae du su, ae du su, ae du su, and ae du wx. These appeared to be higher in reducing sugars than normal. Of these, sh su, ae du su, and ae du su appeared exceptionally high, especially at later kernel development.

Sucrose. Sucrose content in normal decreased from 8.2% at 16 days to 2.2% at 28 days after pollination. The genotypes she, du she, she sul, and ae du wx were exceptionally high in sucrose (7-10 times more than normal) at almost all kernel ages. The genotypes ae, du, sul, ae du, ae sul, ae sul, du sul, du wx, she sul, sul, wx, ae sul sul, du sul, wx, and du sul wx had 2 - 4 times as much as normal. The genotypes ae wx, sul sul, ae du sul, ae du sul, ae sul wx, ae sul wx, ae sul wx, and sul sul, wx had 5-6 times as much sucrose as normal. The gene sul appears to be epistatic over she and she seems to be partially epistatic over du and sul.

Water-soluble polysaccharides. The WSP content of normal appeared to decrease slightly from 3.7% at 16 days to 2.2% at 28 days. The decrease was not significant. Apparently, WSP was not accumulating with kernel development. A slight, but insignificant, increase over normal was noted for ae, sh₂, ae du, ae wx, du sh₂, du su₂, du wx, sh₂ su₁, ae du su₂, ae du wx, ae su₁ wx, and ae su₂ wx. Significant increases over normal were noted for su₁, du su₁, sh₂ su₂, su₁ su₂, su₁ wx, ae du su₁, ae su₁ su₂, du su₁, sh₂ su₂, su₁ su₂, su₁ su₂ wx. The gene su₁ is associated with a dramatic increase in WSP at all 4

stages of kernel development. The gene ae apparently is epistatic or partially epistatic over <u>sul</u>. The genes <u>du</u> and <u>su</u> appear to intensify the accumulation of WSP in combination with the other genes.

Starch. The starch content in normal increased from 39.2% at 16 days to 73.4% at 28 days after pollination. Extreme starch reduction (approximately one-half or less of normal) was associated with the genotypes sh₂, su₁, ae su₁, du su₁, du sh₂, sh₂ su₁, sh₂ su₂, su₁ su₂, su₁ su₂, su₁ su₂, du su₁ wx, and su₁ su₂ wx.

Total sugar and total carbohydrates are also shown in Table 1. Total sugar content is the sum of the reducing sugars and sucrose contents. Total carbyhydrates content is the sum of the contents of all the carbohydrates analysed. There seems to be a decrease in total sugar with kernel development in most instances. An increase in total carbohydrates with kernel development is indicated in all cases except those that are medium to high in sugar and low in WSP and starch.

Table 1. The quantitites of various carbohydrates and total dry matter in entire kernels of thirty-one maize genotypes at four stages of maturity.

Code no.	Geno- type	Kernel age (days)	Reducing sugars	Sucrose %	Total sugar	WSP*	Starch % h	Total carbo- ydrates*	Dry matter
1	normal	16 20 24 28	9.4 2.4 1.6 0.8	8.2 3.5 2.6 2.2	17.6 5.9 4.8 3.0	3.7 2.8 2.8 2.2	39.2 66.2 69.2 73.4	60.5 74.9 76.1 78.6	15.7 27.1 37.2 43.8
2	ae	16 20 24 28	8.6 4.8 3.1 1.9	21.9 13.9 8.3 7.4	30.6 18.7 11.4 9.4	5•7 4•2 3•7 4•4	20.8 37.6 48.9 49.3	57•2 60•5 64•0 62•9	18.4 26.0 34.0 37.5
3	du	16 20 24 28	8.8 4.8 2.8 1.3	15.5 10.5 6.1 6.7	24.2 15.3 9.0 8.0	4.1 2.7 2.4 1.9	25.1 Ци.6 56.5 59.9	53•4 62•6 67•9 69•8	16.2 25.6 33.5 38.9
4	sh ₂	16 20 24 28	6.9 4.9 4.4 3.6	21.4 29.9 24.9 22.1	28.3 34.8 29.4 25.7	5.6 4.4 2.4 5.1	22.3 18.4 19.6 21.9	56.1 57.6 51.4 52.8	16.8 20.3 22.9 26.3

^{1/}Percent of dry matter2/Percent of fresh weight3/ Three replications

^{*}WSP = water-soluble polysaccharides

^{**}Sum of weights of reducing sugar, sucrose, WSP, and starch/dry matter weight.

Code no•	Geno-	Kernel age (days)	Reducing sugars	Sucrose	Total Sugar	WSP*	Starcl \$	Total n carbo hydrate	- matt	;er
5	sul	16 20 24	9.2 5.4 3.6	16.5 10.2 9.5 4.4	25.7 15.6 13.1 8.3	14.3 22.8 28.5 24.2	23•3 28•0 29•3 35•1	70.8	25• 30•	,6 .5
6	su ₂	28 16 20 2h	3.9 7.4 3.5 1.9 1.4	10.5 9.2 2.6 1.9	16.7 12.7 4.5 3.3	3.6 3.1 2.5 1.9	63.	7 61.1	8 24 9 34 8 43	•9 •9 3•6
7	WX	28 16 20 2h	10.1 3.5 2.5 1.6	9.6 5.2 4.5 1.7	19.7 8.7 7.0 3.	2.	3 53 8 61	.9 71. .0 74	6 2: 5 3	1.9 3.9 3.1 7.3
8	ae	20 21	8-7 7-3 4-6	19.9 10.4 6.8	11.	7 7	1 4	1.4 70 5.5 69)•2)•9	20.0 24.6 27.9 33.7
. 9	ae	su ₁ 10 20 20 2	6 6.9 0 3.7 4 2.3	12.6 8.5 5.	$\frac{12}{3}$	•0 •6	3.6	29.3 4 37.2 4 34.4 4	4.9 8.4 5.1	19.3 24.6 31.9 33.9
10	ає	su ₂ 1	16 12. 20 5. 24 3.	2 31. 6 16. 6 13.	և կ։ 3 2: 5 1:	1.9	4.5	35.2 37.6	52.1 61.6 59.1 62.8	16. 24. 28. 35.
11	а		16 6. 20 3.	1 23 8 23 9 17	8 2	9.9 17.0 22.4 15.4	և.2 և.6 5.6 և.6	19.7 26.6 37.1 39.5	53.9 58.2 64.9 59.5	18 23 25 28
12	2 (du sul	16 5 20 2	.3 1 .7 1	7.6 1.1 7.3 5.1	22.9 13.8 9.8 6.8	13.3 24.5 29.5 40.9	21.5 24.8 23.6 18.6	57.7 63.1 62.8 65.5	18 2: 2: 3

Code	Geno-	ernel age days)	Reducing sugars	Sucrese .	Total Sugar	WSP*	Starch % h	Total carbo- ydrates:	Dry matter
13	du sh ₂	16 20 2l ₁ 28	10.7 4.0 2.3 2.9	33.9 33.4 27.1 19.9	44.7 37.8 29.4 22.8	4.1 3.8 5.3 6.4	8.8 16.3 20.9 24.6	57.6 58.0 55.6 53.7	16.6 23.2 24.8 27.7
14	du su ₂	16 20 2h 28	5.1 2.9 1.8 3.8	21.7 10.3 6.8 6.1	26.8 13.2 8.6 9.9	3.3 2.9 3.4 5.1	27.1 41.9 47.1 48.9	57.2 58.0 59.0 60.3	19.5 27.7 32.9 37.7
15	du wx	16 20 24 28	7•3 4•1 3•8 3•0	25.5 15.8 11.6 9.5	32.8 19.9 15.4 12.5	5.5 12.2 11.4 11.6	21.3 34.3 37.9 45.4	59.6 66.4 64.7 69.5	21.1 25.7 30.4 34.8
16	sh ₂ su ₁	16 20 24 28	8.9 8.1 7.1 5.7	24.1 25.4 19.1 20.1	33.1 33.5 27.8 24.5	5.0 4.9 4.6 4.9	7.2 11.7 14.4 15.7	47•3 50•1 46•9 45•4	20.5 23.8 25.2 24.6
17	sh ₂ su ₂	16 20 24 28	10.4 4.0 3.3 2.5	14.6 8.5 7.6 6.8	25.1 12.6 10.9 9.3	6.3 9.5 10.0 13.6	26.8 38.3 38.6 35.1	58.1 60.3 59.5 57.9	18.8 28.3 33.8 38.3
18	su _l su ₂	16 20 24 28	4.9 2.8 2.4 2.5	16.8 11.2 9.6 10.4	21.8 14.1 12.0 12.8	33.7 31.5 31.0 36.9	11.9 20.1 20.5 18.9	67.5 65.6 63.5 68.6	20.1 28.5 31.1 35.4
19	su _l wx	16 20 24 28	4.4 3.4 2.6 3.0	14.7 11.1 7.5 5.7	19.1 14.4 10.1 8.7	19.5 26.4 29.1 30.3	28.1 29.9 32.8 32.5	66.6 70.9 71.9 71.5	21.9 29.5 35.0 37.3
20	su ⁵ ax	16 20 24 28	6.1 3.2 1.5 0.9	12.3 9.7 7.1 3.5	18.4 12.9 8.5 4.4	3•4 4•4 3•5 3•3	30.1 山山.0 62.6 66.3	51.8 61.3 74.7 73.9	17.9 25.7 37.3 42.5

Code no.	Geno-	ernel age ays)	Reducing sugars	Sucrose	Total Sugar	WSP#	Starch % h	Total carbo- ydrates*	Dry matter * %
21	ae du su <u>l</u>	16 20 24 28	12.8 9.2 4.7 4.6	21.6 18.0 15.5 10.6	37.3 27.2 21.3 15.3	9.6 12.4 16.1 18.2	23.6 30.9 32.7 38.0	70.5 70.5 70.0 71.5	17.3 22.6 25.8 27.6
22	ae du su ₂	16 20 21 ₁ 28	7•7 7•7 6•8 5•4	21.7 17.9 10.4 10.4	29.4 25.6 17.2 15.7	7.6 10.2 10.2 10.8	30.8 36.8 45.0 47.5	69.9 72.7 72.4 74.1	22.4 25.8 31.7 32.5
23	ae du wx	16 20 24 28	6.8 4.1 3.6 4.4	39•9 34•6 30•7 23•7	46.7 38.7 34.3 28.1	4.2 3.6 4.5 4.9	15.9 26.6 31.1 32.0	66.7 68.9 69.9 65.1	18.5 24.6 25.8 24.5
2);	ae sul su	16 20 24 28	8.5 3.5 2.7 2.4	23.2 9.7 7.9 8.6	31.7 13.2 10.6 11.0	6.6 10.4 10.6 11.0	23.8 41.6 39.6 41.0	62.0 65.3 61.1 65.9	20.3 27.1 31.5 34.1
25	ae su <u>l</u> wx	16 20 24 28	8.0 5.2 3.5 2.8	28.2 21.9 15.0 11.1	36.2 27.0 18.5 13.9	4.5 8.4 12.2 12.4	22.0 30.7 38.5 38.3	62.7 66.0 69.1 64.5	16.3 21.7 25.8 26.2
26	ae su ₂ wx	16 20 24 28	10.3 7.9 4.0 3.2	22.2 17.1 16.4 12.6	32.4 25.1 20.4 15.8		18.0 40.3 41.7 49.6	55.5 71.3 67.9 70.4	16.4 19.4 27.0 28.4
27	du su _l su	2 16 20 24 28	7•3 4•1 2•9 2•4	16.4 12.3 7.3 5.4	26.9 16.4 10.2 7.8	31.9 34.9	19.4 21.2 24.9 22.8	68.1 69.4 70.1 65.5	20.7 26.5 31.6 33.9
28	du su _l wy		5.9 3.2 2.9 2.3	16.8 10.2 7.8 6.7	21.7 13.4 10.7 9.0	36.1 38.4	14.7 21.4 17.5 15.9	60.8 70.9 66.6 72.3	22.0 27.9 33.4 35.3

Code	Geno-	Kernel age (days)	Reducing sugars	Sucrose	Total Sugar	WSP*	Starch	Total carbo- drates*	Dry matter * %
29	du su ₂ w	nx 16 20 21, 28	9•2 5•2 3•0 2•7	25.7 19.5 10.6 8.9	34.9 24.7 13.3 11.6	4.6 14.8 14.3 16.7	17.2 24.7 33.9 38.1	53.4 64.2 61.5 64.5	15.2 20.8 27.9 30.7
30	sul sus	- 1	6.5 3.4 2.8 2.1	19.6 15.1 11.4 10.6	26.1 18.5 14.3 12.7	22.1 33.9 38.9 40.1	11.9 13.8 16.2 18.2	60.1 66.2 69.3 71.0	18.5 24.8 32.0 34.6
31	Golden Cr Bantam(s Sweet Corn	ross 16 mi) 20 24	8.1 3.2 1.9 1.6	15•4 5•5 3•9 1•9	23.6 8.7 5.9 3.6	7.8 27.0 33.3 34.8	28.7 35.5 38.5 33.9	71.3 77.7 72.3	16.1 26.8 33.5 37.0
LSI	D Genotypo	28 es withi %		10.4··· 13.9		10.4	14.2 18.8	15.3 20.4	2•9 3•9
LS	SD Ages wi	thin Ger	notypes 2.4 3.2	6.0 7.9	5.8 7.1	в 4.8 7 6.3	7.6 10.1		2.9 3.8

A partial symmetric correlation matrix is presented in Table 2. All variables measured, percent dry matter, percent total sugar, percent reducing sugar, percent sucrose, and percent alcohol insolubles (AIS, not shown in Table 1) were either positively or negatively associated. Total sugar, reducing sugar, and sucrose contents were negatively correlated with dry matter content. AIS was positively associated with dry matter content. A correlation value of -0.81 between sucrose with dry matter content indicates, as previous workers content and alcohol insoluble content indicates, as previous workers have shown, that one may obtain increases in sugar content by selecting for types with low AIS. AIS determinations are relatively inexpensive as compared with sugar determinations. This is of value in sweet corn breeding.

Table 2. Symmetric correlation matrix.

Table					1.
	Jariable	1	2	3	
2. T 3. R h. S	ry matter % otal sugar % educing sugar % ucrose % IS %	-0.72** -0.73** -0.62** 0.76**	0.69** 0.94** -0.86**	0•50** -0•62**	-0.81**

1/ AIS Alcohol insolubles (contains WSP, starch, and kernel residue) ***Exceeds the 1% point (r > 0.15)

Sweet corn quality. Some of the most interesting genotypes showing promise for possible use in sweet corn quality improvement are ae su wx, ae wx, and sh. However, it must be pointed out that none of these genotypes, except sh., have been evaluated for other factors that contribute to sweet corn quality besides carbohydrates.

Indicated areas of genetic control in carbohydrate synthesis. These date support the findings of previous workers that the gene sul apparently causes or is associated with a substantial block between WSP and starch. The gene sh2, as reported by Laughnan and as these data indicate, apparently causes a substantial block between sucrose and WSP.

The genes ae, wx, su, and du have been of interest for several years because of their effects on the proportions of amylose and amylopectin starch. Because of this, some have thought that perhaps these genes were operating within the starch fraction affecting the formation of straight and branched chain molecules. However, these data indicate that ae alone also causes a marked increase in sucrose and reduction in total starch. In addition, ae combined with wx and wx du causes a dramatic increase in sucrose. The amylose and amylopectin data (Kramer et al, 1958, and Vineyard and Bear, 1952) combined with the effects of ae and wx on sugar content as shown in Table 1, indicate that ae and wx are in separate pathways of starch snythesis, This is, of course, assuming that the mutants are associated with partial or complete blocks in the biosynthesis of starch. This leads us to propose that the mutant gene ae is associated with a partial block between sucrose and the branched chain polysaccharides which eventually form anylopectin starch, and the mutant gene wx is associated with a substantial block between sucrose and the straight chain polysaccharides which eventually form amylose starch. There is some indication that du and perhaps su 2 may be in a second amylopectin pathway. have not taken space in this report to discuss all the apparent gene interactions. These will be discussed in detail in a later article.

Additional carbohydrate studies are planned with the gene mutations used in this study and additional mutants. Studies of the effects of these genes alone and in combinations on qualitative and quantitative changes in enzymes known to be associated with carbohydrate snythesis are being initiated. Studies to determine the types of carbohydrates produced in each genotype are presently underway.

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l. A case of cytoplasmic control of susceptibility to Helminthosperium leaf spot in corn.

In the 1962 issue of the News Letter, we reported a case of an apparent relationship between cytoplasmic male sterility involving the T plasmatype derived from FiliT and susceptibility to Helminthosporium leaf spot. A case of cytoplasmic control of susceptibility has been hypothesized in the light of the following observations: (1) the extreme susceptibility to the disease of cyto-sterile inbred lines, single crosses and double crosses in contrast with the apparent resistance of their normal counterparts, and (2) the extreme uniformity in the degree of infection of plants within the population of any line carrying the T-cytoplasm. To provide a more conclusive proof of this hypothesis further studies were conducted using populations of reciprocal crosses which differed only in the cytoplasmic background and this was made possible with the use of a "restored-cyto-sterile" parent. Inbred lines differing in the cytoplasmic background and/or in their genetic constitution for the fertility restoring factor were also included.

Each experiment was grown in three replications and the plants were subjected to very severe natural or artificially induced infestations of the disease. Disease reaction of individual plants in each entry were scored in numerical values ranging from 0 to 5, correspondingly from a very neglible infection to a very severe condition.