GENETIC CONTROL OF CARBOHYDRATE SYNTHESIS IN MAIZE ENDOSPERM[1]

ROY G. CREECH

Department of Horticulture, The Pennsylvania State University, University Park

Received June 28, 1965

IN the study of gene function, geneticists and breeders are turning increasingly to an evaluation of gene action in the biological processes of metabolic regulation. This trend opens new horizons for the geneticist who desires to know the mechanisms of genetic influence within higher organisms. As a result, the unification of the disciplines of genetics and biochemistry offers a multitude of possibilities for uncovering fundamental knowledge of interest to both.

The effects of specific mutations on qualitative and quantitative changes in carbohydrates in maize endosperm are now being investigated. Preliminary findings have been summarized and reported (CREECH 1963; CREECH, MCARDLE and KRAMER 1963).

There are many gene mutations in maize which produce endosperm differences. Several genes have been shown to alter carbohydrate type and quantity during kernel development. The gene *wx* (waxy) increases sugars and water-soluble polysaccharides (WSP) in a su_1 (sugary-1) background and alone (ANDREW, BRINK and NEAL 1944). The effects of bt_1 (brittle-1) and bt_2 (brittle-2) were reported by CAMERON and TEAS (1954). Each gene increased sugar and reduced starch content at mid-development and beyond. No change in WSP was observed. LAUGHNAN (1953) reported similar effects at kernel maturity for sh_2 (shrunken-2). The sh_2 kernels contained a high percentage of sucrose and less starch than su_1, but had very little WSP. The double recessive su_1 sh_2 had even higher sugar and less starch than su_1. The WSP content was about the same as sh_2 alone (about 2%).

MANGELSDORF (1947) and CAMERON (1947) reported that in mature kernels the gene *du* (dull), when homozygous with su_1, produced less starch, more WSP, and perhaps more sugars than su_1 alone. HOROVITZ, MARCHIONI and FISHER (1941) reported that su_1 su_2 (sugary-1, sugary-2) produced about 14% sugar in mature kernels in contrast to about 5% for su_1 alone. DVONCH, KRAMER and WHISTLER (1951), DUNN, KRAMER and WHISTLER (1953), and CAMERON and COLE (1959) indicated that in mature kernels starch is lower and WSP is higher in su_1 *du* and su_1 su_2 than in su_1.

The genes *ae* (amylose-extender), su_2, and *du* have been reported to alter the proportion of the two starch fractions, amylose and amylopectin (KRAMER, PFAHLER and WHISTLER 1958; KRAMER and WHISTLER 1949; VINEYARD and BEAR 1952; VINEYARD, BEAR, MACMASTERS and DEATHERAGE 1958; ZUBER, GROGAN, DEATHERAGE and HUBBARD 1958). Amylose is a straight chain molecule and stains blue with iodine. Amylopectin, which has a higher molecular weight than amylose, consists of branched chains with about 24 glucose-units in each branch. WEATHERWAX (1922) was the first to show that waxy (*wx*) maize starch stained red with iodine. SPRAGUE, BRIMHALL and HIXON (1943) showed that the standard waxy endosperm contains no amylose but all amylopectin. CREECH and KRAMER (1961) demonstrated that *ae wx* pollen grains stain blue, indicating the presence of amylose or "amylose-like" polysaccharide.

[1] Paper No. 2973 in the journal series of The Pennsylvania Agricultural Experiment Station. This investigation was partially supported by National Science Foundation Grant GB-1876 and by U.S. Department of Agriculture Regional Research Funds.

According to ZIMMERMAN (1960) 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. Some authorities indicate the amylopectin may be formed by the rearrangement of preformed amylose chains (BADENHUIZEN 1963; PORTER 1962; WHELAN 1958; WHISTLER and YOUNG 1960). However, ERLANDER (1961) claims that the absence of amylose in waxy starch be accounted for by the absence of hypothetical debranching enzyme. PORTER (1962) suggests that there is insufficient evidence to conclude that either amylose or amylopectin is formed first, MANNERS (1962) suggests that, under nonequilibrium conditions, amylose and amylopectin can be synthesized at the same time, and that amylose is not necessarily a precursor of amylopectin. NELSON and RINES (1962) and NELSON and TSAI (1964) have found that *wx* endosperms probably lack starch-granule bound uridine diphosphate-glucose (UDPG) or adenosine diphosphate-glucose (ADPG) transferase activity that is present to a considerable degree in *Wx* endosperms. NELSON and RINES suggest that UDPG-transferase is necessary for amylose synthesis.

MATERIALS AND METHODS

Thirty genetic lines were obtained from DR. H. H. KRAMER, Purdue University in 1961. Their genotypes and phenotypes are shown in Table 1. They are single, double and triple recessives of *ae*, *du*, sh_2, su_1, su_2, and *wx*. All mutants were in a genetic background related to the single cross W23/L317, except Golden Cross Bantam sweet corn (su_1). *Normal* is the single cross W23/L317. The backgrounds are not isogenic; therefore, possible background effects must be kept in mind. A program is under way to establish these genotypes in common backgrounds. These lines were grown in a replicated trial in 1961 and fresh kernel composite samples of 50 grams were taken from 2 or 3 ears at 16, 20, 24, and 28 days after pollination and stored in 200 ml of 95% ethanol within 2 hours after removal from the plant.

Statistical analyses (analysis of variance and multiple correlations) were performed according to LECLERG, LEONARD, and CLARK (1962).

After all samples had been collected, each was processed to separate the alcohol-soluble from the alcohol-insoluble (AIS) fraction. The sample was blended for 30 seconds in a Waring Blender and poured into a 400 ml beaker. The sample was extracted for 45 minutes in hot 80% ethanol, then filtered by means of a vacuum funnel with Whatman 541H filter paper. The residue was resimmered 45 min in 150 ml 80% ethanol and again filtered. The first and second filtrates, which contained the sugars, were combined. The final residue was dried 18 hr in an oven at 70°C, placed in a dessicator for 30 minutes, weighed, and stored in a stoppered glass vial. The residue contained the water-soluble polysacchrides (WSP), starch, cellulose, protein, and other alcohol insoluble materials.

Dry matter content was determined by the weight of the alcohol insolubles plus the alcohol solubles. Chemical analyses were made for reducing sugars, sucrose, WSP, and starch.

Reducing sugars and sucrose were determined by the method of HASSID (1936, 1937) where sucrose is estimated from the difference between reducing sugars preceding and following enzymatic degradation with invertase. The method of CAMERON and COLE (1959) was used to determine the WSP content. The dried alcohol-insoluble residue, described above, was extracted three times for 30-minute periods with 10% ethanol, using a magnetic stirrer and filtering each time. Starch was determined from 3g samples of the remaining residue by a modification of the method of HIXON (1945). The procedure involved heating 3g samples of the 10% ethanol extracted residue in 300 ml of ammonium persulfate solution (0.3 g/100 ml water) for 90 minutes in a boiling water bath, filtering, drying the residue 18 hours at 70°C, and weighing. The difference between the weight of residue and the original sample represents the weight of starch originally present. Total carbohydrate content was estimated by summing the weights of the individual carbohydrates analyzed.

Starch granule size and weight determinations were made by plating a determined weight of granules from 28 day kernels, which had been washed with 80% ethanol, water, and acetone and dried, on a microscope slide. The granules were stained with iodine-KI solution for contrast and mounted in gelatin medium for stability. The number and diameter of the starch

TABLE 1

Phenotypes of kernels of 30 genotypes of maize

Genotype	Phenotype of kernel
normal	normal dent
ae	translucent, tarnished
du	opaque
sh_2	shrunken, opaque
su_1	wrinkled, glassy
su_2	slightly tarnished
wx	opaque
ae du	translucent
ae su_1	translucent
ae su_2	opaque
ae wx	semi-collapsed, translucent
du su_1	wrinkled, glassy
du sh_2	shrunken, opaque
du su_2	translucent
du wx	semi-collapsed, opaque
sh_2 su_1	shrunken, opaque
sh_2 su_2	shrunken, opaque
su_1 su_2	wrinkled, glassy
su_1 *wx*	wrinkled, glassy
su_2 *wx*	opaque
ae du su_1	wrinkled, translucent
ae du su_2	semi-collapsed, translucent
ae du wx	shrunken, opaque
ae su_1 su_2	wrinkled, translucent
ae su_1 *wx*	wrinkled, translucent
ae su_2 *wx*	wrinkled, translucent
du su_1 su_2	wrinkled, glassy
du su_1 *wx*	wrinkled, glassy
du su_2 *wx*	semi-collapsed, opaque
su_1 su_2 *wx*	wrinkled, glassy

granules were determined by counting the granules in six randomly selected areas 100 μ wide and measuring ten randomly selected granules in each area with a calibrated ocular micrometer. These sample measurements were done in triplicate and the mean was used to estimate the number of starch granules per microgram of starch and the average starch granule diameter in microns for each genotype.

RESULTS

Gene interaction for dry matter and carbohydrate contents: Significant differences were noted for all carbohydrate and dry matter analyses between genotypes at all kernel maturities. Highly significant differences were observed between maturities within genotypes for all characteristics measured. These data are presented in Table 2.

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_2 and su_2wx, tended to be significantly less than *normal* at

TABLE 2

The quantities of various carbohydrates and total dry matter† in entire kernels of 31 maize genotypes at four stages of development‡*

Code No.	Genotype	Kernel age (days)	Reducing sugars (%)	Sucrose (%)	Total sugar (%)	WSP (%)§	Starch (%)	Total carbohydrates (%)‖	Dry matter (%)
1	*normal*	16	9.4	8.2	17.6	3.7	39.2	60.5	15.7
		20	2.4	3.5	5.9	2.8	66.2	74.9	27.1
		24	1.6	2.6	4.8	2.8	69.2	76.1	37.2
		28	0.8	2.2	3.0	2.2	73.4	78.6	43.8
2	*ae*	16	8.6	21.9	30.6	5.7	20.8	57.2	18.4
		20	4.8	13.9	18.7	4.2	37.6	60.5	26.0
		24	3.1	8.3	11.4	3.7	48.9	64.0	34.0
		28	1.9	7.4	9.4	4.4	49.3	62.9	37.5
3	*du*	16	8.8	15.5	24.2	4.1	25.1	53.4	16.2
		20	4.8	10.5	15.3	2.7	44.6	62.6	25.6
		24	2.8	6.1	9.0	2.4	56.5	67.9	33.5
		28	1.3	6.7	8.0	1.9	59.9	69.8	38.9
4	sh_2	16	6.9	21.4	28.3	5.6	22.3	56.1	16.8
		20	4.9	29.9	34.8	4.4	18.4	57.6	20.3
		24	4.4	24.9	29.4	2.4	19.6	51.4	22.9
		28	3.6	22.1	25.7	5.1	21.9	52.8	26.3
5	su_1	16	9.2	16.5	25.7	14.3	23.3	65.3	19.9
		20	5.4	10.2	15.6	22.8	28.0	66.5	25.6
		24	3.6	9.5	13.1	28.5	29.2	70.8	30.5
		28	3.9	4.4	8.3	24.2	35.4	69.6	37.6
6	su_2	16	7.4	10.5	16.7	3.6	39.3	59.6	17.5
		20	3.5	9.2	12.7	3.1	50.7	61.8	24.9
		24	1.9	2.6	4.5	2.5	63.9	70.9	34.9
		28	1.4	1.9	3.3	1.9	64.6	69.8	43.6
7	*wx*	16	10.1	9.6	19.7	3.5	34.1	57.2	14.9
		20	3.5	5.2	8.7	2.3	53.3	64.6	23.9
		24	2.5	4.5	7.0	2.8	61.9	71.5	33.1
		28	1.6	1.7	3.3	2.2	69.0	74.5	37.3
8	*ae du*	16	8.7	19.9	28.6	6.5	28.6	63.7	20.0
		20	7.3	10.4	17.7	7.1	43.5	68.4	24.6
		24	4.6	6.8	11.4	7.4	51.4	70.2	27.9
		28	2.8	5.9	8.8	5.7	55.5	69.9	33.7
9	*ae* su_1	16	6.9	12.6	19.6	3.7	18.3	41.5	19.3
		20	3.7	8.3	12.0	3.6	29.3	44.9	24.8
		24	2.2	5.3	7.6	3.6	37.2	48.4	31.5
		28	2.1	5.3	7.4	3.2	34.4	45.1	33.9
10	*ae* su_2	16	12.2	31.4	43.6	4.4	14.1	62.1	16.9
		20	5.6	16.3	21.9	4.5	35.2	61.6	24.3
		24	3.6	13.5	17.1	4.3	37.6	59.1	28.8
		28	2.4	8.9	11.3	3.2	48.2	62.8	35.4
11	*ae wx*	16	6.1	23.8	29.9	4.2	19.7	53.9	18.3
		20	3.8	23.2	27.0	4.6	26.6	58.2	23.5
		24	3.9	17.9	22.4	5.6	37.1	64.9	25.0
		28	3.2	12.3	15.4	4.6	39.5	59.5	28.3
12	*du* su_1	16	5.3	17.6	22.9	13.3	21.5	57.7	18.5
		20	2.7	11.1	13.8	24.5	24.8	63.1	22.7
		24	2.5	7.3	9.8	29.5	23.6	62.8	26.9
		28	1.7	5.1	6.8	40.9	18.6	65.5	32.4

TABLE 2—Continued

Code No.	Genotype	Kernel age (days)	Reducing sugars (%)	Sucrose (%)	Total sugar (%)	WSP (%)§	Starch (%)	Total carbohydrates (%)‖	Dry matter (%)
13	*du sh₂*	16	10.7	33.9	44.7	4.1	8.8	57.6	16.6
		20	4.0	33.4	37.8	3.8	16.3	58.0	23.2
		24	2.3	27.1	29.4	5.3	20.9	55.6	24.8
		28	2.9	19.9	22.8	6.4	24.6	53.7	27.7
14	*du su₂*	16	5.1	21.7	26.8	3.3	27.1	57.2	19.5
		20	2.9	10.3	13.2	2.9	41.9	58.0	27.7
		24	1.8	6.8	8.6	3.4	47.1	59.0	32.9
		28	3.8	6.1	9.9	5.1	48.9	60.3	37.7
15	*du wx*	16	7.3	25.5	32.8	5.5	21.3	59.6	21.1
		20	4.1	15.8	19.9	12.2	34.3	66.4	25.7
		24	3.8	11.6	15.4	11.4	37.9	64.7	30.4
		28	3.0	9.5	12.5	11.6	45.4	69.5	34.8
16	*sh₂ su₁*	16	8.9	24.1	33.1	5.0	7.2	47.3	20.5
		20	8.1	25.4	33.5	4.9	11.7	50.1	23.8
		24	7.1	19.1	27.8	4.6	14.4	46.9	25.2
		28	5.7	20.1	24.5	4.9	15.7	45.4	24.6
17	*sh₂ su₂*	16	10.4	14.6	25.1	6.3	26.8	58.1	18.8
		20	4.0	8.5	12.6	9.5	38.3	60.3	28.3
		24	3.3	7.6	10.9	10.0	38.6	59.5	33.8
		28	2.5	6.8	9.3	13.6	35.1	57.9	38.3
18	*su₁ su₂*	16	4.9	16.8	21.8	33.7	11.9	67.5	20.1
		20	2.8	11.2	14.1	31.5	20.1	65.6	28.5
		24	2.4	9.6	12.0	31.0	20.5	63.5	31.1
		28	2.5	10.4	12.8	36.9	18.9	68.6	35.4
19	*su₁ wx*	16	4.4	14.7	19.1	19.5	28.1	66.6	21.9
		20	3.4	11.1	14.4	26.4	29.9	70.9	29.5
		24	2.6	7.5	10.1	29.1	32.8	71.9	35.0
		28	3.0	5.7	8.7	30.3	32.5	71.5	37.3
20	*su₂ wx*	16	6.1	12.3	18.4	3.4	30.1	51.8	17.9
		20	3.2	9.7	12.9	4.4	44.0	61.3	25.7
		24	1.5	7.1	8.5	3.5	62.6	74.7	37.3
		28	0.9	3.5	4.4	3.3	66.3	73.9	42.5
21	*ae du su₁*	16	12.8	24.6	37.3	9.6	23.6	70.5	17.3
		20	9.2	18.0	27.2	12.4	30.9	70.5	22.6
		24	4.7	15.5	21.3	16.1	32.7	70.0	25.8
		28	4.6	10.6	15.3	18.2	38.0	71.5	27.6
22	*ae du su₂*	16	7.7	21.7	29.4	7.6	30.8	69.9	22.4
		20	7.7	17.9	25.6	10.2	36.8	72.7	25.8
		24	6.8	10.4	17.2	10.2	45.0	72.4	31.7
		28	5.4	10.4	15.7	10.8	47.5	74.1	32.5
23	*ae du wx*	16	6.8	39.9	46.7	4.2	15.9	66.7	18.5
		20	4.1	34.6	38.7	3.6	26.6	68.9	24.6
		24	3.6	30.7	34.3	4.5	31.1	69.9	25.8
		28	4.4	23.7	28.1	4.9	32.0	65.1	24.5
24	*ae su₁ su₂*	16	8.5	23.2	31.7	6.6	23.8	62.0	20.3
		20	3.5	9.7	13.2	10.4	41.6	65.3	27.1
		24	2.7	7.9	10.6	10.6	39.6	61.1	31.5
		28	2.4	8.6	11.0	11.0	41.0	65.9	34.1
25	*ae su₁ wx*	16	8.0	28.2	36.2	4.5	22.0	62.7	16.3
		20	5.2	21.9	27.0	8.4	30.7	66.0	21.7

TABLE 2—Continued

Code No.	Genotype	Kernel age (days)	Reducing sugars (%)	Sucrose (%)	Total sugar (%)	WSP (%)§	Starch (%)	Total carbohydrates (%)‖	Dry matter (%)
		24	3.5	15.0	18.5	12.2	38.5	69.1	25.8
		28	2.8	11.1	13.9	12.4	38.3	64.5	26.2
26	*ae* su_2 *wx*	16	10.3	22.2	32.4	5.1	18.0	55.5	16.4
		20	7.9	17.1	25.1	5.9	40.3	71.3	19.4
		24	4.0	16.4	20.4	5.9	41.7	67.9	27.0
		28	3.2	12.6	15.8	5.0	49.6	70.4	28.4
27	*du* su_1 su_2	16	7.3	16.4	26.9	21.8	19.4	68.1	20.7
		20	4.1	12.3	16.4	31.9	21.2	69.4	26.5
		24	2.9	7.3	10.2	34.9	24.9	70.1	31.6
		28	2.4	5.4	7.8	34.8	22.8	65.5	33.9
28	*du* su_1 *wx*	16	5.9	16.8	21.7	24.4	14.7	60.8	22.0
		20	3.2	10.2	13.4	36.1	21.4	70.9	27.9
		24	2.9	7.8	10.7	38.4	17.5	66.6	33.4
		28	2.3	6.7	9.0	47.5	15.9	72.3	35.3
29	*du* su_2 *wx*	16	9.2	25.7	34.9	4.6	17.2	53.4	15.2
		20	5.2	19.5	24.7	14.8	24.7	64.2	20.8
		24	3.0	10.6	13.3	14.3	33.9	61.5	27.9
		28	2.7	8.9	11.6	16.7	38.1	64.5	30.7
30	su_1 su_2 *wx*	16	6.5	19.6	26.1	22.1	11.9	60.1	18.5
		20	3.4	15.1	18.5	33.9	13.8	66.2	24.8
		24	2.8	11.4	14.3	38.9	16.2	69.3	32.0
		28	2.1	10.6	12.7	40.1	18.2	71.0	34.6
31	Golden Cross Bantam (su_1) Sweet Corn	16	8.1	15.4	23.6	7.8	28.7	60.1	16.1
		20	3.2	5.5	8.7	27.0	35.5	71.3	26.8
		24	1.9	3.9	5.9	33.3	38.5	77.7	33.5
		28	1.6	1.9	3.6	34.8	33.9	72.3	37.0
Least significant difference, genotypes within ages									
	5%	..	3.2	10.4	10.9	10.4	14.2	15.3	2.9
	1%	..	4.2	13.9	14.5	13.9	18.8	20.4	3.9
Least significant difference, ages within genotypes									
	5%	..	2.4	6.0	5.8	4.8	7.6	7.5	2.9
	1%	..	3.2	7.9	7.7	6.3	10.1	9.9	3.8

* Percent of dry matter.
† Percent of fresh weight.
‡ Three replications.
§ WSP = water-soluble polysaccharides.
‖ Sum of weights of reducing sugar, sucrose, WSP, and starch/dry matter weight.

most stages of development. The genotypes that were exceptionally low in dry matter were *sh*$_2$, *ae wx*, *du sh*$_2$, *sh*$_2$ *su*$_1$, *ae du su*$_1$, *ae du wx*, *ae su*$_1$ *wx*, and *ae su*$_2$ *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 quantities of particular carbohydrates reported here as percent-

ages of dry matter. Actual weights and percentages of fresh weights have been omitted 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. All other genotypes possessed approximately the same amount of reducing sugars as *normal* except *sh*$_2$, *su*$_1$, *ae wx*, *du wx*, *sh*$_2$ *su*$_1$, *ae du su*$_1$, *ae du su*$_2$, and *ae du wx*. These appeared to be higher in reducing sugars than *normal*. Of these, *sh*$_2$, *su*$_1$, *ae du su*$_1$, and *ae du su*$_2$ 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. The genotypes *sh*$_2$, *du sh*$_2$, *sh*$_2$ *su*$_1$, and *ae du wx* were exceptionally high in sucrose (7 to 10 times *normal*) at almost all kernel ages. The genotypes *ae*, *du*, *su*$_1$, *ae du*, *ae su*$_1$, *ae su*$_2$, *du su*$_2$, *du wx*, *sh*$_2$ *su*$_2$, *su*$_1$ *wx*, *ae su*$_1$ *su*$_2$, *du su*$_1$ *wx*, and *du su*$_2$ *wx* had 2 to 4 times as much sucrose as *normal*. The genotypes *ae wx*, *su*$_1$ *su*$_2$, *ae du su*$_1$, *ae du su*$_2$, *ae su*$_1$ *wx*, *ae su*$_2$ *wx*, and *su*$_1$ *su*$_2$ *wx* had 5 to 6 times as much sucrose as *normal*. The gene *su*$_2$ appears to be epistatic to *sh*$_2$ and *sh*$_2$ seems to be partially epistatic to *du*, *su*$_1$ and *wx*. The gene *ae* appears epistatic to *su*$_1$. A pattern of epistasis is presented in Figure 1. The arrow points to the epistatic gene.

Water-soluble polysaccharides: The WSP content of *normal* was 3.7% at 16 days and 2.2% at 28 days. Apparently WSP was not accumulating during kernel development. A slight, but insignificant, increase over normal was noted for *ae*, *sh*$_2$, *ae du*, *ae wx*, *du sh*$_2$, *du wx*, *sh*$_2$ *su*$_1$, *ae du su*$_2$, *ae du wx*, *ae su*$_1$ *wx*, *du su*$_2$ and *ae su*$_2$ *wx*. Significant increases over *normal* were noted for *su*$_1$, *du su*$_1$, *sh*$_2$ *su*$_2$, *su*$_1$ *su*$_2$, *su*$_1$ *wx*, *ae du su*$_1$, *ae su*$_1$ *su*$_2$, *du su*$_1$ *su*$_2$, *du su*$_1$ *wx*, *du su*$_2$ *wx*, *and su*$_1$ *su*$_2$ *wx*. The gene *su*$_1$ is associated with a dramatic increase in WSP at all four stages of kernel development. The genes *ae* and *sh*$_2$ apparently are epistatic or partially epistatic to *su*$_1$ and *su*$_1$ is epistatic to *du*, *su*$_2$, and *wx*. The genes *du* and *su*$_2$ appear to intensify the accumulation of WSP in combination with the other genes. A pattern for epistasis is shown in Figure 2.

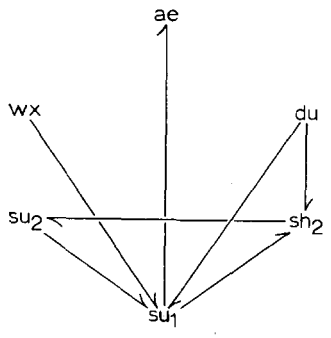

FIGURE 1.—Pattern of epistasis for six genes conditioning sucrose accumulation. The arrow indicates the epistatic gene.

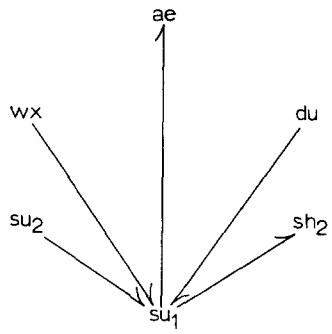

FIGURE 2.—Pattern of epistasis for six genes conditioning water-soluble polysaccharide accumulation. The arrow indicates the epistatic gene.

Starch: The starch content in *normal* increased from 39.2% at 16 days to 73.4% at 28 days. Extreme starch reduction (approximately one half or less of *normal*) was associated with the genotypes sh_2, su_1, *ae* su_1, *du* su_1, *du* sh_2, sh_2 su_1, sh_2 su_2, su_1 su_2, su_1 *wx*, *ae du wx*, *du* su_1 su_2, *du* su_1 *wx*, and su_1 su_2 *wx*. In general, kernels high in sugar are low in starch. The gene sh_2 appears epistatic to *du*, su_1, and *wx*. The gene su_2 appears epistatic to sh_2. The pattern for epistasis is the same as that presented in Figure 1 for sucrose accumulation.

Total sugar and total carbohydrates: Total sugar content is the sum of the reducing sugars and sucrose contents. Total carbohydrates content is the sum of the contents of all the carbohydrates analyzed. It is important to note that these data do not include maltose and other types of sugars. Total sugar and total carbohydrate values are presented for reference. 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.

Correlations: Symmetric correlations of all variables for 31 genotypes are presented in Table 3. These calculations were made on data from analyses of kernel samples at 16, 20, 24, and 28 days after pollination. All variables measured were either positively or negatively correlated. All correlations except one were highly significant. Total sugar, reducing sugar, and sucrose contents were negatively correlated with dry matter and starch content, indicating sugars are precursors of starch. AIS was positively associated with dry matter content. A correlation value of −0.81 between sucrose content and AIS 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.

Starch granule estimates: Starch granule weight and diameter estimates are shown in Table 4. The granules of *ae*, *du*, sh_2, and su_1 appeared to be smaller than *normal*. It was observed that *ae* granules are irregular in shape and *normal* granules are spherical, which agrees with the findings of WOLF, SECKINGER, and DIM-

TABLE 3

Symmetric correlation matrix of seven variables† expressed as percentages on the basis of dry weights

Variable	1	2	3	4	5	6
1. Dry matter	...					
2. Reducing sugar	−0.76**	...				
3. Sucrose	−0.62**	0.46**	...			
4. Total sugars	−0.72**			...		
5. AIS‡	0.73**	−0.60**	−0.81**	−0.87**	...	
6. WSP	0.17**	0.18**	0.22**	−0.24**	0.12*	...
7. Starch	0.56**	−0.45**	−0.58**	−0.61**	0.72**	−0.47**

* Significant at 5% level (r>0.11). ** Significant at 1% level (r>0.15).
† Individual data of 31 genotypes at four maturities in three replications (N=372).
‡ Alcohol insolubles (80% ethyl alcohol).

TABLE 4

*Mean starch-granule weight and diameter estimates in normal and mutant kernels at 28 days after pollination**

Genotype	Mean number of granules per microgram of starch	Mean diameter in microns
normal	1036.7	10.2
ae	1167.5	8.6
du	1534.6	8.1
sh_2	2206.3	5.9
su_1	7023.3	3.7
su_2	1571.1	11.0
wx	1224.1	13.2

* Three replications.

LER (1964). Granules from su_1 were aggregated in clusters and some very small unstained granules were observed. It appears that specific gene mutations are not only causing gross changes in the carbohydrate content of the endosperm but are also influencing starch granule deposition. This is especially true of su_1.

DISCUSSION

The gene sh_2 apparently causes a block between the sugars and the polysaccharides as evidenced by a dramatic increase in sugars as reported by LAUGHNAN (1953). The mutation su_1 is associated with a significant increase in WSP, as reported by CULPEPPER and MAGOON (1924).

The mutations *ae*, *du*, su_2, and *wx* have been of interest to maize geneticists for several years because of their effects on the proportions of amylose and amylopectin starch. These effects have led some workers to propose that the enzymes coded for by these genes were principally concerned with directing the proportions of straight and branched chain molecules within the starch fraction (ERLANDER 1961; WHELAN 1958; WHISTLER and YOUNG 1960). However, our data indicate that this is not the case. The mutant *ae* alone, in addition to changing the amylose content, also causes a substantial increase in sugars and reduction in starch. In addition, *ae* combined with *wx* and *du wx* causes a dramatic increase in sugars and reduction in starch.

The amylose and amylopectin data (KRAMER, PFAHLER and WHISTLER 1958) shown in Table 5, combined with the effects of *ae* and *wx* on sugar and polysaccharide content as shown in Table 2, strongely indicate that *ae* and *wx* are in separate pathways of starch synthesis. This suggests that *ae* is associated with a metabolic block between the sugars and the branched chain polysaccharides and *wx* is associated with a block between the sugars and the straight chain polysaccharides. NELSON and RINES (1962) have reached a similar conclusion concerning *wx*. There is evidence that *du* and su_2 are in separate pathways for the synthesis of branched chain polysaccharides.

The fact of genetic control of polysaccharide and starch synthesis in maize endosperm is amply evident from the preceding data. A detailed chemical analysis is now required and this work must be done before a secure scheme of synthesis

TABLE 5

*Amylose content of mature kernels of 24 genotypes of maize**

Gene combination	Amylose in starch (percent)
normal	27
ae	61
du	38
su_1	29
su_2	40
wx	0
ae du	57
ae su_1	60
ae su_2	54
ae wx	15
du su_1	63
du su_2	47
du wx	0
su_1 su_2	55
su_1 *wx*	0
su_2 *wx*	0
ae du su_1	41
ae du su_2	48
ae su_1 su_2	54
ae su_1 *wx*	13
du su_1 su_2	73
du su_1 *wx*	0
du su_2 *wx*	0
su_1 su_2 *wx*	0

* Adapted from KRAMER, PFAHLER and WHISTLER (1958).

can be presented. It is felt that such studies will provide the necessary evidence for evaluating the relative order and importance of specific synthetic steps within the intact scheme. The interactions of multiple mutations on pathways of starch synthesis offer a further refinement for analyzing the actual pathways involved. Physical studies of the starch granules of the various mutants are under way as an additional tool in evaluating the influence of particular mutations on the synthesis and deposition of starch.

Research has also been initiated to assay enzyme activity and to define in detailed physical and chemical terms the nature and quantities of the carbohydrates in the various mutants.

The significant increase in sugars and sugar retention of certain genotypes suggets possible use in sweet corn quality improvement. The reduction in starch by the gene *ae* poses a severe problem in the breeding of "amylo-maize" strains (high amylose starch). The specific applications of these genes in corn improvement for particular purposes will be discussed elsewhere.

SUMMARY

The effects of the genes *ae*, *du*, sh_2, su_1, su_2 and *wx* have been determined

singly and in combination on qualitative and quantitative changes in carbohydrates in maize endosperm during kernel development. Very significant differences were noted between genotypes for dry matter, reducing sugars, sucrose, water-soluble polysaccharides and starch content at four stages of kernel development. Simplified schemes for polysaccharide synthesis are discussed.

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