Characterization of Starch Structures of 17 Maize Endosperm Mutant Genotypes with Oh43 Inbred Line Background[1]

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ABSTRACT

The characteristics of starches from 17 endosperm mutant genotypes in a common Oh43 inbred background were examined by gel-permeation chromatography (GPC), iodine affinity (IA), and scanning electron microscopy (SEM). The chain-length distributions of amylopectins were determined by an enzymatic-chromatographic method. Each genotype exhibited distinctive GPC elution patterns of its native and isoamylase-debranched starches and distinctive morphology as noted by SEM. The amylose-extender (*ae*), dull-1 (*du1*), and sugary-1 (*su1*) genes were associated with increased amounts of amylose and intermediate fractions compared with normal starch. The waxy (*wx*) gene was epistatic to other genes relative to the accumulation of amylopectin, which was consistent with work done elsewhere. The discrepancy in amylose percentage determined by GPC and IA in some genotypes may have resulted from the presence of a large amount of intermediate materials in those genotypes, which could not always be distinguished from amylose by the IA method. For example, in *ae* starch, most of the intermediate materials were measured as amylose by the IA procedure, whereas in *du1, ae* brittle-1 (*bt1*), and *ae du1* starches, most of the intermediate materials were excluded from IA measurements. The intermediate fractions from each genotype in the GPC elution profiles also differed from each other, suggesting differences in molecular weight and/or branching. The proportions of long B chains and the average chain length of amylopectins were increased when the *ae* gene was present. In contrast, the *du1* gene decreased the proportions of the long B chains of amylopectins. The mutants containing the *ae* gene showed low degrees of branching in amylopectin; mutants containing the *du1* and/or *su1* genes had high degrees of branching. Genetic background played a major role in determining the fine structure of starch components. The effects of interactions between recessive mutant genes on the structures and morphology of different starch genotypes were evident.

Normal maize (*Zea mays* L.) starch is composed primarily of essentially linear (amylose) and branched (amylopectin) components. The amylose content in normal maize starch ranges from 25 to 30% but can vary among cultivars and especially with the presence of mutant genes.

Many recessive mutant genes of maize have been identified that alter the quality and quantity of starch in the endosperm. The amylose-extender (*ae*) genotype has a high apparent amylose content ranging from 50 to 80% (Banks and Greenwood 1975). In contrast, the waxy (*wx*) gene is epistatic to all other known recessive genes for blocking the accumulation of amylose (Ikawa et al 1981; Yeh et al 1981; Inouchi et al 1983, 1987, 1991; Boyer and Liu 1985). The brittle-1 (*bt1*) mutant increased sugar content at the expense of starch accumulation (Creech 1965), and the shrunken-2 (*sh2*) genotype reduced the starch content to about 30% of the normal amount and dramatically increased the sucrose content (Holder et al 1974). The sugary (*su*) mutant synthesizes and accumulates a highly branched polysaccharide, phytoglycogen, to 25% or more of the kernel dry weight (Shannon and Garwood 1984). In other work, Yeh et al (1981) reported 55% amylose in the dull (*du*) starch from a sweet corn background.

Moreover, additional variations can be generated when several recessive genes are combined. Fuwa et al (1987) found the fine structure of amylopectins to be affected by the recessive gene (*ae* or *du*) coupled with the *wx* gene. Holder et al (1974) reported that the presence of *sh2* in multiple recessive genotypes inhibited the effect of the *ae* gene at increasing amylose content.

Elucidation of the fine structure of amylopectin is the subject of many current investigations, involving measurements such as average chain length, the A-B chain ratio, and the chain length of the exterior and interior chains. Fuwa et al (1987) showed that, although the *wx* gene was epistatic to others for amylopectin production, the fine structure of the starches varied with the presence of other recessive genes. Inouchi et al (1983) proposed that the fine structure of amylopectin was under genetic control. For example, the *ae wx* starch had increased long B chains and decreased short B chains, and the *du wx* starch had decreased long B chains and increased short B chains compared with starch of the *wx* genotype.

A potentiometric titration method measuring iodine affinity (IA) has long been employed to determine the amylose content in the endosperm starch. The estimated amylose is termed *apparent amylose* because the occurrence of short chain-length amylose underestimates the amylose content and amylopectin with long external chains overestimates the amylose content (Shannon and Garwood 1984). More recently, gel-permeation chromatography (GPC) has been employed to elucidate the profiles of starch components. The elution profile of native starch disclosed that there was no sharp separation between amylopectin and amylose (Yeh et al 1981, Boyer and Liu 1985). An intermediate material, consisting of a branched molecule with a molecular weight lower than that of amylopectin, was present and eluted between amylopectin and amylose fractions. Whistler and Doane (1961) obtained intermediate materials ranging from 4.5% of the total starch amount for normal commercial corn starch to 6.6–8.7% for high-amylose corn starches. They demonstrated that the properties of the intermediate fractions from different starch types were similar to each other and were between those of amylose and amylopectin. The presence of intermediate materials in amounts greater than just a few percentage points usually is associated with the presence of the *ae* gene (Whistler and Doane 1961; Ikawa et al 1978, 1981; Yeh et al 1981; Inouchi et al 1983; Baba and Arai 1984). The intermediate materials of amylomaize (50% apparent amylose) were characterized as having four or five branches with an average chain length (CL) of approximately 50 glucose units, which were linked to a main linear chain containing 100–150 glucose units (Baba and Arai 1984).

The structure of starch should be considered at both molecular and granular levels (Banks et al 1973). It is the physical and mechanical properties of granular starch that determine many industrial applications. Many researchers have studied elucidating the morphology of various starches using light microscopy (LM) (Alsberg 1938, Badenhuizen 1965). Because starch granules are translucent crystals, they often give images that are difficult to define with LM. The greatest error comes from the diffraction effect of light, which may make the interpretation of the internal and surface structures of starch granules difficult. With scanning electron microscopy (SEM), only the surface structure of the starch granule is revealed. Hall and Sayre (1969, 1970, 1971)

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studied the shapes and surface structures of various starches using SEM, demonstrating the advantages of SEM in determining the shape and detailed surface characteristics of starch granules.

Although much information is now known about the effects of some recessive mutant genes of maize on starch properties, few studies have comprehensively evaluated of the visual and structural properties of the starch granules from many mutant genotypes in a common maize background. The objectives of this study were to characterize the structure and morphology of starches from 17 maize endosperm mutant genotypes in a common Oh43 inbred background to help in understanding the influences of recessive mutant genes on the maize starches.

MATERIALS AND METHODS

Mature kernels of Oh43 inbred (normal) and its single and double mutants (Table I) were harvested from a summer nursery near Ames, IA, in 1991. Development of the genotypes and sampling and storing of the kernels have been previously described (Wang et al, 1992).

Isolation and Preparation of Starch Samples

Starches were isolated according to a small-scale wet-milling procedure described by White et al (1990). Approximately 5 g of kernels from each genotype was used for starch extraction.

After extraction from the kernels, starch was preliminarily defatted by refluxing in 85% methanol for 24 hr and dried at 45°C overnight. Defatted starch granules were then dispersed in 90:10 (v/v) dimethyl sulfoxide-deionized water and stirred in a boiling water bath for 1 hr and at room temperature for another 24 hr to ensure complete dispersion.

GPC

GPC of native starch. Seventy-five milligrams of starch was precipitated from 1.5 ml of dimethyl sulfoxide-dispersed starch solution (50 mg of starch per milliliter) with 10 volumes of absolute ethanol and was collected by centrifugation at 8,700 × *g* for 20 min at 4°C. Precipitated starch was redissolved in 25 ml of boiling water, stirred in a boiling water bath for 30 min, and filtered through Whatman No. 1 filter paper for further purification.

Five milliliters of starch solution (containing 15 mg of starch

TABLE I
Percent Compositions of Polysaccharides[a,b] and Chain Length Distribution[b] of Starches from 17 Mutant Genotypes of Oh43 Inbred

	GPC,[d] Native			GPC, Debranched						
								CL[k] at Peak of		Intermediate
Genotype[c]	Fraction I[e] (%)	Fraction II[f] (%)	IA[g] (%)	Fraction I[h] (%)	Fraction II[i] (%)	Fraction III[j] (%)	Fraction III: Fraction II	Fraction II[i]	Fraction III[j]	Materials[l] (%)
Normal	70.1	29.9	27.8	26.7	19.2	54.1	2.8	43	15	3.2
	(0.6)	(0.6)	(1.1)	(0.3)	(0.1)	(0.1)	(0)	(2)	(1)	
ae	38.7	61.3	56.4	46.0	27.2	26.8	1.0	48	20	15.3
	(0.1)	(0.1)	(1.1)	(0.6)	(0.2)	(0.4)	(0)	(1)	(1)	
btl	73.3	26.7	23.2	24.9	18.9	56.2	3.0	42	14	1.8
	(1.3)	(1.3)	(0.8)	(0.7)	(0.6)	(0.1)	(0.1)	(3)	(0)	
bt2	73.2	26.8	26.9	24.7	22.1	53.2	2.4	41	15	2.1
	(0.6)	(0.6)	(0)	(0.6)	(0.3)	(0.3)	(0)	(1)	(0)	
dul	54.3	45.7	31.4	30.5	10.6	58.9	5.6	51	14	15.2
	(0.1)	(0.1)	(0.4)	(0.2)	(0.4)	(0.3)	(0.2)	(1)	(0)	
h	71.3	28.7	26.4	28.1	19.2	52.7	2.8	41	15	0.6
	(2.5)	(2.5)	(0.8)	(0.1)	(1.0)	(1.1)	(0.2)	(1)	(1)	
sh2	72.6	27.4	28.8	30.1	14.7	55.2	3.8	40	13	−2.7
	(0.6)	(0.6)	(0)	(0.6)	(0)	(0.6)	(0.1)	(3)	(1)	
sul	59.5	40.5	37.4	31.2	12.3	56.5	4.6	41	14	9.3
	(0.4)	(0.4)	(1.5)	(0.6)	(0.1)	(0.7)	(0.1)	(4)	(0)	
wx	100.0	0	0	0	27.7	72.3	2.6	39	15	0
	(0)	(0)	(0)	(0)	(0.4)	(0.4)	(0)	(1)	(1)	
ae btl	45.1	54.9	30.5	32.4	24.5	43.1	1.8	49	16	22.5
	(0.1)	(0.1)	(0.1)	(0)	(0.4)	(0.4)	(0)	(2)	(1)	
ae dul	23.8	76.2	57.8	57.3	19.8	22.9	1.2	49	19	18.9
	(0.1)	(0.1)	(1.4)	(0.1)	(0.1)	(0.1)	(0)	(0)	(1)	
dul sul	53.8	46.2	39.9	34.5	13.0	52.5	4.0	47	13	11.7
	(0.4)	(0.4)	(0.7)	(1.3)	(0.4)	(0.9)	(0.1)	(1)	(0)	
h sh2	70.5	29.5	25.4	26.4	20.0	53.6	2.7	44	14	3.1
	(0.4)	(0.4)	(0.1)	(0.2)	(0.2)	(0.4)	(0)	(2)	(0)	
h wx	100.0	0	0	0	29.2	70.8	2.4	43	16	0
	(0)	(0)	(0)	(0)	(0.1)	(0.1)	(0)	(2)	(1)	
sh2 btl	71.7	28.3	27.6	27.7	15.4	56.9	3.7	49	13	0.6
	(0.2)	(0.2)	(0.4)	(0.2)	(0)	(0.1)	(0)	(1)	(0)	
sh2 wx	100.0	0	0	0	30.6	69.4	2.3	42	13	0
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(2)	(1)	
wx dul	100.0	0	0	0	26.3	73.7	2.8	39	15	0
	(0)	(0)	(0)	(0)	(0.4)	(0.4)	(0)	(0)	(0)	

[a] The division of each fraction is described in Materials and Methods.
[b] Values are the average of two separate determinations, with SD in parentheses.
[c] *ae* = Amylose extender; *bt* = brittle; *du* = dull; *h* = horny; *sh* = shrunken; *su* = sugary; and *wx* = waxy.
[d] Gel-permeation chromatography.
[e] Amylopectin.
[f] Amylose and intermediate materials.
[g] Amylose percentage calculated from iodine affinity.
[h] Amylose.
[i] Long B chains of amylopectin.
[j] A and short B chains of amylopectin.
[k] Average chain length of isoamylase-debranched starch measured at the apex of the peak from each fraction. Expressed as number of glucose units.
[l] Calculated as the difference between fraction II of the native starch and fraction I of the debranched starch GPC elution profiles.

and 0.8 mg of glucose marker) was loaded onto a Pharmacia column (2.6 i.d. × 79 cm) packed with Sepharose CL-2B gel (Pharmacia LKB Biotech., Uppsala, Sweden). The procedure followed that of Jane and Chen (1992), except that the column was eluted with degassed 20 m*M* NaCl solution adjusted to pH 11 with 1*N* NaOH in the ascending mode with a flow rate of 30 ml/hr. Fractions of 4.9 ml of effluent were collected every 9.5 min and subjected to total carbohydrate and amylose content analyses by the anthrone-sulfuric acid method (Wright and Gann 1966) and the iodine staining tests (Juliano 1971), respectively. The minimum value from iodine staining was used to identify the end of the eluted amylopectin fraction; thus, the starch profile could be identified as to amylopectin and amylose fractions. The amylose percentage was calculated by dividing the amount of starch under the amylose peak by the total starch in all fractions according to Jane and Chen (1992). Starch from each genotype was fractionated twice, and the values from the data were averaged. Replicates were very similar to one another.

GPC of isoamylase-debranched starch. The starch was prepared, debranched, and fractionated on a Bio-Gel P-6 column (Bio-Rad Laboratories, Richmond, CA) by the method of Jane and Chen (1992), except that native starch was used instead of amylopectin. Crystalline *Pseudomonas* isoamylase was used (Hayashibara Shoji, Inc., Olayama, Japan).

Fractions (2.3 ml) from the elution were collected and assayed for total carbohydrate by the anthrone-sulfuric acid method (Wright and Gann 1966). The eluted materials were separated into three fractions with divisions made at minimum points according to the total carbohydrate values. Figure 1A shows the elution pattern and fractions of normal starch. The waxy starches, containing 100% amylopectin, consisted only of fractions II and III. Three portions (2.3 ml each) of effluent at the peaks of fractions II and III were assayed for total carbohydrate using the phenol-sulfuric acid method (Dubois et al 1956) and for reducing value using a modified Park-Johnson method (Hizukuri et al 1981, Jane and Chen 1992). The average CL of debranched amylopectin was calculated by dividing total carbohydrate by its reducing value.

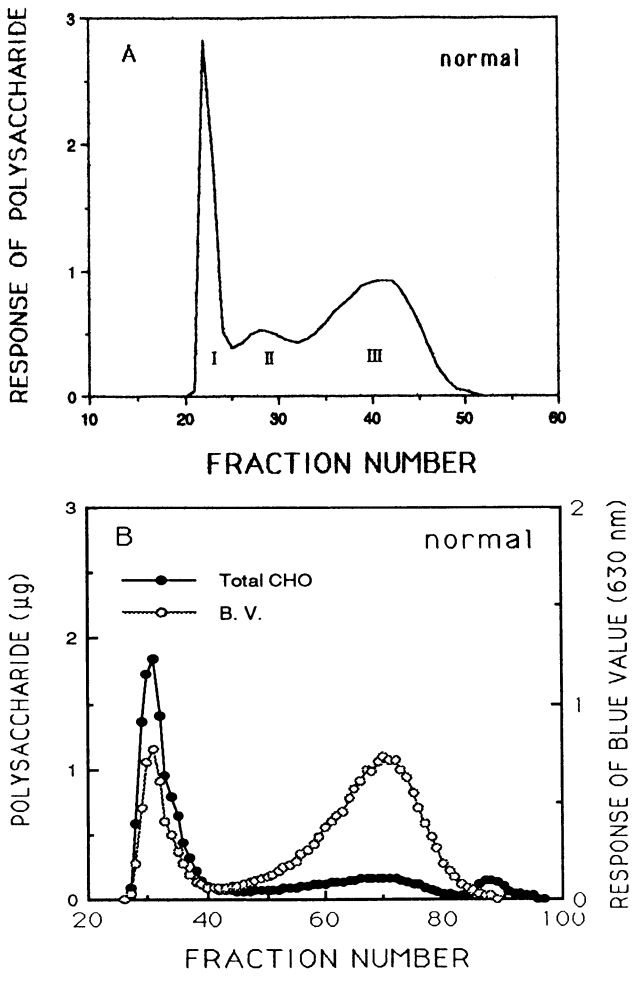

Fig. 1. Gel-permeation chromatography elution profile of Oh43 starch. **A,** Isoamylase-debranched starch. Fraction I was composed of amylose, fraction II included long B chains of amylopectin, and fraction III contained A and short B chains of amylopectin. **B,** Native starch.

IA

Iodine affinities for the defatted starches, expressed as milligrams of iodine bound to 100 mg of starch, were determined in duplicate with amperometric titration (Schoch 1964) at 30°C and the results were averaged. Amylose percentages were extrapolated from the inflection points and calculated by assuming that pure maize amylose has an iodine affinity of 19.0%.

SEM

Each starch sample was stirred carefully to obtain a homogeneous mixture from which a small amount of sample was removed for SEM. Starch granules were sprinkled onto double-stick tape attached to specimen stubs and coated with gold-palladium. The mounted specimens were examined with a JEOL JSM-35 scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 10 kV. Representative micrographs were taken on each genotype at a magnification of ×1,000. The starch granule diameter was estimated by averaging the largest dimension of 15 random starch granules from duplicate micrographs for each starch type.

Statistical Analyses

Duplicate results were obtained and analyzed for correlations among percent compositions and chain-length distribution of polysaccharides with the SAS program (SAS Institute 1990).

RESULTS AND DISCUSSION

Characteristics of Native Starches

The elution profiles of native starches presented in Figures 1B, 2, and 3 show the total carbohydrate content and the blue value response derived from the iodine staining test. In the elution profiles, the first peak (fraction I) corresponds to amylopectin, which, because of its large molecular weight, was excluded from the gel and appeared at the void volume. The second peak (fraction II) is considered to be amylose, and the peak at fraction 88 is glucose, added to mark the end of the elution. Intermediate material eluted between fractions I and II was detected either as a small hump or, more likely, by an elevated baseline between fractions I and II that did not allow clear separation between the two fractions.

The normal starch exhibited typical amylopectin and amylose peaks (Fig. 1B). There was no baseline separation between amylopectin and amylose, which also was observed by Yeh et al (1981) and Boyer and Liu (1985), but the amount of intermediate materials was very little. The *ae* starch (Fig. 2A) had a highly elevated baseline between fractions I and II, indicating a large proportion of intermediate materials, and the blue value response was relatively high in the amylose region. The elution profiles of *bt1, bt2,* and horny (*h*) starches (Figs. 2B, C, and E) were similar to that of normal starch. The *du1* starch (Fig. 2D) contained an amount of intermediate materials similar to that of *ae* starch; however, the blue value response in the amylose region was less than that of *ae* starch. A large amount of intermediate materials in *du1* starch in a dent corn inbred (W64A) also was observed by Boyer and Liu (1985), but Yeh et al (1981) did not observe this in a sweet corn background (Ia5125). It is likely that the genetic background plays a role in determining the fine structure of starch components. The chromatogram of the *sh2* starch (Fig. 2F) was similar to that of normal starch at the amylose portion but had a higher blue value response at the amylopectin portion than did normal starch. Although *su1* starch (Fig. 2G) contained less amylose than did *du1* starch as

measured by GPC of the native starch, its blue value response was greater than that of the *du1* starch, indicating the amylose portion of *su1* starch bound more iodine than did the amylose portion of *du1* starch. There was no amylose peak observed in the profile of *wx* starch (Fig. 2H), indicating the presence only of amylopectin.

An amylopectin peak and a broad, two-component polysaccharide response in the region normally associated with amylose were noted in the profile of *ae bt1* starch (Fig. 3A). The amylose percentage of *ae bt1* starch (54.9% when calculated according to the GPC data) was overestimated because it included both intermediate and amylose components as noted in the Materials and Methods section. Similarly, the *ae du1* and *du1 su1* starches (Fig. 3B and C) contained a large amount of intermediate materials as evidenced by the elevated baseline between fractions I and II. High proportions of intermediate fractions in the elution profiles of *ae du* and *du su2* also were reported by Yeh et al (1981). The blue value response of *ae du1* in the region of amylose was the greatest among all the starches, indicating the high amylose content of *ae du1* starch. The elution profiles of *h sh2* and *sh2 bt1* starches (Fig. 3D and F) were similar to that of normal starch for polysaccharide response, but the blue value response of *sh2 bt1* starch was higher in the amylopectin region and lower in the amylose region than was that of normal starch. A small response of blue value was noted for amylose in the profile of *h wx* starch (Fig. 3E), suggesting the presence of low-molecular-weight (LMW) molecules in *h wx* starch. Starches from the double-mutant genotypes of *sh2 wx* and *wx du1* (Figs. 3G and H) exhibited elution profiles similar to that of the *wx* starch.

The amylose percentages of starch from the 17 genotypes, determined from fraction II of GPC profiles of native starches, are summarized in Table I. The normal starch contained 29.9% amylose, which is in the range (25–30%) usually found in normal maize starch. The amylose contents of *bt1*, *bt2*, *h*, and *sh2* starches were within the range of that of normal starch. The single- and double-mutant genotypes containing *ae*, *du1*, and *su1* (except when present with the *wx* gene) exhibited amylose percentages higher than that found in normal maize, which agrees with previous reports (Yeh et al 1981; Inouchi et al 1983, 1987; Boyer and Liu 1985). The *ae* starch had the highest amylose percentage, 61.3%, among single mutants. The *du1* and *su1* genotypes produced starch with 45.7 and 40.5% amylose, respectively, amounts less than those reported by Yeh et al (1981). Boyer and Liu (1985) reported that dent corns produced mutants with more amylose content than did sweet corns. Their findings were in contrast to results shown in the present study in which amylose contents of *du1* and *su1* starches from a dent background were less than those from a sweet corn background studied by Yeh et al (1981). Results from these two research groups and from our work suggest that the genetic background produces specific variations in the starch properties of maize mutants.

The waxy genotype and the double mutants containing the *wx* gene (*h wx*, *sh2 wx*, and *wx du1*) produced starches consisting of 100% amylopectin, which is in agreement with previous reports that the *wx* gene blocked the accumulation of amylose (Ikawa et al 1981; Yeh et al 1981; Inouchi et al 1983, 1987, 1991; Boyer and Liu 1985). When the *ae* gene was present with another gene, the amylose percentages of the combined mutants increased considerably. The double-mutant combinations of *ae bt1* and *ae du1* resulted in higher amylose percentages (54.9 and 76.2%,

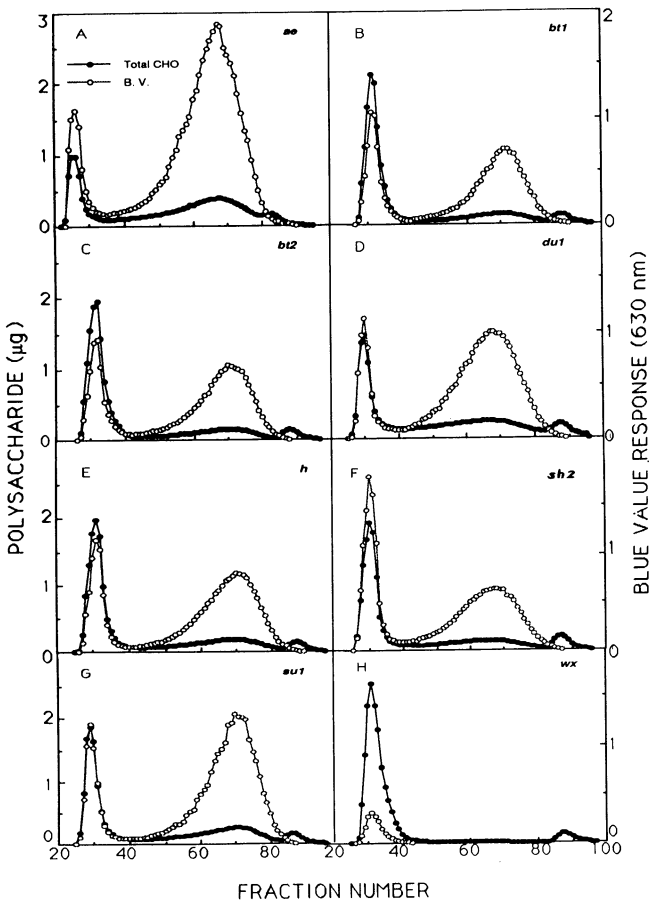

Fig. 2. Elution profile of native starch from single mutant genotypes on Sepharose CL-2B. **A,** Amylose-extender (*ae*) starch. **B,** Brittle-1 (*bt1*) starch. **C,** Brittle-2 (*bt2*) starch. **D,** Dull-1 (*du1*) starch. **E,** Horny (*h*) starch. **F,** Shrunken-2 (*sh2*) starch. **G,** Sugary-1 (*su1*) starch. **H,** Waxy (*wx*) starch. BV = Blue value response; total CHO = total polysaccharide value.

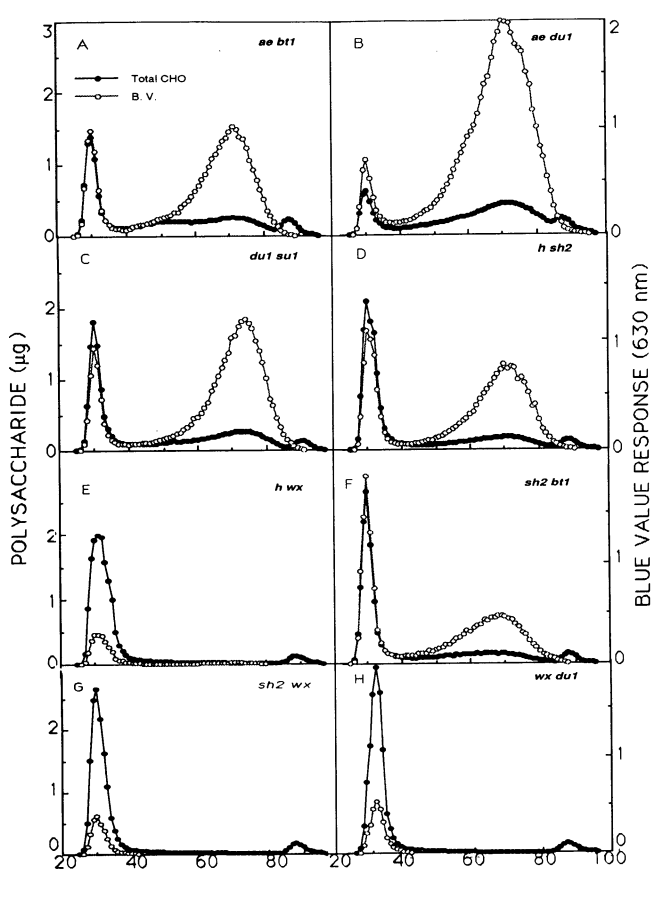

Fig. 3. Elution profile of native starch from double mutant genotypes on Sepharose CL-2B. **A,** Amylose extender (*ae*) brittle-1 (*bt1*) starch. **B,** *ae* Dull-1 (*du1*) starch. **C,** *du1* Sugary-1 (*su1*) starch. **D,** Horny (*h*) shrunken-2 (*sh2*) starch. **E,** *h* Waxy (*wx*) starch. **F,** *sh2 bt1* Starch. **G,** *sh2 wx* Starch. **H,** *wx du1* Starch.

respectively) than did normal, *bt1*, or *du1* starches, which is consistent with other results (Boyer and Liu 1985, Inouchi et al 1991). An additive effect was noted when the *ae* and *du1* genes were combined, which resulted in *ae du1* starch having the greatest amylose content among all starches in this study. This effect, however, was not noted by Yeh et al (1981). The *du1 su1* starch also had a large amylose content, 46.2%, which was more than that of the *du1* or *su1* starches. The amylose contents of the *h sh2* and *sh2 bt1* starches were similar to those of their respective single mutants and to normal starch.

Amylose Percent Determined by IA

The amylose percentages of the 17 starch genotypes determined by iodine potentiometric titration are presented in Table I. In general, the amylose percentage calculated from IA was similar to that measured by column chromatography for all genotypes except for those of *du1, ae bt1, ae du1*, and *du1 su1*. The amylose content derived from the IA method was lower than that calculated from the GPC profile of the native starches of *du1, ae bt1, ae du1*, and *du1 su1*. These starches, at the same time, showed a great amount of intermediate materials by means of GPC, suggesting that the IA method did not measure the intermediate components of the *du1, ae bt1, ae du1*, and *du1 su1* starches. The *ae* starch also contained a significant amount of intermediate component, but there was little difference in amylose percent determined by the two methods. It is suspected that the branched chains of intermediate materials in *ae* starch were longer than those of *du1, ae bt1, ae du1*, and *du1 su1* starches; therefore, they were detected as amylose by the IA method. The results suggest that the intermediate materials in starch from each genotype may be different and should not be grouped as similar materials, which conflicts with the earlier belief that intermediate materials from different starch types were similar (Whistler and Doane 1961). Furthermore, it is hard to predict the amount of intermediate materials simply by using the IA or GPC methods, perhaps because of the diverse nature and, thus, behavior of intermediate materials among the different genotypes. Several research groups demonstrated that the presence of intermediate materials caused a discrepancy in determining amylose content by different methods (Kramer et al 1958, Seckinger and Wolf 1966, Holder et al 1974, Ikawa et al 1978, Yeh et al 1981).

GPC Properties of Debranched Starches

The chromatograms of isoamylase-debranched starch from the 17 maize genotypes are presented in Figures 1A, 4, and 5. The eluted profile was divided into three fractions, and the CL from material collected at the apices of peaks from fractions II and III were determined (see Fig. 1A and Table I). Fraction I was composed mostly of amylose, fraction II included long B chains of amylopectin, and fraction III contained A and short B chains of amylopectin (Hizukuri 1986). The ratio of fraction III to fraction II, as shown, may be used as an index of the extent of branching of amylopectin; the higher the ratio, the higher the degree of branching (Biliaderis et al 1981).

The elution profiles of *bt1, bt2, h, sh2, ae bt1, h sh2*, and *sh2 bt1* starches (Figs. 4B, C, E, F, and 5A, D, and F) are similar to that of normal starch (Fig. 1A), which exhibited three fractions with clear division. The *ae* and *ae du1* starches (Figs. 4A and 5B) had similar elution profiles in which fraction II is hard to separate from fraction I. The starches of *du1, su1*, and *du1 su1* (Figs. 4D, G, and 5C) did not show clear fraction II in the chromatograms. All of the waxy starches—*wx, h wx, sh2 wx*, and *wx*

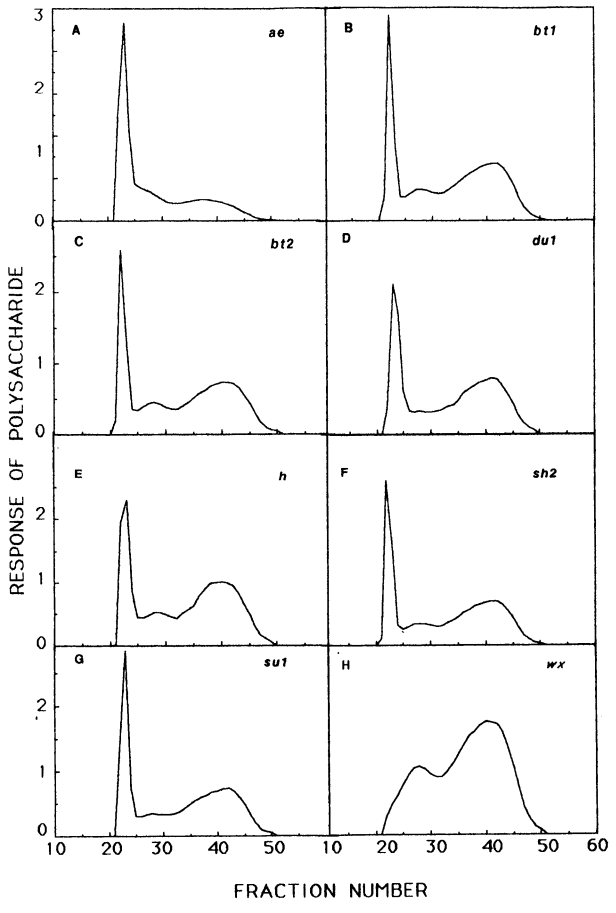

Fig. 4. Gel-permeation chromatography elution profile of isoamylase-debranched starch from single-mutant genotypes on Bio-Gel P-6. **A,** Amylose extender (*ae*) starch. **B,** Brittle-1 (*bt1*) starch. **C,** *bt2* starch. **D,** Dull-1 (*du1*) starch. **E,** Horny (*h*) starch. **F,** Shrunken-2 (*sh2*) starch. **G,** Sugary-1 (*su1*) starch. **H,** Waxy (*wx*) starch.

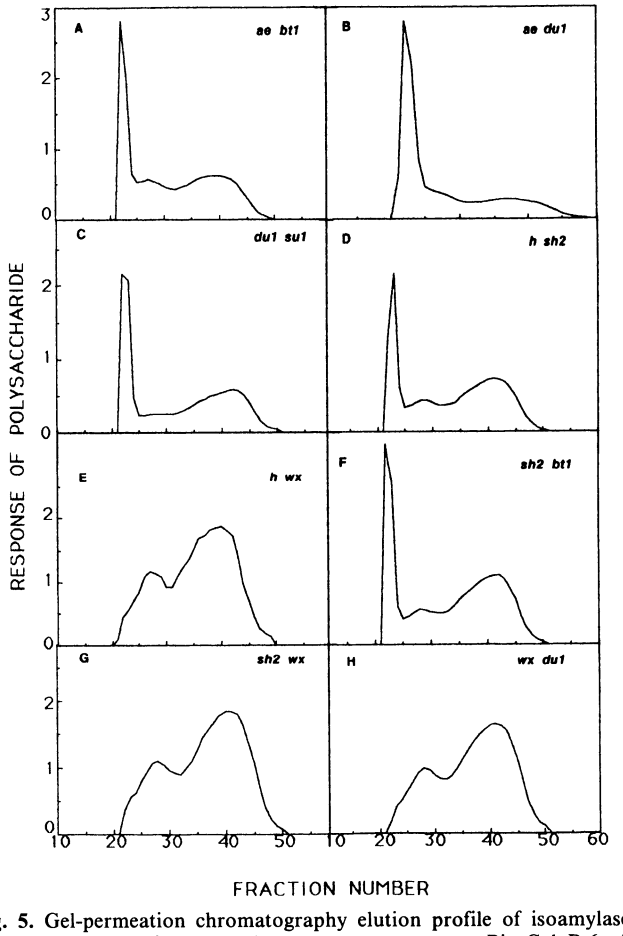

Fig. 5. Gel-permeation chromatography elution profile of isoamylase-debranched starch from double-mutant genotypes on Bio-Gel P-6. **A,** Amylose extender (*ae*) brittle-1 (*bt1*) starch. **B,** *ae* Dull-1 (*du1*) starch. **C,** *du1* Sugary-1 (*su1*) starch. **D,** Horny (*h*) shrunken-2 (*sh2*) starch. **E,** *h* Waxy (*wx*) starch. **F,** *sh2 bt1* starch. **G,** *sh2 wx* starch. **H,** *wx du1* starch.

du1 (Figs. 4H, 5E, G, and H)—had similar elution profiles where no fraction I was present.

Among single mutants, the ratios of fraction III to fraction II were similar for normal, *bt1, h,* and *wx* starches (ratios ranging from 2.6 to 3.0) and close to those of previous reports (Inouchi et al 1983, 1987; Hizukuri 1985; Ninomya et al 1989). The ratios for *ae* and *bt2* starches were lower than those for normal starch at 1.0 and 2.4, respectively, whereas *du1, sh2,* and *su1* starches had ratios higher than 3.8. The *du1* starch had the highest ratio (5.6) among all starches, which was higher than those in previous reports (Inouchi et al 1983, 1987). Among single mutants, the peak CL for fraction II ranged from 39 for *wx* to 51 for *du1,* and the peak CL for fraction III ranged from 13 to 15 except for the *ae* genotype, which had a peak CL of 20 glucose units. These results are consistent with those of Inouchi et al (1987) in that, compared with the amylopectin from normal or *wx* starch, *ae* starch had longer branches and more long B chains (fraction II) and *du1* starch had more A and short B chains of amylopectin (fraction III). In addition, the longest peak CL for fraction II (51 glucose units) among all starches was found in *du1* starch, a feature that is usually associated with *ae* starch.

The ratios of fraction III to fraction II for double mutants ranged from 1.2 for *ae du1* starch to 4.0 for *du1 su1* starch. When the *ae* gene was combined with the *bt1* or *du1* gene, the double-mutant combinations (*ae bt1* and *ae du1*) showed ratios and peak CL similar to those of *ae* starch, suggesting that the *ae* gene is more important than are the *bt1* and *du1* genes in determining the fine structure of amylopectin of *ae bt1* and *ae du1* starches. The starches of *du1 su1* and *sh2 bt1* possessed longer peak CL at fraction II than did the normal starch. The results of the ratios of fraction III to fraction II for *bt1* (3.0), *h* (2.8), *sh2* (3.8), *h sh2* (2.7), and *sh2 bt1* (3.7) suggest that the *h* gene is more important than is the *sh2* gene and that the *sh2* gene is more important than is the *bt1* gene in determining the fraction III-fraction II ratios of *h sh2* and *sh2 bt1* starches. However, *bt1, h,* and *sh2* starches had similar peak CL at both fractions II and III.

The starches with high amylose content (*ae, ae bt1,* and *ae du1*) had low ratios of fraction III to fraction II, indicating low degrees of branching. They also had long peak CL at both fractions II and III. After being debranched by isoamylase, the starch genotypes exhibited chromatograms that were different from each other, demonstrating that the chromatograms of debranched starches are characteristic of the starch genotype in addition to providing fine structure information of amylopectin.

Estimation of Intermediate Fraction from GPC Profiles

Recently, South et al (1991) reported that a more accurate amylose content can be obtained from GPC data of debranched starches than from IA or GPC of native starch. The IA procedure gave reliable estimates of amylose content only when amylopectin had a normal low iodine-binding capacity. When anomalous types of amylopectin with extended external chains were present, false high values for amylose contents were obtained. Similarly, high amylose values were calculated from the elution profile of native starch because the anomalous amylopectin (intermediate materials) was included in the amylose fraction. South et al (1991) estimated that the LMW (anomalous) amylopectin could be calculated as the difference between fraction II of the native starch and fraction I of the debranched starch GPC elution profiles. Accordingly, the intermediate materials corresponding to the anomalous amylopectins in the present study were quantified (Table I).

The *ae, du1, su1, ae bt1, ae du1,* and *du1 su1* starches had large amounts of intermediate materials as also qualitatively noted from GPC of the native starches. When starches containing the *wx* gene were excluded, the amounts of intermediate materials were weakly correlated with the peak CL of fraction II from GPC of debranched starches ($r = 0.73$, $P < 0.01$). These results support the statement of South et al (1991) that the intermediate materials are a type of LMW amylopectin with extended chains and, through the action of the *ae* gene, can give enhanced IA values for starches. The *du1* gene, in contrast, increased amylose and intermediate materials contents but did not increase iodine-binding capacities, which also is consistent with the observation of South et al (1991). The peak CL of fraction II from GPC of debranched *du1* starch was longer than that of normal starch, which, according to South et al (1991), should cause a marked difference between the IA value and the amylose value as measured in fraction I of debranched starch. The discrepancy in amylose content between the IA method and that calculated from fraction I in GPC of the debranched starch was observed for *ae* starch but not for *du1* starch. The *ae bt1* and *ae du1* starches also showed little difference between the two values. The results indicate that other factors besides chain length may be important in controlling the binding between iodine and starch molecules.

SEM of Starch Granules

The morphology of native starch granules from the 17 maize genotypes was captured by means of SEM (Figs. 6–8). All micrographs were taken at a magnification of ×1,000. Table II summarizes the size distribution of starch granules from different genotypes.

Normal starch granules had typical angular and spherical shapes (Fig. 6A), and the diameters of granules varied from 6 to 17 μm, with an average of 11.6 μm (Table II). Starch granules in the floury endosperm are more rounded, whereas those in the horny endosperm are more angular (Watson 1967). The *ae* starch granules were generally spherical with some elongated shapes and had smooth outlines in contrast to the facets of normal starch, which suggests a loose arrangement of granules in the endosperm of the *ae* genotype (Wolf et al 1964) (see Fig. 6B). The appearance of irregular and elongated shaped granules in *ae* starch is well documented (Deatherage et al 1954, Wolf et al 1964, Sandstedt et al 1968, Hall and Sayre 1970, Banks et al 1974, Boyer et al 1976, Gallant and Bouchet 1986). The average size of *ae* starch granules, 7.0 μm, was smaller than that of normal starch, which is in agreement with previous reports (Wolf et al 1964, Boyer et al 1976, Cluskey et al 1980). Granules of *bt1, du1, sh2,* and *su1* starches (Figs. 6C, E, 7B, and C, respectively) were generally smaller than those of normal starch (see Table II). The granular size (range and average) for *bt2* and *wx* starches (Figs. 6D and 7D, respectively) was similar to that for normal starch. The *h* starch granules were large with a smooth surface (Fig. 7A), and the average size (13.8 μm) was the largest among all genotypes. The small granules of *su1* starch (Fig. 7C) were agglomerated with distinct divisions, which first was observed by Sandstedt et al (1968).

The morphology of the starch granules is influenced by the presence of another mutant gene, and additional complexity is created by the interactions of the two genes. When the *ae* gene was combined with the *bt1* or *du1* gene, abnormally shaped granules, characteristic of *ae* starch, were found (Fig. 7E and F). Many researchers have suggested that as the amylose content increases, the irregularity of the starch granule shape increases (Wolf et al 1964, Banks et al 1974, Gallant and Bauchet 1986). On the other hand, Boyer et al (1977) suggested that high amylose content was not a requirement for abnormal starch granule formation in genotypes containing the *ae* gene and that the abnormal granule was indeed a result of a high ratio of linearity to branching in the total starch. No conclusion regarding this relationship can be drawn from the present results.

One of many examples illustrating the effects between two genes comes from *du1 su1* starch (Fig. 8A). The *du1 su1* starch looked similar to *su1* starch with respect to the aggregations of small granules, but the size of the granules (6.9 μm) was between those of *du1* (7.8 μm) and *su1* (5.4 μm) starches. In contrast, the average size of *ae bt1* was slightly smaller than that of *ae* and *bt1* starches, suggesting a combined effect on granular size. Similarly, *sh2 bt1* starch had an average granular size smaller than that of either *sh2* or *bt1* starches. The *h* gene seemed to have more influence than did the *sh2* gene relative to the size of the starch granules when the *h sh2* starch was examined (Fig. 8B). The *wx* gene seemed to be more important than *du1, sh2,* and, perhaps, *h*

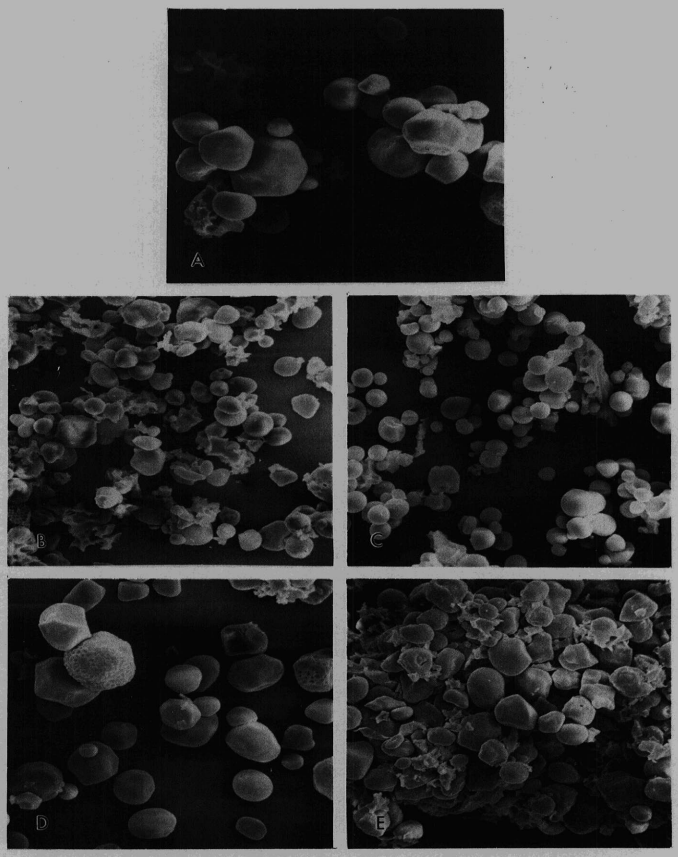

Fig. 6. Scanning electron micrograph of maize starch granules (×1,000). **A,** Normal starch. **B,** Amylose extender (*ae*) starch. **C,** Brittle-1 (*bt1*) starch. **D,** *bt2* starch. **E,** Dull-1 (*du1*) starch.

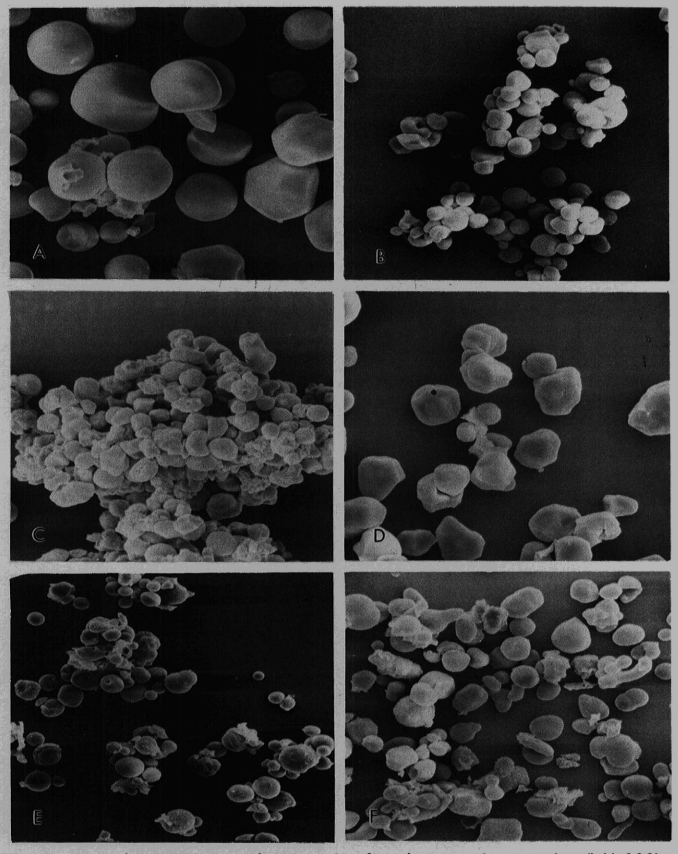

Fig. 7. Scanning electron micrograph of maize starch granules (×1,000). **A,** Horny (*h*) starch. **B,** Shrunken-2 (*sh2*) starch. **C,** Sugary-1 (*su1*) starch. **D,** Waxy (*wx*) starch. **E,** Amylose extender (*ae*) brittle-1 (*bt1*) starch. **F,** *ae* Dull-1 (*du1*) starch.

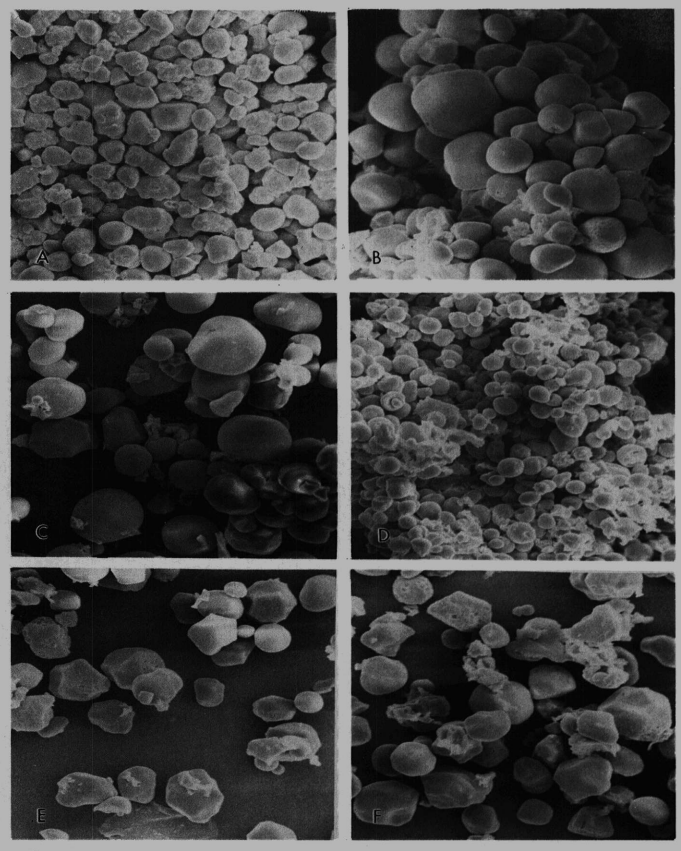

Fig. 8. Scanning electron micrograph of maize starch granules (×1,000). **A,** Dull-1 (*du1*) sugary-1 (*su1*) starch. **B,** Horny (*h*) shrunken-2 (*sh2*) starch. **C,** *h* Waxy (*wx*) starch. **D,** *sh2* Brittle-1 (*bt1*) starch. **E,** *sh2 wx* starch. **F,** *wx du1* starch.

TABLE II
Size Distribution of Starch Granules of 17 Maize Genotypes from Oh43 Inbred Measured from Scanning Electron Micrographs

Genotype[a]	Range (μm)	Average ± SD[b] (μm)
Normal	6–17	11.6 ± 4.5
ae	4–11	7.0 ± 2.1
bt1	4–9	6.1 ± 1.3
bt2	6–19	10.8 ± 3.4
du1	4–11	7.8 ± 2.1
h	8–22	13.8 ± 3.6
sh2	2–9	6.3 ± 1.9
su1	2–10	5.4 ± 2.6
wx	6–14	10.3 ± 2.6
ae bt1	3–10	5.6 ± 1.6
ae du1	4–14	7.4 ± 2.3
du1 su1	3–12	6.9 ± 2.6
h sh2	6–23	11.2 ± 3.8
h wx	3–20	11.0 ± 4.4
sh2 bt1	3–9	5.4 ± 1.6
sh2 wx	4–16	10.2 ± 3.7
wx du1	4–19	10.2 ± 3.6

[a] *ae* = Amylose extender; *bt* = brittle; *du* = dull; *h* = horny; *sh* = shrunken; *su* = sugary; and *wx* = waxy.
[b] Average ± SD of 30 starch granules, 15 each from two micrographs.

genes in the *h wx, sh2 wx*, and *wx du1* genotypes (Figs. 8C, E, and F, respectively) relative to the morphology and size of the starch granules. The granular size of the *h sh2* starch (11.2 μm) (Fig. 8B) was between that of *h* and *sh2* starches. Banks et al (1974) and Cluskey et al (1980) reported an inverse relationship between the size of starch granules and the apparent amylose content of amylomaize starches with different amylose contents and normal maize starch. Only a weak relationship ($r = -0.29$, $P < 0.01$) was found in the present study between the

apparent amylose content (from IA) and the average granular size of the 13 maize genotypes (excluding waxy starches).

CONCLUSIONS

By using GPC and SEM, the structure and morphology of 17 maize endosperm mutant genotypes of Oh43 inbred were elucidated. The starches containing the *ae* gene showed high amylose content, low degree of branching, long branch chain length of amylopectin, and high intermediate materials content, suggesting the important role of the *ae* gene in determining the fine structure of starch. The *du1* starch had increased amylose and intermediate materials contents with the longest peak CL at fraction II of debranched amylopectin among all starches. The *su1* and *du1 su1* genotypes also produced starches with higher amylose and intermediate materials contents than did the normal genotype. The starches of *wx, h wx, sh2 wx,* and *wx du1* had similar structures as measured by GPC of native and isoamylase-debranched starches, although minor differences existed. In general, starches from each genotype were different from each other, and the combination of two genes created additional variations in the structure and shape of starch granules. How these variations affect the starch properties needs further investigation.

ACKNOWLEDGMENT

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Changes in Sorghum Starch During Parboiling[1]

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ABSTRACT

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Sorghum grains varying in grain hardness or endosperm texture (soft and intermediate) and starch composition (nonwaxy and waxy) were parboiled. Whole grain (one volume) and water (three volumes) were boiled, soaked for 12 hr, and brought to boil again (boil-soak-boil process) or, alternatively, soaked overnight and boiled for 10 min (soak-boil process). The grain was dried at room temperature and decorticated. Parboiled kernels were darker, denser, smaller, and harder than nonparboiled kernels. Parboiling decreased starch crystallinity and starch dispersion in hot water. Parboiled grain with soft endosperm texture contained less dispersible and soluble starch than parboiled grain with intermediate endosperm texture. The physical characteristics of the waxy cultivar were changed after parboiling; however, starch solubility and crystallinity decreased only slightly. Pasting properties of waxy sorghum were not changed as dramatically by parboiling as were those of nonwaxy cultivars. Apparently, the absence of amylose in waxy starch substantially decreased retrogradation of starch polymers.

Parboiling of rice is one of the most widespread food processes in the world. The hydrothermal process consists of soaking rough rice in water until it is saturated, draining the excess water, steaming or otherwise heating the grain to partially gelatinize the starch, and drying (Bhattacharya 1985).

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important staple foods in arid regions unsuitable for the cultivation of other crops. In 1989, world sorghum production was estimated at 58.0 million metric tonnes. Asia, North America, and Africa produced 32.7, 27.1, and 24.1% of the world's sorghum, respectively (FAO 1990). Rooney and Serna-Saldivar (1991) indicated that 30% of world production is consumed directly by humans, primarily as traditional foods. Sorghum is used as a substitute for rice in Mali and India (Subramanian et al 1982). In India, the ricelike product called *annam* or *soru* accounts for 10% of the total sorghum grain produced. Similar products have also been reported in Bangladesh (*khicuri*), Botswana (*lehata wagen*), China (*kaohang mi fan*), Ethiopia (*nufio*), and Nigeria (*oko baba*) (Subramanian and Jambunathan 1980). In most African countries, special types of sorghum with very hard, flinty endosperm are dehulled and used as a rice substitute. However, these special sorghums have small kernel size and low agronomic yields. Parboiling could allow consumers to use higher yield sorghums with intermediate or soft endosperm texture.

Young et al (1990) adapted rice technology to produce a parboiled sorghum called SORI (SOrghum RIcelike product). The modified process was simply boiling, soaking for 12 hr, reboiling, and air-drying. Parboiled sorghum was then decorticated. The most important benefits of parboiling sorghum were: 1) increased yield of decorticated sorghum; 2) reduced amounts of broken kernels during decortication; 3) improved texture of the boiled product (i.e., cooked SORI had a firmer, less-sticky texture compared to nonparboiled cooked sorghum); and 4) increased shelf-stability. The major disadvantage of parboiling is the energy the process uses, because the SORI needs longer cooking time than the raw grain. However, the decorticated grain can be cracked into various sizes of grits to produce a wide array of products for use in different food preparations such as thick porridges (*couscous* and *tô*), making it a more diversified, marketable product. Parboiling trials conducted in Mali, at the village level, have shown that well-accepted products can be obtained by soaking the sorghum overnight, boiling it, and drying it. This saves energy and time and makes the process more practical (*unpublished data*).

The physicochemical changes in sorghum starch during parboiling that are responsible for the improved characteristics have not been determined. Therefore, the objective of this study was to evaluate the effects of parboiling on starch crystallinity, birefringence, pasting properties, and dispersion in hot water using sorghum cultivars with different endosperm texture and starch composition.

MATERIALS AND METHODS

Sample Preparation

Three sorghum cultivars with different endosperm texture were used in this study. In sorghum, endosperm texture refers to the proportion of hard to soft endosperm in the kernel. Grain samples of Dorado (nonwaxy, intermediate endosperm texture), P721Q (nonwaxy, higher lysine, soft endosperm texture), and ATx630* R3338 (waxy, intermediate endosperm texture) (Table I) grown at Halfway, TX, in 1987, were parboiled using two methods.

Method I. Soak-boil (SB). Washed, whole grain (one volume) was soaked overnight in tap water (three volumes) and boiled in the same solution for 10 min at a rate of 5°C/min in an 11-L steam-jacketed cooker (model TDC/2-20, Groen Div., Dover Corp., Elk Grove Village, IL).

Method II. Boil-soak-boil (BSB). Washed, whole grain was brought to boil as in method I, soaked overnight, and brought to boil again in the same solution.

The grain samples from both methods were dried for 48 hr

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