SOME OBSERVED SECONDARY EFFECTS OF HIGH-AMYLOSE GENES IN MAIZE[1]

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ABSTRACT

In the material studied, in which the *ae* and *du* genes were each from a single source, generally as amylose content increased, protein content of the methanol-extracted (fat-free) endosperm increased. Likewise, as amylose increased, kernel weight decreased. When the high-amylose starch genes *ae* and *du* were considered individually, there was a high positive correlation between protein and amylose for the *ae* gene; whereas the *du* gene gave a significant negative correlation. Analysis of the phenotypes suspected of being the double recessive *ae du* indicated there was a negative relationship still between protein and amylose but not so large as for the *du* gene alone. There appeared to be a greater decrease in kernel weight as amylose increased for the *du* gene than for the *ae* gene. However, the possibility of developing high-amylose strains with a relatively low endosperm protein appears promising and worthy of special attention by breeders developing hybrids with high-amylose starch. Such hybrids would be especially beneficial to the corn wet-milling industry where current studies have shown considerable difficulty in the separation of gluten and starch from corn containing more than 50% amylose. *Ae* and *du* genes in other genetic backgrounds might give results different from those reported in this study.

The recent interest in breeding high-amylose strains of maize has given added impetus to investigating the secondary effects of high-amylose genes. If such associations were definitely established, breeding procedures might necessarily require modification. Some of the characteristics that may be associated with high-amylose strains are weight, hardness, and brittleness of the kernel and protein content of the endosperm.

The processing of high-amylose corn by the corn wet-milling method at the Northern Utilization Research and Development Division (1) has indicated difficulty in separating protein and starch. The trouble could be the result of close association between high protein and high amylose. If it were possible to develop a high-amylose corn with reasonably low endosperm protein, the task of separating gluten from starch might lessen.

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Materials and Methods

The assumed double recessive combination of the high-amylose genes *ae* (ha_1) (47–48%) and *du* (ha_2) (35–40%) has given F_3 progeny of 70% (6), and several F_5 progeny with 80% amylose. The *ae* source used in this study was from a stock obtained from Kramer in 1955, with the tentative designation of ha_1, which is now known to be the original *ae* of Bear (4); and the *du* source was from selfed progeny of the Cassel open-pollinated variety with the tentative designation of ha_2. Recently, it was proposed by Kramer *et al.* (4) that ha_1 and ha_2 be designated as *ae* and *du*, respectively. The general procedure followed at the Missouri Agricultural Experiment Station has been to convert standard inbreds separately to *ae* and *du*. After an inbred line has been converted to these two genes through adequate backcrossing, the conversions are then crossed and selfed to recover the double recessive.

Currently, numerous inbreds are being converted to *ae* and *du*. The breeding procedure in making the conversions is to self plants in the segregating progeny and backcross these plants to the recurrent inbred parent. The selfed progeny gives segregations of normal and tarnished kernels. The tarnished kernels are analyzed for amylose content, and ears with the highest amylose content are identified. Since these ears are from plants that were backcrossed to the recurrent inbred parent, this backcrossed progeny is planted in the following generation. The procedure is to be repeated until an inbred has been backcrossed five or more generations.

The results from this breeding operation afforded an opportunity to observe some of the secondary effects of the high-amylose genes *ae* and *du*. The amylose content was determined by potentiometric titration with iodine. Endosperm was separated by hand, ground to pass a 40-mesh screen, and extracted for 24 hours with 85% methanol to remove oil which interferes with the amylose determination. Some nitrogenous materials were removed during the methanol extraction. The methanol-extracted (fat-free) endosperm was washed with distilled water, dried, and reground. Moisture was determined by drying 5 hours in a vacuum oven at 105°C. Nitrogen was determined by the Kjeldahl-Wilfarth-Gunning method (2) with collection of the sample in boric acid as originally done by Wagner (5). The weight of endosperm taken for titration to determine amylose content of the starch was corrected for moisture and protein contents; the amount of fiber present was ignored, since it has been found to be small (not over 5%) and relatively constant. The amylose determinations were made as previously described (3).

The protein content ($N \times 6.25$) referred to throughout this paper

is that of the methanol-extracted (fat-free) endosperm. Part of the protein had been removed by the extraction.

Results and Discussion

Observations made from conversions to *ae* and *du* in 1956 are reported in Table I and summarized in Table II. Each comparison of normal and tarnished kernels in Table I is on an individual ear basis. Although the number of observations was limited, these are believed

TABLE I

COMPARATIVE KERNEL WEIGHT, PERCENTAGE PROTEIN,[a] AND PERCENTAGE AMYLOSE FOR NORMAL AND TARNISHED KERNELS SELECTED FROM SEGREGATING EARS BEING CONVERTED TO EITHER *ae* OR *du* (1956 DATA)

CULTURE No.	NORMAL KERNELS			TARNISHED KERNELS		
	Average Weight	Protein	Amylose	Average Weight	Protein	Amylose
	g	%	%	*g*	%	%
			ae Conversions			
44–1	0.289	7.88	25.0	0.257	9.25	47.2
44–5	.202	7.69	26.8	.180	9.19	45.4
45–13	.293	8.13	25.7	.276	9.31	47.0
46–2	.283	7.00	30.0	.259	7.69	41.1
73–11	.242	9.19	26.8	.215	10.44	41.3
74–9	.196	7.31	32.0	.177	8.56	46.0
76–2	.246	8.06	27.0	.213	9.13	50.0
77–5	.265	8.25	27.7	.222	9.94	48.8
78–6	.242	8.00	26.7	.211	8.94	45.8
83–4	.269	8.19	25.9	.243	9.88	51.3
99–2	.238	9.50	29.5	.213	11.38	45.8
101–4	.251	7.75	30.5	.226	8.19	45.8
104–10	.201	9.00	25.6	.179	10.06	50.2
112–6	.248	10.06	25.7	.217	11.63	60.5
117–3	.248	7.56	26.6	.211	9.06	53.7
187–6	.192	7.88	26.4	.168	9.94	47.7
189–9	0.236	8.56	28.1	0.204	10.25	49.4
Mean	0.244	8.24	27.4	0.216	9.59	48.1
			du Conversions			
38–8	0.228	6.63	27.3	0.202	7.00	33.7
39–3	.213	9.00	26.9	.193	9.88	34.2
58–7	.210	9.56	28.3	.193	10.81	34.4
62–3	.251	8.00	31.8	.227	8.63	32.3
66–7	.304	8.19	25.9	.295	9.69	31.2
68–6	.292	8.81	26.4	.273	9.69	32.6
124–4	.255	9.63	27.7	.242	9.56	34.2
128–4	.236	10.50	25.6	.199	10.25	35.6
135–1	.265	8.38	29.6	.240	7.44	35.0
136–8	.273	7.94	27.0	.242	8.88	35.4
139–6	.275	7.25	26.1	.244	7.50	33.1
142–2	0.298	6.38	26.6	0.267	6.69	32.9
Mean	0.258	8.36	27.4	0.235	8.84	33.7

[a] Protein in endosperm after extraction with 85% methanol.

to be sufficient to indicate a trend in the association of average kernel weight and protein percentage of the methanol-extracted (fat-free) endosperm with amylose content for normal and tarnished kernels, if it is assumed that a significant positive correlation exists between protein of the methanol-extracted (fat-free) endosperm and protein content of the endosperm without extraction.

The mean difference for average kernel weight shows that the normal kernels were significantly heavier than the tarnished among the *ae* conversions; whereas the difference was not significant for the *du* conversions even though the normal kernels were generally heavier. The protein content in the methanol-extracted endosperm was higher for the tarnished selections, especially for the *ae* conversions. The mean difference between normal and tarnished kernels for amylose content was significant for both *ae* and *du* conversions.

TABLE II

MEAN DIFFERENCES FOR AVERAGE KERNEL WEIGHT, PROTEIN,[a] AND AMYLOSE PERCENTAGES OF NORMAL AND TARNISHED SELECTIONS FOR *ae* AND *du* CONVERSIONS (1956 DATA)

	AVERAGE KERNEL WEIGHT		PROTEIN		AMYLOSE	
	ae	*du*	*ae*	*du*	*ae*	*du*
			%	%	%	%
Normal kernels	0.244	0.258	8.24	8.36	27.4	27.4
Tarnished kernels	0.216	0.235	9.59	8.84	48.1	33.7
Difference	0.028*	0.023	1.35**	0.48	20.7**	6.3**

[a] Protein in endosperm after extraction with 85% methanol.

The correlation coefficients given in Table III between kernel weight and protein indicate no relationship for either *ae* or *du* conversions. Kernel weight appears to be negatively associated with amylose content among the tarnished kernels for the *du* conversions and percent protein is positively associated with the amylose content of the tarnished kernels of the *ae* conversions.

TABLE III

CORRELATION COEFFICIENTS FOR AVERAGE KERNEL WEIGHT VS. PROTEIN PERCENTAGE,[a] AVERAGE KERNEL WEIGHT VS. AMYLOSE PERCENTAGE, AND PROTEIN PERCENTAGE VS. AMYLOSE PERCENTAGE FOR NORMAL AND TARNISHED SELECTED KERNELS FROM *ae* AND *du* CONVERSIONS (1956 DATA)

COMPARISON	CORRELATION COEFFICIENTS			
	Normal Kernels		Tarnished Kernels	
	ae	*du*	*ae*	*du*
Average kernel weight vs. percent protein	−0.06	−0.42	−0.20	−0.23
Average kernel weight vs. percent amylose	−0.19	−0.25	0.08	−0.63*
Percent protein vs. percent amylose	−0.34	−0.05	0.48*	0.15

[a] Protein in endosperm after extraction with 85% methanol.

The 1956 results indicate special attention should be given to selecting for low protein in *ae* conversions and for a high kernel weight in the *du* conversions. It is assumed that a low kernel weight would adversely affect the yield of a high-amylose hybrid.

The influence of these various characteristics in *ae du* strains is of special interest. A limited number of comparisons were made in 1957 between protein content and amylose percentage. These results are summarized in Table IV.

TABLE IV

MEAN PROTEIN[a] AND AMYLOSE PERCENTAGE, AND THE REGRESSION AND CORRELATION COEFFICIENTS BETWEEN PROTEIN AND AMYLOSE FOR EARS REPRESENTING VARIOUS RANGES IN AMYLOSE CONTENT (1957 DATA)

AMYLOSE RANGE	NO. OF EARS	MEAN		PROBABLE GENOTYPES	COEFFICIENT	
		Protein	Amylose		Correlation	Regression
%	*No.*	%	%			
21–30	65	9.38 ± 1.65	27.1 ± 2.55	*Ae Ae Du Du*	–0.103	–0.159
31–40	197	9.71 ± 1.36	36.0 ± 2.40	*Ae Ae du du*	–0.599**	–1.054
41–50	131	9.82 ± 1.13	46.2 ± 2.98	*ae ae Du Du*	0.197*	0.522
51–60	118	10.82 ± 0.37	55.6 ± 2.90	*ae ae Du Du*	0.481**	3.735
61–70	81	11.19 ± 1.38	65.2 ± 2.78	*ae ae du du*	–0.362**	–0.729
71–80	7	11.28 ± 1.17	74.0 ± 2.34	*ae ae du du*	–0.373	–0.748
Total	599					

[a] Protein in endosperm after extraction with 85% methanol.

A total of 599 ears was classified into six groups on the basis of amylose content. The mean protein and amylose percentages and standard errors of the means were computed for each of these six groups. The probable genotypes are given for each group. However, the genotype for each ear cannot be positively established without backcrossing to the *ae* and *du* stock. In most instances, the single recessive genotype of *ae* or *du* is known, as these ears are from inbreds being converted to either one of these two genes. Most of the doubtful genotypic classifications are those in the 61–70 and 71–80 percentage groups.

When the six groups were considered individually, the mean protein and mean amylose content increased simultaneously. Although this is an important aspect, a matter of more concern is whether it is possible to select strains with high amylose and low protein content within the six groups. To obtain information on this phase, the regression and correlation coefficients between protein and amylose percentages were computed. Within the amylose ranges, the results show that, whenever the single recessive gene *ae* is predominant, a significant positive correlation between protein and amylose exists, but when *du* is predominant, a significant negative correlation occurs. These results for

ae corroborate the correlation coefficients obtained between protein and amylose percentages in the 1956 study. A negative relationship occurred between protein and amylose for the 61–70% and 71–80% amylose groups where the majority of genotypes are assumed to be the double recessive, *ae du*.

The results of these studies indicate that the gene *ae* from the single source used increases amylose and protein simultaneously with decreased kernel weight but that the *du* gene has a less pronounced effect on the relationship between protein and amylose. It is concluded from these studies that it would be more difficult to select a high-amylose strain with a low protein among the *ae* conversions. However, when the *du* gene used is added to the genotype to give the double recessive *ae du*, the likelihood of selecting a high-amylose strain with a low protein is increased.

It appears that the protein content of the endosperm will be higher and the kernel weight will be lower in high-amylose corn than in normal dent corn, regardless of whether *ae*, *du* (from the sources studied), or the combination is present in a given genotype. Therefore, breeders of high-amylose strains of corn should give special attention to protein content of the endosperm as well as to its amylose content.

Preliminary findings at the Missouri Agricultural Experiment Station suggest that under adverse growing conditions amylose content decreases and protein of the methanol-extracted (fat-free) endosperm increases. Therefore, the genetic-environmental interaction also must be taken into consideration in the breeding of high-amylose strains with a low endosperm protein.

Since the *ae* and *du* genes used in this study each trace to a single source, it is possible that different sources for these two genes might give results differing from those reported in this paper.

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