GENETICALLY CONTROLLED ELECTROPHORETIC VARIANTS OF A STARCH-DEGRADING ENZYME IN *ZEA MAYS**

By D. J. FINNEGAN†

The developing endosperm of young maize (*Zea mays*) kernels has proved to be a rich source of readily extracted enzymes suitable for electrophoretic analysis. Isozymes have already been described for alcohol dehydrogenase (Schwartz 1966), catalase (Beckman, Scandalios, and Brewbaker 1964*a*), esterase (Schwartz 1960), and leucine amino peptidase (Beckman, Scandalios, and Brewbaker 1964*b*). This communication reports the discovery of electrophoretic variants of a starch-degrading enzyme in extracts of this tissue, and some preliminary breeding tests to establish their mode of inheritance.

The only previously published work on amylase isozymes in maize is that of Scandalios (1966), who studied the enzyme from germinating kernels by means of starch-gel electrophoresis. Since starch is the natural substrate for amylase, polyacrylamide gels were used in the present work. Plants were grown in 10-in. pots in a heated glasshouse (minimum temperature 60–65°F) thoughout the South Australian winter. Preliminary investigations showed that under these conditions the starch-degrading enzymes in the endosperm reached maximum activity 30–35 days after pollination and thereafter decreased. Dry kernels immediately after harvesting were found to have low levels of activity but none could be detected in kernels more than 12 months old. Kernels were harvested 30–35 days after pollination and were either extracted immediately or stored in sealed plastic bags at −20°C. Individual kernels were macerated in 0·4 ml of extraction fluid (0·001M disodium EDTA, 0·05M NaCl, and 0·005M cysteine hydrochloride) and the extract centrifuged to remove the cell debris. The supernatant was decanted and used as the enzyme extract.

Electrophoresis was carried out in vertical water-cooled gel trays (30 by 15 cm by 5 mm) with the sample slots 7·5 cm from the cathode end. The gels were of 5% acrylamide [1·5 g *N*,*N*′-methylenebisacrylamide, 25 g acrylamide, 500 ml buffer, 0·5 ml *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, and 8 ml of 5% $(NH_4)_2S_2O_8$ solution], and the discontinuous Tris–citrate buffer system of pH 8·6 described by Ashton and Braden (1961) was used.

The gels were stained by a technique which was a modification of that used by Boettcher (personal communication) for human salivary amylase. After electrophoresis the gel was cut in two and incubated at 37°C for 40 min in a 1% starch solution buffered to pH 6·0 with a 0·1M phosphate buffer. This pH had been found to give optimum enzyme activity in trials of starch solutions buffered from pH 3·0 to 9·0. The starch solution was removed, and the gel washed three times in tap water, then irrigated with a KI–I_2 solution (30 g KI and 13 g I_2 in 1 litre H_2O). The starch solution penetrates the gel during incubation but is degraded in regions of enzyme activity. The enzyme bands can thus be seen as cleared areas on a blue background.

Two types of band could be distinguished in extracts of all kernels, a single clear band and several bands which stained rust-red with iodine solution. A clear band would be expected if an enzyme were present which degrades starch to maltose units;

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† Department of Genetics, University of Adelaide, S.A. 5001.

α-amylase (EC 3.2.1.1.) has this action and is the most likely enzyme in this band. The rust-red bands could be due to the reaction of limit dextrins with iodine solution. Such limit dextrins would be left after the amylopectin component of starch had been degraded by an enzyme which is unable to attack the polysaccharide chain beyond the 1→6 glycosidic links of the branch points; β-amylase (EC 3.2.1.2.) and α-1,4-glucan:D-glucose 4-glucosyltransferase (EC 2.4.1.3.), which acts as a phosphorylase, are such enzymes.

If these conclusions concerning the nature of the enzyme are correct, then the enzyme of the clear band should be able to degrade dextrin, but the enzyme of the rust-red bands should not. To test this one half of a gel was incubated in a buffered 0·5% dextrin solution while the other half was incubated in a starch solution. When the two halves were compared after staining it could be seen that, as expected, the dextrin had only been degraded in the position opposite the clear band on the half incubated in starch.

TABLE 1

MAIZE STOCKS USED IN THE PRESENT STUDY

Stock numbers refer to the crosses shown in Table 2. Descriptions of the stocks are those supplied by the Maize Genetics Cooperative

Stock No.	Description	Stock No.	Description
C2	Papago Flour Corn	C12	Sh_4/sh_4
C3	Maize Chapolote	C14	ae/ae
C6	bt_2/bt_2	C16	du_1/du_1
C7	Bt_2/bt_2		

The stocks examined were obtained from the Maize Genetics Cooperative as samples of endosperm mutants and races of maize as shown in Table 1. The crossing programme was started before the enzyme variants were identified and as many plants as possible were selfed and also used as pollen parents. The progeny from self pollinations should yield information about the genotype of the parent plant, and the progeny from outcrosses would yield information about the genotype of the female parent in such a cross.

In all the progeny analysed from these crosses the single clear band has exhibited the same mobility. The number of rust-red bands which could be detected has varied from 1 to 5, with the slowest band being considerably more intense than any of the others. The weak bands disappeared if the samples were suitably diluted, whilst the strongest band remained. Thus it appears likely that the variation in the number of faint bands present is due to the strength of the extract rather than being genetically determined.

Three mobility variants of the slowest band have been found and these types are designated A, B, and C in order of decreasing mobility. Each variant was associated with a weaker minor band with a slightly faster mobility and the migration rates of both the major and minor bands varied together. In addition to the phenotype with

a single major band the type AC was found with the major bands A and C but no hybrid band. These phenotypes are illustrated in Figure 1.

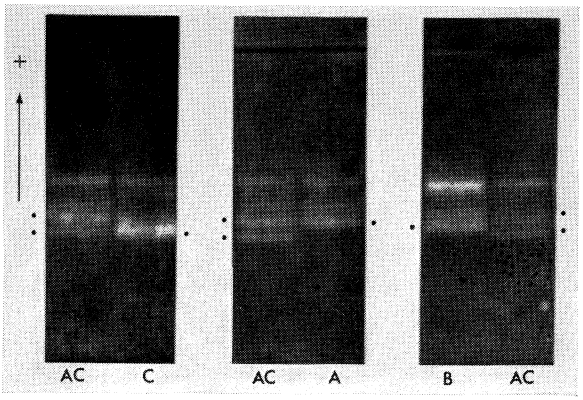

Fig. 1.—Samples of the four enzyme phenotypes mentioned in Table 2. The invariant band is that identified as α-amylase. Each of the major bands of the variant zone is indicated by a black dot.

TABLE 2

RESULTS OF FIRST CROSSES MADE TO INVESTIGATE THE MODE OF INHERITANCE OF THE ENZYME VARIANTS

Cross	No. of Enzyme Phenotypes* in Progenies				Ratio Tested	χ^2	P
	A	AC	C	B			
C14.2 selfed	—	—	18	—	—	—	—
C14.5 selfed	—	—	8	—	—	—	—
C14.1 selfed	—	—	18	—	—	—	—
C3.1 selfed	—	—	18	—	—	—	—
C12.5 selfed	1	5	6	—	1 : 2 : 1	3·5	$0{\cdot}1 < P < 0{\cdot}2$
C6.2 selfed	3	5	3	—	1 : 2 : 1	0·95	$0{\cdot}5 < P < 0{\cdot}7$
C6.1 selfed	1	5	3	—	1 : 2 : 1	1·8	$0{\cdot}3 < P < 0{\cdot}5$
C16.1 × C16.2	4	7	1	—	1 : 2 : 1	1·8	$0{\cdot}3 < P < 0{\cdot}5$
C7.2 × C6.1	13	17	—	—	1 : 1	0·54	$0{\cdot}3 < P < 0{\cdot}5$
C2.3 selfed	—	—	—	10	—	—	—
C2.4 selfed	—	—	—	15	—	—	—

* Phenotypes are shown in Figure 1.

The distribution of enzyme types amongst the progeny of the above crosses are listed in Table 2. Crosses yielding progeny of either type B or C were found, but type A was only detected in progenies which also segregated for type C. Although the numbers analysed are small, the families segregating for types A, AC, and C each fit a ratio of 1 : 2 : 1. The progeny from cross C7.2 × C6.1 is in agreement with a segregation of 1 A : 1 AC.

These results can be most readily accounted for by the hypothesis that each of the variants A, B, and C is controlled by a single allele, and with a heterozygote having two bands corresponding with its two alleles but no hybrid band. These alleles will be notated Sd^A, Sd^B, and Sd^C. *Sd* standing for "starch degradation" is chosen in preference to *Amy* since the latter term is usually used in connection with α-amylase (Sick and Nielsen 1964; Ashton 1965; Bahn 1967), whilst this enzyme system is either β-amylase or the phosphorylase mentioned above.

If this model is correct, then the progeny from the selfing of C14.1 (Table 2) should be homozygous Sd^CSd^C and those from the selfing of C2.3 homozygous Sd^BSd^B. Half of the progeny of the cross C7.2 × C6.1 would be expected to be Sd^ASd^C and half Sd^ASd^A.

To test this hypothesis, plants supposedly having genotypes Sd^CSd^C and Sd^BSd^B were selfed and found to be pure breeding for types C and B respectively, as expected. Three of the progeny from the cross C6.2 × C6.1 were also selfed. The expectation was that half should segregate 1 A : 2 AC : 1 C while half should breed true for the A phenotype. The results agreed with expectation since the progeny of two plants segregated as shown in Table 3, whilst one plant was found to be pure breeding for type A, a form not previously detected. Crosses between Sd^BSd^B and Sd^CSd^C homozygotes yielded the expected BC phenotype.

TABLE 3

RESULTS OF CROSSES DESIGNED TO TEST THE SINGLE LOCUS HYPOTHESIS FOR THE INHERITANCE OF THE ENZYME VARIANTS

Proposed Parental Genotypes	No. of Families	No. of Enzyme Phenotypes in Progenies					χ^2_2 (1 : 2 : 1)	P
		A	AC	C	B	BC		
Sd^CSd^C selfed	5	—	—	43	—	—	—	—
Sd^ASd^C selfed	1	17	26	10	—	—	1·9	$0{\cdot}3 < P < 0{\cdot}5$
Sd^ASd^C selfed	1	8	24	9	—	—	1·2	$0{\cdot}5 < P < 0{\cdot}7$
Sd^BSd^B selfed	1	—	—	—	12	—	—	—
$Sd^CSd^C \times Sd^BSd^B$	1	—	—	—	—	12	—	—
$Sd^BSd^B \times Sd^CSd^C$	1	—	—	—	—	12	—	—
Sd^ASd^A selfed	1	23	—	—	—	—	—	—

The endosperm is a triploid tissue having two doses of the maternal genes to one of the paternal, and this may lead to corresponding quantitative differences between the phenotypes of reciprocal crosses. This dosage effect could be detected in some, but not all gels.

Chao and Scandalios (1968) have recently published an abstract reporting the discovery of three zones of enzyme activity in the developing endosperm. They found fast and slow variants of the second zone which was β-amylase. This probably corresponds to the *Sd* system outlined above.

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