ALLELIC ISOZYMES OF THE pH 7.5 ESTERASE IN MAIZE¹

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SEVEN alleles of the E_1 gene which controls the pH 7.5 esterase in maize have been found to date. Most of the reported investigations on the genetic control of this enzyme were done with the E_1^F , E_1^N , and E_1^S alleles segregating in North American strains. These alleles specify isozymes with different migration rates in starch gel electrophoresis (SCHWARTZ 1960a). The other four alleles which specify isozymes with still different rates of migration (Figures 1 and 2) were reported briefly in an earlier publication (SCHWARTZ 1964a). Two alleles, designated E_1^T and E_1^W were found in a strain of maize from Columbia, South America, and two, E_1^L and E_1^R were discovered in teosinte. The enzyme occurs as a dimer (SCHWARTZ 1960a). The alphabetic superscripts designate the relative positions of the autodimer isozymes specified by the alleles, with the letters closest to the beginning of the alphabet assigned to the most positively charged isozymes. The FF autodimer specified by the E_1^F allele is the most positively charged isozyme, and the WW autodimer formed by the E_1^W allele is the most negatively charged. There are 28 possible positions which the isozymes specified by the seven alleles in homozygous and heterozygous combinations can occupy, since there are 21

FIGURE 1.—Diagrammatic representation of the electrophoretic positions of the seven autodimer isozymes specified by the alleles of the E_1 gene. The arrow designates the origin.

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FIGURE 2.—Esterase zymograms showing the migration rates of the seedling autodimer isozymes specified by the seven E_i alleles. O denotes the origin. The esterase band at the SS position seen in all the zymograms is specified by the nonallelic E_j^F gene (SCHWARTZ 1964b). Two different autodimer isozymes are run side by side on each gel: (a) FF and LL; (b) LL and NN; (c) NN and RR; (d) RR and SS; (e) SS and TT; and (f) TT and WW.

different hybrid or allodimer enzymes which can be formed. However, some of the dimer combinations have identical migration rates (see Figure 3).

The techniques of starch gel electrophoresis and development of the zymograms have been described previously (SCHWARTZ 1960a,b). The horizontal electrophoretic method was used, buffered with borate at pH 8.5. The runs were of about 3-hour duration at room temperature with a voltage drop of 7v/cm along

FIGURE 3.—Overlapping test of seedling extracts from different genotypes run side by side on the same gel: (a) E_i^F/E_i^S versus E_i^N/E_i^N ; (b) E_i^L/E_i^T versus E_i^R/E_i^R ; (c) E_i^R/E_i^W versus E_i^T/E_i^T ; and (d) E_i^F/E_i^W versus E_i^N/E_i^T . O denotes the origin.

the gel. For developing the zymograms the substrate used was 1-naphthyl acetate with Fast Blue RR salt as the dye coupler.

E_i^F , E_i^N , and E_i^S have been shown to be allelic, since they segregate in a strictly Mendelian fashion and the monomeric products hybridize in heterozygotes to form allodimers. By the same criteria, the other four genes E_i^L , E_i^R , E_i^T , and E_i^W are allelic to each other as well as to the North American alleles. If, for example, a plant carrying E_i^F and E_i^T is crossed to plants containing only the E_i^N allele, half of the progeny are E_i^F/E_i^N , and the other half are E_i^T/E_i^N (454 progeny showing the FF, FN, and NN isozymes, and 436 showing the TT, NT, and NN isozymes). In crosses involving heterozygotes the pH 7.5 esterase genes always segregate from each other, regardless of the combination.

All 21 possible heterozygous combinations of the seven alleles have been synthesized and in each case a hybrid enzyme is formed in addition to the two autodimers. In homozygotes there is no indication of dimerization with other nonallelic esterases. Diploid plants never contain more than two different alleles. A tetraploid stock has been synthesized with three different alleles.

The seven genes, although allelic to each other, fall into two different classes. The E_i^F , E_i^N , and E_i^S alleles form a series (SCHWARTZ 1960a) in that the FS allodimer formed in E_i^F/E_i^S heterozygotes has a migration rate identical to the NN autodimer (Figure 2a). This indicates that the difference in charge between the F and N monomers is the same as that between the N and S. F is more positively charged than N to the same extent that S is more negatively charged. Alleles E_i^L , E_i^R , E_i^T , and E_i^W form a second series. The charge difference between L and R is the same as that between R and T or that between T and W. This follows from the finding that the LT allodimer has a migration rate equal to RR, and the RW allodimer migrates at the same rate as the TT autodimer (Figure 2b, c). Furthermore, the magnitude of the minimum charge difference among F, N, and S is identical to that among L, R, T, and W. The FW allodimer migrates at the same rate as the NT allodimer. This condition will hold only if F is more positively charged than N to the same extent that W is more negatively charged than T. When extracts of E_i^F/E_i^W and E_i^N/E_i^T heterozygotes are run side by side on the gel in the overlapping test (SCHWARTZ 1960b), the WF and TN allodimers form a continuous band (Figure 2d).

The seven alleles do not fit into a single series. The LL band lies between NN and FF, but the FN allodimer is not at the position of LL. Similarly, the RR band lies between SS and NN, but the NS allodimer does not have the same migration rate as RR. Also, the RL and RT bands are not at the same position as NN and SS, respectively. The correspondence of migration rates between allodimers formed in heterozygotes involving two "extreme" alleles with autodimers formed by an "intermediate" allele is observed only for the intraseries combinations.

These results suggest that there are two levels of difference between the isozymes specified by the seven alleles. At one level, the isozymes of the F, N, and S series differ from those in the L, R, T, and W series. At another level, similar differences exist between the various isozymes within each series. The nature of

these differences has not been determined, but we have suggested that the intra-series charge differences result from variations in the number of positively charged groups conjugated to the polypeptides (SCHWARTZ 1964a).

SUMMARY

The seven known alleles of the pH 7.5 esterase gene in maize fall into two series, E_1^F , E_1^N , E_1^S , and E_1^L , E_1^R , E_1^T , E_1^W . The isozymes specified by these alleles differ at two levels, since the migration rates of the isozymes in the two series overlap and identical charge differences distinguish the isozymes in both series.

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