

opmental stage. In kernels of B pl x C-I hybrids, phenylalanine deaminase activity as well as the presence of the first precursor, trans-cinnamic acid, have been shown but no coumaric acid or trans-cinnamic acid-4-hydroxylase activity. Also, 10-day-old kernels of B pl x C-I hybrids cultured in vitro did not show coumaric acid or activity of the trans-cinnamic acid-4-hydroxylase enzyme, but cinnamic acid and phenylalanine deaminase activity have been demonstrated just after inoculation.

These results show that dominant inhibitor C-I inhibits the second step of biosynthesis of anthocyanins--i.e., conversion of trans-cinnamic acid into coumaric acid--but is incapable of blocking the first step--i.e., conversion of phenylalanine into trans-cinnamic acid.

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A rapid method of seeking frequency of alleles controlling multimeric enzymes in plant populations

When analyzing the allele frequency controlling the synthesis of multimeric enzymes one can, rather than analyze single plants from the population, carry out electrophoresis of total extract from sampled plants. Accuracy of the definition increases with the enlargement of sampling size.

The basis of the method is the relation between the relative quantity of the enzyme in the band on the electrophoretogram and the frequency of the corresponding allele in the population. For an equilibrium population of diploid plants with two alleles and for an enzyme with multimery equal to k this relation may be expressed by:

$$p = C_1 + 1/2 \sum_{i=2}^k C_i ; \quad q = C_{k+1} + 1/2 \sum_{i=2}^k C_i ; \quad (1)$$

where p and q are the frequencies of two alleles controlling the synthesis of two types of subunits aggregating to form enzyme molecules with different electrophoretic mobility; C_1 and C_{k+1} are the relative quantity of homomultimeric molecules; and C_i is the relative quantity of heteromultimeric molecules (where $i=2, \dots, k$).

For instance, for the dimeric enzyme (alcohol dehydrogenase, ADH) with two alleles, F and S in the population; the relation between the allele frequency and the relative enzyme quantity on the electrophoretogram is expressed by:

$$p = C_1 + 1/2 C_2 , \quad q = C_3 + 1/2 C_2 ; \quad (2)$$

where p is allele frequency F and q is allele frequency S, while C_1 and C_3 are the relative quantities of homodimeric molecules and C_2 is the relative quantity of heterodimeric molecules.

With the electrophoretogram data one can define the frequency of homozygous genotypes (p^2 and q^2) as well as heterozygous ones ($2pq$) in plants:

$$p^2 = C_1 - \frac{1}{2^k - 2} \cdot \sum_{i=2}^k C_i ; \quad q^2 = C_{k+1} - \frac{1}{2^k - 2} \cdot \sum_{i=2}^k C_i ;$$

$$2pq = \frac{2^k}{2^k - 2} \cdot \sum_{i=2}^k C_i \quad (3)$$

--the conventional signs in formula (3) and formula (1) are the same.

For the concrete enzymes the use of the method is limited by the possibility of clear electrophoretic division, by the method of staining and by the accuracy of the scanning apparatus.

The model experiments carried out by us on the ADH of maize have shown that one can identify one grain of $\underline{F}\underline{F}$ genotype among 100 $\underline{S}\underline{S}$ homozygotes; that is, the accuracy of this method for ADH is rather high. In particular, for the dimeric enzyme the relative frequency of $\underline{F}\underline{F}$ (p^2), $\underline{S}\underline{S}$ (q^2) and $\underline{F}\underline{S}$ ($2pq$) genotypes will be defined by:

$$p^2 = C_1 - 1/2 C_2; q^2 = C_3 - 1/2 C_2; 2 pq = 2C_2; \quad (4)$$

The theoretically expected relation between p and q , $p^2:2pq:q^2$, and the relative quantity of protein in bands for dimeric enzymes are cited in Table 1. It is supposed that there is equal activity of alleles and equal specific activity of isoenzymes.

Table 1. Theoretically expected relationships between the allele frequency and genotype in the population and relative quantity of the homo- and heterodimeric isoenzyme.

Allele frequency		Theoretically expected frequency of genotypes in equilibrium population			Theoretically expected relative quantity of homo- and heterodimeric molecules on electrophoretogram		
$P(\underline{F})$	$q(\underline{S})$	$p^2(\underline{F}\underline{F})$	$2pq(\underline{F}\underline{S})$	$q^2(\underline{S}\underline{S})$	$C_1(\underline{F}\underline{F})$	$C_2(\underline{F}\underline{S})$	$C_3(\underline{S}\underline{S})$
0.5	0.5	0.25	0.50	0.25	0.375	0.250	0.375
0.6	0.4	0.36	0.48	0.16	0.480	0.240	0.280
0.7	0.3	0.49	0.42	0.09	0.595	0.210	0.195
0.8	0.2	0.64	0.32	0.04	0.720	0.160	0.120
0.9	0.1	0.81	0.18	0.01	0.855	0.090	0.055

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Minimization of time of search for strains of maize with changed electrophoretic mobility of the enzyme

In searches for inbred strains having rare electrophoretic variants of enzymes, usually each separate strain is analyzed. However, this search may be accelerated if one mixes extracts from many strains and determines whether there are, among many normal strains, some mutants in the mixture. The number of the strains analyzed in one extract mixture depends on the possibility of distinguishing the weakly stained zone of the mutant enzyme close to the bright wide band of the normal enzyme on the electrophoretogram. The experiments carried out by us on alcohol dehydrogenase (ADH) demonstrated that on an electrophoretogram it is possible to pick out the zone of ADH-F molecules when one seed of $\underline{Adh}\text{-F}$ $\underline{Adh}\text{-F}$ genotype is mixed with 30, 50 and even 100 seeds of $\underline{Adh}\text{-S}$ $\underline{Adh}\text{-S}$ genotype. Thus, the mixing procedure allows us to reduce significantly the time for finding one mutant strain among many normal ones. Which exact strain is mutant may be determined by means of sequential dichotomic division of the sample containing the mutant, with subsequent analysis of each half. The mutant strain can also be quickly isolated by means of an algorithm whose principle is given in Table 1.

Table 1. Algorithm* of search for one mutant strain among 62 normal ones.

	** 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

I	+						+	+	+	+	+									
II		+					+					+	+	+						
III			+					+				+				+	+	+		
IV				+					+				+						+	+
V					+					+					+					+
VI						+					+					+				+
	** 21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37			

I		+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
II		+	+	+	+		+	+	+				+	+	+					
III		+					+	+	+				+				+	+		
IV			+				+			+	+					+		+		+
V	+			+				+		+	+				+			+	+	+
VI	+				+				+	+	+				+			+	+	+
	** 38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54			

I					+	+	+	+	+	+	+	+	+	+		+	+	+		
II					+	+	+	+	+	+						+	+	+		
III	+	+	+		+	+	+				+	+	+			+	+	+		
IV	+	+		+	+			+	+		+	+			+	+	+			+
V	+		+	+		+		+		+	+		+	+	+			+	+	+
VI		+	+	+			+		+	+		+	+	+			+	+	+	+
	** 55	56	57	58	59	60	61	62	63											

I			+	+	+	+	+	+	+											
II	+		+	+	+	+			+	+										
III		+	+	+	+			+	+	+										
IV	+	+	+	+		+	+	+	+	+										
V	+	+	+		+	+	+	+	+	+										
VI	+	+		+	+	+	+	+	+	+										

*From strains 1 to 6, one seed from each strain is taken and added to one of the mixtures. From strains 7-21, two seeds from each strain are taken and each seed is added to a different mixture; for each strain of this group, there exists a definite combination of two mixtures. From strains 22-41, three seeds from each strain are taken and each seed is added to one mixture; for each strain of this group there exists a definite combination of three mixtures, etc.

**Line corresponds to the number of the strain.

***Column corresponds to the number of the mixture.

+Means that the seed from the respective strain is added to the respective mixture.

If, after an electrophoretic analysis of the mixtures formed according to this principle, it turns out that in some mixtures there are mutant enzymes, then, by the algorithm of formation of mixtures, one can determine the number of the strain whose seeds are contained only in these mixtures. Thus, the number of the strain with the changed electrophoretic variant of the enzyme is determined.

Our model experiments demonstrated that the algorithm described works well. We took 62 ears of Adh-S Adh-S genotype, and one of Adh-F Adh-F genotype. Having numbered them randomly, we made mixtures of seeds from these ears according to the algorithm. An electrophoretic analysis of the mixtures showed that Adh-F isozyme was present in mixtures III, IV and VI, which points to the fact that the ear of genotype Adh-F Adh-F had the number 39. In this way, the time of determination of the number of the ear is reduced by the factor of 10.

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Analysis of peroxidase isozyme patterns in internodes in haploid and diploid maize

This study was carried out on line W155 and corresponding haploids obtained from W155 by means of "Chase tester" pollination proposed by Chase (Chase, 1947). Haploid plants were exposed by the absence of coloring in embryos at the seed stage, with subsequent cytological control at the seedling stage. The plants were studied at the stage of the formation of the fifth internode, when the growing internode was about half as long as the internode which has developed before it. Isozyme patterns of peroxidase were investigated in small underdeveloped, growing, and mature internodes. The staining of peroxidase isozymes after starch gel electrophoresis was carried out with benzidine. Internode growth in both diploid and haploid plants is associated with definite change in pattern of peroxidase isozymes. Isozyme patterns in diploid and haploid plants were not different. However, haploid plants differed from diploid ones in relative intensity of some individual bands in peroxidase isozyme patterns.

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