cinnamic acid (sinapic); 3,4-dihydroxy cinnamic acid (caffeic); and p-hydroxy cinnamic acid (p-coumaric), showing, thereby, the presence and activity of phenylalanine deaminase. The activity of this enzyme seems to be photocontrolled and is induced by phytochromes, resulting in absence of cinnamic acid in unexposed parts and an accumulation of aromatic amino-acids which could otherwise enter the anthocyanin biosynthetic pathway. The biosynthesis of anthocyanins in unexposed portions does not start even though phenylalanine is not lacking and the enzyme is also present, but the latter, being wavelength dependent, is activated only in the presence of light. This is further confirmed by the fact that the enzyme isolated from unexposed parts does not convert phenylalanine to trans-cinnamic acid during assay.

J. M. S. Mathur

Biosynthesis of anthocyanins in maize: Presence and role of transcinnamic acid-4-hydroxylase enzyme

The second step in biosynthesis of anthocyanins in maize is brought about through the agency of an enzyme, trans-cinnamic acid-4-hydroxylase, which catalyzes the formation of coumaric acid from trans-cinnamic acid.

The crude enzyme has been isolated by homogenizing 5 gm tissue with prechilled (-15 C) acetone, filtering in a Buchner funnel and suspending the acetone powder in cold 0.05 M phosphate buffer (pH 6.6), followed by centrifuging at 10,000xg for 30 min at 4C. The supernatant was used for assay and the activity was followed spectrophotometrically.

All the sun-exposed pigment-bearing portions of B pl plants show its presence in active form, accompanied by accumulation of substantial quantities of coumaric acid. The activity of this enzyme and presence of coumaric acid have not been observed in B pl x C-I hybrids, neither in vivo nor in vitro, although activity of phenylalanine ammonia lyase and presence of trans-cinnamic acids have been demonstrated.

These results further support the view that the dominant inhibitor C-I influences only the second step of biosynthesis in B pl plants and is incapable of blocking the first step of the biosynthetic pathway.

J. M. S. Mathur

Biosynthesis of anthocyanins in maize: Role of dominant inhibitor C-I

Since the dominant inhibitor C-I totally inhibits formation of anthocyanin in the aleurone layer, it was found to be excellent material to study gene action. By studying B pl and C-I (homozygous) and B pl x C-I hybrids (different tissues at different stages of development) both in vitro and in vivo, it was ascertained how C-I inhibits the biosynthesis of anthocyanins and at what stage this blockage occurs. The colored and colorless portions of B pl plants were examined for precursors (phenolic acids) in cob, silk, kernels, stem and colorless portions of leaves and so also in C-I plants. B pl plants showed the presence of the first compound of anthocyanin biosynthesis, cinnamic acid. The phenylalanine ammonia-lyase enzyme is present in all plant parts, and this converts the aromatic amino acid, phenylalanine, into trans-cinnamic acid; thus, biosynthesis of anthocyanins can proceed beyond the first step. However, we have found that the activity of this enzyme is wavelength dependent. This induction of biosynthesis through phytochromes results in formation of anthocyanins only in sun-exposed portions, and unexposed parts do not synthesize anthocyanins even though there is phenylalanine accumulation and enzyme is present. This is because the latter is in an inactive form. This is further confirmed by the fact that we have not been able to demonstrate the presence of trans-cinnamic acid in colorless unexposed portions.

In none of the plant parts from C-I have we been able to identify cinnamic acid or the activity of the enzyme, not even in the presence of light or at any devel-

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:

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

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,...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:

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

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:

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

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

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