carp and endosperm of R-rEm and a-ml mildly decolorizes the aleurone anthocyanins in R-rEm as well as hand-dissected pigmented patches of aleurone from R-rEm with Spf. However, the enzyme complex from colorless portions of spotted aleurone, both in R-rEm with Spf and a-ml with Spm, show very significant diluting capacity for the substrates prepared from pigmented patches of their own aleurone as well as between them.

These results suggest the possibility of increased accumulation and activity of this enzyme complex in colorless portions of aleurone of spotted kernels, which may be genetically related to Spm or Spf or presence of some concomitant pigment promotor such as catechol. The role of Spm and Spf may also be related to breakdown of membrane integrity permitting the mixing of enzyme and vacuolar substrate at certain points, resulting in dilution and causing patched appearance.

J. M. S. Mathur and N. D. Sharma

Biosynthesis of anthocyanins in maize: Presence and role of phenyl-alanine ammonia-lyase enzyme

Phenolic acids play a key role in biosynthesis of the B- ring of the anthocyanidin molecule. Tracer and enzymic studies have shown that cinnamic acid derivatives in higher plants are synthesized from aromatic amino acids via the shikimic-prephenic acid pathway. Phenylalanine is effectively utilized in biosynthesis of the anthocyanin skeleton, forming trans-cinnamic acid by deamination through the agency of the enzyme phenylalanine ammonia-lyase. Demonstration of the presence or accumulation of a particular intermediate would mean the presence of active enzymes synthesizing it.

In B pl plants anthocyanins are synthesized only in sun-exposed parts. Studies were undertaken to decipher whether anthocyanins are not synthesized at all in colorless (unexposed) portions or if the biosynthetic pathway leading to formation of anthocyanins is blocked at any particular stage, and whether the effect is genetic or physiological.

Free phenolic acids were extracted from cob, silk, kernels, anthocyanin-bearing sun-exposed parts and colorless parts of leaves by refluxing with 80% ethanol for 3 hr. The extract was evaporated and the residue was dissolved in distilled water and filtered. The filtrate was adjusted to pH 4 with 6 N HCl, extracted with ether and washed with 25% aq. sodium carbonate solution followed by re-extraction with ether. The ether was again evaporated and the residue moistened with 50% alcohol. Identification was done by two-dimensional descending paper chromatography using benzene-acetic acid-water, 6:7:1 V/V (upper phase) and sodium formate-formic acid-water, 10:1:200 V/V, followed by spraying specific chromogenic reagents and viewing under U.V. light for locating the spots.

For the preparation of enzyme, phenylalanine deaminase, the tissues were ground with 20 ml prechilled (-15 C) acetone. The homogenate was filtered in a Buchner funnel and the residue was washed with cold acetone and dried at room temperature. The dried powder was suspended in 10 ml cold 0.1 M borate buffer at 8.8 and the mixture was stirred for 15 min in an ice bath. The extract was clarified by centrifuging in the cold at 2000xg for 10 min. The crude extract is not stable and was assayed immediately.

The enzyme-catalyzed deamination of phenylalanine was followed by measuring spectrophotometrically the increase in absorption at 290 nm at 20 min intervals for 3 hr. The diluted enzyme contributes negligible absorption at this wave-length. The reaction mixture consisted of 50 μ moles of L-phenylalanine, 200 μ moles of borate buffer (pH 8.8), 1 ml of enzyme extract (200-250 mg of protein) and water to bring the volume to 6 ml. The reaction was carried out at room temperature. No increase in absorption occurs when enzyme or phenylalanine is omitted. Controls consisted of everything else except phenylalanine.

In the sun-exposed pigment-bearing tissues, four substituted cinnamic acids have been identified in addition to trans-cinnamic acid, which was predominant. These acids are 3-methoxy-4-hydroxy cinnamic acid (ferulic); 3,5-dimethoxy, 4-hydroxy

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-