By culturing the kernels at 10 days post-pollination, studies were carried out in vitro on the role of Spm and Spf in causing patched aleurone patterns (a-ml and R-rEm) and also on the 'Anthocyanin Decolorizing Enzyme Complex.' The results confirm our findings from studies in vivo.

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

Biosynthesis of anthocyanins in maize: Presence and role of an 'Anthocyanin Decolorizing Enzyme Complex'

During in vivo and in vitro studies on the role of the regulatory systems Spm and Spf in causing spotted aleurone patterns (a-ml and R-rEm), the presence of an 'Anthocyanin Decolorizing Enzyme Complex' has been demonstrated in maize for the first time. The object was to ascertain whether anthocyanins in colorless patches are not synthesized at all or are decolorized in localized areas only after synthesis, leading to spotted patterns.

The crude enzyme complex was isolated from endosperm of R-rEm, R-sd2 and a-ml; pericarp of R-rEm, R-sd2 and a-ml; and aleurone (colorless portions) of R-sd2 and a-ml by homogenizing about 1 gm tissue in cold (-15 C) acetone. The homogenate was vacuum filtered in a Buchner funnel, and the residue was suspended in cold 0.05 M phosphate buffer (pH 6.6) and centrifuged at 2000xg for 15 min at 4 C. The supernatant was used immediately for assaying activity with substrates prepared from aleurone of R-rEm and pigmented portions of aleurone of a-ml and R-sd2. For preparation of substrates the tissues were extracted with cold 1% methanolic HCl, evaporated to dryness and dissolved in enough dilute HCl to get a solution with maximum 5% transmittance at 515 nm.

The assay mixture consisted of 4 ml of freshly prepared substrate, 2.5 ml of enzyme preparation in 0.05 M phosphate buffer (pH 6.6) and water to bring the total volume to 8 ml. The anthocyanin decolorizing capacity of the enzyme complex was measured spectrophotometrically by observing changes in transmittance at 515 nm after intervals of 45 min, 2 hr, 4½ hr and 24 hr.

The results given in Table 1 indicate that the enzyme complex from aleurone tissue of a-ml dilutes R-rEm colored aleurone substrate, enzyme complex from peri-

Table 1. Activity of 'Anthocyanin Decolorizing Enzyme Complex.'

Source of enzyme complex	Substrate*	% Transmittance				
		0 hr	45 min	2 hr	4½ hr	24 hr
R-rEm endosperm	A	58	60	63	69	49
	C	63	68	60	56	59
R-sd2 endosperm	C	66	50	44	46	38
a-ml endosperm	A	48	52	52	50	44
	B	65	64	69	61	62
	C	60	64	68	68	-
R-rEm pericarp	A	60	65	67	68	68
	C	52	56	56	56	51
R-sd2 pericarp	C	66	71	74	67	70
a-ml pericarp	A	48	48	56	46	42
	B	65	71	68	70	72
	C	62	49	48	52	-
R-sd2 aleurone (colorless portion)	B	80	82	-	-	87
	C	86	89	88	-	85
a-ml aleurone (colorless portion)	A	66	72	73	76	78

*Key for substrates: A = R-rEm aleurone; B = a-ml aleurone (pigmented portion); C = R-sd2 aleurone (pigmented portion)

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.

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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