and expenses of split planting which might otherwise be necessary to consummate a "nick".

Cryptic lateness was observed as a side effect in the recessive phenotypes of one of our routine conversions. The alteration in days to ½-silk was noted in the 1975 Hawaii winter nursery:

Inbred	Normal version days to ½-silk	Homozygous recessive conversion days to ½-silk	Stage of recovery
Fr3	63	56	BC_1I_2
Mo16	67	61	BC_3I_2
K55	66	57	BC_3I_2
659	63	56	BC_3I_2
907	67	60	BC_3I_2
Average	65.2	58.0	

These data indicate that converted lines are 7.2 days earlier to ½-silk and that, extrapolating to a seedfield situation, the use of a converted line could avert the need for a one leaf split in planting male and female.

It should be noted that in making "cryptically late" conversions, recoveries can be made as exactly as for any other normal conversion. This is not the case with "cryptically early" conversions based upon id/id, since this genotype is normally ear-barren, and an undetermined number of complementary loci would have to be transferred along with id in order to restore workable ear-fertility.

D. L. Shaver

DEFIANCE COLLEGE
Defiance, Ohio

Flavonoid analysis of Zea mays tissues at different developmental stages — Much work has been devoted to flavonoids associated with gene action in maize. However, most work has centered on flavonoids found in the aleurone. It is the purpose of this report to survey flavonoids found in three tissues at two different developmental stages.

Flavonoids were extracted from roots, sheaths and leaves of two-week- and four-week-old plants with the following genetic background: R R W22 (A, A2, C, C2, R, Pr). The two-week-old plants were grown in a growth chamber at 22°C. The four-week-old plants were grown in a growth chamber for two weeks and transferred to a greenhouse for the remaining time.

Table 1. Identity, color reactions and spectral maxima of Zea mays flavonoids.

Compound	Identity[a]	Color[b] in U.V.	Color[b] in $+NH_3$	R_f in TBA	R_f in HOAc	Spectra[c] in MeOH	Spectra[c] in $+AlCl_3$	Spectra[c] in +HCl	Spectra[c] in +NAOAc	Spectra[c] in $+H_3BO_3$
1	Quercetin 3,7-diglycoside	DP	YG	.42	.68	343,267, 255	420,328sh, 300sh,274	372,354, 293sh,272	394,266	372,261
2	Kaempferol 3-monoglycoside	DP	YG	.41	.35	344,265	417,296sh, 272	398,274	402,342sh, 268	372,268
3	Flavonol-glycoside	DP	YG	.40	.52	No spectra measured[d]				
4	Vitexin	DP	YG	.25	.48	328,274 266,260	378,344 300,280	372,344, 298,278	370,342, 292	402,354, 321,285
5	Flavonol 7-monoglycoside	DP	O	.28	.12	345,270	358,270	355,269	420,342, 297	360,269
6	Flavonol 7-monoglycoside	DP	O	.16	.23	344,268	359,282	354,267	424,343, 290	358,265
7	Flavonol-glycoside	Y	Y	.48	.29	No spectra measured[d]				
8	Flavonol-glycoside	Y	Y	.53	.16	No spectra measured[d]				
9	Flavonol-glycoside	Y	Y	.63	.27	No spectra measured[d]				
10	Quercetin 3-monoglycoside	DP	YG	.54	.45	354,298sh, 256	404,358sh, 296sh,270	394,354, 296,264	404,310sh, 272	366,296sh, 258
11	Cyanidin 3,5-diglycoside	DP	DP	.35	.54	520,282	555,285			

a - tentative identification

b - DP, dark purple; YG, yellow green; O, orange; Y, yellow

c - spectra measured according to T.J. Mabry, The Systematic Identification of Flavonoids (1970)

d - only trace amounts present

A minimum of 1.0 g of fresh material was cut into small pieces, placed in a 50 ml Ten Broeck homogenizer and ground with 10 ml of 0.5% HCl in methanol (v/v). The homogenate and methanol washings were centrifuged for 5 min at 200 x g, and the methanolic supernatant was evaporated in vacuo to about 1 ml. The total methanolic extract and washings were applied to a 2.5 cm disk of Whatman No. 3 MM chromatography paper suspended on stainless steel pins. The disk was dried with the aid of a hairdryer and then inserted through four parallel slits, 5 mm apart, at the origin of a 46 cm x 57 cm sheet of Whatman No. 3 paper. Two-dimensional descending chromatography was used to separate the flavonoids. Chromatograms were developed in the long direction of the paper in t-butanol, acetic acid and water (3:1:1 v/v), and acetic acid (15%) was used in the second dimension.

Chromatograms were examined under long-wave ultraviolet light, and the flavonoids were readily detected as light-absorbing regions. The flavonoid-containing areas were cut from the chromatograms and extracted into high grade spectral methanol. Five spectra are reported for most compounds. The compounds were identified on the basis of R_f values, color reactions on the paper chromatogram and spectral analysis (Table 1). Anthocyanins were further characterized by hydrolyzing in 2N HCl for one and one-half hours and chromatographing the resulting sugar. The aglycone was also analyzed spectrophotometrically.

It is apparent from the results (Table 2) that there are differences in flavonoid composition between various tissues and that further differences are found

Table 2. Flavonoids in various tissues at different ages.

Tissue		Compound number*										
		1	2	3	4	5	6	7	8	9	10	11
Root	2 wk											+
	4 wk											+
Leaf	2 wk	+	+	+	+	+	+					
	4 wk	+	+		+	+	+					
Pigmented sheath	2 wk					+	+	+	+	+	+	+
	4 wk					+	+				+	+
Sheath	2 wk				+	+	+	+	+			
	4 wk				+	+	+	+	+	+		

\+ present

* corresponds to Table 1 compounds

between two week and four week old plants. The fact that differences exist between tissues is not surprising in view of other research as well as field observations which show that flavonoids are not uniformly distributed within the plant or even in all cells of a tissue. Differences in various developmental stages would seem to imply that their occurrence within a plant is sufficiently controlled to provide a basis for flavonoid regulation of biological systems. These experimental results are important in assigning a primary role for flavonoids, which are generally considered as secondary constituents, not absolutely essential to the life of individual cells or even to the plant as a whole.

John Trautman and B. C. Mikula

Flavonoids in chloroplasts of Zea mays — Early biological interest in the flavonoids was concerned largely with chemical characterization within a framework of genetic and taxonomic studies. Recent work has been concerned with physiological control and biosynthesis. This report is concerned with the localization of flavonoids in specific organelles for further insight into the biosynthetic processes and the likely physiological functions of flavonoids at the ultrastructural level.

A minimum of 100 g fresh weight of leaf material was used for plastid isolation. Midribs and petioles were discarded, the ramaining material washed in distilled water, and the sample chilled to $4^{o}C$.

The tissue was cut into 2 cm pieces and placed in a Waring blender, and from three to four volumes (w/v) isolating medium were added. The aqueous isolating medium contained 1.1 M sorbitol, 0.01 M magnesium chloride and 0.02 M EDTA in 0.15 M pH 6.8 Sorensen's buffer. The tissue was homogenized at about 23,000 rpm by three two-second bursts in a Waring blender. The homogenate was filtered through one layer of cheesecloth and two layers of "Miracloth." The filtrate was centrifuged for one minute at 200 x g at $4^{o}C$. The pellet was discarded and the supernatant recentrifuged at 2000 x g for one minute. The resulting chloroplast pellet was resuspended in a known volume of medium based on the number of centrifuge tubes used, and an aliquot was taken for plastid determination. Plastids were repelleted by centrifugation at 2000 x g for two minutes. This pellet served as the chloroplast preparation. Flavonoids were extracted from the chloroplasts with acidic 50% methanol (0.5% HCl in 50% aqueous methanol v/v) for 12 hours in a cold room. The extract was filtered through Whatman No. 1 filter paper in a Büchner funnel and evaporated to about 1 ml for paper chromatography. A paper chromatogram was also prepared from a standard methanol extract of the leaf (see part 1 of this report).