

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.

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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 remaining material washed in distilled water, and the sample chilled to 4°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°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).

On comparison of the plastid extract chromatogram and the methanol extract chromatogram, it was determined that the flavonoids were the same in both samples. The paired chromatograms were analyzed under ultraviolet light, color changes in ammonia vapor noted, and the flavonoids eluted for spectrophotometric measurements (Table 3).

Table 3. Comparison of leaf and chloroplast flavonoids of Zea mays.

Compound number*	Identity	Leaf	Chloroplast
1	Kaempferol 3-monoglycoside	+	+
4	Vitexin	+	+
5	Flavonol 7-monoglycoside	+	+
6	Flavonol 7-monoglycoside	+	+

+ present

* corresponds to the compounds in Table 1 which are fully characterized

Recent research has shown that flavonoids are not end-products but are continuously synthesized and degraded in healthy tissues. The above evidence indicates that flavonoids are most likely synthesized within the chloroplast and transported to the vacuoles in most cells. It had been thought in the past that flavonoids were stable end-products of metabolism stored in the vacuole and thus removed from enzymatic activities associated with plant growth, differentiation, etc. It appears that this older view must be seriously questioned.

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Allotaxis in maize — We use the word "allotaxis," with the same value as "heterotaxis," to express in a general way that the plant material stands aside from the common or normal arrangement, such as the modifications in a maize stock reported by us on other occasions (IV Jornadas de Genética Luso-Espanholas, Oeiras, Portugal, 1967; Port. Acta Biol. 10: 289-300, 1968; An. Aula Dei 10: 716-723, 1969). This material is not only of the aberrant phyllotaxy type reported by Greyson and Walden (Amer. J. Bot. 59: 466-472, 1972), but also includes more