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THE DISTRIBUTION OF FLAVONOIDS IN CHLOROPLASTS OF TWENTY FIVE SPECIES OF VASCULAR PLANTS*

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Abstract—A survey was made of the major flavonoids in whole leaf extracts and in chloroplast preparations from twenty five species of vascular plants including Anthophyta (20), Coniferophyta (1), Ginkophyta (1), Pterophyta (2), and Arthrophyta (1). The chloroplasts variously contained derivatives of flavones, *C*-glycosylflavones, flavonols, flavanones, isoflavones, 3-deoxyanthocyanidins, and anthocyanins. Twenty three species contain one or more flavonoids in isolated chloroplast, usually in a pattern quite similar to that found in whole-leaf extracts but occasionally showing enrichment of one or more flavonoids in the chloroplasts. Flavonoids are apparently absent from chloroplasts of *Phaseolus aureus* and *Morus alba* although whole-leaf extracts of these species are rich in quercetin derivatives.

INTRODUCTION

Our previous studies [1] have shown that the flavonoid saponarin is present in either etioplasts or chloroplasts of Atlas 68 barley. Experiments conducted with foreign flavonoids indicate that this is not a contaminant of isolation. In the present work we have applied the techniques developed for these studies to a survey of the general occurrence of flavonoids in chloroplasts isolated from a range of vascular plants. The only previous study in which a series of plants was surveyed for chloroplasts flavonoids was that of Montes [2] who, in addition to identifying the flavonoids in chloroplasts isolated from tomato and melon, found compounds with absorbance spectra suggestive of flavonoids in chloroplasts isolated from nine other angiosperm species.

RESULTS

The results of this survey are shown in Table 1. It was surprising to find no flavonoids in chloroplasts isolated from either *Phaseolus aureus* or *Morus alba*, especially as the whole-leaf extracts were rich in flavonols. However, repeated examinations of these species confirmed this observation. In the case of *P. aureus*, plants were grown in the greenhouse and examined periodically up to 45 days of age. While the apparent lack of flavonoids in chloroplasts from these plants may be entirely quantitative, we can detect amounts as small as 25 nmol [1] and chloroplasts isolated from these plants would, at best, contain flavonoid levels orders or magnitude below that found in the plastids of the other 23 species of plants. The failure to recover flavonoids from chloroplasts of *P. aureus* and *M. alba* supports our contention that flavonoids are not general contaminants which adhere to chloroplasts during isolation.

In a few cases there were striking differences in the relative concentrations of certain flavonoids in whole-leaf extracts and in chloroplasts isolated from the same plant sample. For example, in chloroplasts isolated from *Spirodela polyrhiza* luteolin-7-glucoside is prominent and both apigenin-7-glucoside and vitexin are minor constituents. In whole-frond extracts, however, apigenin-7-glucoside and vitexin are major constituents and only a small amount of luteolin-7-glucoside is present. A different pattern of distribution with regard to B-ring substitution is seen in young barley seedlings. Here only the apigenin derivative saponarin is found in plastids while whole-shoot extracts contain both saponarin and the luteolin derivative lutonarin [1].

Early in this survey we examined the flavonoid composition of older, yellowing, corn leaves. While the whole-leaf extracts from these plants contained kaempferol and quercetin derivatives, flavonoids were not recovered from the plastids. When corn plants were grown in a greenhouse, or in environmental chambers, or collected from local farms, the recently matured green leaves yielded plastid preparations with the normal flavonoid pattern found in whole-leaf extracts. Similar results have been obtained with older tobacco plants where rutin is present in chloroplasts of green leaves but absent from plastids isolated from the lower yellowing leaves [3].

DISCUSSION

While our survey included only 25 species, this broad and rather arbitrarily-chosen sample can serve as a basis for a few general conclusions and some speculations. For example, we invariably found the same pattern of flavonoids in the chloroplasts on each occasion as long as the starting material was mature, green, and apparently "normal" tissue. Thus a consistent pattern of flavonoids

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Table 1. Flavonoids identified in isolated chloroplasts

Plant	Chloroplast flavonoids
Anthophyta	
Aceraceae	
Acer saccharum Marsh	Km-3-gly
	Qu-3-gly
	Qu-3-digly
Aristolochiaceae	
Asarum canadense L.	Qu-3,7-digly
	Qu-3-methyly,7-gly
	Km-3,7-digly
Caprifoliaceae	
Viburnum sieboldi Mig	Orientin
	Lut-7-digly
	Ap-7-gly
Chenopodiaceae	
Spinacia oleraceae L	Spinacetin
Curcurbitaceae	
Cucurbita maxima Duchesne	Orientin
	Lu-7-digly
	Qu-3-gly
	Qu-3-digly
Gramineae	
Hordeum vulgare L	Saponarin
Zea mays L.	Km-3-gly
	Qu-3,7-digly
Leguminosae	
Cercis canadensis L	Qu-3-gly
	Qu-3-digly
	Qu-3,7-digly
*Glycine max*L	Km-3-digly
Phaseolus aureus Roxb	none*
P. vulgaris L	Km-3-gly
	Qu-3-digly
Sophora japonica L	Rutin
	Genistein-gly
	Km-3-gly
Lemnaceae	
Spirodela intermedia W Koch.	Orientin
	Vitexin
S. polyrhiza (L) Schleid.	Orientin
	Ap-7-gly
	Lu-7-gly
	Cy-3-gly
Magnoliaceae	
Magnolia soulangeana Hort	Qu-3-digly
Moraceae	
Morus alba L	None†
Phytolaccaceae	
Phytolacca americana L.	Km-3-digly
	Qu-3,7-digly
Polygonaceae	
Fagopyrum esculentum Moench	Rutin
	Isovitexin
	Vitexin
	Orientin
	Homo-orientin
	Cy-3-gly
Solanaceae	
Lycopersicon esculentum Mill.	Rutin
Umbelliferae	
Daucus carota L.	Ap-7-gly
	Lu-7-gly
Arthrophyta	
Equisetaceae	
Equisetum arvense L	Km-3-gly
Coniferophyta	
Pinaceae	
Pinus nigra Arnold	Naringenin
Ginkgophyta	
Ginkgoaceae	
Ginkgo biloba L	Km-3-gly
	Qu-3-gly
Pterophyta	
Adiantaceae	
Adiantum caudatum L.	Km-3-gly
	Qu-3-gly
Polypodiaceae	
Dryopteris erythrosora Eat	Ap-7-gly
	Luteolinidin-3-gly

Abbreviations: Ap = apigenin, Km = kaempferol, Lu = luteolin, Qu = quercetin, Cy = cyanidin.

* Whole leaf tissues of *Phaseolus aureus* contain rutin.

† Whole leaf tissues of *Morus alba* contain Qu-3-gly.

seems to be retained within the functional, mature, chloroplasts. When yellowing tissue was examined, flavonoids were not detected in the plastid fraction but were present in whole-leaf extracts of all ages and in plastids from green tissue. If flavonoids are liberated from the chloroplasts into the general biochemical mileu of a cell, they could clearly hasten the course of senescence by inhibiting enzymes and altering primary metabolism.

Although chloroplasts isolated from most of the species yielded chromatographic patterns essentially identical to those obtained from whole-leaf extracts, there were several striking exceptions to this generalization. In the case of *Spirodela polyrhiza*, the chloroplasts are enriched in luteolin-7-glucoside while the chloroplast flavonoid profile in *S. intermedia* is indistinguishable from that of whole-frond extracts. Also, *Phaseolus aureus* chloroplast appear to be devoid of rutin, which is found in whole-leaf extracts, while *P. vulgaris* contains a quercetin glycoside in both chloroplasts and in whole-leaf extracts.The diversity of flavonoids is well recognized in the area of taxonomic distribution and in the pattern of flavonoid localization at the organ, tissue, and cellular level [4]. This diversity of flavonoid localization apparently extends to the subcellular level as well.

EXPERIMENTAL

Many of the plants were collected on the campus of Miami University; others were obtained from the Botany Department greenhouse or grown in environmental chambers. An attempt was made to include only healthy, recently matured leaves in the survey. In the case of the larger leaves, midribs were discarded and the leaf blades cut into small, *ca* 1 × 1 cm, pieces. About 100 g freshly harvested foliar tissue was homogenized in 4 vols cold isolating medium following the procedure developed for isolating barley plastids for flavonoid investigation [1]. Plastid preparations were monitored for intactness and purity using phase contrast microscopy. Whole-leaf flavonoids were extracted from about 1 g fr. wt of tissue by the technique used for whole barley shoots. Each species was examined on at least three occasions for plastid flavonoids and again for whole-leaf flavonoid profiles. Methanolic extracts from isolated chloroplasts (around 1×10^{10}) or 1 g samples fresh leaves containing the flavonoids were separated by PC and the compounds eluted from the paper in spectral methanol [1]. Identification was based primarily on measuring UV spectra in MeOH with and without the standard diagnostic reagents [5].

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