Yellow Flower Pigments in Magnolia

by Frank S. Santamour, Jr. and Polly Demuth

Most of the yellow and orange flower petal pigments in plants are carotenoids and in only a few species of woody landscape plants have these pigments been fully characterized. We have recently identified the major carotenoids in *Magnolia* and *Liriodendron* (Demuth and Santamour, 1978). The present paper is an attempt to make these data more meaningful to persons involved in magnolia breeding.

Materials and Methods

The plants examined were growing at the U.S. National Arboretum in Washington, D.C. or at the Kitchawan (N.Y.) Research Station of the Brooklyn Botanic Garden. We thank Dr. Lola E. Koerting of the Brooklyn Botanic Garden for allowing us to sample various magnolia hybrids from her research program.

Fresh tepals were extracted for 2 min. in acetone in a Waring blendor, then filtered. The residue was washed with acetone until the filtrate was clear. The filtrate was shaken with an equal volume of light petroleum (b.p. 38° C. - 49.5° C.), then distilled water was added to form two phases. The upper phase was washed with distilled water, saponified with a solution of 4 g. potassium hydroxide in 10 ml. of water, washed with distilled water, and evaporated under vacuum to 25 ml. Samples were eluted stepwise on an alumina-packed column, using as solvents: light petroleum, 1 percent propanol in light petroleum, and 95 percent ethanol. Fractions were run on TLC (thin-layer chromatography) silica gel sheets along with commercial *beta*-carotene, lutein, and column fractions of *Ranunculus acris* L. (buttercup) and *Taraxacum officinale* Weber (dandelion) as standards. TLC solvents were 10 percent, 25 percent, and 50 percent benzene in light petroleum and 10 percent methanol in benzene. Bands were eluted from TLC sheets in ethanol for spectral analyses. Identity of purified compounds was established by spectral and chromatographic comparisons with authentic standards and reference data (Davies, 1976; Goodwin, 1952 and 1955).

Carotenoids in M. Acuminata

The only species of *Magnolia* with pronounced yellow tepals is *M. acuminata* L., including var. *subcordata* (Spach) Dandy. The major carotenoids of this species included the carotenes *beta*-carotene and *alpha*-carotene-5, 6-epoxide and the xanthophylls lutein and lutein-5, 6-epoxide. Lutein-5, 6-epoxide was the predominant pigment. These were also the major compounds in the two species of *Liriodendron* (Demuth and Santamour, 1978). The R_f values (x100) on TLC were: lutein-5, 6-epoxide (35) and lutein (51) in 10 percent methanol/benzene and *alpha*-carotene-5, 6-epoxide (15) in 50 percent benzene/light petroleum.

Beta-carotene ran with the front in all solvent systems, but was readily identified by spectral analyses of the light petroleum column fraction.

Three *M. acuminata* hybrids from the Brooklyn Botanic Garden were also sampled. The most outstanding of these (No. 391) was an F_1 hybrid between *M. acuminata* and *M. heptapeta* (Buc'hoz) Dandy (=*M. denudata* Desrouss.) produced light canary-yellow tepals (Royal Horticultural Society Colour Chart, yellow 9-D) in which lutein-5, 6-epoxide comprised more than 98% of the carotenoid fraction. The other two hybrids contained varying amounts of *M. quinquepeta* (Buc'hoz) Dandy (=*M. liliflora* Desrouss.) germplasm. One (No. 278) was a backcross of *M.* × *brooklynensis* Kalmbacher 'Evamaria' to *M. acuminata* and the other (No. 389) was a second-generation seedling of *M.* × *brooklynensis*. All four of the major carotenoids were present in the tepals of these hybrids and lutein-5, 6-epoxide was the predominant pigment. However, both contained appreciable amounts of *alpha*-carotene-5, 6-epoxide and more than trace quantities of *beta*-carotene. Five different unknown xanthophylls were also found in trace amounts. The tepals of these hybrids (which were not color-coded) were more golden-yellow than those of No. 391. Whether the golden-yellow shades were the result of a higher carotene content or a "blending" of anthocyanins and carotenoids is not known.

Carotenoids in Other Magnolias

Flowers of both *M. heptapeta* and *M. virginiana* L. frequently exhibit a tinge of yellow and we did find carotenoid pigments in these species. The tepals of *M. heptapeta* and the sepaloid tepals of *M. virginiana* contained small amounts of three of the four (no lutein) identified pigments and traces of two unidentified xanthophylls. The petaloid tepals of *M. virginiana* contained small amounts of *alpha*-carotene-5, 6-epoxide only while those of *M. grandiflora* L. had only traces of *beta*-carotene: neither contained any trace xanthophylls. The tepals of *M.* × *loebneri* Kache 'Merrill' contained lutein and two trace xanthophylls. In the anthocyanin-pigmented tepals of *M. sprengeri* Pampan. 'Diva', we found small amounts of *alpha*-carotene-5, 6-epoxide, lutein-5, 6-epoxide and a single trace xanthophyll.

Although our survey was restricted largely to species and cultivars of particular interest to the National Arboretum magnolia breeding program, the results suggest that probably many, and maybe all, magnolia species have the genetic potential to produce some carotenoid pigments in their tepals, even though the amounts produced may not result in visible "yellowness."

Crosses between species with low levels of carotenoids may give highly variable progeny. One individual of the cross between *M.* × *veitchii* W.J. Bean and *M. heptapeta* produced distinctly yellowish tepals in 1977, and we found appreciable quantities of lutein-5, 6-epoxide, a small amount of *alpha*-carotene-5, 6-epoxide and three trace xanthophylls. A sibling of this plant, with whiter tepals, produced *beta*-carotene in addition to the other pigments, but all were in trace amounts. A hybrid of *M. heptapeta* and *M. sprengeri* 'Diva' contained only a trace of a single unknown xanthophyll, even though both parents are capable of producing the major carotenoid pigments.

Conclusions

The major carotenoid pigment in the tepals of *Magnolia* flowers is the xanthophyll lutein-5, 6-epoxide. Of the two carotenes identified, *alpha*-carotene-5, 6-epoxide was always more abundant then *beta*-carotene. Even though the "yellowness" of flower petals is dependent upon the amount of pigment present, the observed distribution pattern indicates that a fairly narrow range of yellow shades can be achieved by hybridization between *M. acuminata* and "white-flowered" species. Thus, since the levels of carotenes, especially *beta*-carotene, are so low, it is highly unlikely that tepals having an orange color similar to the familiar orange band in *L. tulipifera* petals could be produced in *Magnolia* by carotenoids alone.

Orange or golden-yellow shades can also be produced by a combination of plastid carotenoids and cell-sap anthocyanin pigments. Thus, some of the segregates from advanced generation breeding of *M.* × *brooklynensis* (involving *M. quinquepeta*) or the National Arboretum hybrids between *M. acuminata* and *M. sprengeri* 'Diva' (Santamour, 1976) may produce flowers with a wider range of colors.

Frank Santamour is a research geneticist and Polly Demuth a biochemist at the U.S. National Arboretum, Washington, D.C.

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