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Analysis of Normal and Mutant Peanut Chloroplast Pigments By Liquid Chromatography[1]

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ABSTRACT

Chloroplast pigments from young and old leaves of three chlorophyll-deficient peanut mutants (lutescens, auredus, and virescent) and two "normal" peanut genotypes ('Chico' and 'Guanajuato') were analyzed using high performance liquid chromatography. All genotypes showed chlorophylls *a* and *b*, carotene, lutein, neoxanthin, and violaxanthin in young and old leaves. Generally, there were decreases in most pigments as the leaves aged for Guanajuato, Chico, and aureus, whereas the reverse was true for virescent and lutescens except for violaxanthin, for which the latter two mutants showed decreases. Guanajuato and Chico consistently showed larger quantities of all pigments than the mutants at the young age. Guanajuato showed the largest quantities of all pigments at the old age. Aureus contrasted sharply with the other genotypes for carotenoid to chlorophyll ratio in the old leaves, probably accounting for its conspicuous yellow color.

Key Words: *Arachis hypogaea*, carotenoids, chlorophyll-deficient, groundnuts, senescence.

Chlorophyll-deficient peanut plants occur as spontaneous or induced mutants. The inheritance of several of these mutant peanut plants was reviewed by Hammons (8). Tai et al. (12) described the genetic relationship among three types of chlorophyll-deficient mutants: lutescens, aureus, and virescent. Tai and Todd (11), using paper and column chromatography, reported on pigment analyses of some chlorophyll mutations in peanuts. Our objective was to make additional pigment analyses using a modern, highly sensitive HPLC system to confirm earlier work and to more fully elucidate the chlorophyll pigment constituents in the leaves of selected mutant peanuts.

Materials and Methods

Three chlorophyll-deficient mutants (aureus, virescent, and lutescens) and two normal peanut cultivars ('Chico' and 'Guanajuato') were chosen for study. Aureus is a single plant selection from PI266D837 (10) whose young leaves have normal green color which turn golden-yellow as they age. Virescent, a Virginia botanical type peanut, originated as a radiation-induced mutation from NC4 stock (W. C. Gregory, personal communication). It has light yellowish-green leaves in the seedling stage which turn normal green as they mature. Lutescens was isolated as a single plant selection from a plot of PI234422 (13). It has crinkled leaflets which are pale yellow-green throughout its life. Although the latter mutant can reproduce in the greenhouse, it dies in the seedling stage in the field plantings, possibly because of unfavorable light or temperature conditions. Chico (PI268661, 'Apaxuc') is an extremely early maturing Spanish peanut introduction from Russia with normal green color (2). Guanajuato (PI380688), an introduction from Mexico, is a later maturing Virginia botanical peanut (Var. *hirsuta* Kohler, according to Krapovick - as, personal communication) with a dark green color and conspicuous anthocyanin pigments in the stems and leaves. Additional details about these genotypes have been presented by Tai et al. (12), Banks (3), Alberte et al. (1), Tai and Todd (11), and Benedict and Ketring (4).

Seeds of the genotypes were germinated in rolled wet paper towels in a growth chamber set on a 24 hour 29 C day regime. Four days later, the germinated seeds were transplanted to 10-cm clay pots, one per pot, in a sandy loam soil mixture. The plants were grown on a bench in a completely randomized design in a fiberglass greenhouse at 21 - 29 C. The first leaf samples (designated young) were collected 25 days after germination. The first two fully unfolded leaves from the tip of the main axis were collected from each of two plants from the same genotype and were combined into one sample and weighed. Four replicate samples were made for each genotype. After weighing, the leaves were immediately packaged in polyethylene bags, packed in dry ice, and shipped to the USDA, SEA-AR Northern Regional Research Center by air-freight for HPLC analysis. Similar samples were taken of older leaves from the same plants 28 days later. The later samples consisted of 2 to 4 leaves from each of two plants. The older leaves were taken from nodes immediately below or above the original (young) leaf sample. Again, the leaves were weighed and packaged in dry ice and air-freighted to the Peoria lab. HPLC analyses for the chlorophyll pigments were performed by the procedures described by Eskins et al. (7).

Results and Discussion

Leaves of all of the genotypes analyzed showed detectible levels of chlorophyll *a*, chlorophyll *b*, carotene, lutein, neoxanthin, and violaxanthin. Histograms, based on the analytical data for the pigments by genotype, are presented in figure 1. Qualitative differences in the pigments were shown to exist among the different genotypes at young and old stages of leaf development.

Genotypes Guanajuato, Chico, and aureus generally showed decreasing amounts of each of the pigments as the leaves aged. As their leaves aged, virescent and lutescens showed increasing amounts of chorophyll a, chlorophyll b, carotene, lutein, and neoxanthin, but decreasing amounts of the pigment violaxanthin. Except for chlorophyll a, Guanajuato showed the largest amounts of each of the pigments at both the young and old stages. Chico had the largest amount of chlorophyll a at the young stage. Guanajuato and Chico had similar amounts of most pigments at the young leaf stage and were generally significantly different from the other genotypes. However, at the old leaf stage, Guanajuato was significantly different from all genotypes. At the young leaf stage, lutescens contained the smallest amounts of each of the pigments and was significantly different from the other genotypes for all pigments, except for carotene and lutein, for which it was not significantly different from virescent.

Aureus was significantly lower in all of the pigments in old leaves than the other genotypes. This great reduction in pigments, especially chlorophylls a and b, probably helps account for the striking golden color of this mutant at harvest time when most of the leaves are relatively mature. Carotene quantity per se does not appear to be the chief factor contributing to the yellow color in aureus. More likely it is the combination of all the carotenoids in

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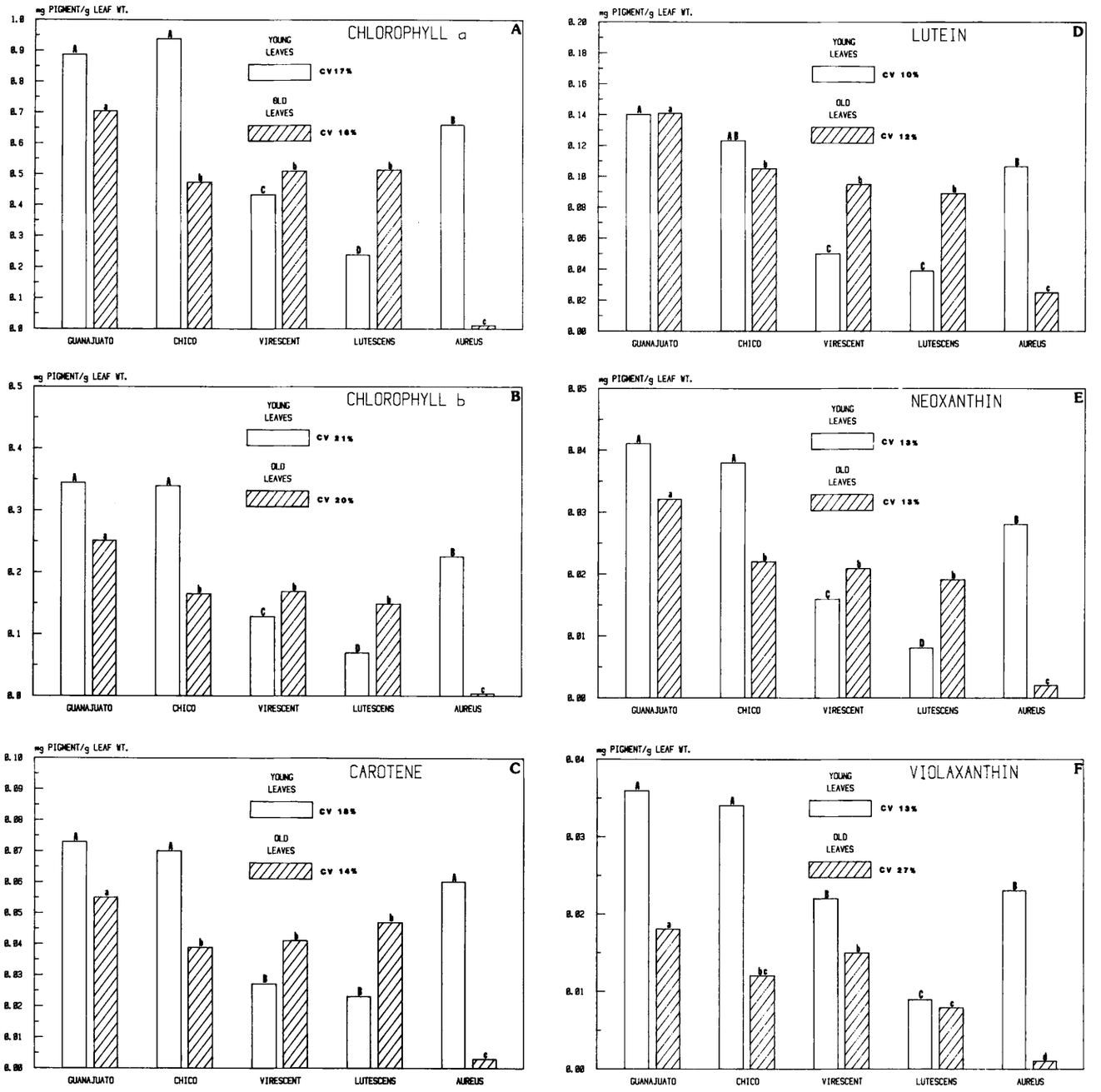

Fig. 1. Comparisons of chloroplast pigments in young and old leaves of five peanut genotypes. Graphs are based on the means of four replications. Within age, values for the histograms with the same letter (caps for young, lower case for old) are not significantly different at the 5% level by Duncan's New Multiple Range Test. Coefficient of Variability (CV) values are rounded to whole numbers.

comparison to total chlorophyll which contributes to the golden color. Chichester and Nakayama (5) suggested that esters of lutein and violaxanthin are responsible for autumn coloration. Schmid (9) suggested that a high ratio of carotenoids to chlorophyll caused the yellow color of mutant tobacco plants. Ratios for chlorophyll a to chlorophyll b and for the carotenoids to chlorophyll for our peanut data are presented in Table 1. The ratios of chlorophyll *a* to chlorophyll *b* in young and old peanut leaves showed ranges of 2.57 in young Guanajuato leaves to 3.48 in both young and old leaves of lutescens. These ratios are raily close to the usual normal ratio of 3:1. The carotenoid to total chlorophyll (i.e. $a \pm b$) ratios in young leaves ranged from 0.205 in virescent to 0.256 in lutescens. However, a striking ratio was found in the old leaves in aureus (2,769) as compared to the old leaves of the other genotypes (0.246 - 0.279).

Although some of the same genotypes were used, general results vary from those of Tai and Todd (11). For example, in their study, the chlorophyll a content of normal peanuts ('Argentine') declined significantly as the leaves aged but chlorophyll b decreased only slightly. Our analyses showed decreases in both chlorophylls with age in both normal genotypes (Fig. 1A - B). Leaves of their lutescens mutants contained smaller amounts of chlorophyll b

Table 1. Comparisons of ratios of chlorophyll *a* to chlorophyll *b* and carotenoids to chlorophyll in young and old leaves of five peanut genotypes.

Genotype	Chl *a*/*b*		Carotenoids/Chl *a*+*b*	
	young	old	young	old
Guanajuato	2.57	2.79	0.235	0.257
Chico	2.76	2.85	0.207	0.279
Virescent	3.38	3.02	0.205	0.253
Lutescens	3.48	3.48	0.256	0.246
Aureus	2.92	3.33	0.245	2.769

than did other plants they tested except for the old aureus leaves which were about the same. In our analyses the lutescens mutant at the young leaf stage was lower in chlorophyll b than the others, but it was much higher in chlorophyll b than aureus at the old leaf stage. They reported the lutein-zeaxanthin content of normal and aureus leaves was quite high at the young stage and increased with age, whereas our results generally showed decreases in lutein for the normal and aureus genotypes. Their test also showed relatively high amounts of carotene in young leaves of both normal and aureus plants, decreasing to a low value in older leaves. We showed moderate decreases of carotene for normal genotypes, but for virescent and lutescens, the carotene content actually increased.

Reasons for the differences between our results and those of Tai and Todd (11) are unknown, but because the plants for both of our experiments were grown in the same greenhouse under similar environments, we believe the differences are probably due to analytical procedures. In our experiments the high sensitivity and the relative uniformity among replications suggest the superiority of high-pressure liquid chromotography over paper and column chromotography for leaf pigment analyses. Similar results have been achieved in comparable mutants of soybean (6).

Aureus is a particularly interesting mutant because of its dramatic color change from essentially normal green to the conspicuous "golden" color. Tai and Todd (11) suggested that this change may be due to premature senescence. We do not believe senescence in the usual sense is the chief reason for the color change, however, because other peanut genotypes undergoing natural senescence do not show this golden color. It would be interesting to study the biochemistry of the aureus mutant to determine the relationship, if any, between senescence and the expression of the "golden" trait.

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