CAROTENOID DISTRIBUTION IN SEEDS OF WILD LEGUME SPECIES: PHYLOGENY *VERSUS* ECOLOGY

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1. Introduction and Aims

Carotenoids are lipophilic pigments synthesised and present in plastids. Carotenoids play pivotal roles in plants including light capture, phytohormone synthesis, pigmentation, membrane stabilization, photoprotection and antioxidant activity. Most work on carotenoids focused on photosynthetic tissues and fruits, whereas **their distribution and functions have been very poorly studied in plant seeds**, except for a few crops of interest to human nutrition, such as corn, soybean, rice or wheat (some carotenoids are essential pro-vitamins to humans). Here, we report on the occurrence and distribution of carotenoids in the seeds of wild species of the Fabaceae, which is one of the largest of all plant families.

Our aims were:

(*i*) to describe the carotenoid composition of Fabaceae seeds
(*ii*) to compare the patterns of carotenoid composition with the accumulation of other family of lipophilic antioxidants (tocochromanols) with which they share functions.
(*iii*) to relate these patterns the biological, ecological and phylogenetical requirements of each species.

Abbreviations: A: antheraxanthin, Car: carotene, Carot: carotenoids, L: lutein, Lx: lutein epoxide, N: neoxanthin, Toc: tocophoerol, T3: tocotrienol, V: violaxanthin, Z: zeaxanthin.

2. Plant material and Methods

Seed material comprising 54 species from the Caesalpinioideae, Mimosoideae, and Papilionoideae subfamilies was provided by the Royal Botanic Gardens, Kew (United Kingdom) and by the Banco de Germoplasma del Jardín Botánico de Olarizu, Vitoria-Gasteiz (Spain). Carotenoids and tocochromanols were analysed in mature dry seeds using HPLC and following the method of (García-Plazaola & Becerril (1999, 2001) for pigments an Bagci et al. (2004) for tocochromanols analyses.

Table 1. List of the analysed species, their subfamilies and their numeric code for thier identification in the figures.

CAESALPINIOIDE		MIMOSOIDEAE		PAPILIONOIDEAE	
Adenolobus pechuelii	22	*Faidherbia albida*	19	*Centrosema pubescens*	20
Colophospermum mopane	30	*Dichrostachys cinerea*	23	*Lupinus angustifolius*	21
Caesalpinia mollis	32	*Amblygonocarpus andongensis*	24	*Vigna adenantha*	25
Pterogyne nitens	33	*Pithecellobium albicans*	27	*Sophora arizonica*	26
Peltophohrum dubium	34	*Leucaena leucocephala*	35	*Xanthocercis zambesiaca*	28
Cassia sieberiana	42	*Havardia pallens*	36	*Philenoptera violacea*	29
Piliostigma reticulatum	43	*Paraserianthes lophantha*	41	*Galactia striata*	31
Daniellia oliveri	44	*Parkia biglobosa*	46	*Luetzelburgia auriculata*	37
Ceratonia siliqua	49	*Entada abyssinica*	47	*Glycyrrhiza echinata*	38
Dialium guineense	50	*Neptunia oleracea*	48	*Virgilia oroboides*	39
Afzelia africana	51	*Acacia angustissima*	54	*Podalyria cuneifolia*	40
Erythrophleum africanum	52			*Pterocarpus erinaceus*	45
				Sesbania sesban	53
				Anthyllis vulneraria	60
				Psoralea bituminosa	61
				Dorycnium pentaphylum	62
				Genista hispanica	63
				Genista scorpius	64
				Genista tinctoria	65
				Lathyrus pratensis	66
				Lotus corniculatus	67
				Lotus pedunculatus	68
				Trifolium pratense	69
				Onobrychis argentea	70
				Melilotus albus	71
				Ononis spinosa	72
				Astragalus glycyphyllos	73

3. Results and Discussion

3.1. Carotenoid composition of Fabaceae family

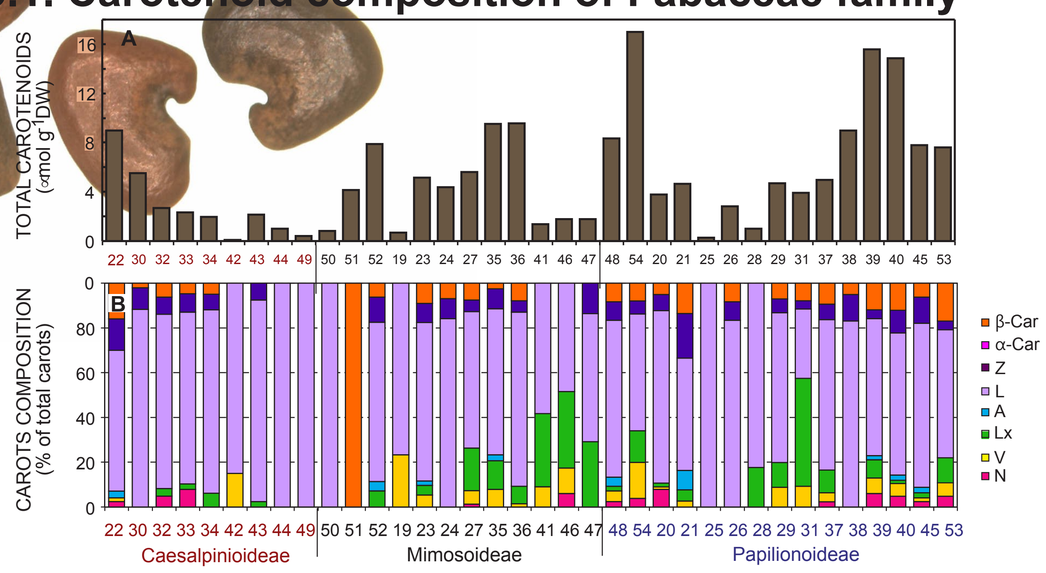


Fig. 1 Concentration (A) and composition (B) of carotenoids in seeds of the three subfamilies of legumes (see Table 1 for number correspondence with species names).

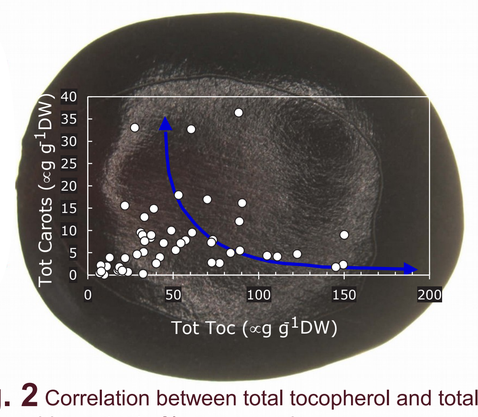


Fig. 2 Correlation between total tocopherol and total carotenoids content of legume seeds.

Lutein (L) was the major carotenoid of seeds and was present in all species except for *Afzelia africana (51),* which contained only β-carotene (β-car) (Fig. 1). Most species also contained considerable amounts of zeaxanthin, lutein epoxide and β-car.
When total carotenoids content was plotted against total tocopherols (Fig. 2) content a trade off in the accumulation of these two kinds of lipophilic antioxidants was observed. Thus, the species containing the highest amount of carotenoids had lower amounts of tocopherols and *vice versa*.

3.2. Phylogenetic distribution

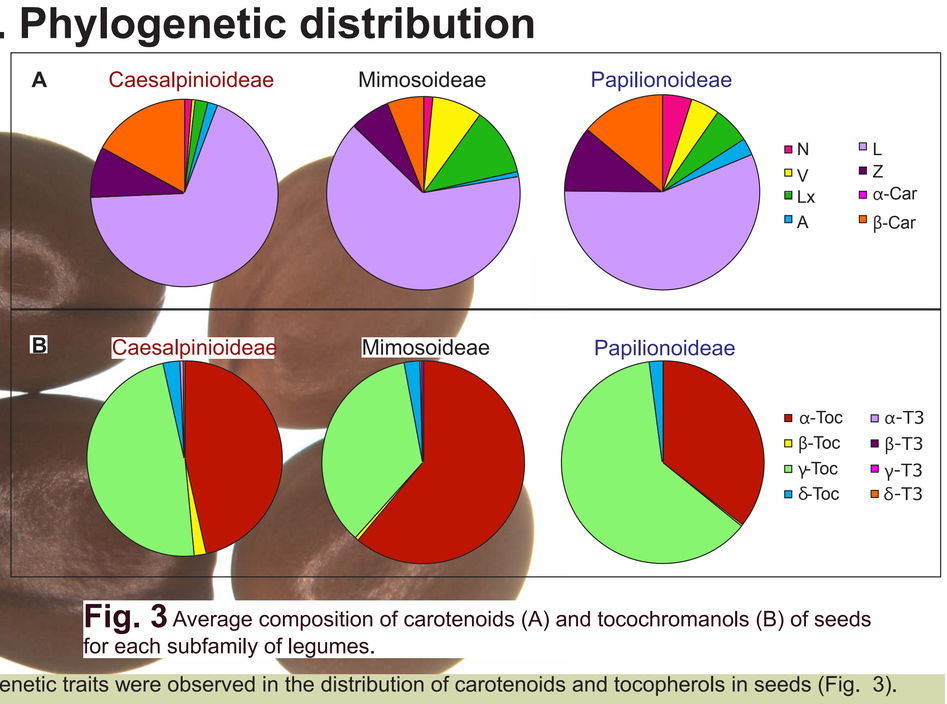
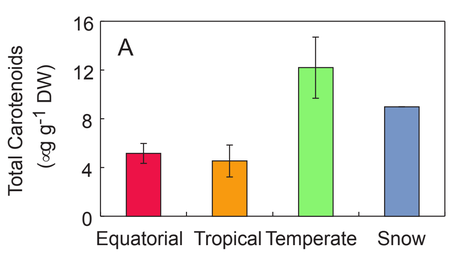


Fig. 3 Average composition of carotenoids (A) and tocochromanols (B) of seeds for each subfamily of legumes.

Phylogenetic traits were observed in the distribution of carotenoids and tocopherols in seeds (Fig. 3). Caesalpinioideae subfamily showed the lowest content of N, V, and Lx, and the highest of L (Fig. 3A). On the other hand, Papilionoideae was the only subfamily which lacked tocotrienols (data not shown) and had γ-Toc as the main isoform of tocopherol (Fig. 3B)

3.3. Ecological factors affectiong carotenoid and tocopherol composition of seeds



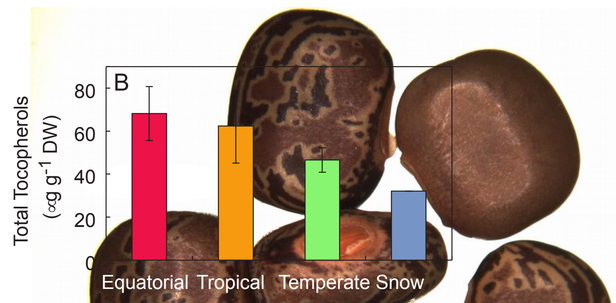


Fig. 4 Mean carotenoids content (A) and tococopherol content (B) of legume seeds according to the climatic origin of each species. Main Climates were choosen following the World Map of Köppen-Geiger Climate Classification (Kottek et al. 2006).

The climatic origin of the species also seemed to have an influence on the preference for the accumulation of different lipophilic antioxidants in seeds (Fig. 4). Carotenoids content was higher in cold regions (Fig. 4A) while tocoopherol concentration was higher in the hottest climates (Fig. 4B).

4. Conclusions

- Lutein is the main carotenoid found in legume seeds and α- and γ-Toc are the main tocochromanols.
- There is a trade off between Carots and Toc accumultaion in legume seeds.
- Both phylogenetic and ecological traits seem to determine the pattern of lipophilic antioxidants accumulation in seeds.

5. Acknowledgements

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