QUALITATIVE DISTRIBUTION PATTERN OF CAROTENOIDS IN THREE SELECTED GYMNOSPERMS

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ABSTRACT

The distribution pattern of carotenoids in the foliage of three forest tree species, viz, *Ginkgo biloba, Pinus roxburghii* and *Cephalotaxus* sp with varying leaf longevities ,representing deciduous and evergreen species was studied. Seventeen carotenoids were identified on average from all the species. A monthly variation in the distribution pattern has been studied and a rapid fluctuation was found in deciduous species as compared to evergreen species. On the basis of their presence during different phenophases, a classification was prepared which indicated that different carotenoid played their role during different phenophases.

INTRODUCTION

THE general distribution of carotenoids in plants has been discussed in detail by Goodwin[1] and in a recent review by Sestak[2]. In the Kumaun Himalayan forest species, the carotenoid distribution has been studied[3,4] in some evergreen and deciduous species. Carotenoid distribution is also well documented in crop plants[5] and fruit plants[6,7]. Carotenoids are well known for their role as accessory pigments because they absorb light efficiently in low wavelength region and increase photosynthetic efficiency. Different carotenoids are known to be responsible for their functions during different growth periods. The present study was carried out to understand their distribution and role during different phenophases.

MATERIALS AND METHODS

Three gymnospermous species viz *Ginkgo biloba* Linn, *Pinus roxburghii* Roxb, and *Cephalotaxus* sp (this monoecious specimen could not be identified) with varying leaf longevities, growing in the Botanical Garden of this University (29°24′ lat. and 79°28′ long. 2050 m elevation), were selected for this study. While *Ginkgo biloba* was a winter deciduous species, *Pinus roxburghii* was evergreen with concentrated summer leaf drop (leaf longevity slightly above one year) and *Cephalotaxus* sp bore leaves of 3–4 years longevity. The leaves of *Cephalotaxus* sp were categorized as new year leaves, preceding year leaves, earlier year category-I leaves and earlier year category-II leaves representing current years leaves, one-year-old leaves, two-year-old leaves and three-year-old leaves respectively. Leaves were sampled randomly at monthly intervals for one year (March 1984–March 1985).

1. *Isolation of chloroplast*: Following the method described by Reeves *et al*[8] the chloroplast was isolated. Following was the composition of grinding and suspending media.

(a) Grinding medium
0.33 m sorbitol
10 ml $Na_4P_2O_7$
3 mM $MgCl_2$
2 mM ascorbic acid
Adjust to pH 6.5 with HCl

(b) Suspending medium
0.33 m sorbitol
2 mM EDTA
1 mM $MgCl_2$
50 mM HEPES
1 mM $MnCl_2$
Adjust to pH 7.6 with NaOH

2. *Isolation of carotenoids*: The method of Jensen and Jensen[9] was employed to extract carotenoids. Isolation, estimation and separation of carotenoids were performed in dim light as carotenoids are very sensitive to light.

The aliquot obtained from acetone extraction (4.7 ml) was further processed by phase-separation with 50 ml of aqueous methanol (90% v/v) and an equal amount of petroleum ether (60–80°c) in a separatory funnel. The lower phase of aqueous methanol which comprised of chlorophyll *a*, chlor-

ophyll *b* and other derivatives was discarded. This was not tested. The upper phase was conserved. This procedure was directly handled to avoid mixing of two phases during elution. The evaporated residue was redissolved in 15 ml of diethyl ether and oven-dried anhydrous sodium sulphate which absorbed traces of chlorophyll *a* and chlorophyll *b*, if any, left during phase separation. The diethyl ether extract was mixed with 0.5 ml acetone and 24.5 ml of benzene and the resulting solution was evaporated to dryness under reduced pressure. This dried material was dissolved in 15 ml of petroleum ether. This solution was used for carotenoid estimation and separation.

3. *Separation and identification of carotenoids*: Carotenoids were separated using thin layer chromatography (TLC) on silicagel-G. A fine slurry was prepared by taking 30 g of silica gel-G in 65 ml of 3% KOH. The slurry was spread over five standard, already washed and cleaned glass plates. (200 mm × 20 mm). Plates were air-dried and then activated in an oven at 120°c.

Exactly 400 ml of carotenoid solution was applied in the form of a band of 5 cm length on each plate. The plates were placed in a chromotographic chamber pre-saturated with solvent system of petroleum ether and acetone (13:7 v/v)[10]. The chromatogram was allowed to run for 40 min. Carotenoid after separation appeared as coloured bands, each of 5 cm length and 0.2 cm width. The bands were scrapped quickly to avoid their destruction by exposure to light and dissolved in 3 ml of either hexane, methanol or petroleum ether, depending upon the nature of the carotenoid and colour was noted.

Although TLC on silicagel-G is a good technique to separate carotenoids some carotenoids were so sensitive that their colour intensity discarded immediately withdrawing the plates from chamber. Some were even lost completely. However, the remaining bands could be preserved by layering the gel with liquid- paraffin solution in petroleum ether (50:50 v/v).

Various carotenoids were identified by comparing the λ_{max} for individual bands obtained with that of λ_{max} given in literature. To measure the λ_{max}, coloured bands were scrapped separately, immediately after chromatography. The carotenoids were eluted in an appropriate solvent which was either hexane, methanol or petroleum ether. Absorbance values for each were recorded by spectrophotometer (Gs B 66 C, Electronics Corporation of India Ltd) starting from 300 mm to 50 nm and λ_{max} were found out. Simultaneously Rf values of all bands were also calculated and compared with those given in literature.

RESULTS AND DISCUSSION

The monthly distribution pattern of carotenoids is presented in table 1 and the total number of carotenoids found and their structures are given in table 2. A classification of carotenoids, on the basis of their distribution pattern at different stages (leaf initiation, maturity and senescence) is given in table 3.

A general trend of distribution pattern is discussed separately in each species.

Ginkgo biloba:

On average thirteen spots were observed on TLC plates, including one unidentified carotenoid (UC_1) table 3. At the leaf initiation stage most of the carotenoids were present except lutein, 5,6-monoepoxy lutein, auroxanthin, and flavoxanthin and UC_1. Among these, lutein and 5,6-monoepoxy lutein were developed at maturity stage, while auroxanthin and flavoxanthin were not found. α-carotene and zeaxanthin were found in trace amount. During senescence lutein and 5,6-monoepoxy lutein disappeared except α-carotene and zeaxanthin which were present only in trace amounts. The presence of UC_1 only during senescence is significant (table 3).

Pinus roxburghii:

A total of eleven spots appeared on TLC plates of which only eight were identified (table 3) β-carotene, cis-Neoxanthin and trans-Neoxanthin were present at all three stages, while cis-Violaxanthin and trans-Violaxanthin which were present at both the leaf initiation and maturity stages but absent at the senescence stage. Lutein was found only at the leaf initiation stage. Auroxanthin, flavoxanthin, UC_1 and UC_2 were observed only during senescence and UC_3 was noted only during leaf initiation.

Cephalotaxus sp:

On average a total of fourteen spots appeared of which nine were identified (tables 1 and 3). In *Cephalotaxus* sp 3 year-old-leaves were present at the

Table 1 *Monthly variation in distribution of carotenoids in foliage of different species*

Carotenoids	Months												
	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March
						1. *Ginkgo biloba*							
1. α-Carotene	+	+	+	*	*	*	*	*	LF				
2. β-Carotene	+	+	+	+	+	+	+	+	LF				
3. γ-Carotene	+	+	+	+	+	+	+	+	LF				
4. Lutein	−	−	+	+	+	+	−	−	LF				
5. 5,6-Monoepoxy lutein	−	−	+	+	+	−	−	−	LF				
6. cis-Violaxanthin	+	+	+	+	+	+	+	+	LF				
7. trans-Violaxanthin	+	+	+	+	+	+	+	+	LF				
8. cis-Neoxanthin	+	+	+	+	+	+	+	+	LF				
9. trans-Neoxanthin	+	+	+	+	+	+	+	+	LF				
10. Auroxanthin	−	−	−	−	−	+	+	+	LF				
11. Flavoxanthin	−	−	−	−	−	+	+	+	LF				
12. Zeaxanthin	+	+	+	+	*	*	*	*	LF				
13. Unidentified carotenoid (UC_1)	−	−	−	−	−	+	+	+	LF				
						2. *Pinus roxburghii*							
1. β-Carotene	+	+	+	+	+	+	+	+	+	+	+	+	+
2. cis-Violaxanthin	+	+	+	+	+	+	+	+		−	−	−	−
3. trans-Violaxanthin	+	+	+	+	+	+	+	+	+	−	−	−	−
4. cis-Neoxanthin	+	+	+	+	+	+	+	+	+	+	+	+	+
5. trans-Neoxanthin	+	+	+	+	+	+	+	+	+	+	+	+	+
6. Lutein	+	+	+	+	−	−	−	−	−	−	−	−	−
7. Auroxanthin	−	−	−	−	−	−	−	−	+	+	+	+	+
8. Zeaxanthin	−	−	−	−	−	−	+	+	+	+	+	+	+
9. UC_1	−	−	−	−	−	−	+	+	+	+	+	+	+
10. UC_2	−	−	−	−	−	−	−	−	+	+	+	+	+
11. UC_3	+	+	+	−	−	−	−	−	−	−	−	−	−
						3. *Cephalotaxus* sp (New leaves)							
1. β-Carotene	+	+	+	+	+	+	+	+	+	+	+	+	+
2. γ-Carotene	+	+	+	+	+	*	*	*	*	*	−	−	−
3. Lutein	−	−	−	−	−	−	−	+	+	+	+	+	*
4. 5,6-Monoepoxy lutein	−	−	−	−	−	−	−	+	+	+	+	+	*
5. cis-Violaxanthin	+	+	+	+	+	+	+	+	+	+	+	+	+
6. trans-Violaxanthin	+	+	+	+	+	+	+	+	+	+	+	+	+
7. cis-Neoxanthin	+	+	+	+	+	+	+	+	+	+	*	*	*
8. trans-Neoxanthin	+	+	+	+	+	+	+	+	+	+	*	*	+
9. Zeaxanthin	*	*	*	*	*	*	+	+	+	+	+	+	+
10. UC_3	+	+	+	−	−	−	−	−	−	−	−	−	−
11. UC_5	+	+	+	−	−	−	−	−	−	−	−	−	−
						Cephalotaxus (Earlier year category I)							
1. β-Carotene	+	+	+	+	+	+	+	+	+	+	+	+	+
2. γ-Carotene	−	−	−	−	−	−	−	−	−	−	−	−	−
3. Lutein	+	+	+	+	+	+	+	+	+	+	−	−	−
4. 5,6-Monoepoxy lutein	+	+	+	+	+	+	+	+	+	+	+	−	−

Carotenoids	Months												
	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March
5. cis-Violaxanthin	+	+	+	+	+	+	+	+	+	+	+	+	+
6. trans-Violaxanthin	+	+	+	+	+	+	+	+	+	+	+	+	+
7. cis-Neoxanthin	−	−	−	−	−	−	−	−	−	−	−	−	−
8. trans-Neoxanthin	+	+	+	+	+	+	+	+	+	+	+	−	−
9. Zeaxanthin	−	−	−	−	−	−	−	+	+	+	+	+	+
10. UC_4	+	+	+	+	+	+	+	+	+	+	−	−	−
Cephalotaxus (Earlier year category II)													
1. β-Carotene	+	+	+	+	+	+	+	+	+	+	+	+	+
2. γ-Carotene	−	−	−	−	−	−	−	−	−	−	−	−	−
3. Lutein	−	−	−	−	−	−	−	−	−	−	−	−	−
4. 5,6-Monoepoxy lutein	−	−	−	−	−	−	−	−	−	−	−	−	−
5. cis-Violaxanthin	+	+	+	+	+	+	+	+	+	+	+	+	+
6. trans-Violaxanthin	+	+	+	+	+	+	+	+	+	+	+	+	+
7. cis-Neoxanthin	−	−	−	−	−	−	−	−	−	−	−	−	−
8. trans-Neoxanthin	−	−	−	−	−	−	−	−	−	−	−	−	−
9. Zeaxanthin	+	+	+	+	+	+	+	+	+	+	+	+	+
10. UC_1	+	+	+	+	+	+	+	+	+	+	+	+	+
11. UC_2	+	+	+	+	+	+	+	+	+	+	+	+	+

Foot Note: +, indicates presence; −, indicates absence; *, indicates presence in trace amount; LF, indicates leaf fall.

Table 2 *Total carotenoids observed on TLC plates (average of all the three species and all the months)*

Name	Structures
1. α-Carotene	β-ε-Carotene
2. β-Carotene	β, β-Carotene
3. γ-Carotene	β, ψ-Carotene
4. Lutein	3,3′-di OH-α-carotene
5. 5,6-Monoepoxy lutein	Lutein, 5-epoxide
6. cis-Violaxanthin	cis-5,6,5′,6′-diepoxy 5,6,5′,6′-tetrahydro-β-β-carotene-3,3′-diol
7. trans-Violaxanthin	trans-5,6,5′6′-diepoxy 5,6,5′6′-tetrahydro-β-β-carotene-3,3′-diol
8. cis-Neoxanthin	cis-5,6′-epoxy-6,7-didehydro-5,6,5′6′-tetrahydro-β-β-carotene-3,5,3′-triol
9. trans-Neoxanthin	trans-5,6′-epoxy-6,7-didehydro-5,6,5′6′-tetrahydro-β-β-carotene-3,5,3′-triol
10. Zeaxanthin	β-β-carotene-3,3′ diol
11. Auroxanthin	5,8,5′,8′-diepoxy-5,8,5′,8′-tetrahydro-β,β-carotene-3,3′-diol
12. Flavoxanthin	5,8-epoxy-5,8-dihydro-β,ε-carotene-3,3′-diol
13. Unidentified carotenoid-I (UC_1)	
14. Unidentified carotenoid-2 (UC_2)	
15. Unidentified carotenoid-3 (UC_3)	
16. Unidentified carotenoid-4 (UC_4)	
17. Unidentified carotenoid-5 (UC_5)	

Table 3 *Classification of carotenoids on the basis of their distribution pattern at different stages (leaf initiation, maturity and senescence)*

Species	Total carotenoids	Stages		
		Leaf initiation	Maturity	Senescence
i) *Ginkgo biloba*	1. α-Carotene	+	*	*
	2. β-Carotene	+	+	+
	3. γ-Carotene	+	+	+
	4. Lutein	−	+	−
	5. 5,6-Monoepoxy lutein	−	+	−
	6. cis-Violaxanthin	+	+	+
	7. trans-Violaxanthin	+	+	+
	8. cis-Neoxanthin	+	+	+
	9. trans-Neoxanthin	+	+	+
	10. Auroxanthin	−	−	+
	11. Flavoxanthin	−	−	+
	12. Zeaxanthin	+	*	*
	13. UC_1	−	−	+
ii) *Pinus roxburghii*	1. β-Carotene	+	+	+
	2. cis-Violaxanthin	+	+	−
	3. trans-Violaxanthin	+	+	−
	4. cis-Neoxanthin	+	+	+
	5. trans-Neoxanthin	+	+	+
	6. Lutein	+	−	−
	7. Auroxanthin	−	−	+
	8. Zeaxanthin	−	−	+
	9. UC_1	−	−	+
	10. UC_2	−	−	+
	11. UC_3	+	−	−
iii) *Cephalotaxus* sp	1. α-Carotene	+	+	+
	2. γ-Carotene	+	−	−
	3. Lutein	−	+	−
	4. 5,6-Monoepoxy lutein	−	+	−
	5. cis-Violaxanthin	+	+	+
	6. trans-Violaxanthin	+	+	+
	7. cis-Neoxanthin	+	+	−
	8. trans-Neoxanthin	+	+	−
	9. Zeaxanthin	+	−	+
	10. UC_1	−	−	+
	11. UC_2	−	−	+
	12. UC_3	+	−	−
	13. UC_4	−	−	−
	14. UC_5	+	−	−

Notes: +, indicates presence; −, indicates absence; *, indicates presence in trace amount

same time when new leaves were initiated. For the maturity and senescence stages, a mixture of preceding year and earlier year leaves and earlier year category-II leaves were respectively considered.

On screening, it was found that β-carotene, cis-Violaxanthin and trans-Violaxanthin were present at all the stages. Lutein and 5,6-monoepoxy lutein and one unidentified carotenoid (UC_4) were present only during maturity stage while Zeaxanthin and the unidentified carotenoid (UC_1) were present during senescence.

It can be concluded (table 3) that some of the carotenoids were found at one particular stage showing their role in maintaining photosynthetic

efficiency as they are well known for their protective role to the chlorophylls. Lutein and 5,6-monoepoxy lutein were found only at the maturity stage in all the three species, while auroxanthin and flavoxanthin were noted during the senescence stage in general and the distribution of other carotenoid was general and common almost at all stages and in all species.

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1. Goodwin, T. W., *Distribution of carotenoids: Chemistry and biochemistry of plant pigments*, Academic Press, London, New York, San Francisco, 1976, Vol. 1.
2. Sestak, Z., *Photosynthetica*, 1982, **16**, 568.
3. Yadav, S., Singh, S. P., *Proc. Second Ann. Conf. Nat. Environ. Sci. Acad.*, 1986, (In press).
4. Yadav, S., Dwivedi, P. D., Tewari, M., Rana, R., *Seasonal variation of carotenoid contents in Maidenhari tree Ginkgo biloba-environment and ecology*, 1985, **3**, 446.
5. Dwivedi, P. D., Yadav, S., b. *J. Plant Nature*, 1986b (In press).
6. Osman, M. E., Mummery, R. S., Valadon, L. R. G., *Ann. Bot.*, 1984, **53**, 21.
7. Valadon, L. R. G., Mummery, R. S., Kates, M., *Plant Sci. Lett.*, 1984, **34**, 253.
8. Reeves, S. G., Hall, D. O., *Methods Enzymol.*, 1980, **69**, 85.
9. Jensen, S. L., Jensen, A., *Methods Enzymol.*, 1971, **23**, 586.
10. Marshell, J. H., Cregeory, *J. Bacteriol.*, 1981, **143**, 900.

NEWS

INFLUENZA VACCINES FOR 1987–1988— MODIFIED COMPOSITION RECOMMENDED

Representatives of the World Health Organization (WHO) Collaborating Centres for Reference and Research on Influenza have completed their yearly meeting to formulate their recommendations concerning the influenza vaccines to be manufactured for the 1987–1988 season. Having studied the prevalence and antigenic character of the various viruses isolated during the current season, they recommended that the vaccine for use in the 1987–1988 season be trivalent and contain the following antigens:

— an A/Singapore/6/86(H1N1)-like antigen
— a B/Ann Arbor/1/86-like antigen, and
— an A(H3N2) antigen, to be recommended

During the 1986–1987 season, influenza A(H1N1) viruses have predominated and in most countries have been the only type of influenza virus to be isolated. Almost all of them were similar to the A/Singapore/6/86-like strains isolated in Asia from April to July 1986, which has been recommended in August 1986 in addition to the three components chosen in February 1986. There were few influenza B viruses isolated and all were similar to the type used in the previous vaccine.

(Press Release WHO/9, 27 February 1987; WHO, Media Service 1211, Geneva 27, Switzerland)