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Full Length Research Paper

Changes in fatty acid composition of polar lipids associated with growth and senescence in leaves of *Catharanthus roseus*

Sanjay Mishra[1]* and Rajender Singh Sangwan[2]

[1]Department of Biotechnology, College of Engineering and Technology, IFTM Campus, Lodhipur Rajput, Moradabad 244 001, U.P., India.

[2]Division of Plant Physiology and Biochemistry, Central Institute of Medicinal and Aromatic Plants (CIMAP), P.O. CIMAP, Lucknow 226 015, India.

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Polar lipids are important membrane components of plant cells. They are known to affect certain membrane properties like permeability, fluidity and active transport. In the present study, individual leaf buds were tagged on the day of appearance and sampling began 7 days later. Additional samples were taken at 7 days intervals until leaf abscission occurred about 77 days after tagging. The experiments were performed from July to October, a time period characterized by a day length of 12 ± 1.5 h and average day and night temperature of approximately 30 and 20°C, respectively. The fatty acid composition of polar lipids from young, mature and senescent leaves of *Catharanthus roseus* was studied in the present study. Green leaves were observed to contain a considerable amount of hexadecatrienoic acid (16:3) in monogalactosyldiglyceride (MGDG), which suggests that *C. roseus* belongs to 16:3 plants. Further, the percentage of linolenic acid (18:3) in the chloroplast lipids was lower in senescent leaves than that of green tissues. Senescent leaves also had lower proportions of MGDG 16:3 and phosphatidyl glycerol (PG) hexadecanoic acid (16:1). Such selective catabolism of molecular species of these lipids may be suggestive of significant alterations in ultra structure of chloroplast membranes, thus probably affecting the accumulation of indole alkaloids in the leaf tissue. Besides, the age dependent alterations in the fatty acid composition of other polar lipids, namely, phosphatidyl choline (PC), phosphatidyl inositol (PI), phosphatidyl ethanolamine (PE), and phosphatidyl serine (PS) indicate the possibility of degradation of the organelles other than chloroplast (principally vacuoles) in the leaf cells.

Key words: *Catharanthus roseus,* fatty acid composition, glycolipids, leaf development, polar lipids, phospholipids, senescence, sterol.

INTRODUCTION

Catharanthus roseus (L.) G.Don, known as the common or Madagascar periwinkle, is a perennial and evergreen herb of the dogbane family (Apocynaceae) that was originally native to the island of Madagascar. It has been widely cultivated for hundreds of years and can now be found growing wild in most warm regions of the world, including the Southern U.S. The plants grow one or two feet high, have glossy, dark green leaves (1-2 inches long) and flowers throughout summer. Vincristine (Oncovin) and vinblastine (Navelbine), the main indole alkaloids produced by this plant, are medicinally important, having long been used as anticancerous agents (Blasko and Cordell, 1990; Verpoorte et al., 1991).

In fact, these secondary metabolites are produced at extremely low levels within the plants and remain resistant to feasible chemical synthesis due to their complex structures (Hughes and Shanks, 2002; Verpoorte et al., 1999) . During last decade, a lot of efforts have been made in view of exploring primary to secondary metabo-

*Corresponding author. E-mail: sanjay_mishra23@rediffmail.com; sanjay_mishra23@hotmail.com.

lite networks for terpenoid indole alkaloid biosynthesis in *C. roseus* (Ayora-Talavera et al., 2002; Canel et al*.,* 1998; Hughes et al., 2002; Hughes et al*.,* 2004; van der Fits and Memelink, 2000; Sangwan et al., 1998; Whitmer et al., 2002a, b).

However, the lipid metabolism viz-a-viz. qualitative and quantitative production and sequestration of medicinally and biotechnologically valuable phytoche-micals in *C. roseus* is still a thrust area to work in.

Recently, Mishra et al*.* (2006) have taken initiatives to work on this aspect and led to a hypothesis that the spatial alterations in lipid profiles may be suggestive of concomitant changes in membrane ultrastructure and functions, putatively leading to perturbation in indole alkaloid sequestration capability of the tissues of a species of pharmaceutical significance.

Further, polar lipids are important membrane constituents of plant cells. They play a pivotal role by participating in membrane properties like fluidity, permeability and active transport. In fact, any change in physiological state as well as biotic and abiotic factors may influence the chloroplast biochemistry in terms of state and status of polar lipids (Sreenivasulu et al*.,* 1977; Powles, 1984; Sharma and Sanwal, 1992; Mishra and Sangwan, 1998; Mishra et al*.,* 1998). Age-dependent changes in individual polar lipids have been reported for green leaves of cucumber (Ferguson and Simon, 1973) and bean (Fong and Heath, 1977).

However, the information on this class of lipids in alkaloid producing plant is very sparse. Koiwai and Kisaki (1979) have reported that glycolipids are degraded more rapidly than phospholipids during flue-cring of tobacco leaves. Later on, Koiwai et al. (1981) studied certain changes in total and polar lipids and their fatty acid composition in tobacco leaves during growth and senescence. To our knowledge, there is no report so far concerning the establishment of putative inter-relationship between lipid status in chloroplast and/or vacuolar membrane and level of alkaloid accumulation in plants. Our earlier studies on *Duboisia myoporoides* R. Br. indicated significant changes in accumulation of the tro-pane phytochemicals accompanying its various develop-mental phases (Mishra and Sangwan, 1996). Further, we have explained this phasic pattern (Mishra et al., 1998) in view of the essence of a defined cellular organization to facilitate and maintain the accumulation or sequestration of the secondary phytochemicals.

Consequently, the temporal trend of various lipid classes and components deserve to be analyzed in this perspective. This paper deals with the age-dependent changes in fatty acid com-position of polar lipids of *C. roseus* during leaf growth and senescence.

MATERIAL AND METHODS

Plant materials

Leaf samples were harvested from plants of *C. roseus* growing at the experimental farm of the CIMAP, Lucknow (26.5° N latitude, 80.5° E longitude, 120 m altitude, and subtropical zone), India. Individual leaf buds were tagged on the day of appearance and sampling began 7 days later. Additional samples were taken at 7 days interval until leaf abscission occurred about 77 days after tagging. The experiments were performed from July to October, a time period characterized by a day length of 12 ± 1.5 h and average day and night temperature of approximately 30 and 20°C, respectively.

Lipid extraction

All solvents were glass distilled prior to use. Total lipids were extracted from powdered leaf samples with the aid of $CHCl_3$-MeOH (2:1, v/v) according to the method employed by Mishra and Sangwan (1998) and purification of lipid fraction was performed with help of 0.9% (w/v) NaCl according to Folch et al. (1957). The purified lipid fraction in chloroform was stored under nitrogen –20°C till further use.

Separation and identification of polar lipids

Polar lipids were separated from the total lipid extract employing 2D- TLC according to Mishra and Sanwal (1994). The plate was developed with chloroform-methanol-aqueous (28%) ammonia (65:35:5, v/v) in first direction followed by chloroform–acetone-methanol-acetic acid-water (5:2:1:1:0.5, v/v) in the second direction. Lipid spots were located under UV light after spraying with Rhodamine 6 G solution. Each spot was identified by chromatography with standards and by spraying with specific reagents for the lipid class (Mishra and Sangwan, 1998).

Preparation of fatty acid methyl esters and gas liquid chromatography

Each florescent lipid area on the TLC was properly scrapped from the plate and transferred to a test tube containing 5 ml of 5% (v/v) sulphuric acid in methanol. Methanolysis was performed at 40°C overnight. The fatty acid methyl esters (FAME) were extracted with n-hexane, separated and detected using an AIMIL- Nucan gas chromatograph fitted with a stainless steel column (1.8 m x 2 mm, i.d.) of 20% di-ethylene glycol succinate (DEGS) on Chromosorb W (100-120 mesh). The operating conditions were as follows: oven temperature, 190^{o}C; flame ionization detector and injector temperature, 220^{o}C; flow rate of H_2 and N_2, 30 ml min^{-1}. Employing authentic reference standards performed the identification of FAME. The peak area was calculated by measuring the height multiplied by the width of the peak at half peak height. The values for each fatty acid are given as percent by weight of total fatty acids according to Mishra and Sanwal (1994).

Values were statistically calculated as mean ± SD of three independent sets of experiments with triplicates in each set and expressed as mol % of total fatty acids of each polar lipid.

RESULTS AND DISCUSSION

The cellular and metabolic regulations and interactions may have direct bearing on the transport and accumulation of indole alkaloids (Table 1) in *C. roseus* leaf. The amount of polar lipids varied grossly in the same fashion as total lipids (Mishra et al., 2006) except that during maturation and senescence initiation the polar lipids declined to a greater extent than the neutral lipids.

Table 1. Comparative account of fatty acid composition of each polar lipid among young mature and senescent leaves of *Catharanthus roseus*.

Lipid	Leaf age	Fatty acid composition (mol %)						
		16:00	16:01	16:03	18:00	18:01	18:02	18:03
MGDG	Young	4.0±0.40	ND	12.1±.1.13	ND	3.0±0.29	1.9±0.10	79.0±3.23
	Mature	2.0±0.10	ND	16.8±1.42	ND	ND	2.1±0.20	79.1±2.39
	Senescent	12.1±1.02	ND	4.9±0.79	4.0±0.50	1.1±0.10	4.8±0.30	73.1±2.10
DGDG	Young	17.0±1.15	ND	ND	2.0±0.21	1.0±0.10	1.0±0.10	79.0±2.32
	Mature	15.2±1.09	ND	ND	1.0±0.10	1.9±0.20	1.9±0.20	80.0±2.52
	Senescent	25.9±1.93	ND	ND	12.0±1.03	7.0±0.39	3.0±0.2	52.1±1.95
SQDG	Young	46.0±2.24	ND	ND	4.0±0.42	3.0±0.15	5.0±0.42	42.0±2.02
	Mature	56.3±2.39	ND	ND	4.0±0.41	2.0±0.10	3.0±0.23	34.7±0.93
	Senescent	48.1±2.10	ND	ND	7.0±0.71	4.0±0.41	5.9±0.45	35.0±0.91
PG	Young	16.0±1.21	33.0±1.28	ND	5.0±0.45	6.0±0.85	9.0±0.76	31.0±0.89
	Mature	22.1±.1.13	31.0±1.26	ND	3.0±0.21	4.0±0.40	11.0±1.05	28.9±0.79
	Senescent	52.0±1.82	6.0±0.91	ND	12.0±1.05	6.0±0.88	9.0±0.82	15.0±1.01
PC	Young	33.2±1.16	ND	ND	7.9±0.92	7.0±0.93	30.0±1.23	21.9±1.07
	Mature	28.0±1.06	ND	ND	5.0±0.43	6.0±0.88	21.0±1.15	40.0±1.75
	Senescent	39.1±1.43	ND	ND	8.0±0.96	7.0±0.90	19.0±1.02	26.9±1.02
PI	Young	25.0±1.20	ND	ND	7.0±0.65	6.0±0.88	32.0±1.16	30.0±1.23
	Mature	28.3±1.01	ND	ND	3.9±0.34	7.9±0.89	23.0±1.34	36.9±1.31
	Senescent	41.0±1.92	ND	ND	7.0±0.74	8.0±0.89	25.0±1.02	19.0±1.03
PE	Young	21.4±1.81	ND	ND	6.9±0.63	3.9±0.20	37.9±1.29	29.9±1.21
	Mature	32.0±1.28	ND	ND	5.0±0.45	6.0±0.88	30.0±1.21	27.0±1.19
	Senescent	37.7±1.08	ND	ND	11.1±1.01	10.1±0.92	16.0±1.02	25.1±1.01
PS	Young	44.0±1.99	ND	ND	8.0±0.89	9.0±0.86	23.0±1.71	16.0±1.04
	Mature	39.3±2.02	ND	ND	9.9±0.95	10.9±0.91	19.0±1.52	20.9±1.34
	Senescent	47.1±.35	ND	ND	4.0±0.39	5.0±0.32	16.0±1.24	27.9±1.26

Values are mean ± SD of three independent sets of experiments with triplicates in each set and are expressed as mol % of total fatty acids of each polar lipid. Young, 28 DAP (Day after proliferation); Mature, 56 DAP; Senescent 77 DAP. 'ND' denotes 'not detected'.

The period of aging and senescence accompanied a slow but consistent trend of decline in polar lipids. Most of polar lipids in *C. roseus* leaves alter concomitantly with changes in chlorophyll content during development and senescence (Mishra et al*.,* 2006).

This study deals with the alterations in fatty acid profile of polar lipids in young, mature and senescent leaves of *C. roseus*. Fatty acid composition of each glycolipid and PG differed little between young and mature leaves (Table 1). Increases in 18:3 and decreases in linolenic acid (18:2) in glycolipids and PG during greening of leaves have been reported for many plants (Ohnishi and Yamada, 1980a). MGDG and digalactosyl diglyceride (DGDG) were found to possess a very high proportion of 18:3 in young and mature leaves when compared to other phospholipids. MGDG was characterized by 16:3. This suggests that *C. roseus* belongs to 16:3 plants (Siebertz and Heinz, 1977; Heinz, 1977; Heinz et al*.,* 1979; Roughan et al., 1979; Koiwai et al*.,* 1981)) and may possess the prokaryotic pathway. Nevertheless, it cannot absolutely preclude the possibility of the employ-ment of the eukaryotic pathway (Roughan et al*.,* 1980; Koiwai et al*.,* 1981) for the synthesis of 18:3-MGDG via oleic acid (18:1)- PC and 18:2-PC have been reported in 18:3 plants such as Avena (Ohnishi and Yamada, 1980a, b). Sulphoquinovosyl diglyceride (SQDG) and PG had relatively high proportions of 18:3 and palmitic acid (16:0) and the latter was characteristic of 16:1. Generally, the relative amount of 18:3 in these four chloroplast lipids dropped considerably in senescent leaves with concomitant rise in 16:0 and stearic acid (18:0), although the contents of all fatty acids declined significantly. The relative amounts of 16:3 in MGDG and 16:1 in PG also decreased in senescent leaves. In fact, these changes are basically consistent with those reported for cucumber (Ferguson and Simon, 1973).

Taken together with our observations and discussion in the previous reports on the medicinal plant lipid profile (Mishra and Sangwan, 1998; Mishra et al., 1998; Mishra et al., 2006), it appears that selective degradation occurring in molecular species of these lipids during senescence might be a manifestation of the changing ultra structure and composition of organellar membranes during the phase. Such changes, particularly those concerning vacuoles etc., in turn, might lead to a perturbation of indole alkaloids in the leaf tissue. Specifically, age-dependent changes in fatty acid profile of polar lipids like PC, PI, PE, and PS might be related to degradation of the organelles mainly vacuoles in the leaf cells in *C. roseus*.

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