IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 1 Ver. II (Jan -Feb. 2015), PP 95-98
www.iosrjournals.org

Alterations in Fatty Acid Profile of Polar Lipids Accompanied By Growth and Senescence in Leaves of Rauwolfia Serpentina

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Abstract: *Polar lipids are essential 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 August to November, a time period characterized by a day length of 12 ± 2.0 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 Rauwolfia serpentina was studied in the present study. Green leaves were observed to contain a considerable amount of hexadecatrienoic acid (16:3) in monogalactosyldiglyceride (MGDG), suggesting R. serpentina to be member of 16:3 plants' family. 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.*

Keywords: *Rauwolfia serpentina, fatty acid composition, glycolipids, leaf development, polar lipids, phospholipids, senescence, sterol.*

I. Introduction

Rauwolfia serpentina (Apocynaceae) is a medicinal plant, commonly known as Indian snake root, chandra, sarpgandha, an evergreen, perennial, glabrous and erect under shrub. Generally it grows up to 15-45 cm in height, but may grow up to 90 cm under very favorable conditions. Leaves grow in whorls of 34, are deciduous, ellipticlanceolate or obviate, pointed, green on the upper surface, pale-green underneath, 7.5 cm long and 3.5 cm broad in size, are aboriginal to India and Bangladesh and is found to grow wild in the Asian continent. It has been reported to contain 50 indole alkaloids that are mainly localized in the root bark (Klushnichenkoet al., 1995). These alkaloids are, reserpine, yohimbine, serpentine, deserpidine, ajmalicine and ajmaline are used to treat hypertension (Von Poser.et al., 1990) and breast cancer (Stanford. et al., 1986), antidote against bite from poisonous reptiles, antidysentry (Bhataraet al., 1997). Reserpine, used as a natural tranquilizer was reported to have several times greater hypotensive activity compared to the crude plant extracts (Pullaiah et al., 2002). *Rauwolfia* root is known to contain 0.7 – 3.0 % of total alkaloids in the dry mass and the amount vary with time and source of collection (Kokate et al., 1998; Poonam et al., 2013).

However, the lipid metabolism viz-a-viz. qualitative and quantitative production and sequestration of medicinally and biotechnologically valuable photochemical in R. serpentine 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 evocative of concomitant changes in membrane ultrastructure and functions, putatively leading to perturbation in indole alkaloid sequestration capacity of the tissues of a species of pharmaceutical significance. Further, polar lipids are important membrane constituents of plant cells. They play a key 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 (Fongand Heath, 1977). However, the information on this class of lipids in alkaloid producing plant is quite sparse. Koiwai and Kisaki (1979) have reported that glycolipids are degraded more speedily as compared to 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 profile 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. The earlier study on *Duboisia myoporoides* R. Br.

Indicated significant changes in accumulation of the tropane phytochemicals accompanying its various developmental 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 alterations in fatty acid profile of polar lipids of *R.serpentina* during leaf growth and senescence.

II. Material and Methods

Plant materials: Leaf samples were harvested from plants of *R. serpentina* growing at 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 7days 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 CHCl3-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 fromthe 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 authenticreference standards performed the identification of FAME. The peak area was calculated by measuring the height multipliedby the width of the peak at half peak height. The values for eachfatty 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.

III. 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 R. serpentina 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 as compared to the neutral lipids. The period of aging and senescence accompanied a slow but steady trend of reduction in polar lipids. Most of polar lipids in *R.serpentina* leaves change concomitantly with changes in chlorophyll content during development and senescence (Mishra et al., 2006).

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

Lipid	Leaf Age	Fatty acid composition (mol %)						
		16:00	16:01	16:03	18:00	18:01	18:02	18:03
MGDG	Young	4.1±0.39	ND	12.3±.1.10	ND	3.5±0.26	1.8±0.11	79.3±3.0
	Mature	2.2±0.11	ND	16.5±1.40	ND	ND	2.3±0.21	78.8±2.34
	Senescent	12.2±1.01	ND	4.5±0.74	4.0±0.49	1.1±0.11	4.7±0.30	73.5±2.10
DGDG	Young	17.1±1.12	ND	ND	2.1±0.20	1.1±0.11	1.1±0.11	78.6±2.30
	Mature	15.0±1.07	ND	ND	0.9±0.11	1.8±0.21	1.8±0.22	80.5±2.50
	Senescent	25.7±1.90	ND	ND	11.8±1.01	6.8±0.36	2.9±0.3	52.8±1.93
SQDG	Young	45.8±2.22	ND	ND	3.8±0.41	3.4±0.13	4.8±0.40	42.2±2.00
	Mature	55.8±2.37	ND	ND	4.3±0.40	2.2±0.12	3.5±0.25	34.2±0.90

	Senescent	48.0±2.09	ND	ND	7.1±0.70	4.1±0.40	5.5±0.42	35.3±0.90
PG	Young	15.6±1.23	33.0±1.26	ND	4.6±0.42	6.0±0.83	8.6±0.73	32.2±0.85
	Mature	22.0±.1.11	31.1±1.24	ND	3.2±0.20	4.3±0.41	11.2±1.03	28.2±0.75
	Senescent	52.1±1.80	6.1±0.94	ND	12.1±1.05	6.1±0.86	9.1±0.81	14.5±1.00
PC	Young	33.0 ±1.14	ND	ND	7.7±0.90	7.3±0.90	30.3±1.20	21.7±1.05
	Mature	28.1±1.04	ND	ND	5.1±0.41	6.1±0.86	21.2±1.14	39.5±1.74
	Senescent	39.1±1.44	ND	ND	8.1±0.97	7.1±0.91	19.1±1.04	26.5±1.03
PI	Young	25.1±1.22	ND	ND	7.1±0.67	6.1±0.85	31.5±1.13	30.2±1.20
	Mature	28.5±1.03	ND	ND	3.7±0.31	7.6±0.87	23.2±1.31	37.0±1.30
	Senescent	40.5±1.90	ND	ND	7.1±0.72	8.1±0.87	25.1±1.01	19.1±1.02
PE	Young	21.5±1.80	ND	ND	6.8±0.64	3.7±0.21	37.7±1.27	30.3±1.23
	Mature	32.1±1.25	ND	ND	5.1±0.43	6.1±0.86	29.5±1.20	27.2±1.16
	Senescent	37.5±1.09	ND	ND	11.0±1.03	10.0±0.90	16.2±1.01	25.3±1.03
PS	Young	43.8±1.95	ND	ND	7.8±0.85	8.8±0.84	23.3±1.70	16.3±1.02
	Mature	39.1±2.01	ND	ND	9.7±0.91	10.7±0.93	19.2±1.50	21.3±1.32
	Senescent	46.9±.31	ND	ND	3.8±0.35	4.8±0.30	16.3±1.21	28.2±1.23

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

This study deals with the alterations in fatty acid profile of polar lipids in young, mature and senescent leaves of *R. serpentina*. Fatty acid composition of each glycolipid and PG differed slight between young and mature leaves (Table 1). Increases in 18:3 and decreases in linolenic acid (18:2) in glycolipids and PG during greening ofleaves have been reported for many plants (Ohnishi andYamada, 1980a). MGDG and digalactosyldiglyceride (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 R. serpentina belongs to 16:3 plants (Siebertz and Heinz, 1977; Heinz, 1977; Heinz et al.,1979; Roughan et al., 1979; Koiwai et al., 1981; Mishra and Sangwan, 2008)) and may possess the prokaryotic pathway. However, it cannot absolutely exclude the possibility of the employment 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 in18:3 plants such as Avena (Ohnishi and Yamada, 1980a,b). Sulphoquinovosyldiglyceride (SQDG) and PG had relatively high proportions of 18:3 and palmitic acid (16:0) and the latter was characteristic of 16:1. Normally, the relative amount of 18:3 in these four chloroplast lipids dropped significantly in senescent leaves with concomitant increase in 16:0 and stearic acid (18:0), while 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 fundamentally steady with those reported for cucumber (Ferguson and Simon, 1973) and *Catharanthus* (Mishra and Sangwan, 2008)

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; Mishra and Sangwan, 2008)), it appears that selective degradation happening in molecular species of these lipids during senescence might be a materialization of the changing ultrastructure and composition of organellar membranes during the phase. Such changes, principally those concerning vacuoles etc., in turn, might direct to a perturbation of indole alkaloids in the leaf tissue. Particularly, age dependent alterations in fatty acid profile of polar lipids like PC, PI, PE, and PS might be associated with degradation of the organelles primarily vacuoles in the leaf cells in *R.serpentina.*

Acknowledgements

An institutional research promotion grant to the School of Biotechnology, IFTM University,Moradabad, U.P., India is duly acknowledged. The authors are grateful to Prof. R.M. Dubey (Vice Chancellor, IFTM University, Moradabad, U.P., India for providing the necessary facilities and encouragement. Besides, all the faculty members of the School of Biotechnology, IFTM University, Moradabad, U.P.,India are thanked for valuable discussion.

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