CHEMICAL ANALYSIS OF EPICUTICULAR WAX OF X-RAY-INDUCED THICK LEAF AND BIG SEED MUTANT OF *BETA PALONGA*

K. K. MUKHERJEE, R. K. BASU and A. GHOSH
Cytogenetics Laboratory and Lipid Chemistry Laboratory, Bose Institute, Calcutta 700 009, India.

ABSTRACT

Ester was the major constituent of leaf surface wax of *Beta palonga* and its x-ray-induced mutant, TLBS. In the two plants the chain lengths of fatty acids and combined alcohols ranged from C_{14} to C_{24} and C_{18} to C_{32} respectively. Only the relative proportion of the components of fatty acids and alcohols varied between the control and the mutant.

INTRODUCTION

A TRUE breeding morphological mutant having thick glossy leaves and big seeds (TLBS) against thin leaves and small seeds in the control was isolated from a population of *Beta palonga* (Chenopodiaceae). The leaves of the mutant plant had abundant cube-shaped epicuticular wax, while the control had scanty wax deposition which were irregular in shape. The inheritance of mutant characteristics has been reported earlier[1]. Electron microscopic study of epicuticular wax in the leaf of *Beta vulgaris*, a closely related species of *B. palonga*, has been reported[2]. The waxy deposits on the external surface of various parts of higher plants contain a wide range of organic compounds. Alcohols, acids, hydrocarbons, esters, β-diketone, its hydroxy and oxo derivatives usually constitute the wax. The major components of epicuticular waxes, however, vary with plant species and even in different parts of the same plant[3,4]. Reports on changes in chemical composition of waxes in plants grown after treatment with mutagens are scanty[5-8]. In the present paper chemical analysis of the major constituent of the epicuticular waxes in the leaves of the mutant TLBS and its control is presented.

MATERIALS AND METHODS

A true breeding x-ray-induced mutant (TLBS) and its control, *Beta palonga* Basu and Mukherjee, of Chenopodiaceae, formed the materials of the present investigation. Epicuticular wax from leaf surfaces of the two materials was extracted following the methods of Tulloch[9]. TLC of the waxes showed that esters formed the major constituent in both the cases. The methyl esters of the acids and alcohol fractions of the esters were separated by column chromatography after methanolysis. The alcohols were derivatized[10] (–OTMS) using BSA/Py. The methyl esters of fatty acids and alcohols were analyzed by GLC using a Pye Unicam (model GCD) gas chromatograph with dual column and dual flame ionization detector. Chromatograms were taken on 1.8 m×3 mm 10% DEGS for fatty acids and 3% OV-17 for alcohol-OTMS on 80–100 mesh Gas-chrom Q column. Carrier gas was nitrogen (60 ml/min) and the column temperature for fatty acids was 180°C with an increment of 4°C/min up to 220°C. For alcohol –OTMS, the temperature was programmed from 226° to 270°C (2°C/min). The peaks were identified by comparing the retention times with those of known samples and the compositions were calculated by the method of triangulation.

RESULTS AND DISCUSSION

The total wax extracted represented 0.6% of the dry weight of leaves in the control and 1.2% in the mutant (table 1). Column chromatography and preparative TLC were used to separate the different fractions and the results are presented in table 1. The esters being the major component of the epicuticular waxes in both control and mutant, this fraction was further analyzed after hydrolysis.

Table 1 *Composition and yield* (%) of leaf epicuticular wax in control and mutant of B. palonga*

Components	Control	Mutant
Ester	43	62
Triglyceride	13	8
Free fatty acid	15	6
Sterol	12	9
Unidentified material	17	15
Yield (% dry weight)	00.6	1.2

* In wt % determined by column chromatography.

Table 2 *Per cent composition of acids and alcohols obtained by hydrolysis of esters from waxes in control and mutant (TLBS) of Beta palonga*

Chain length: Number of double bond	Hydrolysis products of esters			
	Control		Mutant (TLBS)	
	Acid	Alcohol	Acid	Alcohol
14:0	3.05		6.08	
15:0	0.60		0.76	
16:0	21.41		28.79	
16:1	3.08		1.53	
17:0	0.64		1.53	
18:0	2.67	2.83	2.41	6.41
18:1	20.21		28.73	
18:2	13.13		14.82	
18:3	9.04		9.20	
20:0		4.71		3.20
22:0	7.87	2.19	2.90	1.28
22:1	3.53		1.52	
23:0		Trace		Trace
24:0	8.77	8.37	1.63	3.99
25:0		1.67		1.60
26:0		23.53		15.04
27:0		3.35		5.35
28:1so		3.76		2.70
28:0		15.70		18.53
29:0		3.97		3.13
30:0		6.28		4.99
31:0		12.56		7.05
32:0		10.99		26.66

GLC analysis of methyl esters of fatty acids and alcohols showed that the mutant differed quantitatively from the control in both acid and alcohol fractions of the esters. Fatty acid components with chain lengths from C_{14} to C_{24} were common to both the control and the mutant. Fatty acids with chain lengths from C_{19} to C_{21} were not recorded. The mutant was, however, conspicuous in having increased amounts of C_{14}, C_{17} and $C_{18:1}$ and less of $C_{16:1}$, $C_{22:1}$ and C_{24} acids than the control (table 2). The combined alcohols showed a wide range of chain lengths from C_{18} to C_{32} with total absence of C_{19} to C_{21} alcohols. Compared to control, the mutant had strikingly more of C_{18} and C_{32} and less of $C_{24}C_{26}$ and C_{31} alcohols (table 2).

The mutant can be phenotypically distinguished from the wild type due to glossy appearance of the leaves and chemically due to preponderance of ester which was the most affected compound by mutation. Similar observation was recorded in glossy II mutant of maize[11]. Mutational study revealed that long chain molecules of wax are synthesized in maize by decarboxylation complexes. The mutant TLBS provide opportunity for further chemical analysis of wax synthesis route also in *B. palonga*. Increased production of epicuticular wax on the surface of the leaves of the mutant will enable this mutant to withstand stressed condition particularly water shortage. It was reported[12] that epicuticular wax synthesis enhanced in stressed leaves, compared with its control. The mutant having double the amount of epicuticular wax will have greater advantage over the normal plants under stressed situation.

25 August 1986

1. Mukherjee, K. K. and Basu, R. K., *Indian J. Genet. Plant Breed.*, 1985, **45**, (in press).
2. Bystrom, B. G., Glater, R. B., Scott, F. M. and Bowler, E. S. C., *Bot. Gaz.*, 1968, **129**, 133.

3. Eglinton, G. and Hamilton, R. J., *Science*, 1967, **156**, 1322.
4. Tulloch, A. P., Baum, B. R. and Hoffman, L. L., *Can. J. Bot.*, 1980, **58**, 2602.
5. Bianchi, G., Avato, P. and Salamini, F., *Maydica*, 1975, **20**, 165.
6. Macey, M. J. K. and Barber, H. N., *Phytochemistry*, 1970, **9**, 13.
7. Wettstein-Knowles, P. Von, *FEBS Lett.*, 1974, **42**, 187.
8. Nodskov, Giese, B., *Hereditas*, 1976, **82**, 137.
9. Tulloch, A. P., *Phytochemistry*, 1984, **23**, 1619.
10. Klebe, J. F., Finkbeiner, H. and White, D. M., *J. Am. Chem. Soc.*, 1966, **88**, 3390.
11. Avato, P., Bianchi, G. and Salamini, F., *Phytochemistry*, 1985, **24**, 1997.
12. Weete, J. D., Leek, G. L., Peterson, C. M., Currie, H. E. and Branch, W. D., *Plant Physiol.*, 1978, **62**, 677.

NEWS

INDIAN NATIONAL SCIENCE ACADEMY MEDAL FOR YOUNG SCIENTISTS

Fifteen young scientists have been awarded the INSA medals. The recipients are as follows: 1. Dr C. Varadhachari, Senior Research Fellow, Department of Agriculture, Calcutta University for chemistry and process development. 2. Dr Biman Bagchi, Indian Institute of Science, Bangalore for his contributions to theoretical studies of liquid structure and liquid-solid transitions. 3. Dr Amarjit Singh Basara, Punjab Agricultural University, Ludhiana for his original contribution in mechanisms underlying elongation of cotton fibres. 4. Dr Kamal Bhatacharya, Indian Association for the Cultivation of Science, Jadavpur for his work in quantum chemistry especially in the area of perturbation formalism. 5. Dr Kandala Venkataramana Chary, Tata Institute of Fundamental Research, Bombay for his study on the structure of DNA segments by 2D-Ft NMR. 6. Dr Jayaram Gowrishankar, Centre for Cellular and Molecular Biology, Hyderabad for his work on genetics of osmoregulation. 7. Dr Dipankar Home, Saha Institute of Nuclear Physics, Calcutta for his work on fundamental aspects of quantum mechanics. 8. Dr Shiban Kishen Koul, Indian Institute of Technology, Delhi for his innovative analysis of micro-strip-like transmission lines and its application to design and development of microwave integrated circuits. 9. Ravi Mehrotra, National Physical Laboratory, New Delhi for his work on the quantum Hall effect. Theoretical studies on Visco-elastic effect in the 2D-electron liquid and for his prediction of the existence of sher waves in 2D-electron liquid. 10. Dr Biswajit Mishra, Indian Institute of Technology, Kharagpur for his ingenuity in applying thermodynamics to problems of sulphide-ore genesis. 11. Dr Echur Varadadesikan Sampathkumaran, Tata Institute of Fundamental Research, Bombay for his extensive experimental studies of valency fluctuation in rare earth systems. 12. Dr Jainigesh Akkaraju Sekhar, Defence Metallurgical Research Laboratory, Hyderabad for his discovery of rapid pressurization as a new solidification technique and impressive contributions to rapid solidification processing. 13. Dr Yarasam Chandra Sekharudu, University of Wisconsin, Madison for his work on computational study of lectin-carbohydrate interactions. 14. Dr Anurag Sharma, Indian Institute of Technology, New Delhi for his novel technique for the development of graded index official imaging systems. 15. Dr Ram Sanmukh Upadhyay, Centre for Advanced Study in Botany, Banaras Hindu University, Varanasi for his original contribution towards biological control of fusarium wilt of pigeon pea.