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A COMPARATIVE STUDY OF CYCAD MUCILAGES

P. DE LUCA, A. MORETTI, S. SABATO and G. SINISCALCO GIGLIANO*

Istituto di Botanica, Facoltà di Scienze, Università di Napoli, Via Foria 223, Napoli, Italy; *Orto Botanico, Facoltà di Scienze, Università di Napoli, Via Foria 223, Napoli, Italy

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Key Word Index—Cycadales; cycads; chemotaxonomy; monosaccharides of the mucilages.

Abstract—The patterns of monosaccharide distribution of the mucilages of Cycadales are characteristic at the generic level. Arabinose, fucose, galactose, mannose, rhamnose and methylrhamnose were identified in the hydrolysed mucilage of *Bowenia, Ceratozamia, Cycas, Dioon, Encephalartos, Lepidozamia, Macrozamia, Microcycas* and *Zamia. Stangeria* contains no rhamnose and methylrhamnose, and *Ceratozamia* contains galactose only in traces. American genera may easily be distinguished from the others by means of their different monosaccharide composition. *Lepidozamia* appears to be well separated from *Macrozamia.*

INTRODUCTION

The cycads are of great interest since they have remained relatively unchanged morphologically since the Mesozoic age. Together with *Ginkgo*, they are the last vestiges of the ancient group of the prephanerogams [1]. Today only 10 genera of cycads are extant and these are confined to the tropical and subtropical regions of both hemispheres. *Cycas* is distributed from Madagascar, throughout south-east Asia and tropical Australia to the western Pacific; *Bowenia, Lepidozamia* and *Macrozamia* are endemic to Australia; *Encephalartos* and *Stangeria* are African; *Ceratozamia, Dioon, Microcycas* and *Zamia* are American.

The cycads are characterized by a number of peculiar morphological [2] and biochemical features [3]. In particular, they contain an abundant system of mucilage ducts. The mucilages, analysed in one female specimen of *Encephalartos, longifolius* Lehm. are complex polysaccharides [4]. The amounts of each monosaccharide obtained on hydrolysis of the mucilage are constant in individuals of *E. longifolius* irrespective of age, sex and growth conditions [5]. Furthermore, an identical pattern of monosaccharide distribution has been found in 14 species of *Encephalartos* [6].

In the present work the mucilages of representative species of all ten genera of cycads have been analysed in order to determine the distribution of the monosaccharides previously found in *Encephalartos.*

RESULTS AND DISCUSSION

The monosaccharide compositions of the mucilages show that differences occur at generic and higher levels. Fig. 1 shows the monosaccharide content for one or two species in each genus. Arabinose, fucose, galactose, mannose, rhamnose and methylrhamnose are the monosaccharides of the polysaccharide in *Bowenia, Ceratozamia, Cycas, Dioon, Encephalartos, Lepidozamia, Macrozamia, Microcycas* and *Zamia.* However, *Ceratozamia* contains galactose only in traces. The only genus which lacks some of the monosaccharides is *Stangeria*, which shows the complete absence of methylrhamnose and rhamnose (Fig. 1). The monosaccharides occur in the mucilages in quantities varying between 32% (w/w) in *Dioon mejiae* and 94% (w/w) in *Macrozamia secunda.* Fucose is the most abundant monosaccharide in *Dioon*, rhamnose in *Ceratozamia* and *Zamia*, galactose in *Encephalartos, Lepidozamia* and *Microcycas*, arabinose in *Bowenia, Cycas, Macrozamia* and *Stangeria.*

The monosaccharide distribution patterns are characteristic at the generic level. *Stangeria* of the monotypic family Stangeriaceae is the most distinct, whereas *Lepidozamia* and *Encephalartos* are the only two genera to show similar patterns. The polysaccharides of the African, Australian and Australasian genera contain mainly arabinose and galactose, whereas those of the American genera contain higher proportions of fucose and galactose in *Dioon*, rhamnose and arabinose in *Ceratozamia*, rhamnose, fucose and methylrhamnose in *Zamia*, galactose and methylrhamnose in *Microcycas.*

These results may well reveal phylogenetic relationships. According to Johnson [7], the order Cycadales may be classified into three families: Cycadaceae (*Cycas*, African and Australasian), Stangeriaceae (*Stangeria*, African) and Zamiaceae, which includes Australian, African and American genera. Stangeriaceae and Cycadaceae are well separated from Zamiaceae morphologically and chemically [8]. Zamiaceae otherwise appears as a heterogenous group which needs further subdivision [7, 8]. From our data it is possible to distinguish Stangeriaceae from the other two families. Furthermore, we show that the American genera of the Zamiaceae may be distinguished from the other members of the family by their different monosaccharide composition. This may indicate an evolutionary divergence at an early stage in the history of this taxon.

The data regarding *Lepidozamia, Macrozamia* and *Zamia* are also of taxonomic interest. Thus *Lepidozamia*, a genus established by Regel [9] and later included by most taxonomists in *Macrozamia* [10],

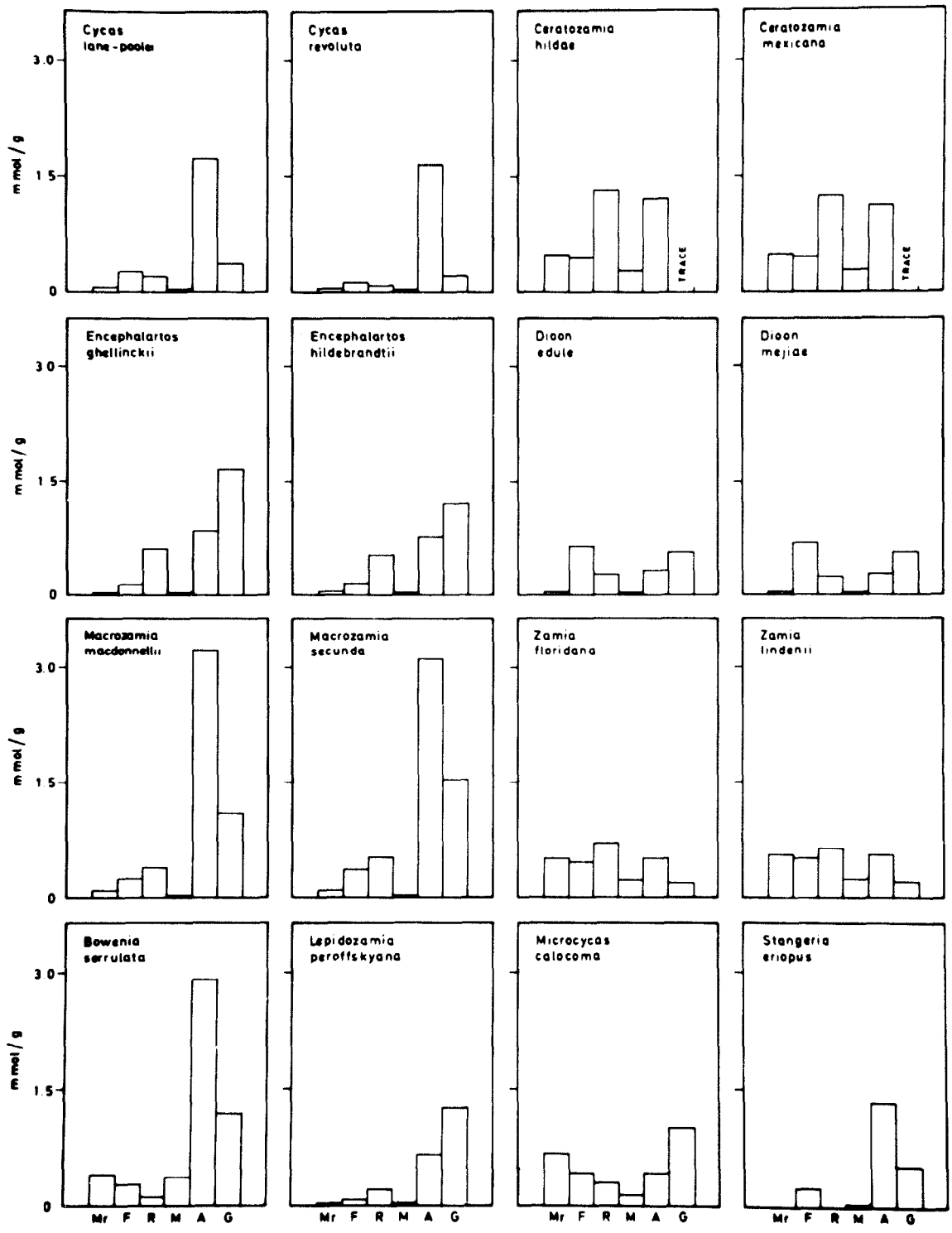

Fig. 1. Monosaccharide distribution histograms of hydrolysed cycad mucilages. Mr, methylrhamnose; F, fucose; R, rhamnose; M, mannose; A, arabinose; G, galactose.

appears well separated from *Macrozamia* on the basis of their different monosaccharide compositions (Fig. 1). In contrast, the monosaccharide composition of the mucilage of *Zamia* does not support the subdivision of this genus into *Zamia* and *Aulacophyllum* as proposed by Regel [11], because the monosaccharide composition is identical in species attributed both to *Zamia* (*Z. furfuracea*, *Z. floridana*) and to *Aulacophyllum* (*Z. lindenii*).

EXPERIMENTAL

Plant materials. Plants were grown in Naples Botanic Garden, Italy. The mucilages were obtained after incision of

the rachis. The species examined were: *Bowenia serrulata* (W. Bull) Chamberlain, east Australia; *Ceratozamia hildae* Landry and Wilson, north-east Mexico; *C. mexicana* Brongn., central Mexico; *Ceratozamia* sp., Tuxtla Gutierrez, Chiapas, Mexico; *Cycas lane-poolei* C. A. Gardner, north-west Australia; *C. revoluta* Thunb., Japan; *C. thouarsii* R. Brown, Madagascar; *Dioon edule* Lindley, central Mexico; *D. mejiae* Standl. and Williams, Honduras; *Dioon.* sp., Temixco, Guerrero, Mexico; *Encephalartos ghellinckii* Lem., South Africa; *E. hildebrandtii* A. Br. and Bouché, central Africa; *E. tegulaneus* Melville, central Africa; *Lepidozamia peroffskyana* Regel, south-east Australia; *Macrozamia macdonnellii* (F. Muell.) A. DC., central Australia; *M. riedlei* (Fisch.) C. A. Gardn., south-west Australia; *M. secunda* C. Moore, south-east Australia; *Microcycas calocoma* (Miq.) A. DC., Cuba; *Stangeria eriopus* (Kunze) Nash, South Africa; *Zamia floridana* A. DC., Florida, U.S.A.; *Z. furfuracea* L. f., Mexico and *Z. lindenii* Regel, Peru.

The Australian plants were collected in the field and supplied by the Terrara firm (Gilgandra, Australia). The South African plants were collected in the field and supplied by Umlaas Aloe Nursery (Umlaas Road, South Africa). The Mexican plants were collected in the field by the authors. *Cycas thouarsii, Microcycas calocoma, Zamia floridana* and *Z. lindenii* were collected in the field and donated respectively by Professor M. Giannattasio (Faculty of Agronomy, University of Naples, Italy), Professor O. Muñiz (Academia de Ciencias de Cuba), Professor K. Norstog (Fairchild Tropical Garden of Miami, Florida, U.S.A.) and L. Giugnolini (Botanic Garden of Florence, Italy). The other plants came from the Califano Collection (Naples, Italy).

Extraction. The mucilage (0.5 g) was solubilized at 40° in 20 ml H_2O and stirred to complete dissolution. The resulting syrup was filtered on a sintered disc. EtOH (95%) was added gradually to the filtrate until pptn of the polysaccharide was complete. The polysaccharide was centrifuged at 700 *g* for 10 min, washed ×5 with H_2O and EtOH in the same proportions used during the pptn to complete elimination of free sugars in the ppt, and dried *in vacuo* at 40°.

Hydrolysis. The polysaccharide (10 mg) was hydrolysed by adding 1 ml 1 M HCl and heating at 100° for 30 min. Sorbitol (1 mg/ml) was added as int. standard.

Trimethylsilylation. The hydrolysate was dried *in vacuo* at 40°, 200 μl of trimethylsilylation reagent (Sigma SIL–A) was added, and after 10 min it was centrifuged at 700 *g* for 10 min. Samples (1–2 μl) were injected directly into the gas chromatograph.

Analysis. GC was carried out in a 2 m × 4 mm glass column packed with 3% OV–1, 100–120 mesh, isothermal 180°. The carrier was N_2 at 40 ml/min. The temp. of the injector and FID detector was 230°. The rhamnose, fucose, arabinose, mannose and galactose peaks (α- and β-anomers) were identified using pure standards (Sigma). The 3-*O*-methylrhamnose peaks were identified using a pure standard generously given by Professor A. M. Stephen, University of Cape Town, South Africa.

REFERENCES

1. Emberger, L. (1968) *Les Plantes Fossiles Dans Leurs Rapports Avec Les Vegetaux Vivants*, 2nd edn, pp. 385–504. Masson & Cie, Paris.
2. Pant, D. D. (1973) *Cycas and the Cycadales.* Central Book Depot, Allahabad.
3. De Luca, P., Moretti, A., Sabato, S. and Siniscalco Gigliano, G. (1980) *Phytochemistry* **19**, 2230.
4. Stephen, A. M. and De Bruyn, D. C. (1967) *Carbohydr. Res.* **5**, 256.
5. Siniscalco Gigliano, G. (1980) *Delpinoa* **21**, 63.
6. Moretti, A., Sabato, S. and Siniscalco Gigliano, G. (1981) *G. Bot. Ital.* **115** (in press).
7. Johnson, L. A. S. (1959) *Proc. Linn. Soc. N. S. W.* **84**, 64.
8. Dossaji, S. F., Mabry, T. J. and Bell, E. A. (1975) *Biochem. Syst. Ecol.* **2**, 171.
9. Regel, E. (1857) *Bull. Soc. Nat. Mosc.* **1**, 182.
10. Schuster, J. (1932) in *Das Pflanzenreich IV* (Engler, A. and Diels L., eds). 1966 edn, Vol. 1, No. 99. Leipzig.
11. Regel, E. (1876) *Acta Hortic. Petropolitani* **4**, 275.