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TAXONOMIC SIGNIFICANCE OF METHYLAZOXYMETHANOL GLYCOSIDES IN THE CYCADS

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Abstract—Cycasin and macrozamin, the main methylazoxymethanol glycosides, found in cycads, have been analysed in seeds of representative species of all 10 genera of this group Macrozamin concentration is taxonomically significant at the generic level The taxonomic and evolutionary significance of the results is discussed

INTRODUCTION

Cycasin, macrozamin and neocycasins are naturally occuring, toxic MAM (methylazoxymethanol) glycosides [1, 2] found in cycads, a relict group of ancient gymnosperms All have the same toxic aglycone [3] while they differ in their sugar moieties The sugar component of macrozamin is primeverose [4], that of cycasin is β-D-glucose [5], while neocycasins show different glucose based units [6]

Recently [7] we have stated that cycasin is characteristic of and exclusive to the cycads, being present in all 10 genera of this group, it is absent from other gymnosperm taxa and from the fern *Marattia salicina* Moreover, we have identified macrozamin in Australasian cycads, where it occurs generally in quantities much higher than cycasin [8] Neocycasins have been isolated only in *Cycas* spp where they occur in small quantities [6]

Today only 10 genera of cycads (Cycadales) are extant and these are confined to the tropical and subtropical regions of both Hemispheres The classification followed in the present work is that of Johnson [9], who divided the order Cycadales into three families Cycadaceae, with *Cycas* (20 sp) distributed from Madagascar and throughout south-east Asia and tropical Australia to the western Pacific, Stangeriaceae, with *Stangeria* (1 sp) distributed in Africa, Zamiaceae, with *Bowenia* (2 sp), *Lepidozamia* (2 sp) and *Macrozamia* (14 sp) endemic to Australia, *Encephalartos* (40 sp) endemic to Africa and *Ceratozamia* (5 sp), *Dioon* (10 sp), *Microcycas* (1 sp) and *Zamia* (30 sp) endemic to America, the Zamiaceae family was further subdivided into three tribes

Because of the phylogenetic interest in these plants and the observation that MAM glycosides are restricted to cycads, in the last five years we have analysed such compounds in seeds of cycads from field and living collections This report is a part of a chemotaxonomic study of this order for which we have also provided reports concerning monosaccharide distributions of mucilages [10, 11]

RESULTS AND DISCUSSION

The results in Table 1 show that macrozamin, as for cycasin [7], is ubiquitous in cycads, being present in all the examined species Macrozamin is generally more abundant than cycasin and it occurs in quantities varying between 0 2% in *Cycas cairnsiana* to *ca* 5% in *Bowenia spectabilis*, while cycasin varies between very low quantities, in most of the examined species, to 0 7% in *C lanepoolei*

While cycasin concentration is not taxonomically significant, macrozamin concentration shows differences at the generic level Macrozamin levels in *Cycas* are 0 20–0 45%, *Bowenia* 4 33–5 04%, *Lepidozamia* 1 11%, *Macrozamia* 2 41–3 88%, *Microcycas* 0 13%, *Encephalartos* 2 09–2 86%, *Stangeria* 4 70%, *Ceratozamia* 1 01–1 06%, *Dioon* 0 62–0 68% and *Zamia* 1 01–1 25% Likewise, monosaccharide distribution in cycad mucilages is characteristic at the generic level [11]

The present work further supports the view that Zamiaceae is a heterogenous family [9] and, moreover, indicates that the subdivision in tribes is not well-established In particular, the tribe Zamieae, which includes *Bowenia* (Australian) and *Ceratozamia*, *Microcycas* and *Zamia* (American) is heterogeneous in that *Bowenia*, on the basis of macrozamin content and monosaccharide distribution pattern [11], is well separated These results strengthen the view that the present members of Zamiaceae have doubtless to be considered relict forms with many lost relatives [9] Moreover, our results support the distinctiveness of the Cycadaceae on account of its very low macrozamin quantities, sometimes lower than those of cycasin, on the other hand both the Cycadaceae and the Stangeriaceae are well separated from Zamiaceae on the basis of biflavone content [12] Furthermore, the observation that *Bowenia* and *Stangeria*, which are distinguishable from the other genera in their types of foliage, contain the highest quantities of MAM glycosides might be of phylogenetic importance

The occurrence of an azoxy group in the aglycone of MAM glycosides strengthens the exclusiveness of such

Table 1 Macrozamin and cycasin percentages in ripe seeds of cycads

Species examined	Macrozamin % (fr wt)*	Cycasin % (fr wt)*
Cycadaceae		
Cycas basaltica C A Gardner†	0 29	0 12
C cairnsiana F Muell †	0 20	0 10
C circinnalis L	0 42	0 10
C lane-poolei C A Gardner†	0 45	0 72
C prunosa Maconochie†	0 33	0 10
C revoluta Thunb †	0 26	0 28
C thouarsii R Brown	0 31	0 06
Stangeriaceae		
Stangeria eriopus (Kunze) Nash	4 70	0 02
Zamiaceae		
Tribe Dioeae		
Dioon edule var *angustifolium* Miq	0 64	0 12
Dioon sp (Guerrero, Mex)	0 62	0 13
Dioon sp (Nayarit, Mex)	0 65	0 01
Dioon sp (Sonora, Mex)	0 68	0 01
Tribe Encephalarteae		
Encephalartos altensteinii Lehm	2 11	0 07
E ferox Bertol f	2 10	0 05
E lebomboensis Verdoorn	2 16	0 05
E umbeluziensis R A Dyer	2 86	0 08
E villosus Lem	2 09	0 08
Lepidozamia peroffskyana Regel†	1 11	0 21
Macrozamia diplomera (F Muell) L Johnson†	2 41	0 16
M fawcettii C Moore	2 49	0 06
M heteromera C Moore	2 59	0 10
M miquelii (F Muell) A DC †	3 88	0 09
M moorei F Muell †	3 72	0 08
Tribe Zamieae		
Bowenia serrulata (W Bull) Chamberlain†	4 33	0 26
B spectabilis Hook f †	5 04	0 42
Ceratozamia matudai Lundell	1 05	0 08
C mexicana Brongn	1 01	0 02
Ceratozamia sp (Hidalgo, Mex)	1 06	0 01
Ceratozamia sp (Oaxaca, Mex)	1 02	0 03
Zamia latifoliolata Prenl	1 02	0 02
Zamia sp (Nayarit, Mex)	1 25	0 01
Zamia sp (Oaxaca, Mex)	1 16	0 16
Zamia sp (Oaxaca, Mex)	1 23	0 15
Zamia sp (Veracruz, Mex)	1 01	0 28
Microcycas calocoma (Miq) A D C	0 13	0 07

* Each result is the average of three measurements
† Data already published in ref [8]

glycosides to cycads The azoxy group, in fact, is very rare in organisms and it is known only in three other naturally occurring compounds which have been isolated from two species of bacteria [13, 14] and one species of fungi [15] All these compounds also have antibacterial and/or antifungal action [14] and, therefore, MAM glycosides may have played an important ecological role during the long evolutionary history of the cycads, whose fossil records extend to the Permian period [16] This could account for the fact that modern genera of cycads, which descend from different early Tertiary ancestors, all retain this chemotaxonomic character

EXPERIMENTAL

Plant material Seeds of *Bowenia serrulata* and *B spectabilis* were collected in the field by Mr Brigden (Casuarina, Australia), seeds of *Cycas basaltica*, *C cairnsiana*, *C lane-poolei*, *C prunosa*, *Lepidozamia peroffskyana*, *Macrozamia diplomera*, *M fawcettii*, *M heteromera*, *M miquelii* and *M moorei* were collected in the field and supplied by Terrara (Gilgandra, Australia), seeds of *Encephalartos altensteinii*, *E ferox*, *E lebomboensis*, *E umbeluziensis*, *E villosus* and *Stangeria eriopus* were collected in the field and supplied by Mrs C Giddy (Natal, South Africa), seeds of *Cycas revoluta* came from specimens grown in the Naples Botanical Garden, Italy, seeds of *Cycas thouarsii* were collected in

Madagascar by Dr G Pinto (Faculty of Science, University of Naples, Italy), seeds of *Cycas circinnalis* and *Microcycas calocoma* were generously donated by Dr J Popenoe of the Fairchild Tropical Garden, Miami, U S A, seeds of *Zamia latifoliolata* were collected in the field and generously donated by Dr J L Vivaldi of the Department of Natural Resources, Puerto Rico, seeds of *Dioon edule* var *angustifolium*, *Dioon* spp, *Ceratozamia matudai*, *C mexicana*, *Ceratozamia* spp and *Zamia* spp were collected in the field by the authors

Cycasin determination Cycasin was determined according to ref [7]

Macrozamin determination Macrozamin was determined according to ref [8]

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