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Fatty acid composition analysis: a new approach to chemotaxonomy of cycads

GESUALDO SINISCALCO GIGLIANO, ROBERTO NAZZARO

Dipartimento di Biologia Vegetale, Facoltà di Scienze, Università di Napoli Federico II, Via Foria 223, 80139 Napoli, Italy.

Riassunto

Sono stati identificati nei semi di undici taxa di *Ceratozamia*, *Dioon* and *Zamia* (Zamiaceae, Cycadales) i seguenti acidi grassi: miristico, palmitico, palmitoleico, eptadecanoico, stearico, oleico, linoleico, linolenico e arachidico. I modelli di distribuzione degli acidi grassi sono simili a livello intraspecifico e intragenerico, ma ben differenziati a livello intergenerico. Il modello di *Dioon* si distingue per la elevata quantità di acido oleico, mentre i modelli di *Ceratozamia* and *Zamia* appaiono simili tra di loro condividendo alte quantità di acido linoleico. Le affinità e le differenze risultate ai vari livelli tassonomici confermano precedenti dati fitochimici sui tre generi.

INTRODUCTION

Cycads (Cycadales) are a group of ancient plants with a gymnospermous reproductive biology whose fossils date back to the Permian Period (ZHIFENG and THOMAS, 1989). They had worldwide distribution during the Mesozoic Era (DELEVORYAS, 1982), but are represented today by a few scattered taxa restricted to tropics and subtropics (STEVENSON, 1990).

Due to their antiquity, the search of relationships among extant genera is difficult. In recent years, several studies have been carried out in the attempt to elucidate phylogenetic relationships among genera (CRANE, 1988; STEVENSON, 1990; CAPUTO *et al.*, 1991). Phytochemical data usefully contributed in providing information on this subject. Studies on biflavonoids (DOSSAJI *et al.*, 1975; DOSSAJI and BELL, 1973a), free aminoacids (VEGA and BELL, 1967; DOSSAJI and BELL, 1973b), MAM-glycosides (MORETTI *et al.*, 1983; SINISCALCO GIGLIANO, 1990), mucilages (DE LUCA *et al.*, 1982; SINISCALCO GIGLIANO, 1990; SINISCALCO GIGLIANO *et al.*, in press;

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STEVENSON and SINISCALCO GIGLIANO, 1989), and waxes (OSBORNE *et al.*, 1989) have proven to be of chemotaxonomic interest in cycads.

In the quest for other compounds potentially useful in cycad chemotaxonomic studies, in the present study we tested fatty acids in the three neotropical cycad genera *Ceratozamia, Dioon* and *Zamia* (Zamiaceae).

MATERIALS AND METHODS

Plant material

Analysis of fatty acid composition was carried out on seeds. The seeds were either collected from plants cultivated at the Botanical Garden of Naples or supplied by other botanical institutions and private collectors. The list of taxa examined and their origin, as well as sources of seeds is reported in Table I.

Lipid extraction and gas chromatographic assay of fatty acids

Lipids were extracted following LITCHFIELD's method (1972). Seeds (1-2 g) were shaken in 50 ml chloroform and methanol (2:1) for 12 hrs at room temperature. The extracts were filtered through Whatman 40 paper and the filtrate washed twice with chloroform and methanol (2:1). Fractions were pooled and methanol was separated from chloroform by adding water in a separating funnel. Water was gradually added until stratification was complete. The lower layer (containing chloroform and lipid) was separated and washed twice with water. Chloroform-lipid phase was concentrated *in vacùo* to a very small volume, transferred to an ampoule, and completely evaporated.

In order to allow gas chromatographic analysis of fatty acids, these were methylated, following MORRISON and SMITH's method (1964). To this purpose, 2 ml methylating reagent (boron fluoride 10% methanol solution) was added to the dry product obtained after evaporation and the ampoule was sealed and heated at 100 °C for 30 min. After cooling, pentane and water (2:1) were added; the ampoule was then vigorously shaken and centrifuged at 700xg for 5 min in order to allow stratification of two phases (pentane and methylating reagent-water). After centrifugation, the upper phase (containing pentane and methyl esters) was recovered, further washed with pentane, brought to a known volume, and 1-2 µl injected into the gas chromatograph.

Gas chromatography was carried out in a 1.5 m x 4 mm glass column packed with PEGA 10%, isothermal 175 °C. The carrier gas was N_2 at 40 ml/min. Temperature of the injector and FID detector was 190 °C. Peaks of methyl esters of myristic, palmitic, palmitoleic, heptadecanoic, stearic, oleic, linoleic, linolenic, and arachidic acids were identified using pure standards (Sigma).

Tests were carried out in triplicate using three seeds for each taxon. Data shown in Table I and Fig. 1 represent the average of the three measurements. Accuracy was within 1% in all cases.

RESULTS AND DISCUSSION

Myristic, palmitic, palmitoleic, heptadecanoic, stearic, oleic, linoleic, linolenic, and arachidic acids are the fatty acids identified in all specimens. For each specimen, amounts of identified fatty acids are reported in Table I. Fatty acid compositions from all taxa are also illustrated in form of histograms in Fig. 1.

Histograms (Fig. 1) show that fatty acid composition patterns are similar at intraspecific and intrageneric levels, but different at the intergeneric level.

Minor interspecific differences (e. g., the difference between *Z. furfuracea* pattern and the rest of *Zamia* species patterns; Fig. 1C) are not regarded of chemotaxonomic value because similar differences were found also at varietal level (e. g., between *C. mexicana* and *C. mexicana* var. *latifolia*; Fig. 1A) and even between specimens from the same taxon (e. g., in *Dioon edule*; Fig. 1C). Intrageneric uniformity of present data supports previous reports on *Ceratozamia* and *Dioon*; biflavonoids (DOSSAJI *et al.*, 1975), monosaccharide composition of mucilages (DE LUCA *et al.*, 1982; SINISCALCO GIGLIANO *et al.*, in press), and macrozamin content (MORETTI *et al.*, 1983) are in fact very similar in the two genera. Intrageneric uniformity, in contrast, does not support previous findings in *Zamia*; this genus shows interspecific variation, albeit low, in biflavonoid and mucilage studies (DOSSAJI *et al.*, 1975; SINISCALCO GIGLIANO *et al.*, in press).

As far as intergeneric differences are concerned, specific quantitative ratios among fatty acids characterize the three genera. *Dioon* has the most distinctive pattern showing high amounts of oleic acid (Fig. 1B), whereas *Ceratozamia* and *Zamia* patterns are similar in showing high amounts of linoleic acid (Fig. 1A, C). Intergeneric differences among fatty acid composition patterns in the three genera (Fig. 1A, B, C) generally confirm previous phytochemical data. In fact, intergeneric differences were found in studies on biflavonoids, mucilages, and macrozamin (DOSSAJI *et al.*, 1975; DE LUCA *et al.*, 1982; SINISCALCO GIGLIANO *et al.*, in press; MORETTI *et al.*, 1983). Diversity of *Dioon* pattern (Fig. 1B) and

Table I - Cycad taxa in study and amounts of fatty acids identified in each taxon

Species	Origin	Seed source	Fatty acid (% of Total)*									Total* (% fr. wt. seed)
			MYR	PAL	PAO	HEP	STE	OLE	LIO	LIN	ARA	
1. *Ceratozamia mexicana*	Veracruz, Mexico	a	0.49	18.72	0.39	0.59	1.56	21.64	53.69	2.14	0.78	0.51
2. *C. mexicana*	Veracruz, Mexico	b	0.21	16.09	0.10	0.46	1.30	23.20	54.84	3.09	0.71	0.48
3. *C.mexicana* var. *latifolia*	Veracruz, Mexico	c	0.29	18.63	0.21	0.52	2.07	19.25	53.86	4.55	0.62	0.48
4. *Dioon edule*	Veracruz, Mexico	d	0.27	17.12	0.43	0.86	5.08	43.19	28.94	2.65	1.46	0.19
5. *D. edule*	Veracruz, Mexico	e	0.24	18.13	0.42	0.44	3.92	42.66	29.70	3.24	1.25	0.46
6. *D. spinulosum*	Oaxaca, Mexico	f	0.27	17.08	0.43	0.86	5.07	43.40	28.83	2.61	1.45	0.37
7. *Zamia amblyphyllidia*	Puerto Rico	f	1.02	21.99	0.50	0.76	6.14	24.00	41.00	1.52	3.07	0.34
8. *Z. furfuracea*	Veracruz, Mexico	a	1.29	20.38	0.29	0.57	5.45	26.40	40.46	1.72	3.44	0.36
9. *Z. integrifolia*	Florida, U.S.A.	f	1.02	22.00	0.51	0.75	6.14	24.04	40.94	1.53	3.07	0.29
10. *Zamia* sp.	Campeche, Mexico	a	1.02	21.97	0.51	0.77	6.13	24.02	40.98	1.53	3.07	0.20
11. *Zamia* sp.	Amazonas, Venezuela	e	1.02	22.00	0.51	0.75	6.14	24.04	40.94	1.53	3.07	0.37

*The amount of a single fatty acid is expressed as percentage of the total amount (last column) of fatty acids identified in each taxon. The acronyms indicate myristic (MYR), palmitic (PAL), palmitoleic (PAO), heptadecanoic (HEP), stearic (STE), oleic (OLE), linoleic (LIO), linolenic (LIN) and arachidic (ARA) acids.

a: collected in the field by G. Vallariello (Orto Botanico di Napoli); b: collected in the field by M. Vazquez Torres (Universidad Veracruzana, Mexico); c: collected by authors from a plant cultivated at the Orto Botanico di Napoli; d: supplied by Peter Down firm, New Zeland; e: collected in the filed by A. Moretti (Dipartimento di Biologia Vegetale di Napoli); f: collected by E. Williamson (North Carolina, U.S.A.) from a cultivated plant.

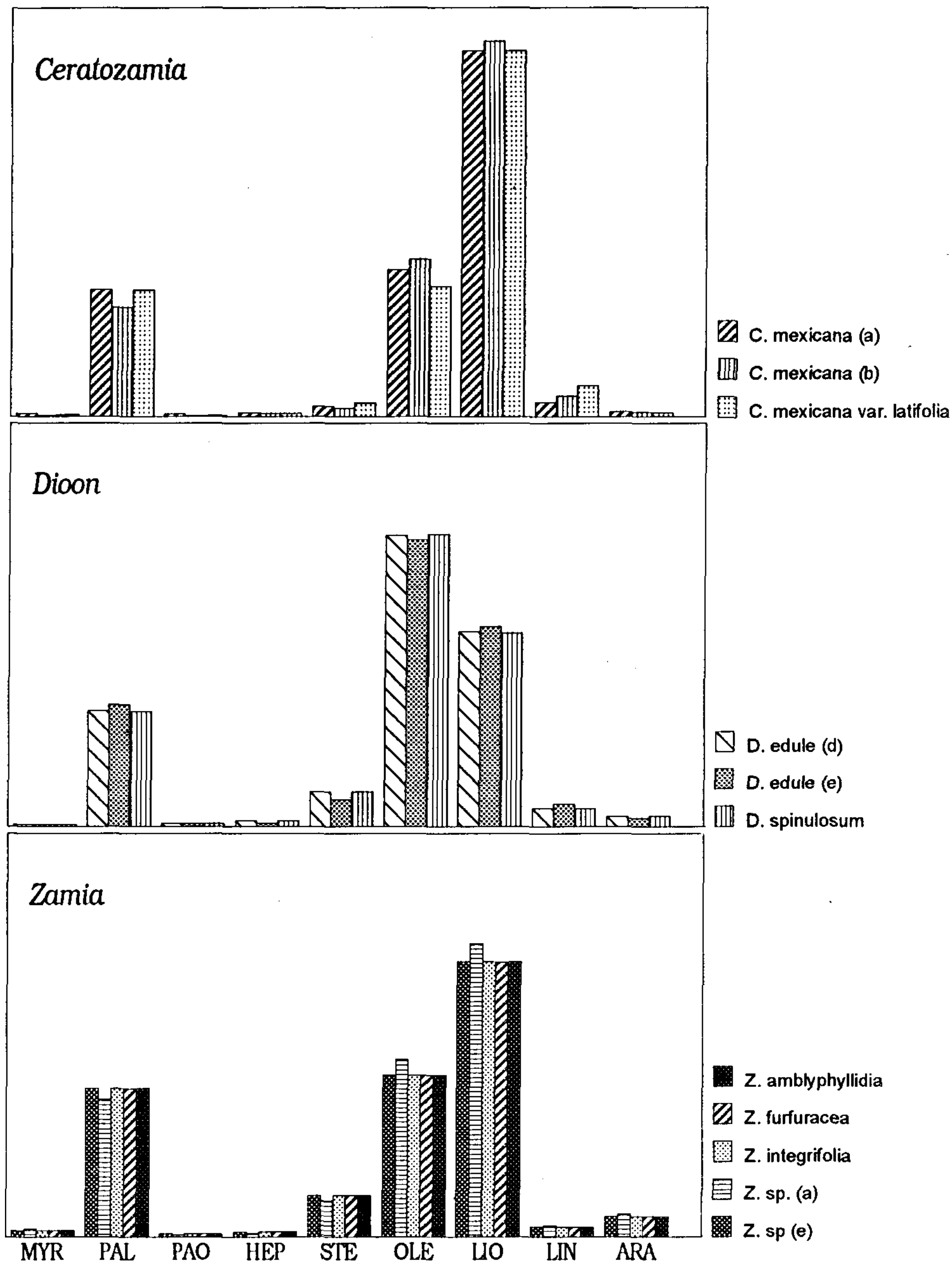

Fig. 1 - Histograms of fatty acid composition in *Ceratozamia*, *Dioon* and *Zamia*. In order to allow an easier comparation among fatty acid composition patterns, histograms express normalized values (total of identified fatty acids in each taxon = 100). Letters in parentheses refer to seed origin as reported in Table I.

similarity between *Ceratozamia* and *Zamia* patterns (Fig. 1A, C) are further supported by past studies on mucilages and macrozamin (De Luca *et al.*, 1982; Siniscalco Gigliano *et al.*, in press; Moretti *et al.*, 1983), but are in contrast with data reported for biflavonoids; the data on biflavonoids suggested relationships between *Dioon* and *Zamia* rather than between *Ceratozamia* and *Zamia* (Dossaji *et al.*, 1975).

Conclusions

Fatty acid composition analysis in *Ceratozamia*, *Dioon* and *Zamia* has proven to be of chemotaxonomic interest. Results appear to be generally congruent with phytochemical data previously reported for the three genera. The minor differences found between present and previous phytochemical data will be more widely discussed when the examination of a greater number of species belonging to the three genera is carried out.

In the meantime, a temptative interpretation of the uniformity among *Zamia* patterns (Fig. 1C), contrasting with previously reported interspecific variation, can be suggested by examining the geographical distribution of this genus. With the exception of *Zamia* sp. from Venezuela, all the species here examined are from the Atlantic coasts of north-eastern America (see Table I), a restricted and ecologically uniform area as compared with the wide distribution of the genus, which covers both tropical belts in America, and with the high variety of habitats known for *Zamia* species (Stevenson, 1990). Thus, uniformity in fatty acid compositions in the species examined, unless due to paucity of sampling, may depend on the uniformity of environmental conditions in the habitats and/or on a common phyletic origin. *Zamia* sp. from Venezuela, although separated in the distribution from the rest of examined species, shows relationships with two of them, namely with *Z. integrifolia* in the mucilage monosaccharide composition pattern (Siniscalco Gigliano *et al.*, in press) and with *Z. furfuracea* in the chromosome number and karyotype (Moretti, 1990, and personal communication). Broader studies on South American *Zamia* species are needed to better clarify such proposed relationships among these three species.

Affinity between *Ceratozamia* and *Zamia* patterns (Fig. 1A, C), in contrast with the affinity between *Dioon* and *Zamia* resulted in studies on biflavonoids (Dossaji *et al.*, 1975), seems to be supported by other studies on these genera. Among the five Neotropical cycad genera, in fact, only *Ceratozamia* and *Zamia* are morphologically similar (Stevenson, 1990; Vovides *et al.*, 1983); cladistic analyses, based both on morphology (Stevenson, 1990) and chloroplast DNA restriction fragment length polymorphism (Caputo *et al.*, 1991), also showed that *Ceratozamia* is close to *Zamia*; finally, karyological analysis showed that all *Ceratozamia* species have the same karyotype and that this was very similar to the karyotype described in some *Zamia* species (e. g., species from West Indies and other North American areas) (Moretti, 1990).

In regard to *Dioon*, phytochemical (De Luca *et al.*, 1982; Morettt *et al.*, 1983; Stevenson and Siniscalco Gigliano, 1989), morphological (Stevenson, 1990), structural (Dehgan and Dehgan, 1988), biomolecular (Caputo *et al.*, 1991), and karyological (Moretti, 1990) data support distinctiveness of this genus from *Zamia*.

Such a large body of evidence confirming our results on *Dioon* represents an additional support to the usefulness of the new chemotaxonomic approach here used.

Abstract

Myristic, palmitic, palmitoleic, heptadecanoic, stearic, oleic, linoleic, linolenic, and arachidic acids are the fatty acids identified in seeds of eleven taxa belonging to *Ceratozamia*, *Dioon* and *Zamia* (Zamiaceae, Cycadales). Fatty acid composition patterns are similar at intraspecific and intrageneric levels, but different at the intergeneric level. *Dioon* has the most distinctive pattern showing high amounts of oleic acid. *Ceratozamia* and *Zamia* patterns show similarity in sharing high amounts of linoleic acid. Both similitarities and differences resulted at the different taxonomic levels support most previous chemotaxonomic data on the three genera.

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