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TAXONOMIC PATTERNS IN AMINO-ACIDS OF *ACACIA* SEED GLOBULINS

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Abstract—Amino-acid analyses of seed globulins from 14 Australian *Acacia* species show a taxonomic pattern involving variation in the proportions of glutamic acid, isoleucine, threnone and valine. The results support recent taxonomic treatments of the genus, and imply that amino-acid patterns may be usefully predictable in other legumes.

INTRODUCTION

It has been assumed that plant protein amino-acid profiles will vary with physiological conditions and environment and, since the protein amino-acids occur universally and any variation will be merely quantitative, they are thought to hold little taxonomic interest [1,2]. Nevertheless, Byers [3] has demonstrated that amino-acid patterns even of total leaf protein extracts from different leaves of the same species are quite constant, though there is variation from species to species. Taira [4,5] has revealed appreciable and constant variation in protein amino-acid patterns among seeds from different grass genera, which fit in clearly with modern views on grass taxonomy, and he has attributed it to quantitative and qualitative variation in particular protein fractions [6]. His work has been neglected, but Watson and Creaser [7], in analysing extensive compiled data, have confirmed his conclusions for total protein amino-acids of cereal grains and have in addition found statistically-significant large-scale taxonomic correlations (involving several amino-acids) in total-protein analyses of dicotyledonous leaves. In view of the biological and nutritional importance of plant proteins, it is desirable to establish how widely and at what levels quantitative variation in their amino-acids is taxonomically predictable. We report here another case of systematic variation in protein amino-acid patterns, this time at intrageneric level, involving the seed globulins of the legume genus *Acacia*.

RESULTS AND DISCUSSION

Table 1 summarizes the results of amino-acid analyses of seed globulins extracted from 14 species of Australian *Acacia*, Section Heterophyllum. Considering they were derived from crude globulin extracts, independent analyses of seed samples of *A. baileyana* collected in different years from different trees yielded a fairly consistent profile. Gaps for valine reflect unsatisfactory standards and unavailability of seed for confirmatory analyses, or presence of a contaminating peak (an amino sugar, probably glucosamine, in *A. armata*). The sample is small, but represents two main taxonomic series of Australian acacias, whose recognition involves placing bipinnate-leaved Botryocephalae (e.g. *A. baileyana*, *A. decurrens*) alongside unnerved phyllodinous species (Group I), and separating these widely from both the plurinerved phyllodinous species and from the bipinnate-leaved Pulchellae (e.g. *A. drummondii*) in Group II. These are the chief groupings to emerge from a recent morphological, anatomical and numerical-taxonomic study [10]

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Table 1 Amino-acid contents* of globulins from seeds of Australian *Acacia* species

Acacia Species	Alanine	Arginine	Aspartic acid	Glutamic acid	Glycine	Histidine	Isoleucine	Leucine	Lysine	Phenylalanine	Proline	Serine	Threonine	Tyrosine	Valine
Group I															
A baileyana (1)	65	59	110	160	71	27	43	92	72	40	62	63	41	39	59
A baileyana (2)	72	62	102	176	76	27	39	102	72	42	71	74	43	42	
A decurrens	73	57	107	181	72	24	30	108	64	40	79	80	46	39	
A armata	88	47	76	181	96	21	31	101	61	38	73	97	54	36	
A rubida	64	57	108	161	62	25	44	99	69	41	65	65	38	45	56
A cultriformis	68	64	97	164	65	28	31	89	75	41	63	72	45	49	49
A alata	71	64	107	177	69	25	34	97	91	39	46	73	43	29	42
A diffusa	66	64	102	166	69	27	42	93	73	39	58	66	40	38	56
A verticillata	67	69	97	170	67	31	31	92	78	39	63	82	38	38	39
A restiacea	74	61	99	156	84	24	33	92	77	40	60	73	49	33	44
Mean group I ($\bar{x}_1$)	71	60	100	**168**	73	26	**35**	96	73	40	63	75	**44**	38	**49**
Group II															
A drummondii	70	49	119	215	71	20	27	108	65	36	64	83	37	37	
A doratoxylon	70	56	127	187	80	26	19	79	70	34	68	85	35	37	26
A longissima	74	51	107	189	81	20	27	104	56	41	79	90	41	38	
A implexa	69	61	111	177	79	22	27	106	61	40	87	83	38	40	
A oxycedrus	59	78	93	211	61	35	28	85	93	33	47	70	41	32	35
Mean group II ($\bar{x}_2$)	68	59	111	**196**	74	25	**26**	96	69	37	69	82	**38**	37	**31**
($\bar{x}_1 - \bar{x}_2$) se	07	02	17	**34**	03	04	**38**	01	06	18	08	16	**26**	07	**35**

* Expressed for each species as percentages of the total estimated amino-acids *A baileyana* (2) omitted from the statistical calculations

and they are very much in accord with an independent classification published recently by Vassal [9].

Some seed globulin amino-acids (alanine, arginine, glycine, histidine, leucine, lysine, proline, serine, tyrosine) are seen to be at rather consistent levels in all these *Acacia* species, while aspartic acid is at a constant level save for very low values in *A armata* and *A oxycedrus* and a high value in *A. doratoxylon*. Phenylalanine tends to be lower in Group II, but the figures are not statistically significant The remaining acids analysed however (glutamic acid, isoleucine, threonine, valine), while individually rather constant within Groups I and II, show statistically significant differences between them. Of these patternised amino-acids, only glutamic acid consistently shows higher values in Group II, the rest reaching higher values in Group I The trend for the nutritionally essential amino-acid isoleucine is particularly clear, with no overlap between the Groups.

Regarding bipinnate-leaved acacias, Table 1 supports the proposed wide separation of *A. decurrens* and *A. baileyana* from *A. drummondii* [9,10], and lends support to the suggestion [10] that the latter is closer to the plurinerved-phyllodinous species *A oxycedrus*, *A verticillata* and *A diffusa* were long associated in Bentham's Pungentes [11], a grouping now disbanded [9,10]. This change also gains support here, since *A diffusa* and *A. verticillata* exhibit similar amino-acid patterns typical of Group I species, while *A. oxycedrus* has the low isoleucine and phenylalanine and high glutamic acid levels characteristic of Group II. However, relative taxonomic isolation of *A oxycedrus* in this sample is indicated by its remarkably low leucine, tyrosine, aspartic acid and proline and its high lysine, arginine and histidine levels, these all being amino-acids which are very consistent in proportions throughout Table 1 as a whole. Similar considerations apply to *A alata*, here assigned tentatively to Group I

CONCLUSIONS

Seed globulin amino-acid profiles lend support to recently proposed changes in the classification

of Australian acacias, so they are no less interesting taxonomically than are non-protein amino-acids, which also show systematic variation in this genus [12]. Moreover there may be fundamental interest in the fact that protein amino-acids give clear patterns among such closely related organisms, and it would be interesting to know precisely what proteins are involved. Since it is known that the relative amounts of major seed globulins can vary in different varieties and lines of the same species, and since different globulins have different amino acid compositions, the amino acid profiles of total globulin extracts will also vary within species. This variation has nowhere been investigated in detail, but in *Acacia* at least it is apparently insufficient to obscure the overall taxonomic pattern The homologies of legume seed proteins in general need investigating in depth, before the theoretical and practical implications of this finding (and of another, demonstrating taxonomic predictability at tribal level of globulin gel-electrophoretic patterns [8]) can be properly evaluated. Nevertheless this result for *Acacia*, when viewed alongside similar ones involving major assemblages of grass genera [4,5,7], emphasises that protein amino-acid patterns ought to be sought and investigated among legumes seeds on a far more ambitious scale Those detected here may well represent taxonomically orderly variation in proportions of different proteins and/or in amino-acid compositions of homologous proteins [cf. grass seeds, 6] rather than (say) differences in extractability of the same proteins from different species. If so, extensive amino-acid surveys of legume seeds, including esculent forms, and employing taxonomically balanced samples of species and genera, might provide useful information for plant breeders and nutritionists, as well as contributing to increased understanding of the functions and evolution of particular proteins.

EXPERIMENTAL

Selected, mature dry seeds obtained from King's Park Botanic Gardens, Perth and Canberra Botanic Gardens, and collected in the field, were stripped of the remains of funicles and reduced to flour by grinding (in 5 min spells to avoid heating) in a Glen Greston M280 Seed Mill

Globulin extracts were prepared following the procedure of Boulter *et al* [8] Seed meals (1 part) were extracted with 5 parts of a 5% wt to vol potassium sulphate in 0 1M Pi buffer (pH 7) by stirring for 1 hr at 2°, and the resultant slurry squeezed through 2 layers of muslin and centrifuged at 1000 *g* for 20 min The supernatant was decanted into dialysis tubing and dialysed overnight against running tap H_2O, then dialysed for half a day against dist H_2O Contents of the dialysis tubing were then centrifuged at 1000 *g* for 30 min and the supernatant (albumins) removed The residue (globulins) was taken up in the original vol of extractant dil 5 × and allowed to stand for 30 min, any remaining ppt being then removed by centrifugation at 1000 *g* for 20 min The globulins were obtained by freeze-drying the supernatant All operations were carried out at 2° Amino-acid analyses were carried out on a Beckman 120C amino acid analyser

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