Phytochemistry, 1975, Vol. 14, pp. 1211–1217. Pergamon Press. Printed in England.

NON-RANDOM VARIATION OF PROTEIN AMINO-ACID PROFILES IN GRASS SEEDS AND DICOT LEAVES

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(*Received* 2 *July* 1974)

Key Word Index—Gramineae; Leguminosae; angiosperms; plant protein amino-acids; taxonomic patterns; cereal proteins; leaf proteins.

Abstract—Re-organization and analyses of extensive published data on protein amino-acid compositions of cereal grains and dicotyledonous leaves have elicited taxonomically-intelligible patterns. In cereal grains, leucine and alanine levels are lower in festucoids than in other grasses, while those of lysine and glycine are higher. Chloridoid grasses, in the middle of grass classifications, have intermediate levels of leucine and alanine. In dicot leaves there is patternization of quantitative data on *iso*leucine, lysine, cystine, phenylalanine, alanine, aspartic acid, glutamic acid, glycine and serine. The leaf patterns conform at statistically-significant levels with the taxonomic distinction between Crassinucelli and Tenuinucelli, and are further consistent with subordinate groupings (e.g. caryophylloids, legumes, acanthoids).

INTRODUCTION

Flowering plants show taxonomically significant variation in amino-acid sequences of their cytochrome-*c* [1], and it is reasonable to assume that other plant proteins may vary systematically in like fashion. If so, comparative analyses of other proteins and of total protein extracts from plant organs might also be expected to show taxonomic patterns. However, it is generally supposed [2] that non-protein amino-acids have greater taxonomic potential, and Taira's [3] claim that amino-acid patterns in grass seed proteins are consistent with modern classificatory views on grasses has created little interest. Unlike their non-protein counterparts, the protein amino-acids are of universal occurrence, so that variation is quantitative and less amenable to easy taxonomic interpretation. Nevertheless it seems important to confirm whether such patterns do indeed exist and to establish how widely they occur, with a view to pursuing them in detail, since they are potentially of practical interest to nutritionists and plant breeders and are relevant to speculation on the evolution of plant proteins. On the other hand the requisite large-scale taxonomic surveys, involving appropriate sampling of materials and standard, comprehensive amino-acid analyses, are unlikely to commend themselves to biochemists unless it can be shown convincingly that the results will justify so large an investment of labour.

We wish to draw attention to published evidence which, if we interpret it correctly, confirms the predictability of the amino-acid patterns of cereal grains as revealed by Taira; suggests that similarly meaningful patterns characterize dicotyledonous leaves; and implies that such patterns might profitably be sought in other materials.

RESULTS

Amino-acid data

The literature on the amino-acid composition of plant proteins has expanded enormously since the introduction of automated analytical systems. Little of it has any direct taxonomic orientation, however, and plant samples chosen with other aims in view seldom allow much scope for taxonomic interpretation. Any attempt at compiling published amino-acid analyses for this purpose will be unsatisfactory, for a variety of reasons. Many different methods of protein hydrolysis have been used, some of which adversely affect the determination of one or more amino-acids. Some analysts give no indication of the accuracy of determinations, and results are expressed in a variety of ways. It is a pity from our standpoint that nutritionists, who are the main source of information, usually express amino-acid contents only against total (plant or organ) nitrogen, and not directly against extracted protein. Variation in amino-acids expressed in *mg/g total N* could reflect differences in non-protein sources of nitrogen rather than in proteins, and since such substances as non-protein amino-acids, alkaloids and nitrates sometimes patternize taxonomically (e.g. Bell [4]) they can be expected to confuse the picture. Nevertheless, the FAO booklet, "Amino-acid content of foods and biological data on proteins" (Cresta and Odendaal, 1970 [5]) is a compilation which seems highly promising for preliminary plant-taxonomic evaluation. The authors have attempted an exhaustive review of comparable data on amino-acids in foods including cereals, with data for what amount to a good taxonomic sample of grass seeds and "vegetables", which comprise an extensive range of angiosperm leaves. Average values for individual amino-acids are given for each food, expressed both in *mg/g total N* and (less usefully, to us) in *mg/100 g food*; total amino-acid figures are also given where available. Sample sizes from which means are derived, along with standard deviations, ranges and coefficients of variability are provided as well. Figures for methionine, cystine and tryptophan often have high coefficients of variability for alternative analyses of the same material, so that means for these amino-acids in particular should evidently be interpreted cautiously. Results obtained by the column-chromatography method predominate in these tables. They are distinguished from those derived via the microbiological method, and from those obtained using the chemical method of Spies and Chambers [6] (quoted only for tryptophan). Indeed this publication is admirable in so many ways that it is depressing for a plant taxonomist to find that even here the virtues of his own speciality are unappreciated, the tabulated information being presented in alphabetical order according to colloquial names of foodstuffs.

Taxonomic analyses: grass seeds

The FAO data on cereals give fairly comprehensive information on seeds of species from 20 grass genera. When these are assigned to main taxonomic assemblages of grasses (Table 1) it emerges that the coverage is quite good, only bamboos and the arundinoid-danthonioids being unrepresented. The *essential amino-acid/total food content* mean figures reveal that high essential amino-acid contents are not confined to any particular group. More interestingly, when the systematized *mg/g total N* figures for all 18 amino-acids are examined, we find the following: (1) all the grass groups are internally reasonably consistent for amino-acids, when the latter are taken both individually and in combination as "profiles"; (2) leucine is decidedly low in festucoids, rather low in *Oryza*, high in panicoids and andropogonoids and "intermediate" in chloridoids; (3) lysine is decidedly higher in festucoids and *Oryza* than is usual elsewhere; (4) mean values for methionine are consistently low in festucoids and (less consistently so) in andropogonoids; and means for cystine are consistently low in andropogonoids and *Oryza*. The high variability

Table 1. Taxonomic classification of grasses, listing species for which extensive seed protein amino-acid data are given by Cresta and Odendaal [5]

Bamboos	(42/520)*	None
Oryzoids†	(10/35)	*Oryza* spp.
Festucoids	(110/1800)	*Hordeum vulgare*, *Avena sativa*, *Secale cereale*, *Triticum* spp., *Triticale*
Chloridoids	(90/890)	*Dactyloctenium aegyptiacum*, *Eleusine coracana*, *Eragrostis tef*
Arundinoid-danthonioids‡	(32/590)	None
Panicoids s.str.	(41/1300)	*Cenchrus biflorus*, *Digitaria exilis*, *Digitaria iburua*, *Echinochloa crus-galli*, *Setaria italica*, *Paspalum commersonii*, *Pennisetum* spp., *Panicum miliaceum*,
Andropogonoids	(65/1000)	*Coix lacryma-jobi*, *Euchlaena mexicana*, *Sorghum* spp., *Zea mays*

* Approximate world totals of genera and species per group in parentheses.

† The oryzoids are a small isolated group, seemingly closest to bamboos and festucoids.

‡ A grouping which includes (e.g.) *Phragmites*.

Table 2. Essential amino-acid contents* of cereal grains

	*Iso-*leucine	Leucine	Lysine	Methionine	Cystine	Phenylalanine	Tyrosine	Threonine	Tryptophan	Valine
Oryzoids										
Oryza spp.	3·8	8·1	3·8	2·3	1·1	5·1	3·5	3·9	—	5·4
Festucoids										
Hordeum vulgare	3·8	7·1	3·7	1·8	2·4	5·4	3·3	3·5	—	5·3
Avena sativa	4·0	7·8	4·0	1·8	2·9	5·4	3·5	3·6	—	5·5
Secale cereale	3·8	6·7	3·7	1·6	2·1	4·8	2·1	3·6	—	5·2
Triticum spp.	3·4	6·9	3·0	1·6	2·6	4·7	3·1	3·0	—	4·6
Chloridiods										
Eleusine coracana	4·4	9·6	2·9	3·1	2·6	5·2	3·6	4·2	1·5	6·7
Eragrostis tef	4·3	8·2	3·1	2·8	2·6	5·4	2·4	3·8	1·7	5·6
Paniceae										
Digitaria exilis	3·9	9·6	2·6	5·5	2·8	5·1	3·5	3·9	1·4	5·7
D. iburua	4·1	11·2	1·8	2·8	2·1	6·4	3·6	3·1	1·7	4·9
Pennisetum spp.	4·2	9·8	3·5	2·5	2·4	4·9	3·3	3·9	2·0	5·6
Echinochloa crus-galli	4.4	11·0	1·6	1·7	2·7	5·5	2·3	3·5	0·0	5·9
Andropogonoids										
Coix lacryma-jobi	4·1	12·9	1·9	2·2	1·3	4·3	3·1	2·8	0·0	5·8
Zea mays	3·8	12·9	2·7	2·0	1·6	5·1	3·9	3·7	0·0	5·0
Euchlaena mexicana	4·8	17·0	1·3	1·2	1·1	5·8	4·5	3·0	0·0	4·9
Sorghum spp.	4·1	13·8	2·1	1·4	1·6	5·1	2·8	3·1	1·3	5·2

* Expressed as percentages of total amino-acids (*mg/g total N* figures). Records for *Avena sativa* and *Secale cereale* based on "wholemeal"; all other based on husked or hulled whole grain. Data re-calculated from Cresta and Odendaal [5].

often exhibited by these two amino-acids in different analyses of the same material should however be kept in mind; (5) alanine is consistently lower in festucoids than in all other groups, while chloridoids and *Oryza* exhibit alanine levels intermediate between those of festucoids and the much higher ones of panicoids and andropogonoids; (6) glycine is higher in festucoids and chloridoids than in Paniceae and andropogonoids, and *Oryza* has the highest value of all; (7) *Oryza* further stands out through its extremely high levels of aspartic acid and arginine, and low proline.

Table 3. Non-essential amino-acid contents* of cereal grains

	Arginine	Histidine	Alanine	Aspartic acid	Glutamic acid	Glycine	Proline	Serine
Oryzoids								
Oryza spp.	8·2	2·5	5·9	10·1	20·3	4·9	4·6	5·4
Festucoids								
Hordeum vulgare	5·0	2·2	4·2	6·0	24·9	4·1	11·5	4·3
Avena sativa	6·7	2·3	4·9	8·3	22·5	5·0	5·6	5·0
Secale cereale	5·0	2·4	4·6	7·8	26·3	4·7	10·2	4·7
Triticum spp.	4·8	2·4	3·8	5·1	30·9	4·1	10·3	4·8
Chloridoids								
Eleusine coracana	4·5	2·2	6·2	6·5	20·4	4·0	7·1	5·1
Eragrostis tef	3·8	2·1	5·4	6·8	28·0	4·1	5·5	4·7
Paniceae								
Digitaria exilis	3·7	2·1	8·9	6·4	19·9	3·1	7·0	5·0
D. iburua	3·1	2·1	9·4	5·8	23·0	2·7	6·9	5·4
Pennisetum spp.	5·4	2·5	8·1	8·2	19·0	3·8	6·3	5·0
Echinochloa crus-galli	3·5	1·8	9·6	5·8	22·7	2·3	9·6	5·4
Andropogonoids								
Coix lacryma-jobi	3·9	2·1	9·0	6·0	22·2	2·8	10·8	4·3
Zea mays	4·3	2·8	7·7	6·4	19·4	3·8	9·2	5·1
Euchlaena mexicana	2·9	1·9	8·6	5·4	20·1	2·2	9·7	5·3
Sorghum spp.	3·2	2·2	9·7	6·7	22·0	3·1	8·4	4·3

* Expressed as percentages of total amino-acids (*mg/g total N* figures). Records for *Avena sativa* and *Secale cereale* based on "wholemeal", all other based on husked or hulled whole grain. Data re-calculated from Cresta and Odendaal [5].

Copies of the re-arranged *mg/g total N* figures from which these conclusions are derived are available from us on request. The taxonomic pattern they reveal (i.e. 1–7 above) is completely in accord with that described by Taira [3, 7] who had examined a bigger and better sample of grass species including non-cereals, but whose data were derived via the microbiological method and are mostly omitted from the FAO compilation. He too represented amino-acid proportions in terms of *amino-acids/total seed nitrogen*, but had found direct evidence [8] that the patterns result both from taxonomically-correlated quantitative variation in proportions of main protein fractions, and from taxonomically-correlated variations among prolamin fractions from different groups. Tables 2 and 3 give figures re-calculated by us from the FAO data, with means for individual amino-acids expressed as percentages of the mean-totals for each species. The lists of species are there reduced to the 15 for which a total amino-acid figure is available. Nevertheless, the same taxonomic trends are apparent, without need to resort to statistical tests; and it seems safe to conclude that there is indeed striking variation in the amino-acid profiles of grass seed proteins, which is perfectly intelligible in taxonomic terms.

Taxonomic analyses: dicotyledonous leaves

Of those "vegetables" on which the FAO booklet provides extensive amino-acid data, 74 (representing 70 genera and 34 families) are leaves from species of dicotyledons (Table 4). Taxonomists might ask whether amino-acid patterns correlate with orthodox groupings of genera into families, but with the exceptions of Compositae and legumes, the sample includes too few representatives per family for these to be usefully sought. On the other hand fashionable "phylogenetic" classifications of dicot families are so complex and conjectural as to make the prospect of using them to try and interpret these data seem both tedious and unpromising. Instead we have employed the phenetic classification of Young and Watson [9], derived via numerical analyses of extensive anatomical and morphological data. This recognizes a primary distinction between crassinucellate and tenuinucellate dicotyledons, coinciding largely with the division into Archichlamydeae and Sympetalae in 19th-century classifications; a distinc-

Table 4. Taxonomic arrangement (after Young and Watson [9]) of the dicotyledonous vegetables for which extensive leaf protein amino-acid data are given by Cresta and Odendaal [5]

Crassinucelli

(1) Caryophylloids
    *Aerva**, *Amaranthus**, *Basella**, *Boerhavia**, *Beta**, *Brassica** (cabbage), *Brassica* (Brussels sprouts), *Brassica* (cauliflower), *Brassica** (turnip), *Celosia**, *Chenopodium**, *Polygonum*, *Rorippa*, *Spinacia**, *Talinum*.*
(2) Legumes
    *Abrus**, *Albizzia**, *Alternanthera*, *Arachis**, *Cassia**, *Leucaena**, *Lupinus**, *Medicago**, *Onobrychis*, *Pisum*, *Sesbania*, *Tamarindus**, *Trifolium**, *Trigonella*, *Vicia*.
(3) Magnolioids
    Boscia, *Cadaba*, *Capparis**, *Crataeva**, *Cucurbita**, *Gynandropsis*, *Lagenaria*.*
(4) Celastroids
    *Afraegle**, *Lannea*.*
(5) Remaining Crassinucelli
    Adansonia, *Bombax**, *Ficus**, *Fleurya**, *Hibiscus**, *Malva*, *Manihot**, *Moringa*, *Morus*, *Piper**, *Tribulus*, *Triumfetta**, *Tropaeolum**, *Urera*.

Tenuinucelli

(1) Compositae
    *Bidens**, *Cichorium**, *Crassocephalum*, *Emilia**, *Inula*, *Lactuca*, *Vernonia*.
(2) Asclepioids
    Leptadenia, *Pavetta*.
(3) Acanthoids
    Barleria, *Ceratotheca*, *Origanum*, *Sesamum**, *Solenostemon**, *Thymus*, *Vitex*.*
(4) Remaining Tenuinucelli
    Capsicum, *Ipomoea**, *Solanum*, *Valerianella*.

* Asterisks indicate cases where availability of total amino-acid figures have enabled individual amino-acids to be re-calculated as percentages of these totals (see Table 6).

Table 5. Summarized results of analysing *mg/g total N* protein amino-acid figures for dicotyledonous leaves [5], in terms of the taxonomic classification set out in Table 4

	$(\bar{x}_1)$	$(\bar{x}_2)$	$\frac{(\bar{x}_1 - \bar{x}_2)}{\text{s.e.}}$
(A)	Mean Crassinucelli	Mean Tenuinucelli	
Lysine	322·2	253·7	3·9
Alanine	332·3	303·7	2·3
Aspartic acid	580·8	704·1	2·6
Serine	261·5	243·9	2·5
(B)	Mean caryophylloids	Mean other Crassinucelli	
Phenylalanine	277·2	335·0	3·3
Aspartic acid	502·8	610·1	3·4
Glutamic acid	732·3	636·3	3·6
(C)	Mean legumes	Mean other Crassinucelli	
Lysine	353·0	310·5	2·5
(D)	Mean acanthoids	Mean other Tenuinucelli	
Glycine	370·4	287·1	2·0

tion which is implicit (but often more or less obscured) in purported phylogenetic systems, in which tenuinucellate families are mainly regarded as more or less "advanced". Within Crassinucelli the caryophylloid families, the legumes and the magnolioids are recognized as clear-cut groupings, while in Tenuinucelli an asclepioid and an acanthoid series are distinguished, with Compositae regarded as an isolated family.

The genera having been arranged accordingly (Table 4), we first examined the *mg/g total N* dicot leaf amino-acid figures for all 18 amino-acids. From this exercise it emerged that most amino-acids (isoleucine, methionine, cystine, phenylalanine, threonine, tryptophan, arginine, glutamic acid, proline) have closely similar means for Crassinucelli and Tenuinucelli. Four (leucine, tyrosine, valine, histidine) exhibit marked differences in means, but these are not statistically significant. However (see Table 5) the mean for the essential amino-acid lysine is significantly higher for crassinucellate leaves; alanine and serine also average significantly higher in Crassinucelli than in Tenuinucelli; and aspartic acid is significantly high in Tenuinucelli.

Salient taxonomic groupings within the major series also show amino-acid peculiarities (Table 5). Caryophylloids when contrasted with other crassinucelli have on average low levels of phenylalanine and aspartic acid (and a low *total essential amino-acid/total food* content), but high levels of glutamic acid. Legumes average higher proportions of lysine than do other crassinucelli, while their isoleucine level tends to be high and their cystine low. Acanthoids tend to be richer than other Tenuinucelli in glycine; and threonine seems high in Compositae, although the data here are too scanty to reach statistical significance.

This information is evidently of taxonomic interest, whatever the biochemical explanation. However it would be dangerous to assume, by possibly false analogy with grass seeds, that patterns discernible when amino-acid values are set against total nitrogen necessarily reflect patternized variations in leaf protein amino-acids. Again therefore, we have recalculated the *mg/g total N* figures for each amino-acid, to express them as percentages of the amino-acid totals. The size of the available sample of species is now drastically reduced to those asterisked in Table 4, even after resorting to an approximation to cover shortage of data on tryptophan. It is therefore not surprising that some taxonomic trends apparent in the more extensive *mg/g total N* figures disappear; some of them may in any case have been reflecting variations from group to group not in proteins, but in N-containing compounds such as non-protein amino-acids and alkaloids. Some of the differences nevertheless persist at statistically significant levels (Table 6), even in the much curtailed re-calculated figures. The difference between Crassinucelli and Tenuinucelli in average lysine content seemingly does reflect variation in leaf protein amino-acids, as do differences in aspartic acid and glutamic acid values distinguishing caryophylloids from legumes, from other Crassinucelli and from Tenuinucelli.

DISCUSSION

The framework of grass classification summarized in Table 1 and on which Tables 2 and 3 are based, while founded in comparative morphology and anatomy, has wide ranging usefulness. It has predictive capacity in connection with (for example) day-length requirements for flowering, the chemical constitution of carbohydrate reserves [10], details of photosynthetic pathways [11], allergenic properties of the pollen [12] and susceptibilities to fungal [13] and viral [14] parasites. The taxonomic patternization of amino-acids in grass seeds, revealed by Taira [3] and abundantly confirmed here, need therefore occasion no surprise. The sequence of the large groups given in Tables 1–3 (i.e. with the festucoids furthest removed from the andropogonoids, and with the chloridoids and panicoids occupying intermediate positions nearest the festucoids and the andropogonoids respectively) directly reflects phenetic relationships (e.g. Clifford *et al.* [15]) but conforms in essence with most modern taxonomic treatments. The sequence is itself no less meaningful than are the constitutions of the individual groups, and the sequential changes in leucine (low in festucoids, highest in andropogonoids, intermediate in chloridoids and panicoids; Table 2) and alanine (Table 3) contents also conform exactly with the well-established taxonomic pattern.

While grass seed amino-acid figures help to confirm the general validity of taxonomic views of these plants, they may have greater significance for plant breeders. Modern discoveries in genetics will lead to the introduction of powerful new crop-improvement techniques. It seems likely that natural sterility barriers will be circumvented with increasing ease, and that gene-transfer may become procurable across wide taxonomic gaps. Nevertheless it may always be easier to transfer genes among forms that are taxonomically more closely related, so that genetic interchange between (say) maize at one extreme of the grass family, and wheat at the other, might involve stepwise transfer via intermediate forms (c.f. Jones and Sneath [16]). Information of the kind found in Tables 2 and 3 may become relevant here, suggesting the most appropriate immediate sources of genes for (say) increased lysine in *Sorghum*, or increased leucine in wheat; or at appropriate stepwise sequences for gene transfer across the family. In this context as elsewhere (e.g. cereal pathology [14]) chloridoid grasses, arundinoid-danthonioids and Stipeae assume special significance, since they occupy key positions in the middle of the system. These appear to merit thorough study, therefore, despite the fact that few of them are grown as cereals. Further work on the variations in individual seed proteins which amino-acid patterns reflect should also be undertaken, paying due attention to genera representing "intermediate" grass groups.

Gerloff *et al.* [17] demonstrated variation in lysine content among purified leaf proteins from eight flowering plant species, and Byers [18] has

Table 6. Summarized results of analysing taxonomically the amino-acid contents of dicotyledonous leaves, expressed as percentages of total amino-acid content*

	$(\bar{x}_1)$	$(\bar{x}_2)$	$\frac{(\bar{x}_1 - \bar{x}_2)}{\text{s.e.}}$
	Mean Crassinucelli	Mean Tenuinucelli	
Lysine	6·0	5·0	3·3
Aspartic acid	10·9	13·9	1·8
	Mean caryophylloids	Mean other Crassinucelli	
Aspartic acid	9·9	11·4	2·1
Glutamic acid	14·9	11·5	3·2
	Mean caryophylloids	Mean Tenuinucelli	
Aspartic acid	9·9	13·9	2·5
Serine	5·0	4·6	1·9

* Re-calculated from *mg/g total N* figures given by Cresta and Odendaal [5] for the species asterisked in Table 4. Where no figure for tryptophan was available, calculations are based on a "total amino-acid content" which includes the mean value for tryptophan (87·1 *mg/g total N*), derived from those leaves for which this amino-acid has been quantified.

found protein differences between barley, lupin and Chinese cabbage leaves in aspartic acid, alanine and methionine, indicating that these amino-acids "might be species-dependent". However, it has generally been supposed [19, 20] that the amino-acid composition of unfractionated leaf protein of all leaves is of the same nutritional quality. The seeming distinctions which have now emerged between crassinucellate and tenuinucellate dicotyledons and among subordinate series of genera (Tables 4–6) derive from unsatisfactory data. Nevertheless although the differences are not absolute, depending on analyses of wide samples of species for their detection, some of them are quite clear and reach high levels of statistical significance. The facts are not irreconcilable with proposed phylogenies (e.g. Cronquist's [21]) and they support suggestions [9] that Crassinucelli and Tenuinucelli can usefully be recognized as discrete groups of dicotyledons and that Cruciferae might be related to the caryophylloids.

It seems very probable that these amino-acid patterns in dicotyledonous leaves represent taxonomically orderly variation in leaf protein complements, although free protein amino-acids may be involved and there remains the remote possibility that they may reflect varying extractabilities of the same proteins from different leaves (a phenomenon which, if demonstrated, would itself be of interest). In any event it seems desirable that amino-acid surveys of leaves should be undertaken with the express purpose of investigating the meaning of these taxonomic correlations, and hopefully of discovering others. There is a need for balanced taxonomic sampling; currently it is not possible to make effective comparisons between leaves of monocotyledons and dicotyledons, nor to seek variation among monocotyledonous families, and there is no comparable information on bryophytes, ferns, horsetails, cycads or conifers. Such deliberately orientated surveys would call for standardized protein-extraction and analytical procedures, and some effort to standardize on the physiological state of leaves sampled. If it is true that amino-acid patterns of leaves directly reflect variation in their proteins, it will be important to discover which proteins are involved, and to investigate the nature of the variation. For example, is taxonomic variation in the lysine content of dicot leaves mainly attributable to the abundant RuDP carboxylase (see Kawashima and Wildman [22]) or to other proteins, quantitatively minor but very rich in lysine? The answers to such questions could be of both fundamental, physiological and nutritional interest.

Acknowledgement—We thank Professor D. J. Carr for criticism and helpful comments.

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