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TAXONOMIC VARIATION IN TOTAL LEAF PROTEIN AMINO ACID COMPOSITIONS OF GRASSES

HOCK-HIN YEOH and LESLIE WATSON

Taxonomy Unit, Research School of Biological Sciences, The Australian National University, P.O. Box 475, Canberra City, A.C.T. 2601, Australia

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Abstract—Leaves of greenhouse grown grasses had free protein amino acid contents of generally less than 5 % total amino acids, while field collected grasses averaged 14.7 % free protein amino acid contents. Taxonomic patterns are detectable in the total leaf amino acid profiles of grasses from both sources, those of pooids being distinguishable from those of chloridoids and panicoids, and those of danthonioids showing an intermediate pattern. Leaf profiles of *Oryza*, Stipeae, and Ehrharteae resemble one another, and are more like those of pooids than those of panicoids. Variations in Thr and Leu are apparently associated with differences in photosynthetic pathway. Grass leaves are generally low in total amino acid contents (2.2 ± 1.0 g % fr. wt), with Ile, Val and Met + Cys identified as the limiting essential amino acids. However, the nutritional 'chemical scores' of grass leaf proteins are high (> 75 %, based on the WHO scoring pattern).

INTRODUCTION

Analyses of compiled data on amino acid compositions of leaves of dicotyledonous flowering plants [1] have revealed systematic variations, these being consistent with classifications of the families into high level groupings (e.g. Crassinucelli/Tenuinucelli; caryophylloids/legumes/acanthoids [2]; and differences exist between barley, lupin and chinese cabbage in terms of both unfractionated and fractionated (i.e. chloroplastic and cytoplasmic) leaf proteins [3]. However, Byers' detailed work [3] covered only three species, while the systematic conclusions of Watson and Creaser [1] were necessarily equivocal, because the available data involved an alarming diversity of analytical procedures and represented a poor sample from the standpoint of taxonomic analysis. For the same reasons, published data on amino acid compositions of grass leaves [4–6] are not amenable to taxonomic analysis.

Since initial exploratory analyses of a small but taxonomically diverse sample of grass leaves showed some differences in protein amino acid compositions, it seemed worthwhile to conduct a more extensive systematic survey of the family in tandem with other studies [7–9]. This article presents the results of protein amino acid analyses of leaves from 41 grass species grown in the greenhouse and of 47 species collected from the field, representing most major grass groupings and tribes. Leaf protein contents have been estimated, some assessment is made of the leaf free protein amino acid components and total leaf amino acid profiles are compared. Comparisons are also made with caryopsis and RuBP-carboxylase analyses, and nutritional 'chemical scores' are given.

RESULTS AND DISCUSSION

Greenhouse grown material

Overall leaf protein amino acid compositions represent not only enzymes and structural proteins, but also free protein amino acids. Considering greenhouse grown grass material, however, the free protein amino acids apparently have little influence on the total leaf amino acid profiles. In a sample of 36 species (Table 1), they constitute only 0.9–12.3 % of the total leaf protein amino acids; the mean value is less than 5 %, and in only four cases does it exceed 7 %. The total leaf amino acid profiles for greenhouse grown material given in Table 2 are apparently dominated by leaf proteins.

Results of total leaf protein amino acid analyses of 41 grass species (30 genera) grown in the greenhouse are set out in Table 2, where they are arranged under major groupings (≃ subfamilies) and tribes according to current information on taxonomic relationships [10, 11]. The close similarity of all grass leaf blades in amino acid compositions is apparent in Table 2, by contrast with corresponding information on caryopsis proteins [7], which are much more variable in this respect. Nevertheless, there is evidence here of taxonomically orientated variation in the leaf amino acid compositions. Group by group comparisons among pooids, chloridoids and panicoids (Table 2, summarized in Table 3) show that a pooid pattern is distinguishable from a panicoid one by its significantly higher (at the 5 % probability level) Thr, Val and Lys and significantly lower Ser, Ala and Leu; and from a chloridoid one by its significantly higher Thr, Val and Lys and lower Ala. The amino acid profiles of the chloridoids and panicoids show no differences significant

Table 1. Free protein amino acid contents of grass leaves

Species	% Free protein amino acids in total amino acid content	Species	% Free protein amino acids in total amino acid content
POOIDS		BAMBUSOIDS, etc.	
Triticeae		Oryzoid	
Hordeum vulgare	5.4	*Oryza sativa*	1.8
Secale cereale	4.9	DANTHONIOIDS	
Bromeae		*Monachather paradoxa*	8.9
Bromus molliformis	7.0	*Danthonia pallida*	5.2
Bromus unioloides	1.5	*Triraphis mollis*†	2.1
Agrostideae		CHLORIDOIDS	
Agrostis avenacea	2.9	*Chloris gayana*†	3.0
*Agrostis tenuis**	15.4	*Eragrostis benthamii*†	4.5
Ammophila arenaria	6.3	*Eragrostis curvula**†	8.8
*Anthoxanthum odoratum**	15.6	*Sporobolus virginicus*†	0.9
*Deyeuxia quadriseta**	28.2	PANICOIDS *sensu lato*	
Holcus lanatus	4.4	Eu-panicoids	
Phalaris amethystina	2.8	*Digitaria sanguinalis*†	3.3
Phalaris arundinacea	5.6	*Echinochloa crus-galli*†	2.5
Phalaris californica	7.0	*Entolasia marginata**	9.1
Phalaris tuberosa	4.4	*Isachne globosa**	8.8
*Polypogon monspeliensis**	28.6	*Oplismenus aemulus**	5.9
Aveneae		*Panicum antidotale*†	1.7
Amphibromus neesii	3.6	*Panicum milioides*	7.9
Avena sativa	2.4	*Paspalum dilatatum*†	3.0
Meliceae		*Paspalum paspalodes*†	4.6
Glyceria declinata	2.9	*Pennisetum typhoides*†	12.3
Poeae		*Setaria glauca*†	3.3
Briza maxima	5.5	*Spinifex hirsutus*†	7.2
*Cynosurus echinatus**	26.9	Andropogonoids	
Lolium perenne	4.3	*Hemarthria uncinata**†	6.4
Festuca arundinacea	5.0	*Sorghum bicolor*†	1.2
Poa helmsii	7.0	*Themeda australis**†	7.7
		Zea mays†	1.3

* Field collected material.
† C_4 species.

at the 5% probability level except for Ala, which is significantly higher in the chloridoids. Viewed against the data as a whole, and in terms of the amino acid levels which seem to be distinguishing samples from the main grass groups, the two danthonioids (*Monachather paradoxa* and *Triraphis mollis*) are intermediate, having the higher Ala and Leu of the panicoids but the higher Lys of pooids.

Among the pooids, *Bromus* spp., Agrostideae, Aveneae, *Glyceria* and Poeae have yielded very similar amino acid compositions. However, the two Triticeae (i.e. *Hordeum vulgare* and *Secale cereale*) share with *Bromus molliformis* the lowest Phe values for the entire sample (cf. MacFarlane's grouping together of Bromeae and Triticeae in his supertribe Triticanae [10]).

Oryza and *Stipa*, whose possible taxonomic relationships with one another and with the bamboos are indicated by certain peculiarities of spikelet morphology and leaf anatomy [11], are represented in Table 2 by species whose leaf amino acid compositions closely resemble each other, although the *Oryza* is higher in Ala and lower in Val. Comparing them with the patterns of the main grass assemblages, they come closest to the pooids by virtue of low Ser and Leu values.

Material collected from the field

In leaves of 11 grass species collected from the field Table 1, asterisked), the free protein amino acids vary from 5.9 to 28.6%; i.e. there is much more variation in field collected than in greenhouse grown grasses, and the mean of 14.7% is significantly higher. The values are noticeably higher in the subfamily Pooideae (15.4–28.6%) than in the chloridoid and the five panicoids (5.9–9.1%). It would appear that although the total protein amino acid profiles of field collected leaves will still largely be dominated by the protein contribution, some (especially among the pooids) may be detectably influenced by the free protein amino acid component. The latter might significantly affect some total leaf amino acid compositions, and might also be expected to introduce more variability.

The total leaf protein amino acid analyses of the field collected leaves (47 species/39 genera, Table 4) are compared directly with those representing greenhouse grown plants (Table 2) in Table 6. This shows that field collected pooids (which include greenhouse and field collected representatives of the same species; cf. Tables 2 and 4, asterisked) average significantly higher levels of Ser and Ala, and significantly lower levels of Gly, Val, Leu and

Lys. The field collected panicoids, however, differ significantly from greenhouse grown panicoids only in their higher Phe, while a comparison of field collected and greenhouse grown chloridoid leaves shows no significant differences at all in terms of these small samples. While it is likely that different environmental conditions may affect protein levels and proportions, these differences may well be reflecting, at least in part, the variation in free protein amino acids [12].

With this in mind, it is perhaps surprising to find that some aspects of the taxonomic patterns found in leaves of greenhouse grown grass species are still detectable in field collected plants. Thus, the pooids (see summary of group means from Table 4, given in Table 5) are still distinguished from the panicoids by higher Thr and Glx, and lower Leu and Tyr, and from the chloridoids by higher Thr and Phe and lower Leu. The taxonomic group differences found in greenhouse grown plants involving Ala, Val and Lys are lost, owing to a relative increase in Ala level and a lowering of Val and Lys levels in the field grown pooid material (see Table 6). Regarding the smaller groups, the danthonioids sampled from the field are intermediate between the pooids and panicoids in terms of their Glx and Tyr levels; and there is no basis here for commenting on Stipeae because of the small sample size. However, the Ser levels of *Microlaena* and *Ehrharta* (i.e. Ehrharteae; see Table 4) are the highest in the sample as a whole.

Total leaf protein amino acid compositions and photosynthetic pathways

It is pertinent to ask whether the taxonomic differences are a direct reflection of differences in photosynthetic pathway (cf. ref. [13]), given that the C_3 and C_4 pathways themselves show taxonomically correlated distributions. The figures in Table 2 in fact show significant correlations between levels of Thr, Ala, Val, Leu and Lys and photosynthetic pathways. However, they are misleading, in that Table 2 includes only four non-pooid C_3 forms; furthermore, it is noticeable that in the one case here where the sample includes plants which are taxonomically closely related but differ in photosynthetic pathway, the species concerned (i.e. *Monachather paradoxa*, C_3; *Triraphis mollis*, C_4) have closely similar amino acid profiles. Table 4, which includes 13 non-pooid C_3 forms (including C_3 eu-panicoids) is more informative in this context; and the figures there show that of the taxonomically distinguishing amino acids (i.e. Thr, Glx, Leu, Tyr and Phe), only Thr and Leu show significant correlation with photosynthetic pathway. Furthermore, the relatively high levels of free Ala characteristic of C_4 grass leaves collected from the field [12] are masked in the total protein amino acid patterns. It seems fair to conclude that while biochemical differences consequent upon possession of different photosynthetic pathways may be contributing to the taxonomic patterns, other factors must also be involved.

Comparisons of amino acid compositions of total leaf proteins, caryopsis proteins and grass RuBP carboxylase

The total leaf protein amino acid compositions differ consistently from those of caryopses, having higher Asx, Thr, Gly, Lys and (except for andropogonoids) Ala and lower Glx [7]. Nor do the leaf patterns show any resemblance to those of grass RuBP-carboxylase, the former being consistently higher in Asx, Thr, Glx Pro and Ala and lower in Met, Tyr, Phe, Trp and Arg [12]. This is not unexpected, because RuBP-carboxylase, although constituting 25% to 59% of the soluble proteins in C_3 plants and 8–23% in C_4 plants [14], in fact represents no more than 10% of the total leaf proteins.

Total leaf protein amino acid compositions and 'chemical scores'

Growing grasses constitute an important food resource for domestic and wild animals. Moreover, grasses and by-products of 'grass' crops (e.g. rice straws and sugar cane tops) have actual and potential value as animal feed and in production of unconventional protein food (e.g. leaf protein concentrate and hydrolysed leaf protein) for animals and humans [15–19]. However, in circumstances where the priority of land utilization is for food crops and where fertiliser is expensive, livestock will have to depend on natural resources [20]; and one notes in this context that since the major grass groupings have different world distributions and different ecological preferences (e.g. temperate pooids; tropical–subtropical andropogonoids; [21–24]), taxonomic differences in grass leaf amino acid profiles may lead to differences between grasslands and pastures. It therefore seems worthwhile briefly examining the data in this context, given that the taxonomic patterns demonstrated in grass leaves involve some of the essential amino acids (i.e. Val, Leu, Tyr, Phe and Lys).

The total leaf amino acid contents for 88 grass species grown in the greenhouse or collected from the field range from 0.6 to 5.9 g% fr. wt (Tables 2 and 4), with an average of 2.2 ± 1.0 g% fr. wt. The values reported here predominantly reflect the protein contents of the leaves, and are similar in range to those given elsewhere for crude leaf protein contents of grasses and other higher plants. Out of 126 species of higher plants reported upon elsewhere, 63% have a leaf protein content of less than 3 g% fr. wt, 30% have protein contents ranging from 4 to 5 g%, and only 7% have protein contents greater than 6 g% (compiled from ref. [25]). Thus, grasses certainly fall among the species with low protein content, and indeed pasture and fodder grasses have previously been regarded as deficient in this respect [26, 27].

Amino acid requirements of humans and livestock are quantitatively different [28–30]. In particular, ruminants show less dependence on the biological value (i.e. essential amino acid content) of dietary proteins than do non-ruminants, because bacteria present in the rumen can convert protein and non-protein nitrogen into available proteins [29, 30]. Nevertheless, the 10 amino acid essential to humans are also needed by other mammals, so it seems justifiable to assume that leaf protein 'chemical scores' calculated in the context of human nitrution may also have some relevance for them.

Data from Tables 2 and 4 were pooled for calculation of nutritional 'chemical scores', the field collected representatives of duplicated species being omitted. Table 7 presents the 'chemical scores' obtained by two different procedures, (a) based on comparisons of essential amino acids with those of the WHO [28] reference for 'ideal protein' and (b) based on comparisons of A/T (for leaf essential amino acids): A/T (for egg protein reference [31]). Only three amino acids are found to be limiting by either standard, i.e. Ile, Val and Met + Cys; and even these are present at relatively high levels in grass leaves (minimum 'chemical score' 75% by WHO; 57% by egg

Table 2. Total leaf amino acid compositions of grasses grown in the greenhouse

Species	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg	Total amino acid (protein content) g % fr. wt.
	Amino acid compositions (g % total amino acid)																		
POOIDS																			
Triticeae																			
Hordeum vulgare	13.5	5.5	5.2	14.3	5.3	5.5	6.9	2.0	4.8	1.7	3.2	8.7	4.0	5.5	2.0	7.1	0.5	4.2	4.1
Secale cereale	11.8	5.5	5.2	14.0	5.4	6.1	7.1	2.3	4.8	2.1	3.3	9.2	4.5	5.7	2.1	6.8	0.7	3.3	5.9
Secale cereale	10.0	5.6	5.1	15.4	5.6	5.8	7.2	1.6	5.2	2.4	3.5	8.1	4.6	5.7	2.2	6.6	0.6	4.8	
Bromeae																			
Bromus arenarius	11.1	5.4	5.4	14.2	5.8	5.5	7.0	0.8	5.1	2.5	3.6	9.0	4.7	5.9	2.3	6.6	0.4	4.8	2.8
Bromus molliformis	12.3	5.4	5.4	14.6	5.5	5.7	6.9	1.9	5.4	1.8	3.8	9.3	4.3	5.0	2.2	6.5	0.0	3.3	1.7
Bromus unioloides	12.7	5.5	6.3	12.9	6.0	6.0	7.8	1.0	4.4	1.8	3.4	9.2	4.8	5.5	2.0	7.0	0.2	3.5	2.7
Bromus unioloides	12.7	5.7	5.8	13.4	5.7	6.4	7.9	1.0	4.3	1.9	3.0	8.9	4.6	5.8	1.9	6.5	0.8	3.7	
Bromus unioloides	11.0	5.3	5.1	13.1	5.9	5.6	7.0	1.1	5.3	2.4	3.5	9.2	4.7	6.0	2.4	6.8	0.8	4.7	
Agrostideae																			
*Agrostis avenacea**	10.8	5.4	5.4	15.8	5.3	5.5	7.6	1.6	5.4	2.1	3.6	8.9	4.3	5.6	2.2	6.7	0.0	3.7	3.4
*Ammophila arenaria**	12.3	5.8	5.8	13.6	5.8	5.7	7.2	2.3	5.1	2.0	3.4	9.2	4.5	5.9	2.1	6.5	0.0	2.6	1.9
*Holcus lanatus**	12.3	5.6	5.4	14.4	5.3	5.6	7.0	1.8	5.7	1.9	3.9	9.3	4.0	6.1	2.1	6.7	0.0	3.1	1.8
Lagurus ovatus	11.3	5.3	5.3	14.3	5.8	5.5	6.9	0.8	5.1	2.4	3.5	8.9	4.2	5.8	2.3	6.7	0.7	4.1	1.1
Phalaris amethystina	12.2	5.4	5.8	15.0	5.4	6.0	7.9	1.2	4.3	1.8	3.1	8.7	4.4	5.7	1.9	6.6	0.7	4.1	2.5
Phalaris arundinacea	12.0	5.6	5.8	12.8	6.1	6.0	7.7	0.6	4.4	2.1	3.3	9.2	4.6	5.8	2.2	7.6	0.0	4.2	0.9
Phalaris californica	11.4	5.5	5.9	12.9	6.0	6.0	7.8	1.4	4.6	1.5	3.5	9.2	4.7	5.7	2.2	7.7	0.0	4.1	1.0
Phalaris aquatica	12.1	5.7	5.8	13.6	6.0	5.8	7.9	0.6	4.5	2.1	3.4	9.2	4.5	5.8	2.1	7.3	0.0	3.7	0.9
Aveneae																			
*Amphibromus neesii**	11.6	5.3	5.6	14.2	5.4	5.4	6.9	2.1	4.8	1.8	3.6	9.2	4.4	5.8	1.8	6.6	1.0	4.5	2.8
*Amphibromus neesii**	11.0	5.4	5.1	13.4	6.1	5.6	7.1	0.9	5.3	2.2	3.6	9.4	4.6	6.1	2.3	6.9	0.2	4.8	
Avena sativa	12.5	5.9	5.4	12.8	8.1	5.7	7.9	1.0	4.5	1.7	3.2	8.6	4.4	5.7	1.9	6.8	0.5	3.4	2.9
Meliceae																			
*Glyceria declinata**	12.3	5.4	6.3	14.1	5.7	6.0	7.4	1.4	4.6	2.1	3.4	9.5	4.7	5.9	2.1	6.4	0.2	3.2	2.4
Poeae																			
Briza maxima	11.4	5.5	5.5	14.0	5.6	5.8	7.4	1.5	4.7	1.2	3.5	9.6	4.7	6.1	2.2	7.2	0.0	4.0	0.9
Lolium perenne	11.9	5.6	5.7	12.7	6.0	6.1	7.7	0.8	4.7	2.0	3.5	9.4	4.6	6.0	2.1	7.0	0.1	3.9	1.4
Lolium perenne	11.2	5.3	5.1	13.0	5.9	5.7	7.1	0.8	5.3	2.2	3.6	9.3	4.6	6.2	2.4	6.7	0.8	4.7	
*Festuca arundinacea**	11.8	5.4	5.2	14.5	5.3	5.5	6.8	1.7	5.7	2.1	3.8	9.1	4.4	6.1	2.2	6.9	0.0	3.5	2.0
*Poa helmsii**	11.2	5.7	5.5	15.9	5.2	5.6	7.4	1.6	5.5	1.6	3.6	9.0	4.3	5.9	2.2	6.6	0.0	3.4	2.0
Poa pratensis	11.2	5.3	5.2	14.4	5.9	5.5	7.0	0.8	5.1	2.5	3.5	9.0	4.6	6.0	2.2	6.6	0.4	4.7	2.7

Table 2—*continued*

Species	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg	Total amino acid (protein content) g % fr. wt.
	Amino acid compositions (g % total amino acid)																		
BAMBUSOIDS, etc.																			
Oryzoids																			
Oryza sativa	12.6	5.6	5.5	14.0	5.6	6.4	8.3	1.2	4.0	2.1	2.8	8.8	4.8	5.4	2.1	6.9	0.4	3.5	3.8
Oryza sativa	12.7	5.5	6.1	13.1	6.0	6.1	7.8	1.1	4.1	1.6	3.2	9.1	4.9	5.5	2.0	6.8	0.1	4.2	
Oryza sativa	10.7	5.2	4.9	12.7	5.9	5.7	7.3	1.9	5.2	2.3	3.6	9.3	4.9	5.8	2.4	6.7	0.2	5.0	
STIPEAE																			
Stipa mollis	10.8	5.4	5.6	13.8	6.0	5.5	7.0	1.7	5.2	2.4	3.5	9.0	4.7	5.7	2.3	6.5	0.3	4.8	3.2
Stipa nitida	12.4	5.6	5.9	12.7	5.9	5.9	7.6	0.7	4.4	2.2	3.1	9.1	4.8	5.9	1.7	6.7	0.1	5.4	2.2
Stipa nitida	15.2	5.2	5.3	14.4	5.2	5.3	6.9	1.6	5.1	2.2	3.5	8.6	4.2	5.4	2.1	6.2	0.0	3.5	
DANTHONIOIDS																			
Monachather paradoxa	12.5	5.7	6.3	12.6	6.2	6.1	7.9	0.6	4.5	2.1	3.3	9.3	4.6	5.7	2.1	7.1	0.0	3.4	0.9
Triraphis mollis†	11.8	5.3	5.8	13.2	5.9	5.7	7.8	1.6	4.8	2.1	3.5	9.6	4.5	5.5	2.2	6.9	0.0	3.8	2.8
CHLORIDOIDS																			
Cynodon dactylon†	12.9	5.3	5.6	14.1	5.9	5.4	7.8	1.0	4.6	1.7	3.6	9.3	4.5	5.6	2.0	6.8	0.1	3.9	4.1
Eleusine coracana†	11.2	5.1	5.3	14.3	5.8	5.4	8.9	0.7	5.0	2.4	3.6	9.0	4.4	5.3	2.1	6.4	0.5	4.6	2.1
Eragrostis curvula†	11.9	5.4	6.3	13.5	6.0	5.9	8.6	0.8	4.6	1.8	3.5	9.7	4.7	5.8	1.6	6.4	0.0	3.7	2.6
Eragrostis dielsii†	12.3	5.3	6.2	13.1	5.8	5.7	8.4	0.9	4.4	2.1	3.3	9.3	4.7	5.6	1.9	6.7	0.1	4.3	1.9
PANICOIDS *sensu lato*																			
Eu-panicoids																			
Digitaria sanguinalis†	12.3	5.4	6.3	13.7	6.2	6.0	7.7	0.8	4.4	1.9	3.6	9.8	4.5	5.7	1.9	6.5	0.0	3.4	0.6
Echinochloa crus-galli†	11.8	5.2	6.0	14.5	5.9	5.7	7.8	0.8	4.7	1.9	3.5	9.2	4.4	5.6	2.0	6.7	0.3	4.2	1.2
Panicum antidotale†	14.0	4.9	5.7	13.2	6.0	5.4	8.1	1.0	4.5	2.1	3.3	9.0	4.4	5.4	2.0	6.4	0.7	4.2	2.3
Panicum milioides	11.9	5.5	5.8	12.8	5.6	5.7	6.9	2.9	5.1	1.9	3.6	9.3	5.0	5.8	2.3	6.5	0.0	3.3	1.4
Paspalum dilatatum†	12.3	5.6	6.3	13.4	6.2	6.0	7.7	1.0	4.5	1.7	3.5	9.8	4.8	5.8	2.0	6.0	0.0	3.3	1.0
Pennisetum typhoides†	11.0	4.8	5.8	14.1	5.5	5.4	7.9	1.9	4.8	2.3	3.7	9.3	4.4	5.5	2.2	6.3	1.0	4.2	1.3
Setaria geniculata†	11.8	5.0	5.8	13.4	7.0	5.6	8.5	1.9	4.2	2.0	3.2	9.2	4.4	5.4	2.3	6.9	0.0	3.6	1.6
Setaria glauca†	12.6	5.2	6.8	13.9	6.1	5.9	7.6	0.8	4.4	1.8	3.4	9.5	4.5	5.8	2.1	6.9	0.0	2.7	0.6
Setaria verticillata†	11.2	5.2	5.5	12.8	6.1	5.5	7.3	1.0	5.2	2.3	3.8	9.9	4.8	6.0	2.0	6.5	0.3	4.5	1.5
Andropogonoids																			
Sorghum bicolor†	11.6	5.5	5.7	13.7	5.9	5.6	7.9	0.8	4.8	2.0	3.5	9.6	4.7	5.7	2.0	6.5	0.3	4.3	2.5
Zea mays†	12.4	5.2	5.9	14.2	5.6	5.6	8.8	0.9	4.6	2.0	3.4	9.2	4.3	5.4	1.9	6.3	0.5	4.0	2.4

* Cf. analysis of field collected material, Table 4.
† C_4 species.

Table 3. Total leaf amino acid compositions of grasses grown in the greenhouse (taxonomic group means of data in Table 2)

Major groups/tribes	Amino acid composition (g % total amino acids)																	
(no. spp./no. genera)	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg
POOIDS (21/15)	11.8	5.5	5.5	14.1	5.8	5.7	7.3	1.4	5.0	2.0	3.5	9.1	4.7	5.8	2.2	6.8	0.3	3.9
Triticeae (2/2)	12.2	5.6	5.2	14.5	5.4	5.8	7.1	2.0	4.9	2.0	3.3	8.7	4.3	5.4	2.1	6.9	0.6	4.2
Bromeae (3/1)	11.8	5.4	5.5	14.0	5.7	5.7	7.2	1.2	5.1	2.1	3.6	9.1	4.6	5.6	2.2	6.6	0.3	4.0
Agrostideae (8/5)	11.8	5.5	5.7	14.1	5.7	5.8	7.5	1.3	4.9	2.0	3.5	9.1	4.4	5.8	2.1	7.0	0.2	3.8
Aveneae (2/2)	11.9	5.7	5.4	13.3	7.0	5.6	7.5	1.3	4.8	1.9	3.4	9.0	4.5	5.9	2.0	6.8	0.6	4.1
Meliceae (1/1)	12.3	5.4	6.3	14.1	5.7	6.0	7.4	1.4	4.6	2.1	3.4	9.5	4.7	5.9	2.1	6.4	0.2	3.2
Poeae (5/4)	11.4	5.5	5.4	14.3	5.6	5.7	7.2	1.3	5.2	1.9	3.6	9.2	4.5	6.0	2.2	6.8	0.2	4.0
BAMBUSOIDS, etc.																		
Oryzoids (1/1)	12.0	5.4	5.5	13.3	5.8	6.1	7.8	1.4	4.4	2.0	3.2	9.1	4.9	5.6	2.2	6.8	0.2	4.2
STIPEAE (2/1)	12.3	5.4	5.6	13.7	5.8	5.6	7.2	1.5	5.0	2.3	3.4	9.0	4.6	5.7	2.1	6.5	0.2	4.7
DANTHONIOIDS (2/2)	12.2	5.5	6.1	12.9	6.1	5.9	7.9	1.1	4.7	2.1	3.4	9.5	4.6	5.6	2.2	7.0	0.0	3.6
CHLORIDOIDS (4/3)	12.1	5.3	5.9	13.8	5.9	5.6	8.4	0.9	4.7	2.0	3.5	9.4	4.6	5.6	1.9	6.6	0.2	4.1
PANICOIDS *sensu lato* (11/8)	12.1	5.2	6.0	13.6	6.0	5.7	7.8	1.3	4.7	2.0	3.5	9.4	4.6	5.7	2.1	6.5	0.3	3.8
Eu-panicoids (9/6)	12.1	5.2	6.0	13.5	6.1	5.7	7.7	1.3	4.6	2.0	3.5	9.4	4.6	5.7	2.1	6.5	0.3	3.7
Andropogonoids (2/2)	12.0	5.4	5.8	14.0	5.8	5.6	8.4	0.9	4.7	2.0	3.5	9.4	4.5	5.6	2.0	6.4	0.4	4.2

Table 4. Total leaf amino acid compositions of grasses collected from the field

Species	Amino acid composition (g % total amino acid) Asx	Thr	Ser	Glx	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg	Total amino acid (protein content) (g % fr. wt.)
POOIDS																			
Agrostideae																			
*Agrostis avenacea**	11.8	5.7	5.8	14.5	7.0	5.7	7.9	0.9	4.5	2.0	3.2	8.9	4.5	6.0	1.9	6.4	0.1	3.3	4.2
Agrostis tenuis	14.8	5.5	6.0	13.2	6.2	5.6	7.7	0.9	4.3	1.7	3.0	8.5	4.3	6.0	1.8	6.4	1.1	3.1	3.7
*Ammophila arenaria**	11.8	5.8	5.7	14.2	6.3	5.6	7.5	0.9	4.7	2.1	3.4	9.0	4.8	5.9	2.0	7.0	0.0	3.4	1.2
Anthoxanthum odoratum	11.0	5.5	5.3	14.4	5.9	5.4	7.2	0.8	5.3	2.2	3.5	8.9	4.5	5.7	2.4	6.7	0.5	4.8	2.3
Deyeuxia quadriseta	11.1	5.5	5.8	14.6	5.7	4.0	8.3	1.8	5.1	1.4	3.8	9.6	4.5	6.0	1.8	6.6	0.0	4.3	1.7
Hierochloe redolens	13.1	5.6	6.1	14.3	5.4	5.5	7.8	1.2	4.8	1.9	3.4	8.5	4.5	5.8	2.2	6.7	0.2	3.1	2.5
*Holcus lanatus**	12.0	5.6	6.0	13.8	5.6	5.2	8.1	1.0	5.0	1.8	3.7	9.4	4.5	6.1	1.8	6.3	0.4	3.8	1.2
Polypogon monspeliensis	14.1	5.6	6.9	14.3	7.8	5.4	6.9	1.1	4.4	1.7	3.2	8.2	3.9	5.7	1.8	5.8	0.4	2.9	2.1
Aveneae																			
*Amphibromus neesii**	12.2	5.4	6.5	14.4	6.8	5.3	7.8	0.8	4.5	1.8	3.3	8.3	4.3	6.0	2.0	6.7	0.1	3.5	3.3
Meliceae																			
*Glyceria declinata**	12.5	5.3	6.7	13.8	5.8	6.3	7.8	0.7	4.2	2.0	3.2	9.5	4.7	5.7	1.8	6.3	0.3	3.5	2.4
Poeae																			
Cynosurus echinatus	11.7	5.5	6.2	13.6	6.7	5.7	7.7	1.5	4.6	1.9	3.3	8.5	4.4	6.0	2.2	6.9	0.0	3.5	1.6
*Festuca arundinacea**	13.6	5.5	6.1	14.5	5.7	5.7	8.9	0.8	4.6	1.8	3.3	8.7	4.5	5.8	2.1	6.6	0.2	1.4	2.0
Festuca hookerana	13.1	5.4	6.2	14.0	5.7	5.4	7.7	0.9	4.6	1.6	3.4	8.8	4.5	6.0	2.0	6.9	0.0	4.0	1.0
Festuca littoralis	10.6	5.5	5.0	12.8	5.8	5.7	7.8	2.3	4.9	2.2	3.5	9.0	4.7	6.0	2.3	7.3	0.0	4.6	2.3
*Poa helmsii**	11.4	5.8	6.5	14.8	6.3	5.6	7.7	1.0	4.6	1.8	3.4	8.7	4.4	6.0	2.2	6.4	0.0	3.4	2.0
Poa labillardieri	13.1	5.9	6.8	16.2	6.1	5.3	7.3	1.0	4.8	1.4	3.3	8.2	3.9	5.6	2.2	6.5	0.0	2.7	1.3
BAMBUSOIDS, etc.																			
Ehrharteae																			
Ehrharta erecta	11.4	5.4	8.0	12.7	9.5	5.8	7.5	1.2	4.5	1.9	3.1	9.4	4.1	5.5	1.9	6.1	0.2	2.8	1.6
Microlaena stipoides	13.3	5.5	8.0	12.7	5.8	5.7	7.2	1.9	4.4	1.4	3.0	8.3	4.3	5.6	2.0	7.2	0.1	3.7	3.3
Tetrarrhena juncea	12.8	5.2	6.6	14.6	5.5	5.9	7.1	2.0	4.6	1.9	3.0	8.3	4.3	5.5	2.3	7.0	0.0	3.3	1.8
STIPEAE																			
Anisopogon avenaceus	11.3	5.8	5.8	13.2	6.6	5.6	8.3	1.0	4.8	1.7	3.3	9.0	4.8	5.9	2.1	6.8	0.0	4.0	2.5
Nassella trichotoma	11.3	5.4	6.8	14.9	8.3	5.4	7.5	1.2	4.1	1.8	3.1	8.6	4.3	5.6	2.1	6.5	0.0	3.2	1.6

Table 4—*continued*

Species	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg	Total amino acid (protein content) (g % fr. wt.)
	Amino acid composition (g % total amino acid)																		
DANTHONIOIDS																			
Arundo donax	11.5	5.6	5.5	13.4	6.1	5.7	7.5	1.5	4.5	2.1	3.3	9.1	5.0	5.8	2.1	7.1	0.0	4.2	3.5
Cortaderia selloana	11.4	5.3	5.6	14.2	5.7	5.6	7.2	1.8	4.8	2.4	3.5	9.1	4.8	5.6	2.2	6.9	0.0	4.0	1.5
Danthonia pallida	9.8	5.2	5.6	13.1	6.9	6.3	8.2	0.9	4.8	1.9	3.5	9.5	5.2	6.0	2.1	7.0	0.0	3.9	2.7
Phragmites australis	12.1	5.5	6.1	13.8	6.5	5.7	7.8	1.1	4.1	1.7	3.1	9.0	4.8	5.6	1.9	7.1	0.4	3.7	3.5
CHLORIDOIDS																			
Chloris gayana†	11.5	5.2	5.4	13.5	6.0	5.4	7.3	1.0	5.0	2.3	3.7	9.4	4.7	5.8	2.1	6.6	0.4	4.6	2.0
Eleusine indica†	12.2	5.3	5.7	15.3	6.1	5.5	8.3	0.8	4.3	2.0	3.3	9.0	4.4	5.3	1.8	6.7	0.5	3.6	2.9
Eleusine tristachya†	12.1	5.4	5.7	14.7	6.3	5.7	8.6	1.2	4.3	1.7	3.4	9.3	4.4	5.5	1.6	6.5	0.2	3.3	1.6
Eragrostis benthamii†	14.0	5.0	6.2	13.4	5.7	5.5	8.1	0.9	4.4	1.9	3.3	9.4	4.6	5.6	1.6	6.4	0.1	3.8	3.8
Sporobolus virginicus†	13.7	5.6	5.7	13.1	6.7	5.5	7.4	0.8	4.6	1.7	3.5	9.3	4.5	5.6	1.7	6.8	0.1	3.6	3.3
Zoysia macrantha†	11.3	5.4	6.0	14.2	5.9	5.4	7.3	1.5	4.8	2.1	3.7	9.8	4.6	5.7	2.1	6.8	0.0	3.4	1.7
PANICOIDS *sensu lato*																			
Eu-panicoids																			
Axonopus affinis†	11.0	5.2	5.7	13.5	5.9	5.5	7.3	1.7	5.0	2.1	3.4	9.8	4.7	5.9	2.3	6.9	0.0	4.1	1.0
Entolasia marginata	11.7	5.3	6.3	13.2	5.8	5.5	7.4	1.5	4.5	1.9	3.6	9.2	4.9	5.8	2.4	6.7	0.0	4.3	3.3
Entolasia stricta	11.4	5.4	6.0	14.8	6.4	5.5	7.4	1.6	4.4	1.1	3.4	9.0	5.0	6.0	2.2	6.4	0.0	4.0	3.0
Eriochloa pseudoacrotricha†	14.3	5.1	5.8	15.5	5.5	5.1	7.2	1.9	4.5	1.4	3.0	8.6	4.4	5.6	2.2	6.4	0.2	3.3	2.1
Isachne globosa	12.8	5.4	5.9	13.5	5.9	5.9	7.9	0.9	4.2	2.0	3.0	9.1	5.0	6.0	1.8	6.4	0.2	3.9	3.2
Oplismenus aemulus	11.7	5.5	5.7	13.3	6.0	5.9	7.3	1.3	4.8	2.9	3.6	9.6	4.7	6.1	2.2	7.1	0.0	3.3	2.2
Paspalum dilatatum†	12.3	5.6	6.3	13.4	6.2	6.0	7.7	1.0	4.5	1.7	3.5	9.8	4.8	5.8	2.0	6.2	0.0	3.3	1.0
Pennisetum alopecuroides†	11.9	5.4	6.3	13.2	6.4	5.8	7.6	1.4	4.2	1.4	3.4	9.8	4.8	5.9	2.1	6.9	0.2	3.4	1.3
Pennisetum clandestinum†	11.4	5.3	6.3	12.7	6.7	5.5	7.4	3.0	4.5	1.5	3.6	9.6	4.6	5.7	1.8	6.9	0.2	3.3	1.9
Pennisetum macrourum†	12.3	5.2	6.4	14.4	5.9	6.1	8.1	0.7	4.9	1.8	3.6	9.1	4.2	5.5	2.3	6.5	0.0	3.1	1.2
Spinifex hirsutus†	10.9	5.2	5.9	12.6	6.4	5.4	8.4	2.9	4.5	1.9	3.6	9.4	4.7	5.7	1.8	7.0	0.0	3.6	1.8
Stenotaphrum secundatum†	11.8	5.5	6.3	12.7	6.8	5.7	7.5	1.2	4.6	1.7	3.6	9.9	4.7	6.2	1.8	6.8	0.1	3.4	1.2
Andropogonoids																			
Bothriochloa macra†	12.5	4.9	6.0	14.2	5.4	5.8	7.3	1.5	4.8	1.9	3.8	9.6	4.6	5.7	2.2	6.7	0.0	3.2	2.1
Hemarthria uncinata†	12.0	5.3	6.4	13.8	6.4	5.9	7.8	0.6	4.3	2.1	3.3	9.8	4.7	5.8	2.0	6.7	0.0	3.4	1.4
Imperata cylindrica†	11.5	5.5	6.2	13.8	6.3	5.7	7.5	0.7	4.7	1.6	3.8	9.9	4.6	6.0	1.9	6.5	0.0	3.8	2.0
Themeda australis†	11.5	5.5	5.9	13.3	5.8	5.3	7.7	0.8	4.7	1.9	3.7	10.1	4.8	5.9	2.0	6.8	0.1	4.2	2.2

* Cf. analysis of greenhouse grown material, Table 2.
† C_4 species.

Table 5. Total leaf amino acid compositions of grasses collected from the field (taxonomic group means of data in Table 4)

Major groups/tribes	Amino acid composition (g % total amino acid)																	
(no. spp./no. genera)	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg
POOIDS (16/12)	12.4	5.6	6.1	14.2	6.2	5.5	7.8	1.1	4.7	1.8	3.4	8.8	4.4	5.9	2.0	6.6	0.2	3.5
Agrostideae (8/7)	12.5	5.6	6.0	14.2	6.2	5.3	7.7	1.1	4.8	1.9	3.4	8.9	4.4	5.9	2.0	6.5	0.3	3.6
Aveneae (1/1)	12.2	5.4	6.5	14.4	6.8	5.3	7.8	0.8	4.5	1.8	3.3	8.3	4.3	6.0	2.0	6.7	0.1	3.5
Meliceae (1/1)	12.5	5.3	6.7	13.8	5.8	6.3	7.8	0.7	4.2	2.0	3.2	9.5	4.7	5.7	1.8	6.3	0.3	3.5
Poeae (6/3)	12.3	5.6	6.1	14.3	6.1	5.6	7.9	1.3	4.7	1.8	3.4	8.7	4.4	5.9	2.2	6.8	0.0	3.3
BAMBUSOIDS, etc.																		
Ehrharteae (3/3)	12.5	5.4	7.5	13.3	6.9	5.8	7.3	1.7	4.5	1.7	3.0	8.7	4.2	5.5	2.1	6.8	0.1	3.3
STIPEAE (2/2)	11.3	5.6	6.3	14.1	7.5	5.5	7.9	1.1	4.5	1.8	3.2	8.8	4.6	5.8	2.1	6.7	0.0	3.6
DANTHONIOIDS (4/4)	11.5	5.3	5.5	13.9	5.9	5.5	7.3	1.4	4.9	2.4	3.6	9.3	4.8	5.7	2.2	6.8	0.2	4.3
CHLORIDOIDS (6/5)	12.5	5.3	5.8	14.0	6.1	5.5	7.8	1.0	4.6	2.0	3.5	9.4	4.5	5.6	1.8	6.6	0.2	3.7
PANICOIDS *sensu lato* (16/13)	12.0	5.3	6.1	13.6	6.1	5.6	7.6	1.5	4.6	1.7	3.5	9.5	4.7	5.8	2.0	6.7	0.1	3.6
Eu-panicoids (12/9)	12.0	5.3	6.0	13.6	6.1	5.6	7.6	1.7	4.5	1.7	3.4	9.4	4.7	5.8	2.1	6.7	0.1	3.6
Andropogonoids (4/4)	11.9	5.3	6.1	13.8	6.0	5.7	7.6	0.9	4.6	1.9	3.7	9.9	4.7	5.9	2.0	6.7	0.0	3.7

Table 6. Comparisons of group means between greenhouse and field collected grasses*

Amino acids	Major grass groups: Pooids	Chloridoids	Panicoids *sensu lato*
Asx	ns[1]	ns	ns
Thr	ns	ns	ns
Ser	GH <[2]	ns	ns
Glx	ns	ns	ns
Pro	ns	ns	ns
Gly	GH >[3]	ns	ns
Ala	GH <	ns	ns
Cys	ns	ns	ns
Val	GH >	ns	ns
Met	ns	ns	ns
Ile	ns	ns	ns
Leu	GH >	ns	ns
Tyr	ns	ns	ns
Phe	ns	ns	GH <
His	ns	ns	ns
Lys	GH >	ns	ns
Trp	ns	ns	ns
Arg	ns	ns	ns

* Data from Tables 2 and 4 are tested for significant differences at 5% probability level. 1, Non-significant; 2, greenhouse-grown material lower in value; 3, greenhouse-grown material higher in value.

Table 7. Limiting essential amino acids and protein chemical scores for grass leaves

Grass tribes (no. spp./no. genera)	Limiting amino acids* (chemical scores %): (a) WHO 1973†	(b) Egg reference‡
Pooids (30/20)	Ile (88)	Ile (64)
	Met + Cys (91)	Met + Cys (69)
	Val (98)	Val (81)
Bambusoids, etc.		
Oryzoids (1/1)	Ile (80)	Ile (59)
	Val (84)	Val (73)
	Met + Cys (97)	Met + Cys (74)
Ehrharteae (3/3)	Ile (75)	Ile (57)
	Val (90)	Val (77)
	Met + Cys (91)	Met + Cys (77)
Stipeae (4/3)	Ile (83)	Ile (61)
	Met + Cys (94)	Met + Cys (72)
	Val (94)	Val (78)
Danthonioids (6/6)	Ile (85)	Ile (61)
	Val (92)	Met + Cys (71)
	Met + Cys (94)	Val (75)
Chloridoids (10/6)	Met + Cys (86)	Met + Cys (65)
	Ile (88)	Ile (65)
	Val (92)	Val (77)
Panicoids (*sensu lato*)		
Eu-panicoids (21/13)	Ile (88)	Ile (64)
	Val (92)	Met + Cys (72)
	Met + Cys (94)	Val (76)
Andropogonoids (6/6)	Met + Cys (80)	Met + Cys (61)
	Ile (90)	Ile (66)
	Val (94)	Val (77)

* Values are based on the means for each species given in Tables 2 and 4, omitting field collected representatives of the same species.

† With reference to the WHO 1973 provisional scoring pattern [28].

‡ With reference to egg composition [31].

protein reference). By comparison with the WHO scoring pattern, leaves of pooids, *Oryza*, Ehrharteae, Stipeae, danthonioids and eu-panicoids have Ile as the most limiting essential amino acid; in the chloridoids and andropogonoids it is Met + Cys, although the difference is minimal. The story is the same when the essential amino acids are scored against the egg protein reference, except that Ile and Met + Cys appear to be equally limiting in the chloridoids. Byers [3] has generalized that unfractionated leaf proteins are rich in Lys compared with seed proteins, and that the average Lys content exceeds that of the WHO reference protein. In conformity with this, and by contrast with grass caryposis proteins, Lys is not a limiting essential amino acid in grass leaves, and 'chemical scores' are generally higher than those of caryopsis proteins [7].

Although taxonomic patterns have been detected in the protein amino acids of grass leaves, these reflect very small variations, and total leaf protein amino acid patterns are very similar across the family. This being so, and since the limiting essential amino acids in grass leaves are not those involved in the taxonomic patterns, it is most unlikely that the patterns have any nutritional significance for mammals. However, it is perhaps surprising that a single plant family should have yielded any detectable patterns at all; and this result does not preclude the possibility that the taxonomic patterns one could now confidently expect to find via systematic surveys of a wide range of plant groups (cf. ref. [1]), may indeed hold nutritional interest.

EXPERIMENTAL

Plant material. Grasses were grown from seeds in the greenhouse (plants watered daily; maximum/minimum temperature cycle, 24°/16°) or collected locally from the field. Identities were carefully checked, with reference to appropriate regional Floras.

Preparation for amino acid analysis. Leaf blades of mature, healthy and green culm leaves (avoiding flag leaves) were excised from their sheaths at the ligule. They were either hydrolysed immediately, or frozen in liquid N_2 for short-term storage. Leaf blades (representing several leaves except in *Arundo*, *Cortaderia* and *Phragmites*) were finely cut up and 100–200 mg material was hydrolysed in 0.5 ml 3 N mercaptoethane–sulphonic acid in a sealed tube at 110° for 22 hr [7]. After hydrolysis, 0.5 ml 2 N NaOH was added to the sample which was then diluted with 2 ml dist. H_2O, and filtered to remove any residues. The sample was then washed through a column of Bio-Rad AG-50W-X2 (100–200 mesh) cation-exchange resin as described in [7]. The sample was analysed on a Beckman amino acid analyser 119CL.

Replicate analyses representing different collections carried out for six species gave very consistent results (see Table 2), and these species were represented by mean values in subsequent calculations.

Total leaf amino acids. Total leaf amino acid contents were calculated from the amino acid analyses, and expressed as g % fr. wt leaf samples.

Free protein amino acid analysis. 1 g leaf blade prepared as above was ground in liquid N_2 and homogenized with 5 ml 3 % (w/v) sulphosalicylic acid. The homogenate was allowed to stand for 30 min at 0° before centrifugation at 27 000 g for 30 min. The supernatant was washed through a column of Bio-Rad AG-50W-X2 (100–200 mesh) cation-exchange resin as described in [7]. 10-μl and 100-μl samples were analysed on a Beckman amino acid analyser 119CL. The total free protein amino acid contents were calculated from the analyses.

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