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AMINO ACIDS IN *HORDEUM DISTICHON* AND *PANICUM MILIACEUM* GROWN IN SAND CULTURE

KENNETH F. EDGAR and SIMON R. DRAPER

School of Agriculture, University of Aberdeen, Aberdeen, Scotland, AB9 1UD

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Key Word Index—*Hordeum distichon*, barley; *Panicum miliaceum*, millet; Gramineae; amino acids; protein; translocation.

Abstract—In comparison with barley, Proso millet was characterized by high levels of alanine and glutamic acid in the leaf free amino acid fraction. In both species, analyses of root amino acids and xylem exudates suggest the existence of specific mechanisms for the transfer of certain amino acids into the xylem vessels.

INTRODUCTION

THE RECENT development of maize varieties with improved protein quality in relation to human and animal nutrition[1] has prompted the initiation of breeding programmes aimed at modifying the amino acid content of other cereal species.[2,3] The mechanisms underlying the deposition of storage proteins in developing seeds have not been fully elucidated and this lack of knowledge may be an obstacle to the genetic improvement of cereal proteins.[3] There is some evidence that the amino acid content of cereal grains is influenced by the proportions of free amino acids which are transferred from other parts of the plant to the ripening seeds, rather than on the pattern of local synthesis.[4] In the present work, a sand culture system has been used to study the distribution and movement of amino acids in barley and Proso millet. The latter species is of particular interest as of the minor millets it has a relatively high level of lysine, the amino acid limiting the value of most cereal proteins as dietary components.

RESULTS AND DISCUSSION

Satisfactory growth in the sand culture system was observed with both species. The only problem encountered was a slight scorch of the barley leaf tips. This occurred if the volume of nutrient solution used was insufficient to displace the residual salts which became concentrated due to loss of water by evaporation and transpiration.

In comparison with the millet, barley leaves showed higher levels of free and protein-amino acids, while the roots showed a lower level of amino acids bound in the protein fraction and low levels of free amino acids (Table 1). Although no significant differences were observed in the proportions of individual amino acids present in the leaf-protein of the two species, there were marked differences in the free-amino acid fractions, the millet

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having significantly higher proportions of alanine ($p < 0.001$) and glutamic acid ($p < 0.01$) and consequently smaller proportions of other amino acids (Table 2).

TABLE 1. DISTRIBUTION OF AMINO ACIDS IN SEEDLINGS OF *Hordeum distichon* AND *Panicum miliaceum* (μmol/g fr. wt)

Plant part		Barley	Millet	LSD ($p < 0.05$)
Leaf	Protein	149·5	129·2	14·9
	Free	10·8	8·9	1·4
Root	Protein	38·0	62·8	13·5
	Free	2·9	3·7	0·9
Xylem exudate (μmol/ml)		5·2	2·6	2·9

TABLE 2. AMINO ACID CONTENT OF LEAF TISSUE (μmol/100 μmol TOTAL AMINO ACIDS)

	Barley		Millet		
Compound	Protein	Free	Protein	Free	s.e.
Lysine	6·84	3·44	6·62	2·54	0·69
Histidine	2·18	2·61	2·21	2·04	0·38
Arginine	4·80	1·61	5·10	2·34	0·40
Aspartic	9·05	17·30	9·26	16·55	1·89
Asparagine	9·05	14·15	9·26	5·54	1·89
Threonine	5·38	6·06	5·41	3·21	0·42
Serine	5·85	15·75	6·38	8·79	0·50
Glutamic	9·79	7·75	10·08	11·25	1·11
Glutamine	9·79	5·94	10·08	3·89	1·11
Glycine	9·70	2·49	9·06	3·17	0·41
Alanine	8·97	10·39	8·66	24·95	0·93
Valine	6·67	2·90	5·50	2·01	0·51
Methionine	1·59	0·68	2·22	0·80	0·47
Isoleucine	4·48	1·12	4·80	1·12	0·31
Leucine	8·99	1·98	9·69	1·73	0·32
Tyrosine	3·59	0·85	3·10	0·82	0·13
Phenylalanine	4·87	1·44	4·66	0·85	0·19
Proline	6·03	3·45	5·12	3·40	0·94

It is unlikely that the high level of alanine could be due to its involvement in a 'CO_2-shuttle' as described for a number of plants possessing the C-4 photosynthetic cycle,[5] since other members of the Panicoideae which have been examined are known to possess the malate, rather than the aspartate transfer system.[5]

In order to obtain some indication of the role of the roots of barley and millet as sources of organic nitrogen for the shoot, xylem exudates were collected and examined for amino compounds. Analyses indicate that both plants export appreciable quantities of amino acids from the roots (Table 1). In both species, glutamine, lysine, arginine and asparagine are all relatively more important as constituents of exudates than as components of the root free amino acid fraction (Table 3). A possible explanation is that some selective transport process exists for the transfer of different amino acids from their site of synthesis in the root cells, into the xylem vessels. Mechanisms for selective amino acid transport are well documented for bacterial cells.[6]

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TABLE 3. FREE AMINO ACID CONTENT OF ROOT TISSUE AND XYLEM EXUDATES (μmol/100 μmol TOTAL AMINO ACIDS)

Compound	Barley		Millet		s.e.
	Root	Xylem	Root	Xylem	
Lysine	4·34	6·70	5·05	7·94	0·88
Histidine	2·40	3·25	3·35	3·98	0·62
Arginine	1·16	4·41	2·34	6·30	0·55
Aspartic	15·29	4·36	18·13	6·33	1·24
Asparagine	8·33	12·11	5·42	6·99	1·85
Threonine	4·29	5·26	3·99	4·86	0·34
Serine	11·36	10·73	10·30	11·85	0·80
Glutamic	12·76	7·16	17·49	9·35	0·91
Glutamine	11·00	27·93	3·88	22·34	2·20
Glycine	3·02	2·22	3·63	3·40	0·49
Alanine	12·48	4·81	9·71	4·62	2·51
Valine	3·04	4·92	3·57	4·13	0·53
Methionine	0·63	0·27	1·39	0·66	0·18
Isoleucine	1·56	1·57	1·78	1·48	0·29
Leucine	2·53	1·89	3·38	1·82	0·29
Tyrosine	1·14	0·76	1·35	0·68	0·18
Phenylalanine	1·65	0·89	1·39	1·40	0·17
Proline	1·84	0·46	3·79	1·14	0·29

EXPERIMENTAL

Plant material. Plants of barley (*H. distichon* cv. Ymer) and Proso millet (*P. miliaceum*) were raised from seed in plastic trays, arranged randomly in a growth cabinet with day temp. 22·5° and night temp 15·0°. The photoperiod was 18 hr (20 000 lx). Samples were taken 21 days after sowing. Sand (horticultural grade) was washed to remove fine particles. The nutrient soln was similar to that described by Hewitt.[7] The constituents and quantities in g/100 l. were as follows: KNO_3 (20·2); K_2SO_4 (17·4); Ca $(NO_3)_2$ (32·8); $CaCl_2$ (22·2); $MgSO_4.7H_2O$ (36·8); $NaH_2PO_4.2H_2O$ (20·8); ferric citrate $5H_2O$ (3·35); $MnSO_4.4H_2O$ (0·223); $CuSO_4.5H_2O$ (0·025); $ZnSO_4.7H_2O$ (0·029); H_3BO_3 (0·31); NaCl (0·59); $NaMoO_4.2H_2O$ (0·012).

Protein amino acids. Fresh plant material (5 g) was ground in a pestle and mortar with 80% EtOH (120 ml) and a little quartz sand. The insoluble residue was removed by filtration and washed with ice-cold 10% trichloroacetic acid. Following acid hydrolysis (6 M HCl/20 hr) 100° (N_2), amino acids were estimated by automated column chromatography.[8]

Free amino acids. The ethanolic filtrate was reduced to dryness *in vacuo* (40°). Pigments were partitioned into petrol. (b.p. 40–60°) and the amino acids taken up in 0·1 M HCl prior to column chromatography. Amides were determined by measuring the increase in parent amino acids following hydrolysis.[8]

Xylem exudates. The leaves were excised 1·5 cm above the surface of the sand, and root exudates were collected at the cut surfaces of the leaf-sheath bases with a syringe. The collection period was 2 hr, ending at midday. Amino acids were extracted into 80% EtOH.[9]

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