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Aminoacid Profiles in the Study of Phylogenetic Relationships in the Genus *Oryza*

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Summary. The amino acid profiles in seeds of thirteen different species of *Oryza*, including two cultivated rices, *O. glaberrima* and *O. sativa* and the two major geographical races *indica* and *japonica* were studied using an automatic amino acid analyser to assess differences in the profiles of cultivated species and their wild progenitors. The polygon graphic method was employed to envision the species relationship. Essential amino acid profiles in different species were also compared with those of the Food and Agriculture Organization (FAO) standards. The results suggest a wide range of variability among *Oryza* species for lysine (up to 4.4% as against 3.5% in cultivated rices) and other essential amino acids. This will be of considerable interest to rice breeders, when after overcoming genetic barriers, the possible utilization of these species in rice breeding becomes feasible.

Key words: *Oryza*-aminoacid profiles – Polygon patterns – Essential aminoacids – Phylogenetic relationships

Introduction

Though phylogenetic relationships of certain species in the genus *Oryza* have been studied by conventional cytogenetic methods, there have been very few attempts to use biochemical traits such as free amino acids (Vaidyanath et al. 1974) and amino acid profiles of seed proteins to gain further insight into phylogenetic relationships. However, in other plant genera amino acid profiles have been employed in studying species relationships (Buzzati-Traverso and Rechnitzer 1953; Bell, 1962; Reuter 1957; Alston and Turner 1963; Sarvella and Stojanovic 1968; Webrew and Matzinger 1968). In the present investigation an attempt has been made (a) to study amino acid profiles in different species of *Oryza* in order to elucidate species relationship, (b) to analyse the changes entailed in the essential amino acid profiles of 'A' genome species, especially cultivated taxa and their wild progenitors during the course of their evolution and differention, (c) to compare profiles of tetraploid and component diploid species of 'BC' genome taxa.

Materials and Methods

Nine diploid species, *Oryza sativa (AA)*; *O. nivara* (*AA*); *O. glaberrima* (A^gA^g); *O. barthii* (A^bA^b); *O. longistaminata* (A^lA^l); *O. punctata* (*BB*); *O. officinalis* (*CC*); *O. australiensis* (*EE*); *O. perrieri* (*PP*) and four tetraploid species, *O. minuta* (*CCBB*); *O. schweinfurthiana* (*CCBB*); *O. latifolia* (*CCDD*); and *O. ridleyi* (*RRRR*?) were used in the present study. The letters A, B, C, D, E, etc; mentioned in the parenthesis indicates their genomic constitution. All the species were grown under uniform conditions in a nethouse. Fully mature seeds of different species were dehusked, ground to a fine powder and defatted with hexane in a soxlet apparatus prior to hydrolysis with 6 N HCl. The separation and analysis of amino acids was accompolished on a Spinco automatic amino acid analyser, Model 120 C; prior to hydrolysis nitrogen and protein was determined by the microkjeldahl method. An analysis of variance of 2 way classification was made (Fisher 1954) to assess the differences in amino acid profiles of different species. To compare and envision the affinities among the species, polygon graphs were plotted using the quantities of each amino acid for each species. Each axis in a polygon represents one of the amino acids. Distances from the centre to periphery are based on the percentage of each amino acid present in the sample of a given species.

Observations

Differences in Profiles

Amino acid profiles of different species differed from each other considerably (Table 1). The values ranged from 0.40 to 20.93 per cent. The two cultivated subspecies, *O. sativa L.* ssp. *indica* (Var. 'TK') and *O. sativa L.* ssp. *japonica* (Var. 'chowsung') closely resembled each other in

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Table 1. Aminoacid profile in seed of some wild and cultivated species of genus *Cryza* (gm/100 gm protein)

Amino acid	*O. sativa* sub sp. *indica* var. 'Tk'	*O. sativa* sub sp. *japonica* var. Chowsung'	*O. nivara* Ac. No. 100189-a	*O. glaberrima* Ac. No. 100297	*O. barthii* Ac. No. 100120	*O. longistaminata* Ac. No. 101200	*O. australiensis* Ac. No. 101410	*O. officinalis* Ac. No. 100112	*O. punctata* Ac. No. PGWR 204	*O. minuta* Ac. No. PGWR 205	*O. schweinfurthiana* Ac. No. PGWR 206	*O. latifolia* Ac. No. 100170	*O. ridleyi* Ac. No. PGWR 212	*O. perrieri* (*L. perrieri*) Ac. No. PGWR 212	FAO Pattern for essential amino acids
	AA	*AA*	A^pA^p	A^gA^g	A^bA^b	A^lA^l	*EE*	*CC*	*BB*	*CCBB*	*CCBB*	*CCDD*	?	?	
Lysine	3.40	3.50	2.92	2.86	2.74	3.31	3.59	2.94	3.03	3.39	3.75	3.00	4.40	3.98	4.2
Histidine	2.11	2.18	2.23	2.17	1.77	2.10	2.07	1.59	1.95	2.42	2.30	1.90	2.06	2.07	2.4
Arginine	8.50	7.59	7.83	7.68	6.73	7.36	8.43	7.18	7.20	7.49	7.66	7.14	7.17	7.99	2.0
Asparticacid	8.85	9.35	4.96	8.66	7.34	8.67	4.00	7.50	8.42	8.84	7.20	9.27	8.75	9.33	–
Threonine	3.16	3.17	3.09	2.89	2.49	2.71	3.05	2.76	2.62	2.62	2.35	3.12	2.73	3.08	2.8
Serine	4.41	4.81	4.61	4.25	3.78	4.03	4.86	4.80	4.03	4.28	3.80	5.30	3.53	3.87	–
Glutamicacid	18.76	18.87	20.84	20.02	18.33	18.53	20.75	20.93	18.65	19.01	19.69	19.04	18.66	19.46	–
Proline	3.90	4.19	4.92	4.82	5.62	7.12	6.74	6.66	7.34	6.87	7.68	3.53	7.77	6.94	–
Glysine	4.10	4.35	3.78	3.61	3.43	3.51	4.10	3.49	3.36	3.62	3.43	3.78	3.88	3.78	–
Alanine	5.66	5.70	5.97	5.49	5.13	6.48	5.54	5.35	4.92	5.04	4.85	6.11	9.58	4.75	–
Cystine	0.97	1.34	0.92	1.00	0.40	0.59	0.67	0.92	0.44	0.56	0.99	0.73	0.60	0.68	2.0
Valine	5.38	5.23	5.52	5.13	4.63	5.26	4.77	4.75	4.81	5.45	7.11	5.65	7.02	4.46	4.2
Methionine	1.64	1.17	1.32	1.25	2.40	1.16	1.33	0.95	0.91	0.92	0.83	1.23	0.76	1.08	2.2
Iso-Leucine	3.55	3.48	3.76	3.58	6.01	3.77	3.69	3.44	3.63	3.43	3.51	4.84	3.57	3.75	4.2
Leucine	7.85	7.99	9.09	8.68	11.97	8.17	8.39	8.84	9.11	8.35	8.43	7.84	7.69	7.43	4.8
Tyrosine	3.38	2.59	3.71	3.51	3.21	3.05	3.26	3.51	4.04	4.28	2.42	3.37	2.24	2.85	–
Phenylalanine	4.33	4.53	4.85	4.39	4.03	4.20	4.78	4.42	4.69	4.33	4.02	4.37	4.58	4.49	2.8
Protein %	10.56	12.06	14.13	13.16	13.19	9.50	15.39	13.13	12.0	13.25	10.69	11.81	11.06	12.50	–

their amino acid profiles. However, minor quantitative differences were observed in the contents of aspartic acid, serine, proline, cystine and tyrosine. The Asian cultivated taxon, *O. sativa*, when compared to its wild relative *O. nivara*, differed less markedly in their profiles, the minor differences observed being in the contents of lysine, aspartic acid, glutamic acid, proline, glycine and isoleucine. Similarly, the African cultivated rice, *O. glaberrima* differed with its wild species, *O. barthii* and *O. longistaminata* in contents of such amino acids as lysine, histidine, arginine, glutamic acid, proline, alanine, cystine, valine, methionine, isoleucine and leucine (Table 1).

The taxa, *O. officinalis* (*CC*) and *O. punctata* (*BB*); *O. minuta* (*CCBB*); *O. schweinfurthiana* (*CCBB*) and *O. latifolia* (*CCDD*) not only displayed the differences in amino acid profiles among themselves, but also differed with species having '*AA*' genomes. The species, *O. officinalis* and *O. punctata* differed from each other with respect to such amino acids as aspartic acid, glutamic acid, proline, cystine, and lysine (Table 1). Similarly, the taxa, *O. officinalis, O. punctata, O. minuta* and *O. schweinfurthiana* were found to differ in amino acids lysine, aspartic acid, serine, glutamic acid, proline, cystine, valine, leucine and tyrosine (Table 1). The allotetraploid taxa, *O. minuta, O. schweinfurthiana* and *O. latifolia* differed from each other in the quantity of such amino acids as lysine, aspartic acid, serine, proline, alanine, methionine, isoleucine and leucine (Table 1). The taxon, *O. ridleyi* differed from other species in the content of the amino acids lysine, proline, alanine, valine, leucine and tyrosine. Similarly, the species *O. perrieri*, which is now removed to another genus *Leersia*, does not differ markedly with other species except for having differences in the contents of lysine and proline (Table 1). The analysis of variance for amino acid profiles in seed of different *Oryza* species suggested clear significant differences from species to species (F value = 4.93 with 13 d.f. significant at 1% level).

Polygon Pattern

Though the polygon patterns of different species indicated subtle differences (Fig. 1), the two major subspecies of *O. sativa* (*indica* and *japonica*) resembled each other very closely in their polygon patterns. The polygon patterns of the Asian cultivated species, *O. sativa* and its wild

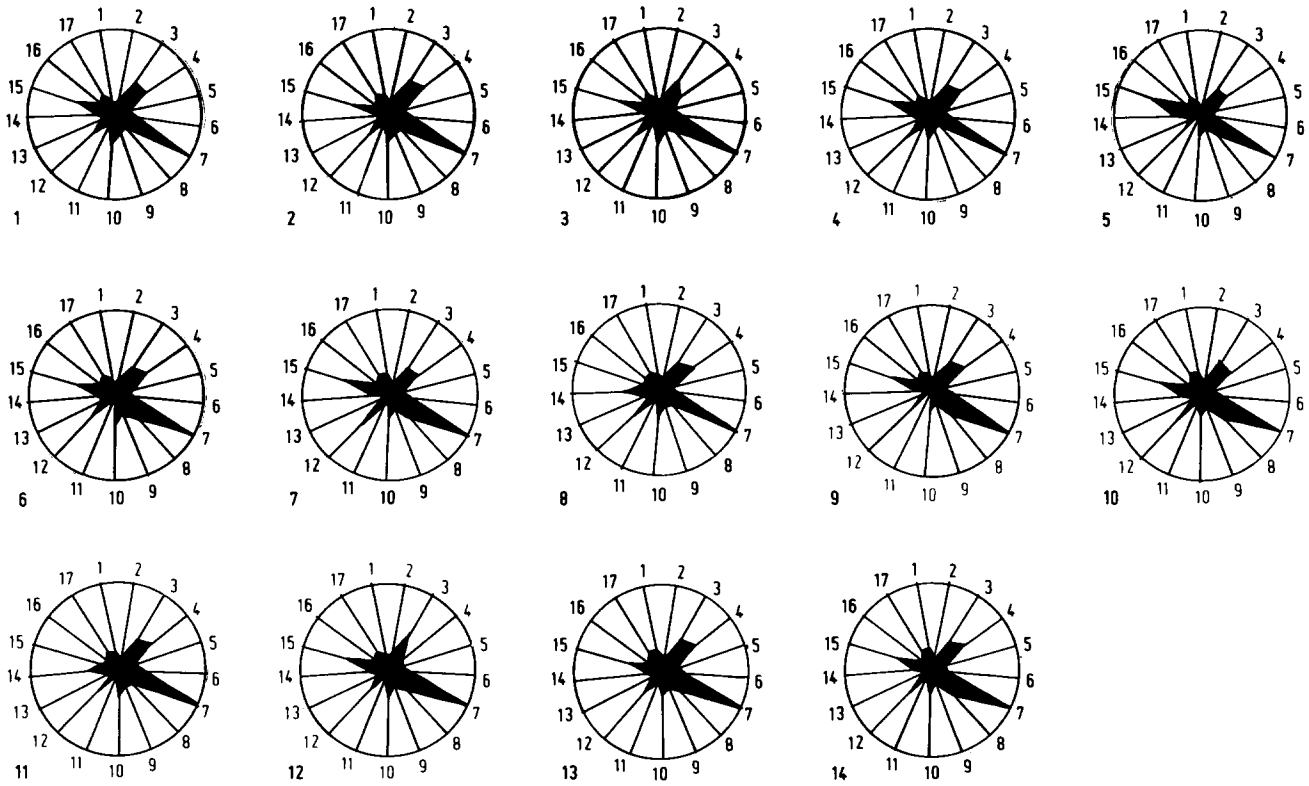

Fig. 1. Amino acid polygon pattern of seed proteins of different species of *Oryza*. **1** *O. sativa* ssp. *indica*; **2** *O. sativa* ssp. *japonia*; **3** *O. nivara*; **4** *O. glaberrima*; **5** *O. barthii*; **6** *O. longistaminata*; **7** *O. punctata*; **8** *O. officinalis*; **9** *O. minuta*; **10** *O. schweinfurthiana*; **11** *O. latifolia*; **12** *O. australiensis*; **12** *O. perrieri*; **14** *O. ridleyi*. Numbers on circle against each radii indicate amino acids: 1) Lysine; 2) Histidine; 3) Arginine; 4) Aspartic acid; 5) Threonine; 6) Serine; 7) Glutamic acid; 8) Proline; 9) Glycine; 10) Alanine; 11) Cystine; 12) Valine; 13) Methionine; 14) Isoleucine; 15) Leucine; 16) Tyrosine; 17) Phenylalanine

relative *O. nivara* also differed markedly (Fig. 1, 1-3). Similarly the African cultivated taxon, *O. glaberrima* compared with its wild relatives, *O. barthii* and *O. longistaminata* also exhibited subtle differences. (Fig. 1, 4-6).

In the *Officinalis* group, the species *O. officinalis* and *O. punctata* differed subtly from each other in their polygon pattern. However, the two taxa when compared to their allopolyploid species, *O. minuta, O. schweinfurthiana* and *O. alta*, showed a broad similarity in their polygon patterns. (Fig. 1, 7-11).

The taxon, *O. australiensis* had a distinctly different polygon pattern when compared to other species (Fig. 12). Similarly the taxa *O. ridleyii* and *O. perrieri* were also found to be distinct in their polygon patterns. (Fig. 1, 13 and 14).

Essential Amino Acid Contents

The essential amino acid contents of each species was compared with that of Food and Agriculture Organization (FAO) standards (Fig. 2). It was observed that the essential amino acid profiles in the taxa were almost equal to or above the FAO standards for most of the amino acids. Lysine and methionine seem to be the only limiting amino acids in the entire genus. However, the species *O. ridlevii*, did have more lysine content than any species and even exceeded the FAO standards.

On comparing the two cultivated species, *O. sativa* and *O. glaberrima* with their respective wild alleles, it was observed that *O. sativa*, differed from its wild relative *O. nivara* in having a reduced quantity of leucine. On the other hand, lysine, methionine and threonine contents were lower in the wild taxon, than the cultivated one; whereas amino acids, isoleucine, phenyl-alanine, tyrosine and valine were more or less present in equal quantities. Similarly, when *O. glaberrima* was compared with its wild relatives, *O. barthii* and *O. longistaminata* for essential amino acid content, it was observed that the trend was exactly opposite to that of the Asian cultivated species and its wild relative.

In general, the content of such amino acids as leucine, isoleucine, lysine, methionine, tyrosine and valine was higher in wild taxa when compared to cultivated species. To gain a further insight into the relationships between diploid and allotetraploid species of the *O. officinalis* group a comparison was made of the quantitative differences in the amino acids. It was observed that allopolyploid species have noticeable deviations from the mean of diploid values (Table 2).

Table 2. Heterotic effects for amino acid contents shown by the allopolyploid species when compared to their plausible diploid progenitors

Amino acid	Amino acid content in diploid species		Mean of diploids	Deviation of mean amino acid content of allopolyploids from mean of diploids	
	O. punctata (*BB*)	*O. officinalis* (*CC*)		*O. minuta* (*CCBB*)	*O. schweinfurthiana* (*CCBB*)
1	2	3	4	5	6
Lysine	3.03	2.94	2.98	0.41	0.77
Histidine	1.95	1.59	1.77	0.65	0.43
Arginine	7.20	7.18	7.19	0.30	0.47
Asparatic acid	8.42	7.50	7.96	0.88	–0.34
Threonine	2.62	2.76	2.69	–0.07	–0.34
Serine	4.03	4.80	4.41	–0.13	–0.61
Glutamic acid	18.65	20.93	19.79	–0.78	–0.10
Proline	7.34	6.66	7.00	–0.13	0.68
Glysine	3.36	3.49	3.42	0.20	–0.01
Alanine	4.92	5.35	5.13	–0.09	–0.28
Cystine	0.44	0.92	0.68	–0.14	–0.31
Valine	4.81	4.75	4.78	0.67	2.43
Methionine	0.91	0.95	0.93	–0.01	–0.10
Isoleucine	3.63	3.44	3.53	–0.10	0.02
Leucine	9.11	8.84	8.97	–0.62	–0.54
Tyrosine	4.04	3.51	3.77	0.51	1.35
Phenylalanine	4.69	4.42	4.55	–0.22	0.53

Discussion

The material used in the present investigation was grown under uniform conditions while the analysis was conducted using a group of randomly chosen, genomically divergent species. The presupposition was that any differences in the protein hydrolysate might reflect their genetic and/or genomic effects and be capable of unfolding clues to their affinities. Besides, such a comparison, especially of essential amino acids in cultivated species and their immediate wild progenitors, might throw light on the nature of biochemical transformation that occurred during their mobilization. Further, the study of variability for amino acids in the genus could possibly indicate the source of limiting amino acids and lead to the improvement of the essential amino acid pattern of cultivated species by inter-specific hybridization, after over-coming crossability barriers through somatic cell hybridization.

The differences in the concentrations of common amino acids have been used as criteria in the study of species affinities by several workers (Reuter 1957; Sarvella and Stojanovic 1968; Weybrew and Matzinger 1968). Though the quantitative differences for different amino acids observed were small, they were statistically significant. The variability for limiting such amino acids as lysine and methionine was found to be 2.74 to 4.40 and 0.76 to 2.40%, respectively. The highest values for lysine was 3.98% and 4.40% in species *O. ridleyii* and *O. perrieri*, respectively. Similarly, *O. barthii* exhibited the highest value for methionine (2.40%). From the data it may be concluded that wild species of the genus *Oryza* possesses potentiality for the synthesis of high quality reserve proteins in the seed. However, the practical realization is conditioned by the existence of genetic barriers which restrict wide crosses within the *Oryza* species.

The polygon graphic method of representation of amino acid contents to visualise objective comparisons between the species suggested subtle differences, reflecting their genomic differences (Fig. 1, 1-14). Taira, (1962), studying the adaptability of amino acid patterns of seed proteins as a unit of plant taxonomy in various sub-families of *Gramineae*, reported satisfactory consistency in the patterns of each species.

The specific comparison drawn between the component species of *Sativa* and *Glaberrima* complexes suggested clues to their phylogenetic relationships and also helped in assessing the pattern of evolution of quantitative variation for these amino acids among different species. In

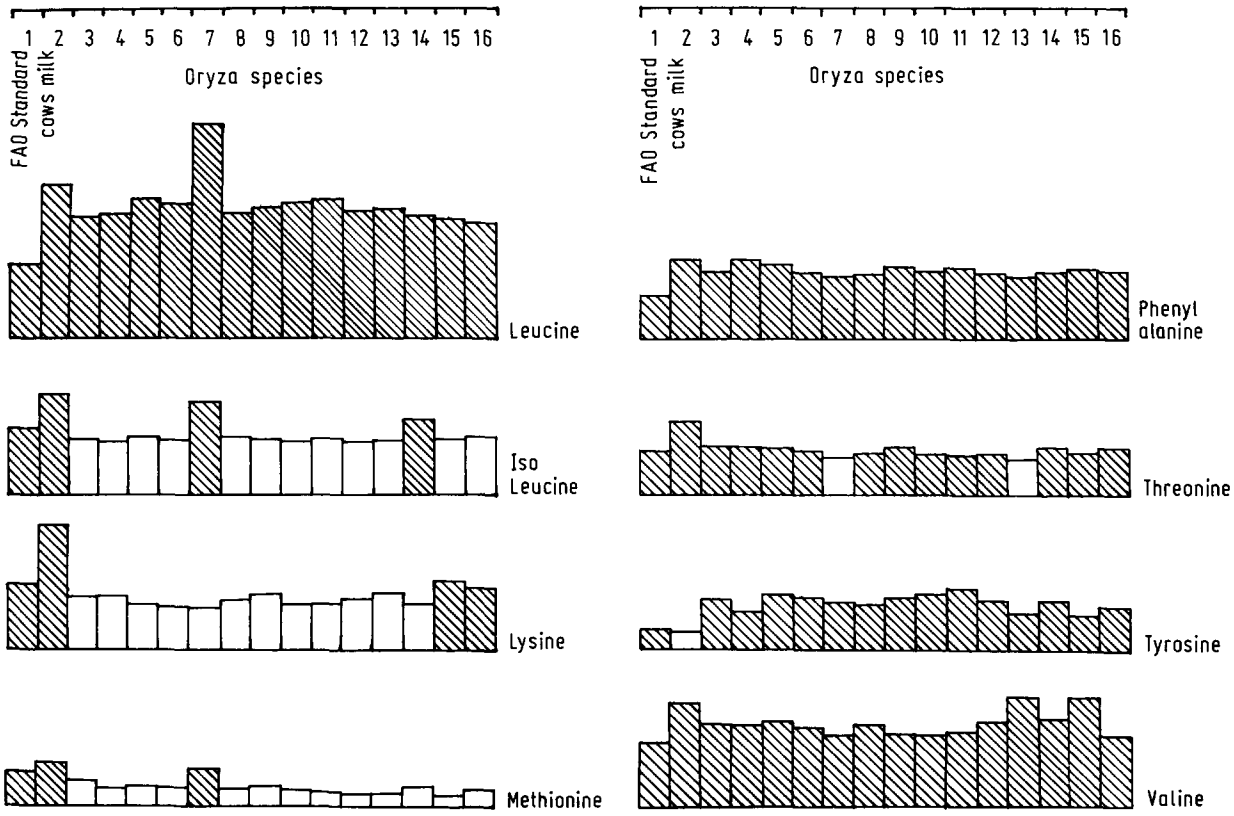

Fig. 2. Essential amino acid profiles of different species of *Oryza* (deficient amino acids relative to FAO standards are shown as blanks). 1 FAO Standard; 2 Cow's milk; 3 *O. sativa* ssp. *indica*; 4 *O. sativa* ssp. *japonica*; 5 *O. nivara*; 6 *O. glaberrima*; 7 *O. barthii*; 8 *O. longistaminata*; 9 *O. australiensis*; 10 *O. officinalis*; 11 *O. punctata*; 12 *O. minuta*; 13 *O. schweinfurthiana*; 14 *O. latifolia*; 15 *O. ridleyi*; 16 *O. perrieri*

the *Sativa* complex, the most successful cultigen *O. sativa* has lower levels of leucine and higher contents of other essential amino acids when compared to its wild annual progenitor *O. nivara*, the taxon *O. nivara* is distinguished by lower levels of lysine, methionine and threonine. In the *Glaberrima* complex, the wild species were found to have higher levels of lysine, methionine, leucine, tyrosine and valine, when compared to the cultivated species *O. glaberrima*. The trend in the *Glaberrima* complex (cultivated vis a vis wild species) appears to be exactly the reverse of what is found in the *Sativa* complex, though all of them have the same basic *AA* genome (Morinaga and Kuriyama 1960; Nezu et al. 1960). The reverse pattern in the levels of some of the essential amino acids in the cultivated rices vis a vis their wild progenitors may be due to their peculiar evolutionary history. The Asian cultivated rice *O. sativa* is very widely distributed and has been differentiating for over nine thousand years (Chang 1976); consequently it has been vigorously subjected to selection by Man and Nature far longer time than the African cultivated rice, *O. glaberrima*, which is comparatively recent in its origin (Porteres 1956). Perhaps in rice a series of mutations have taken place which increased the levels of essential amino acids to the present level. Such differences in the content of amino acids in cultivated and wild species were also noted by Baldi and Salamini (1973) in *phaseolous* species.

The protein quality of cereals is inversely related to their prolamine content (Nelson 1974). Though quantitative fractionation studies of proteins have not been made, from the amino acid profiles of wild and cultivated species of *Sativa* complex, a facile assumption can be made; the prolamine fraction might have gradually been reduced from a wild species level to that of cultivated species in the *Sativa* group. The same may not be true in the case of the *Glaberrima* complex, as evidenced by the essential amino acid profiles (Table 1) of cultivated and wild species. This hypothesis is open to verification by estimating the protein fractions in all species of the *Sativa* and *Glaberrima* complexes. The reason for the poor pattern of *O. glaberrima* when compared to its wild relatives may presumably be due to its recent origin and limited distribution (Porteres 1956).

The species belonging to the *Officinalis* group have also shown distinct differences in their levels of amino acids. Of particular significance is the level of amino acids in allopolyploids and their putative parental diploids. The levels of amino acids in the taxa, *O. officinalis* (*CC*); *O. punctata* (*BB*) can be compared with those of *O. minuta* (*CCBB*) and *O. schweinfurthiana* (*CCBB*). It is very interesting to note that some of the amino acids show positive, and others negative, deviation from the mean of the diploid species. The deviation of a F_1 hybrid from the mean of its parents is evidence of either positive or negative heterosis. This clearly suggests their nature of origin.

The present study clearly demonstrates that the amino acid profiles of different species are useful in assessing species relationships and that the comparison of wild and cultivated species help in the evaluation of their pattern of evolution and origin. As amino acids are building-blocks of proteins which align in proper sequence under the control of a genetic code of particular DNA moiety, the study of amino acids not only serves in understanding species relationships but also provides background information as a prelude to much more sophisticated analyses to be used in studying evolutionary relationships at a molecular level.

Literature

Alston, R.E.; Turner, B.L. (1963): Biochemical systematics. New Jersey: Prentice-Hall

Baldi, G.; Salamini, F. (1973): Variability of essential amino acid content in seeds of 22 *Phaseolus* species. Theor. Appl. Genet. **43**, 75-78

Bell, E.A. (1962): Association of ninhydrin reacting compounds in the seed of 49 species of *Lathyrus*. Biochem. J. **83**, 225-229

Buzzati-Traverso, A.A.; Rechnitzer, A.B. (1953): Paper partition chromatography in taxonomic studies. Science **177**, 58-59

Chang, T. (1976): The origin, evolution, cultivation, dissemination and diversification of Asian and African rices. Euphytica **25**, 425-441

Morinaga, T.; Kuriyama, H. (1960): Interspecific hybrids and genomic constitution of various species in the genus *Oryza*. Nogyo Oyobi Engei. **35**, 773-776, 935-938, 1091, 1094, 1245-1247

Nelson, O.E., Jr. (1974): Interpretive summary and review, presented at the workshop on, 'Genetic improvement of seed proteins' National Academy of Sciences, Washington DC.

Nezu, M.; Katayama, T.C.; Kihara, H. (1960): Genetic study of the genus *Oryza* I. crossability and chromosomal affinity among 17 species. Seiken Ziho. **11**, 1-11

Porteres, R. (1956): Taxonomic agrobotanique des riz cultives *O. sativa* Linn. et *O. glaberrima* Steud. J. Agr. Trop. Bot. Appl. **3**, 341-384, 541-580, 627-700, 821-856

Reuter, C. (1957): Die Hauptformen des löslichen Stickstoffs in vegetativen pflanzlichen Speicherorganen und ihre systematische Bewertbarkeit. Flora **145**, 326-338

Sarvella, P.; Stojanovic, B.J. (1968): Amino acid analyses of the species in the genus *Gossypium*. Can. J. Genet. Cytol. **10**, 362-368

Taira, H. (1962): Amino acid pattern of seed proteins as standard in the plant taxonomy. Bot. Mag. Tokyo **75**, 80-81

Vaidyanath, K.; Raju, K.K.; Reddy, G.M. (1974): Free amino acid pattern and species relationship in genus *Oryza* Theor. Appl. Genet. **45**, 72-76

Weybrew, J.A.; Matzinger, D.F. (1968): The free and protein bound amino acids of certain *Nicotiana* species and hybrids. Tobacco Science **168**, 22-29

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