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Free Aminoacid Pattern and Species Relationship in Genus *Oryza*

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Summary. Using the two-dimensional paper-chromatographic technique, free-aminoacid analysis was made on nine diploids and five allotetraploid species of *Oryza*, to study the biochemical and physiological bases of species relationships. The relationship between different species was inferred on the basis of similarity or differences in polygon patterns of aminoacids, drawn using Ellison's paired affinity values. Among the diploid species, *O. sativa*, *O. perennis* subsp. *balunga*, *O. glaberrima* and *O. breviligulata* showed similar polygon patterns, suggesting close relationships. The other diploid species differed in their polygon pattern, reflecting species differences. In the tetraploid species, *O. alta* and *O. latifolia*, and *O. minuta*, *O. schweinfurthiana* and *O. malampuzhaensis*, showed similar polygon patterns. Polygon maps of *O. punctata* failed to show any similarity with *O. officinalis*, but some similarity was observed between *O. punctata* and *O. schweinfurthiana*.

In recent times, numerous biochemical parameters such as variation in proteins, isoenzymes, phenolic compounds and aminoacids have been used to elucidate phylogenetic and interspecific relationships in various genera, including *Oryza* (Alston and Irwin 1961; Johnson and Hall, 1965; Hart and Bhatia, 1967; Chu 1967; Chu and Oka, 1967; Shahi *et al.*, 1969; and Siddiq *et al.*, 1972). The genus *Oryza* is a small group composed of 24 species, of which *O. sativa* and *O. glaberrima* are cultivated. Based on chromosomal homology, six basic genomic groups, AA, CC, EE, FF, CCBB, and CCDD, have been recognised in 16 species of this genus. The systematic classification, nomenclature, and validity of certain species still remain uncertain. The present investigation of qualitative differences in the free aminoacids of different species of *Oryza* was undertaken to study the biochemical and physiological bases of species relationships.

Materials and Methods

Nine diploid species, *Oryza sativa* (AA), *O. glaberrima* (A^gA^g), *O. perennis* subsp. *balunga* (AA), *O. breviligulata* (A^gA^g), *O. punctata* (BB?), *O. australiensis* (EE), *O. brachyantha* (FF), *O. officinalis* (CC) and *O. perrieri*, and five allotetraploids, *O. alta* (CCDD), *O. latifolia* (CCDD), *O. minuta* (CCBB), *O. malampuzhaensis* (CCBB) and *O. schweinfurthiana*, (CCBB) were used in the present study.

The plants were collected at anthesis and divided into four tissues — panicle, leaf blade, leaf sheath, and internode. Two grams of dried tissue were ground to a fine powder and extracted with 80% ethyl alcohol (4:1 W/V). To the measured volume of the extract, three times the volume of chloroform was added and mixed thoroughly. The aqueous supernatant layer containing free aminoacids was collected and stored in the deep freeze. The two-dimensional chromatographic technique with solvent mixtures of butanol:acetic acid:water (25:6:25) and phenol:water (3:1) was used. After development, the chromatograms were dried and sprayed with 0.2% ninhydrin in acetone. By comparing ninhydrin-positive spots with the standardised Rf values of the authentic samples, the individual aminoacids were identified. The Rf values for each spot were computed from the mean of two independent observations drawn from separate chromatograms for each sample analysed.

Ellison's (1962) paired affinity (P.A.) and group affinity (G.A.) values were enumerated to derive the relationship between any two species based on chromatographic data. The paired affinity test synthetically quantifies the chromatographic data and enables one to express the relationship between two species based on chromatographic affinity. The ninhydrin-positive spots were assigned numerical values depending upon Rf value, the same number being assigned when one or more species were found to have the same Rf value. The P.A. values are calculated as follows:

$$\text{P.A. value} = \frac{\text{Spots common in A and B}}{\text{Total spots in A and B}} \times 100 .$$

Qualitative free aminoacid data can be quantified and expressed as polygon patterns using the P.A. values. The group affinity (G.A.) test is mainly aimed at determining the validity of the concerned species in a single genus. The G.A. value is a numerical expression of all P.A. values of a given species with respect to the other species.

Observations

The qualitative pattern of free aminoacids of the panicle, analysed from all representative species, is given in Table 1. The general chromatographic pattern varied from species to species, although some consistencies in chromatographic pattern were noted between *O. sativa* and *O. perennis* subsp. *balunga*, *O. glaberrima* and *O. breviligulata*, *O. minuta*, *O. schweinfurthiana* and *O. malampuzhaensis*. However, chromatographic patterns in different tissues of the same species — internode, panicle, leaf blade and leaf sheath — were identical. In the case of panicle, the number of ninhydrinpositive spots varied from 11 to 17, *O. australiensis* having 11 and *O. officinalis* 17. The number of aminoacids identified ranged from 8 to 12. The species *O. malampuzhaensis* showed 8,

Table 1. *Free aminoacids (alcohol soluble) of panicle from fourteen different Oryza species*

	O. sativa	*O. perennis* subsp. *balunga*	*O. glaberrima*	*O. breviligulata*	*O. officinalis*	*O. alta*	*O. latifolia*	*O. minuta*	*O. malampuzhaensis*	*O. schwein-furthiana*	*O. punctata*	*O. brachyantha*	*O. perrieri*	*O. australiensis*
	AA	AA	A^gA^g	A^gA^g	CC	CCDD	CCDD	CCBB	CCBB	CCBB	BB?	FF	?	EE
1. Glycine	+	+	+	+	+	+	−	+	−	+	+	−	−	+
2. Alanine	+	−	−	−	−	+	+	−	−	+	−	−	−	−
3. Valine	−	−	−	−	+	−	−	−	−	−	+	−	−	−
4. Isoleucine	−	+	−	−	+	+	+	−	+	+	+	−	+	+
5. Leucine	+	−	−	+	+	+	+	+	−	−	−	−	−	−
6. Serine	−	−	−	−	−	+	−	−	−	+	+	−	+	−
7. Threonine	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8. Aspartic acid	−	+	+	+	−	−	−	−	−	−	−	−	−	−
9. Glutamic acid	−	+	+	+	−	−	−	−	−	−	−	−	−	−
10. Lysine	−	+	−	−	−	−	−	−	−	−	+	+	+	−
11. Arginine	+	−	−	+	−	+	−	+	+	+	+	+	+	−
12. Asparagine	+	+	+	+	+	−	−	−	+	−	+	+	+	+
13. Glutamine	+	−	−	−	+	+	+	+	−	+	+	+	+	−
14. Cysteine	−	−	−	+	+	−	−	−	+	−	−	−	−	+
15. Methionine	−	−	+	+	+	+	+	+	−	+	−	−	−	+
16. Tryptophan	−	−	+	−	−	+	−	+	+	−	−	−	−	−
17. Phenylalanine	+	−	−	−	+	−	+	−	−	+	−	−	+	−
18. Tyrosine	+	+	+	−	−	+	+	+	−	−	+	−	−	+
19. Histidine	+	−	+	−	+	−	+	+	+	−	+	−	+	+
20. Proline	−	+	−	+	+	+	+	+	+	+	−	−	−	−
21. Ninhydrin positive spots	2	3	7	4	5	2	6	2	5	4	2	3	2	3

whereas *O. officinalis* and *O. alta* had 12. The most commonly identified aminoacids were threonine, asparagine, glycine, isoleucine, arginine, proline, glutamine, tyrosine, histidine, and methionine. Threonine was present in all the species. Aspartic acid and glutamic acid were consistently absent in *O. officinalis* (CC), *O. minuta* (CCBB), *O. schweinfurthiana* and *O. malampuzhaensis* (CCBB), while proline was absent in *O. perennis* subsp. *balunga* (AA) and *O. breviligulata* (A^gA^g), which are the nearest wild relatives of the cultivated rice, *O. sativa* (AA) and *O. glaberrima* (A^gA^g). In *O. officinalis* (CC), *O. alta* (CCDD), *O. latifolia* (CCDD) and *O. schweinfurthiana* (CCBB), leucine, threonine, methionine and proline were found to be common. The group affinity values for all the 14 species are presented in Table 2. The values ranged from 663 to 888, expressing a more than 70% relationship between them. Paired affinity and paired affinity index (PAI) values are presented in Table 3. The polygon patterns are shown in Fig. 1. The polygon patterns were found to be similar in closely related species. The species, *O. sativa* and *O. perennis* subsp. *balunga*, *O. glaberrima* and *O. breviligulata*, showed identical polygon patterns. The maximum affinity was observed between *O. alta* and *O. latifolia*.

Table 2. *Group affinity values in fourteen different species of genus Oryza*

Species	Panicle *X/1400	Leaf blade *X/1400	Internode *X/1300	Leaf-sheath *X/1200
1. *O. sativa*	757.1	855.1	709.8	627.0
2. *O. perennis* subsp. *balunga*	718.5	736.0	668.0	645.8
3. *O. glaberrima*	747.0	733.0	715.8	572.8
4. *O. breviligulata*	666.0	669.3	454.4	701.5
5. *O. australiensis*	862.0	878.8	840.1	676.7
6. *O. perrieri*	704.51	801.8	—	—
7. *O. brachyantha*	877.4	663.8	821.0	699.8
8. *O. officinalis*	669.7	888.9	697.2	632.5
9. *O. alta*	911.9	837.5	761.4	709.3
10. *O. latifolia*	815.0	802.4	725.3	707.8
11. *O. minuta*	729.0	858.9	764.5	—
12. *O. malampuzhaensis*	812.0	778.1	716.1	666.4
13. *O. schweinfurthiana*	819.0	807.5	725.6	693.7
14. *O. punctata*	853.7	848.6	806.4	692.2

* X = Group affinity value.

Discussion

Consistent similarity in the chromatographic patterns of free aminoacids in the groups, *O. sativa* and *O. perennis* subsp. *balunga*, *O. glaberrima*, *O. breviligulata* and *O. schweinfurthiana*, and *O. minuta* and *O. malampuzhaensis*, suggest close interspecific relationships within them. Alston and Turner (1962)

Table 3. *Paired affinity values of panicle*

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Oriza sativa as base	1 PA	100	42.1	52.6	42.0	63.0	70.0	55.5	63.6	60.8	57.0	70.0	44.4	60.0	66.6
	PAI	20	8	10	8	13	14	11	13	12	11	14	9	12	13
Oryza perennis subsp. *balunga* as base	2 PA	42.1	100	66.6	44.4	66.6	52.6	35.2	47.6	47.6	42.1	42.1	47.0	42.1	60.0
	PAI	11	13	20	11	13	8	5	9	9	8	10	9	6	12
Oryza glaberrima as base	3 PA	52.6	66.6	100	55.5	66.6	42.0	23.5	47.6	45.4	42.0	52.4	47.0	31.5	60.0
	PAI	11	13	20	11	13	8	5	9	9	8	10	9	6	12
Oryza breviligulata as base	4 PA	42.1	44.4	55.5	100	55.5	100	35.2	57.1	45.4	31.5	52.5	47.0	52.5	40.0
	PAI	8	9	11	20	11	2	7	11	9	6	10	9	10 e	8
Oryza australiensis as base	5 PA	63.1	66.6	66.6	55.5	100	52.5	35.2	76.0	54.1	63.1	63.1	70.1	52.5	60.0
	PAI	13	13	13	11	20	11	7	15	11	13	13	14	11	12
Oryza perrieri as base	6 PA	70.0	52.0	42.0	10.5	52.4	100	66.6	54.5	52.1	60.0	50.0	55.5	60.0	76.0
	PAI	14	10	8	2	10	20	13	11	10	12	10	11	12	15
Oryza brachyantha as base	7 PA	55.5	35.2	23.5	35.2	35.2	66.6	100	60.0	28.5	33.3	33.3	50.0	44.4	63.1
	PAI	11	7	5	7	7	13	20	12	6	7	7	10	9	13
Oryza officinalis as base	8 PA	63.6	47.6	47.6	57.1	76.0	54.5	60.0	100	76.0	72.7	63.6	66.6	63.6	60.8
	PAI	13	10	10	11	15	11	12	20	15	15	13	13	13	12
Oryza alta as base	9 PA	60.8	47.6	45.4	45.4	54.0	52.0	28.5	76.0	100	69.5	78.2	52.6	69.5	58.3
	PAI	12	9	9	9	11	10	6	15	20	14	16	10	14	12
Oryza latifolia as base	10 PA	57.0	42.0	42.1	31.5	63.0	60.0	33.0	72.0	69.5	100	70.0	44.4	70.0	47.6
	PAI	11	8	8	6	13	12	7	14	14	20	14	9	14	9
Oryza minuta as base	11 PA	70.0	42	52.5	52.5	61.1	50.0	33.3	63.6	78.2	70.0	100	66.6	60.0	57.1
	PAI	14	8	10	10	13	10	7	15	16	14	20	13	12	11
Oryza malampuzhaensis as base	12 PA	44.0	47.0	47.0	47.5	70.5	55.5	50.0	66.6	52.6	44.4	66.6	100	44.4	42.1
	PAI	9	9	9	9	14	11	10	13	10	9	13	20	9	8
Oryza schweinfurthiana as base	13 PA	60.0	42.0	31.5	52.5	52.5	60.0	44.4	63.6	69.5	70.0	60.0	44.4	100	57.1
	PAI	12	8	6	10	10	12	9	13	14	14	12	9	20	11
Oryza punctata as base	14 PA	66.6	60.0	60.0	40.0	60.0	76.0	63.0	60.8	58.3	47.6	57.1	42.0	57.0	100
	PAI	13	12	12	8	12	15	13	12	12	9	11	8	11	20

PA = Paired affinity value expressed percentage
PAI = Paired affinity value expressed in centimetres.

made similar observations on related species in their comparison of free aminoacids in seeds of *Baptisia* species. The group affinity values of the 14 *Oryza* species exhibit a more than 70% relationship, justifying their classification into a single genus *Oryza*.

The polygon graphic method to represent the results of qualitative aminoacid studies in different species was found to be advantageous. The polygon patterns of free aminoacids in internode, leaf sheath, leaf blade and panicle suggest that species with common genomes exhibit similar patterns. Among the diploids, the four species, *O. sativa*, *O. perennis* subsp. *balunga*, *O. glaberrima* and *O. breviligulata*, seem to be closely related as evidenced by their similar polygon patterns (Fig. 1 — 1, 2, 3, and 4). In the *sativa* complex, the Asiatic forms, *O. sativa* and *O. perennis* subsp. *balunga*, exhibited a slight variation in free aminoacids. However, the polygon patterns of these species were found to be identical. On the other hand, the African forms, *O. glaberrima* and *O. breviligulata*, exhibited close affinities in polygon pattern as well as in free aminoacid composition. The dissimilarities reflected in polygon patterns in other diploid species, namely *O. officinalis*, *O. australiensis*, *O. brachyantha* and *O. perrieri*, suggest that these species are not closely related to other diploid species and may have different genomes (Fig. 1 — 5, 6, 7 and 8).

The close similarities in polygon pattern observed in the allotetraploid species, *O. alta* and *O. latifolia*, suggest that these species have a close relationship and may share a common genome (Fig. 1 — 9 and 10). Similarly, identical polygon patterns and group affinity value in *O. minuta*, *O. schweinfurthiana* and *O. malampuzhaensis* suggest that these species may share the same genome (Fig. 1—11, 12 and 13). The P. A. values above 60% and the general 50% relationship in polygon maps of *O. officinalis* (diploid) with *O. alta* and *O. latifolia* on one hand, and *O. minuta*, *O. schweinfurthiana* and *O. malampuzhaensis* on the other hand, suggest that all these species are related and might share at least one common genome (Table 2, Fig. 1—8, 9, 10, 11, 12 and 13).

Kihara, Nezu and Katayama (1959, 1960, 1961) and Yeh and Henderson (1962) observed regular chromosomal pairing in F_1 hybrids involving *O. sa-*

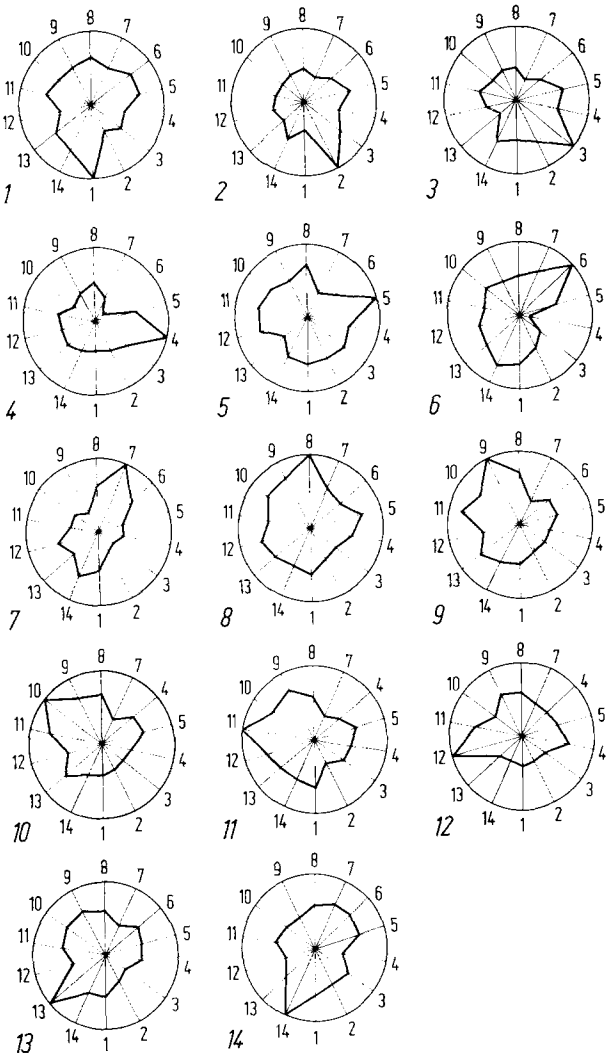

Fig. 1. Free aminoacid polygon patterns of different species of genus *Oryza*

Panicle: 1. *O. sativa*, 2. *O. perennis* subsp. *balunga*, 3. *O. glaberrima*, 4. *O. breviligulata*, 5. *O. australiensis*, 6. *O. perrieri*, 7. *O. brachyantha*, 8. *O. officinalis*, 9. *O. alta*, 10. *O. latifolia*, 11. *O. minuta*, 12. *O. malampuzhaensis*, 13. *O. schweinfurthiana*, 14. *O. punctata*

tiva, *O. perennis* subsp. *balunga*, *O. breviligulata* and *O. glaberrima* and assigned the genome designation "AA" to all four species. The similar polygon maps of free aminoacids for these four species independently confirm this genomic grouping. Nezu *et al.* (1960) and Morinaga *et al.* (1961) observed no chromosomal homology in crosses involving *Oryza officinalis* "CC", *O. australiensis* "EE" and *O. brachyantha* "FF"; the polygon patterns of free aminoacid of these three species (Fig. 1–5, 7 and 8) exhibited significant differences, reflecting the genomic dissimilarities. The F_1 hybrids between *O. alta* × *O. latifolia* revealed that these species had a common genome "CCDD" (Nezu *et al.*, 1960). The close agreement in polygon pattern of free aminoacids of these two species in our studies corroborates their cytogenetic data (Fig. 1–9 and 10). Observations involving crosses between *O. minuta*, *O. schweinfurthiana* and *O. malampuzhaensis* suggest the presence of a similar genome "CCBB" in these species and the identical polygon patterns confirm this observation (Fig. 1–11, 12 and 13). Because of morphological similarities between *O. officinalis* (CC) and *O. punctata* (BB?), previous workers suggested that both these species might have the same genome "CC". However, the present observations on polygon maps of *O. punctata* do not support this contention. On the contrary, these species exhibit some similarity with *O. schweinfurthiana* "CCBB" (Fig. 1–13 and 14).

The present study of species relationships in *Oryza* based on free aminoacid polygon patterns independently confirms some of the earlier cytogenetic studies. The problem of species and their phylogenetic relationships can be further resolved by applying other biochemical parameters, such as phenolic compounds, isoenzymes and the electrophoretic pattern of proteins, etc.

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