

Taxonomic status of *Oryza glumaepatula* Steud. I. Comparative morphological studies of New World diploids and Asian AA genome species

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Abstract

This study was carried out to clarify the taxonomic status of the New World diploid wild rice species, *Oryza glumaepatula*. The morphological variation of 26 diploid rice accessions in the International Rice Genebank at IRRI from South America and Cuba was compared with that of *O. rufipogon* and *O. nivara* from Asia. The 28 morphological characters included 16 spikelet and grain, eight leaf and culm, and four panicle characters, and were analyzed using principal component analysis (PCA) and hierarchical agglomerative cluster analysis. The first two principal components accounted for 53.6% of the total variation. The first component was characterized by leaf and sterile lemma characters, 5-panicle dry weight, and grain length, and the second by anther length and its ratio to grain length, grain width, thickness, and 20-hulled grain weight. The characters effectively differentiated the wild rices from Surinam, French Guiana, and the lower Amazon River basin in Brazil, which showed high negative scores along both axes, from other accessions from Venezuela, Colombia, and Cuba, and the Asian species. Most of the accessions from South America are quite distinct from *O. rufipogon*, with which they have often been grouped as a single species in some taxonomic treatments. Groupings obtained from cluster analysis corresponded closely with the results from PCA. This study supports a distinct taxonomic status of a group of diploid wild rices from South America as *O. glumaepatula*.

Introduction

Four wild *Oryza* species are found in the New World. Three of these, *O. alta* Swallen, *O. latifolia* Desv., and *O. grandiglumis* (Doell) Prod. are tetraploid and have been assigned the CCDD genome. The other species is a diploid, and is generally accepted as a close ally of the AA genome species of Asia and Africa, but its taxonomic status has been the subject of contention in recent years. It was originally classified as *O. glumaepatula* Steud.

Tateoka (1962) reported that *O. glumaepatula* had no clear morphological distinction from *O. rufipogon* Griff., and therefore they must be considered as conspecific. In a dichotomous key for wild rices, Chang (1976) used the character 'found in South America' to differentiate *O. glumaepatula*. Vaughan (1994) treated *O. glumaepatula* as an American form of *O. rufipogon*, although he had considered it a distinct species in an

earlier review of the genus *Oryza* (Vaughan, 1989). *O. glumaepatula* is reported to form an intrafertile group (Chu et al., 1969) but is isolated reproductively from the other AA genome forms by F₁ sterility (Chu et al., 1969; Morishima, 1969). Morishima (1969) previously treated it as the American form of *O. perennis* (= *O. rufipogon*), but recently adopted the name *O. glumaepatula* (Morishima 1994).

In the collection of the International Rice Genebank at the International Rice Research Institute (IRRI), Los Baños, Philippines, there are at least thirty diploid accessions from South America and the Caribbean. We conducted this study to clarify the taxonomic status of these samples from the Americas relative to *O. rufipogon* and *O. nivara* Sharma et Shastry from Asia, using comparative morphological variation.

Table 1. Diploid wild rices from the New World scored for morphological characters.

IRGC accession number	Country	District/Province	Latitude & Longitude	Site
100924	Brazil	Manaus/Amazonas	–	–
100970	Brazil	Manaus/Amazonas	3° 00' S 60° 00' W	along Rio Solimoes
100971	Brazil	Manaus/Amazonas	3° 00' S 60° 00' W	river side, Rio Negro
101960	Brazil	–	–	–
104387	Brazil	–	–	–
105662	Brazil	Macapa/Amapa	0° 7' N 51° 05' W	swamps formed by streams
105663	Brazil	Monte Alegre/Para	2° 15' S 54° 10' W	edge of small stream
105665	Brazil	Caucau pereri/Amazonas	3° 10' S 60° 00' W	river edge
105666	Brazil	Caceiro/Amazonas	3° 10' S 59° 45' W	small pond
105667	Brazil	Ariau/Amazonas	3° 15' S 60° 30' W	stream
105668	Brazil	Ariau/Amazonas	3° 15' S 60° 30' W	edge of lake
105670	Brazil	Manacapuru/Amazonas	3° 15' S 60° 40' W	lake edge
105672	Brazil	Manacapuru/Amazonas	3° 15' S 60° 40' W	lake edge
105686	Brazil	Monte Alegre/Para	2° 15' S 54° 10' W	varzea lake
105687	Brazil	Marcuri/Para	2° 15' S 54° 10' W	varzea lake
105688	Brazil	Santarem/Para	2° 10' S 54° 10' W	edge of Amazon lake
105689	Brazil	Caceiro/Amazonas	3° 10' S 59° 45' W	edge of lake
105692	Brazil	Makapa/Amapa	0° 00' S 51° 12' W	varzea lake
105561	Colombia	Meta/–	4° 10' N 74° 00' W	swampy pasture near shallow pond
100961	Cuba	–/Sta Clara	22° 30' N 80° 00' W	muddy swamp adjacent to rice field
105465	French Guiana	–	–	–
100968	Surinam	45 km S of Paramaribo/–	6° 00' N 55° 00' W	pond
100969	Surinam	45 km S of Paramaribo/–	6° 00' N 55° 00' W	swamp
103810, 103811, 103812	Venezuela	–	–	–

Table 2. Origin of IRGC accessions of *O. nivara* and *O. rufipogon*.

Country	<i>O. nivara</i>	<i>O. rufipogon</i>
Bangladesh	102468, 103830, 105895	105890
Cambodia	105721	105720
China, People's Republic of	103821	105402
India	101967, 106185	100208, 101965, 106135
Indonesia	–	105567, 105953
Lao PDR	106148, 106154	106161
Malaysia	–	106036
Myanmar	106345	–
Nepal	–	105696
Papua New Guinea	–	106275, 106278
Philippines	–	103305
Sri Lanka	103407, 105431	105214
Taiwan	100593	100588, 100678
Thailand	105391, 105815, 105834	104714, 105759, 105942
Vietnam	–	106166, 106168

Table 3. Morphological characters of diploid *Oryza* species from the New World and Asia scored at specific growth stages.

Time of scoring	Character
30 days after transplanting	number of tillers
During flowering	stigma length
	style length
	anther length
	apiculus length
	awn length
15 days after flowering	flag leaf length
	flag leaf width
	second leaf length
	second leaf width
	second leaf ligule length
	culm length
	culm diameter
	number of panicles
	panicle length
	After harvest
grain length	
grain width	
grain thickness (dorso-ventral)	
anther length/grain length	
1st sterile lemma length	
1st sterile lemma width	
second sterile lemma length	
second sterile lemma width	
20-grain weight (unhulled)	
20-grain weight (hulled)	
panicle fertility	
5-panicle dry weight	

Table 4. The eigenvectors of the correlation matrix for 28 characters on 64 accessions including New World diploids, *O. nivara*, and *O. rufipogon*.

Variable	Principal Components ^a	
	1st (9.776; 34.9%)	2nd (5.249; 18.7%)
2nd sterile lemma width	-0.274	0.025
1st sterile lemma width	-0.274	0.049
second leaf length	-0.258	-0.016
flag leaf width	-0.254	-0.100
2nd sterile lemma length	-0.252	-0.079
1st sterile lemma length	-0.247	-0.075
grain length	-0.247	-0.141
second leaf width	-0.244	-0.141
panicle dry weight	-0.244	0.175
culm diameter	-0.228	0.038
second leaf ligule length	-0.220	-0.117
20-unhulled grain weight	-0.220	0.270
panicle length	-0.211	-0.085
awn diameter	-0.196	-0.005
20-hulled grain weight	-0.192	0.305
spikelet fertility	-0.188	0.027
flag leaf length	-0.166	0.076
number of tillers	0.151	0.206
grain width	-0.132	0.334
number of panicles	0.106	0.298
culm length	-0.103	-0.215
grain thickness	-0.095	0.351
anther length	-0.071	-0.358
apiculus length	-0.042	0.014
awn length	-0.041	-0.053
style length	0.030	-0.017
anther length/grain length	0.017	-0.341
stigma length	0.014	-0.223

^a Values in parentheses correspond to the eigenvalue and proportion of total variation accounted for by each component.

Materials and methods

Plant materials

We included 25 diploid wild rice samples from five countries in South America, and one from Cuba (Table 1). Twenty-two accessions of *O. rufipogon* and 16 accessions of *O. nivara* were randomly sampled from the International Rice Genebank collection to cover the wide geographical range of these species across Asia and Papua New Guinea (PNG) (Table 2).

Seed dormancy was broken by heat treatment at 50 °C for 7 days, and hull removal. Accessions with weak seeds were germinated on MS medium (Murashige and Skoog, 1962), transferred to culture solution (Yoshida et al., 1976) at the 2-leaf stage, and allowed to grow in a cabinet under controlled con-

ditions (21 °C/29 °C, 70% RH, 12h/12h 1000 $\mu\text{E m}^{-2}\text{s}^{-2}$ light intensity). Accessions with high viability seeds were germinated at room temperature (28 ± 1 °C) in petri dishes lined with moist filter paper, and transferred to seed boxes immediately after germination. All seedlings were transplanted singly to 30 cm clay pots 24 days after seeding, and maintained in a screenhouse. Normal control of pests and diseases was applied throughout the growing period from September to April, the optimum time to cultivate the wild species in Los Baños.

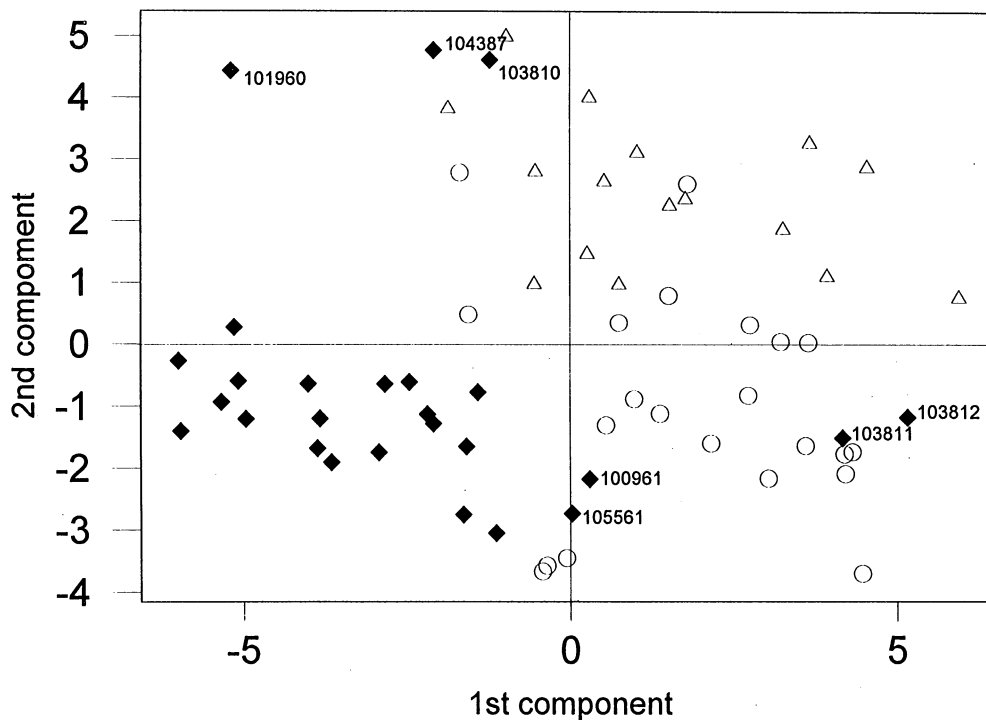


Fig 1. Scatter plot expressing the morphological variation of 64 diploid *Oryza* accessions from the New World and Asia, based on mean values, obtained from the first two components of Principal Components Analysis (◆ = diploids from the New World; ○ = *O. rufipogon*; △ = *O. nivara*).

Experimental design, morphological characterization, and analysis of data

Each accession was replicated four times with each replicate being a single plant arranged in randomized complete block design, with pots spaced 85 cm apart. Each plant was scored for 28 morphological characters at the appropriate growth stage, or after harvest (Table 3). Principal component analysis (PCA) was carried out using the MINITAB statistical package (MINITAB, 1994) and cluster analysis was performed using NTSYS-pc (Rohlf, 1994). Mean values per accession were used and standardized prior to analysis. Cluster analysis was carried on the dissimilarity matrix of Euclidean distances with UPGMA as the clustering algorithm.

Results

Nineteen of the 26 New World diploid accessions were clearly distinct from *O. rufipogon* and *O. nivara* as revealed by Principal Components Analysis (PCA).

PCA showed that the first seven principal components had eigenvalues greater than 1 accounting for 82.0% of the total variation. The first and second principal components accounted for 34.9 and 18.7% of the total variation, respectively (Table 4). The first component was characterized by the length and width of the pair of sterile lemmas, second leaf length, flag leaf width, and grain length. The second component was heavily weighted by anther length and its ratio to grain length, grain width and thickness, and 20-hulled grain weight.

Figure 1 shows the plot obtained from the first two vectors of PCA. Most diploid accessions from Brazil, the two accessions from Surinam and the single accession from French Guiana formed a distinct group with negative scores along both axes. These accessions showed a tendency towards long and flat grains, a long and wide pair of sterile lemmas, a long second leaf, and a wide flag leaf. Two accessions, IRGC 100961 from Cuba and IRGC 105561 from Colombia, also had negative scores along the second axis although they had low positive scores along the first. These two accessions also had similar anther length and grain width as the main group of South American diploids, but a

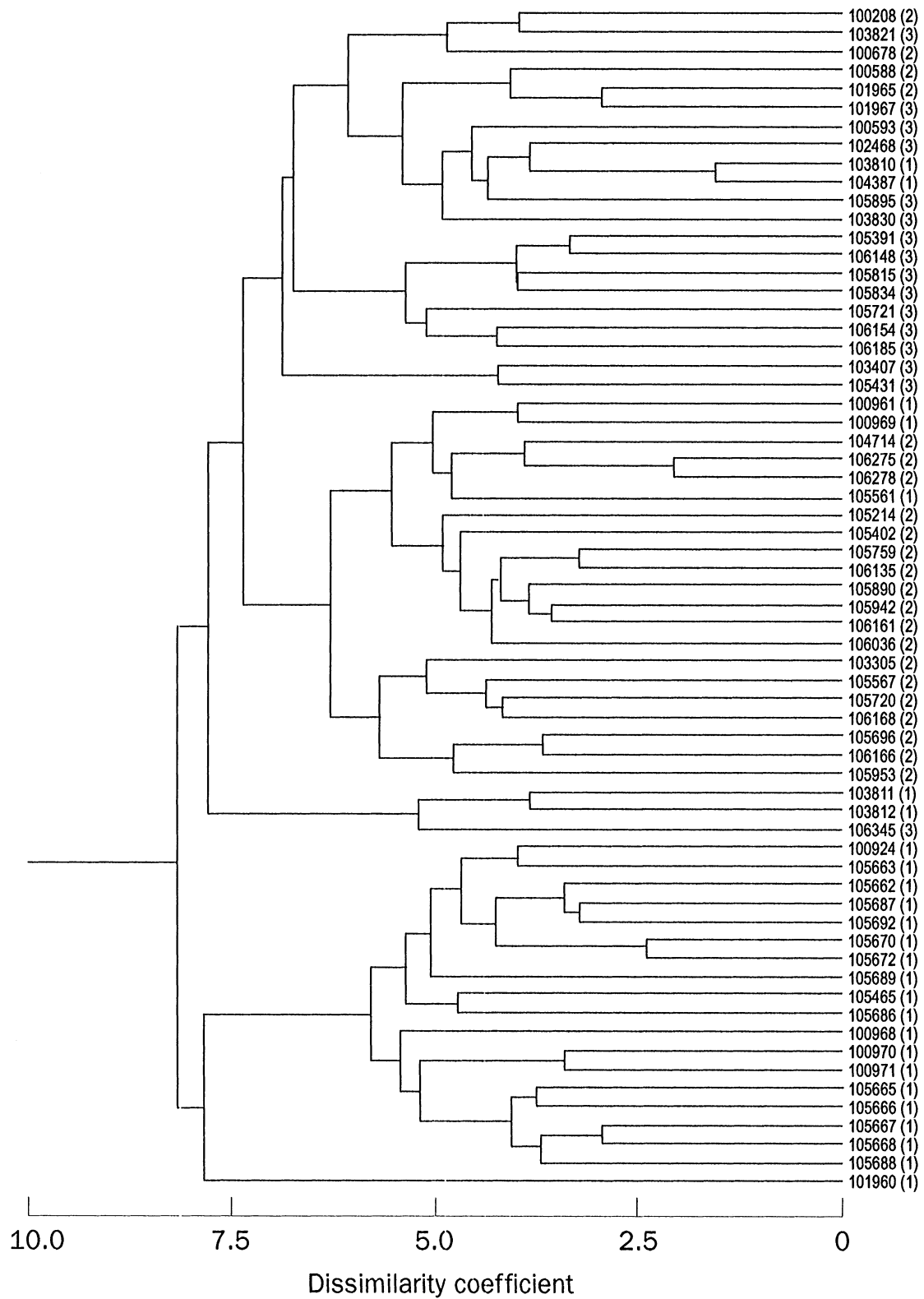


Fig 2. Dendrogram obtained for 64 diploid *Oryza* accessions from the New World and Asia after Cluster Analysis of morphological data from 28 characters. (1) = New World diploids; (2) = *O. rufipogon*; (3) = *O. nivara*.

shorter and narrower pair of sterile lemmas, and a narrower flag leaf. The two Venezuelan accessions, IRGC 103811 and IRGC 103812, had high positive scores along the first axis but negative scores on the second. They had very short anthers and small grains. The Brazilian accession IRGC 104387 and the Venezuelan accession IRGC 103810 also had negative scores along the first axis, but had high positive scores on the second along with the *O. nivara* accessions from Bangladesh, IRGC 102468 and IRGC 105895. These accessions had short anthers, and wide, thick, and short grains. One accession from Brazil (IRGC 101960) was clearly distinct from all other accessions, both South American and Asian, and had a high negative score on the first axis and high positive score along the second axis. It had long, wide, and thick grains, as well as long and wide sterile lemmas.

Most *O. rufipogon* accessions had positive scores along the first axis and negative scores on the second. They had narrow grains, short and narrow sterile lemmas, long anthers, and a narrow second leaf. Three *O. rufipogon* accessions IRGC 104714 (Thailand), and 106275 and 106278 (PNG) showed very low negative scores along the first axis and high negative scores on the second, and had characteristically long anthers, and a high anther length to grain length ratio. The *O. rufipogon* accessions IRGC 100208, 100678, 105214, 105759, 105890, and 106166 had positive scores along both axes, and overlapped with most of the *O. nivara* accessions. They generally had short anthers, wide, thick, and short grains, and short and narrow sterile lemmas.

In the cluster analysis, five clusters can be identified at a dissimilarity level of 7.341 (Figure 2). Only one accession, IRGC 101960 from Brazil, formed the first cluster. The second cluster was made up of 16 accessions from the lower Amazon River basin in Brazil, and an accession each from Surinam and French Guiana. The third cluster consisted only of the two Venezuelan accessions IRGC 103811 and 103812, and one *O. nivara* accession IRGC 106345. Eighteen *O. rufipogon* accessions, together with an accession each from Cuba (IRGC 100961), Surinam (IRGC 100969), and Colombia (IRGC 105561) comprised the fourth cluster. The fifth cluster consisted of 15 *O. nivara* accessions, four *O. rufipogon* accessions, and an accession each from Venezuela (IRGC 103810) and Brazil (IRGC 104387).

Discussion

We employed multivariate analysis to compare the morphological variation in diploid wild rices from the New World with that of *O. rufipogon* and *O. nivara* from a wide geographical range in Asia. Our study has shown that the majority of the South American samples, particularly those from the lower Amazon River basin in Brazil, and coastal Surinam and French Guiana are morphologically distinct from *O. rufipogon* and *O. nivara*.

The original description of *O. glumaepatula* by Steudel in 1854, as cited by Chevalier (1952), states that the type specimens came from Dutch and British Guiana (= Surinam and Guyana, respectively) and had compact panicles about 20 cm long, flat glumes bearing white sterile lemmas about 3 mm long and awns 4–5 cm long. Our results confirm the existence of a group of diploids, mainly from Brazil that match the original description, defined by the first two vectors of the PCA, and as a distinct group in the cluster analysis. Although the accessions from Brazil showed intermediate types of panicles, those from French Guiana and Surinam showed more or less compact panicles. All accessions in this group showed a tendency towards flat grains, with long and wide sterile lemmas, a wide flag leaf and long and wide second leaves. A well-defined group consisting of similar accessions was obtained by cluster analysis using RFLP markers (Doi et al., 1996) and RAPD markers (Martin et al., 1997).

In the lower Amazon River basin, forms described as *O. glumaepatula* grow in close proximity with the tetraploid species *O. alta* and *O. grandiglumis* in sunny locations on the edges of seasonally-flooded depressions, or *varzea* lakes, and along rivers and streams in 1–2 m of white water (nutrient-rich) or black water (nutrient-poor) (Nowick and Groth, 1987). Similar forms have been reported along the Araguaia River in central north-east Brazil (Marcio Ferreira, personal communication). Morishima (1994) also reported the unique ability of *O. glumaepatula* for tiller separation in water due to its brittle culm, allowing extensive vegetative propagation. However, the mode of reproduction is mainly by seeds which occurs when the water level subsides. Morphological differentiation in *O. glumaepatula* is apparently accompanied by a distinct mode of propagation and ecological preference.

The variation observed among the diploid American rices was detected earlier at the molecular level by Pental and Barnes (1985) and Ishii and co-workers (1996). IRGC accessions 104387 and 103810 (Cluster

5) had wide, thick, and short grains characteristic of *O. nivara* but had the wide sterile lemmas characteristic of *O. glumaepatula*. In a hybridization study (Naredo et al., 1998), IRGC 103810 and IRGC 104386 (an accession from Brazil not included in this study) formed fertile hybrids with the Asian species, but were isolated by an F₁ sterility barrier from the main *O. glumaepatula* group. These accessions might have been produced by hybridization between *O. sativa* and indigenous American diploid rices following the introduction of the cultivated form to the Americas. A similar observation has also been reported to us in this respect (H. Morishima, personal communication). Rice was introduced by the Spaniards to Central America and some parts of South America and by the Portuguese to Brazil (Grist, 1965), and was cultivated mainly in Brazil as a dry-land crop by slash and burn methods (Grigg, 1974).

IRGC 103811 and 103812 (Cluster 3) varied from the 'typical' *O. glumaepatula* by having shorter anthers and shorter and narrower leaves, grains and sterile lemma. IRGC 100961, 105561, and 100969 (Cluster 4) had flat and narrow grains with narrow and short sterile lemmas that were morphologically similar to *O. rufipogon*. Second (1985) also reported similarities of the American diploids and Asian AA genome forms at the isozyme level, leading him to suggest that they had not evolved independently. He concluded that the American form was a recent introduction to the Americas. However, these accessions cannot be considered as true *O. rufipogon* because, in hybridization studies, they are reproductively isolated from the Asian AA genome species but not from the 'typical' *O. glumaepatula* (Naredo et al., 1998).

In this study we have shown that a group of diploid wild rices from South America, particularly from the lower Amazon River basin, are different from the Asian *O. rufipogon*, and their recognition as *O. glumaepatula* is justified. Further studies must be conducted to verify the taxonomic status of the other South American diploids that are morphologically different from *O. glumaepatula*.

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References

- Chang, T.T. 1976. Manual on genetic conservation of rice germplasm for evaluation and utilization. International Rice Research Institute, P.O. Box 933, Manila, Philippines.
- Chevalier, A. 1952. Nouvelle contribution a l'étude systématique des *Oryza*. Rev. Bot. Appl. Agric. Trop. 12: 1014–1032.
- Chu, Y.E., H. Morishima & H.I. Oka. 1969. Reproductive barriers distributed in cultivated rice species and their wild relatives. Japan J. Genetics. 44: 207–223.
- Doi, K., A. Yoshimura, M. Nakano, N. Iwata & D.A. Vaughan. 1996. Classification of A genome species in the genus *Oryza* using nuclear DNA markers. Int. Rice Res. Notes. 21: 8–10.
- Grigg, D.B. 1974. The Agricultural Systems of the World – An Evolutionary Approach. Cambridge University Press, London.
- Grist, D.H. 1965. Rice. Longmans, Green and Co. Ltd., London.
- Ishii, T., T. Nakano, H. Maeda & O. Kamijima. 1996. Phylogenetic relationships in A-genome species of rice as revealed by RAPD analysis. Genes Genet. Syst. 71: 195–201.
- Martin, C., A. Juliano, H.J. Newbury, B-R Lu, M.T. Jackson & B.V. Ford-Lloyd. 1997. The use of RAPD markers to facilitate the identification of *Oryza* species within a germplasm collection. Genet. Res. and Crop Evol. 44: 175–183.
- MINITAB 1994. MINITAB Statistical Software Release 10 for Windows. MINITAB Inc.
- Morishima, H. 1969. Variations in breeding system and numerical estimation of phylogeny in *Oryza perennis*. Japan J. Genetics 44: 317–324.
- Morishima, H. 1994. Background information about *Oryza* species in tropical America. In: Investigations of plant genetic resources in the American basin with emphasis on the genus *Oryza*: Report of 1992/93 Amazon Project: Monbusho International Scientific Res. Prog.
- Murashige, T. & F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant. 15: 473–497.
- Naredo, M.E.B., A.B. Juliano, B-R. Lu & M.T. Jackson. 1998. Taxonomic status of *Oryza glumaepatula* Steud. II. Hybridization between New World diploids and AA genome species from Asia and Australia. Genet. Res. and Crop Evol.
- Nowick, E. & D. Groth. 1987. Plant exploration report. March 15 – April 20, 1987. (mimeographed paper).
- Pental, D. & S.R. Barnes. 1985. Interrelationship of cultivated rices *Oryza sativa* and *O. glaberrima* with wild *O. perennis* complex. Theor. Appl. Genet. 70: 185–191.
- Rohlf, F.J. 1994. NTSYS-pc Numerical Taxonomy and Multivariate Analysis System Version 1.80. Exeter Software New York.
- Second, G. 1985. Evolutionary relationships in the *Sativa* group of *Oryza* based on isozyme data. Genet. Sel. Evol. 17: 89–114.
- Tateoka, T. 1962. Taxonomic studies of *Oryza* II. Several species complexes. Bot. Mag. Tokyo 75: 455–461.
- Vaughan, D.A. 1989. The genus *Oryza* L.: Current status of taxonomy. IRRI Research Paper Series. 138, Manila, Philippines.
- Vaughan, D.A. 1994. The Wild Relatives of Rice: A Genetic Resources Handbook. IRRI, Manila Philippines.
- Yoshida, S., D.A. Forno, J.H. Cock & K.A. Gomez. 1976. Routine procedures for growing rice plants in culture solution. In: Laboratory Manual for Physiological Studies of Rice, IRRI, Manila, Philippines.