Assessment of the Genetic Diversity in Argentine Rice Cultivars with SSR Markers

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ABSTRACT

Genetic diversity of rice (*Oryza sativa* L.) cultivars that are historically significance to rice breeding and production in Argentina were evaluated at the DNA level. Sixty-nine accessions were surveyed with 26 simple sequence repeat (SSR) markers revealing the genomic relationship among cultivars. A total of 219 polymorphic bands were detected. Cluster analysis based on pair-wise comparisons of cultivar genetic similarities resolved the *O. sativa* accessions into two major *O. sativa* groups, *indica* and *japonica*, and the *japonica* group into the subgroups, *tropical* and *temperate*. These clusters agree with the pedigree information available on the accessions and almost all Argentina-released cultivars grouped within the *japonica* cluster. Application of DNA polymorphism analysis revealed genomic relationships in Argentine rice germplasm, generating a database useful for cultivar identification, local germplasm conservation, and breeding programs.

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Abbreviations: GS, genetic similarity; HG, accession heterogeneity; PIC, polymorphism information content; SSR, simple sequence repeat; UPGMA, unweighted pair-group method with arithmetic averages.

A detailed understanding of the extent and structure of crop genetic diversity is necessary for effective management and use of crop germplasm resources (Brown and Kresovich, 1996). DNA-based markers such as SSR markers (or microsatellites), which are codominant and highly polymorphic, offer an easy, accurate, and quantifiable measure of the genetic variation within crop plants (Litt and Luty, 1989; Tautz, 1989). For rice there are nearly 15 000 SSRs now available (www.gramene.org; verified 22 Nov. 2006) and these are currently being used to develop high-density genetic maps, genotype rice accessions, determine the genetic structure and diversity patterns, optimize the assembly of core collections, and for marker-assisted breeding (McCouch et al., 2002; Yu et al., 2003; Garris et al., 2005).

Rice is grown worldwide and is frequently identified by the subspecies *indica* and *japonica* (Jackson, 1997; Khush, 1997). In Argentina, rice is cultivated in the Northeast region of the country between 27°S and 33°S latitude, predominantly, in the provinces of Entre Ríos and Corrientes. Rice breeding began in the early 1940s, when new cultivars (e.g., Blue Rose M.A.) adapted to the

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Table 1. Description of the rice cultivars used to study simple sequence repeat (SSR) marker variations, including origin, pedigree, and percentage of heterogeneity (HG) by accession across 26 SSR markers.

No.	Name	Pedigree†	Origin†	Grain type§	Average GS by cultivar‡	HG (%)
1	Altamirano P. A.	Dawn//Zenith/Yeruá P. A.	Argentina	LW	0.40 (0.49)	0
2	Argo	Raffael/Stirpe 224//Stirpe 244	Italy	M	0.29	10.3
3	Ariete	Unknown	Italy	LF	0.15	3.4
4	Arroyo Grande P. A.	Unknown	Argentina	LW	0.19 (0.41)	20.7
5	Artico	Unknown	Italy	M	0.30	0
6	Bluebelle	Rexark Rogue//Century Patna 231/CI9122	U.S.A	LF	0.39	0
7	Bluebonnett 50- INTA	Bluebonnet (Rexoro/Fortuna) selection	Argentina	LF	0.40 (0.43)	0
8	Calá P. A.	L. P. Mocoretá F. A.//Zenith/ Blue Rose	Argentina	MW	0.34 (0.44)	0
9	Colonia Mascias	Unknown	Argentina	LF	0.23 (0.15)	27.6
10	Colonia Mascias 5 C. A.	L. P. Gualeyán F. A./Dawn	Argentina	LF	0.40 (0.45)	0
11	CT6919	IRGA 409/Lemont//BG374–1	Brazil	LF	0.21	3.4
12	Cumeman F. A.	Blue Rose/Bertone	Argentina	M	0.37 (0.48)	13.8
13	Cupalén P. A.	Gulfrose//Rizzotto/L. P. Yuquerí	Argentina	MW	0.34 (0.43)	3.4
14	Cypress	L 202/Lemont	USA	LF	0.34	0
15	Chacarero P. A.	Blue Rose/Bertone	Argentina	M	0.32 (0.42)	0
16	Chajarí P. A.	Norim 17/L. P. Ayuí F. A.	Argentina	S	0.31 (0.37)	0
17	Dawn	Century Patna 231/HO 12–1–1	USA	LF	0.38	10.3
18	Don Juan INTA	Arroyo Grande P. A./Lemont	Argentina	LF	0.42 (0.48)	0.0
19	El Paso L-144	IR930–2/IR665–31–2–4	Uruguay	LF	0.26	0.0
20	El Paso L-227	CI 9902/Labelle	Uruguay	LF	0.38	3.4
21	Embrapa 6 Chui	IR 930–53/IR 665–31–24	Brazil	LF	0.27	0
22	Embrapa 7 Taim	Unknown	Brazil	LF	0.23	0
23	Entrerriano P. A.	L. P. Ayuí F. A./Fujizaka N° 5	Argentina	M	0.30 (0.36)	0
24	Epagri 106	P3085-F4–54/CT6771	Brazil	LF	0.26	3.4
25	Epagri 108	CT7347/IR21015–72–3–3–3–1	Brazil	LF	0.22	0
26	Epagri 109	CT7347/IR21015–72–3–3–3–1	Brazil	LF	0.21	0
27	Fortuna INTA	Selection from Fortuna	Argentina	LW	0.36 (0.45)	0
28	Guaviraví	Unknown	Argentina	LF	0.32 (0.39)	0
29	Guayquiraró P. A.	IR224–54–3–3–1/Gulfrose// Rikuu 132/L. P. Ayuí F.A.	Argentina	LF	0.29 (0.32)	0
30	Illabong	St. Andrea/M7	Australia	LW	0.32	17.2
31	INIA Tacuarí	Newbonnet/Newrex L 79	Uruguay	LF	0.40	0
32	Iñacorá sel. Drew	Unknown	Argentina	LW	0.35 (0.43)	17.2
33	IR1529	IR305–3–17–1–3/IR24	Philippines	LF	0.26	3.4
34	IRGA 409	IR930–2/IR665–31–2–4	Brazil	LF	0.27	13.8
35	IRGA 414	IR930–2/IR665–31–7–4	Brazil	LF	0.25	0
36	IRGA 417	New Rex/IR19743–25–2–2// IRGA 409	Brazil	LF	0.24	0
37	Japones de Aragón	Unknown	Unknown	S	0.26	0
38	Katy	Bonnet 73/CI9722//Startbonnet/Tetep/3/Lebonnet	USA	LF	0.35	0
39	L. P. Gená F. A.	Cumeman/Zenith	Argentina	S	0.35 (0.46)	0
40	L. P. Gualeyán F. A.	Cumeman/Zenith	Argentina	M	0.37 (0.48)	0
41	L. P. Itapé F. A.	Cumeman/Zenith	Argentina	M	0.33 (0.42)	0
42	L. P. Mocoretá F. A.	Cumeman/Zenith	Argentina	M	0.35 (0.45)	0
43	L. P. Mochi F. A.	H180–9–3–2//H161- 18/ unknown/80–16–2	Argentina	S	0.35 (0.42)	3.4

northern subtropical climate (ca. 27°S latitude) were obtained from selections in rice cultivars coming from Brazil and the USA (M.A. Marassi, unpublished). Subsequently, cultivars were developed from germplasm introduced mainly from Italy for the southern regions (33°N latitude). More recently Argentine rice breeding programs have concentrated on improving grain quality, tolerance to adverse soil conditions, and disease resistance.

Cuevas-Pérez et al. (1992) studied the genetic base of irrigated rice in Latin America and the Caribbean, and noted that materials released in Argentina between 1971 and 1989 have a very low coefficient of parentage compared with materials from the other countries in the Latin America region. Even though studies to asses the genetic diversity within *O. sativa* collections utilizing isozyme, restriction fragment length polymorphism (RFLP) and SSR markers have been conducted (Glaszmann, 1987; Yang et al., 1994; Xu et al., 2004; Lu et al., 2005; Garris et al., 2005) there has been no reported systematic survey of Argentine rice germplasm at the DNA level. The objectives of this study was to use SSR markers to (i) quantify the allelic diversity, (ii) estimate the genetic diversity, and (iii) determine the genetic relationship between 38 Argentine rice cultivars and 29 plant introductions in the Argentine national rice collection.

MATERIALS AND METHODS

Plant Material

Sixty-seven rice accessions of historical significance to rice breeding and production in Argentina were selected for SSR screening (Table 1). This material includes most rice cultivars from Argentina registered in the National Seed Property Register (INASE) and released in the last six decades, progenitors of modern commercial cultivars, breeding lines, plant introductions, and materials that carry traits of particular interest for rice improvement. Also 'IR36' and one accession of the African cultivated rice, *O. glaberrima* Steud. were included in this study (Table 1). Seeds were kindly provided by the National Institute of Agricultural Technol-

Table 1. Continued

No.	Name	Pedigree†	Origin†	Grain type§	Average GS by cultivar‡	HG (%)
44	L. P. Yuquerí F. A.	Blue Rose/Bertone	Argentina	S	0.32 (0.42)	0
45	L-1435	Unknown	Uruguay	LF	0.25	6.9
46	La Candelaria F. A.	Ñancay P.A./Dawn//Basmati 370	Argentina	LF	0.40 (0.47)	0
47	Lampo	Selection from Kenya rice	Italy	LF	0.16	3.4
48	Lemont	Lebonnet//CI9881/IR659–10–8–3	USA	LF	0.40	3.4
49	Lucas P. A.	CI9453/CI9187//Blubonnet 50 INTA	Argentina	LF	0.37 (0.44)	0
50	Mandisoví P. A.	Chocoto/(IR1103–15–10/Calady 40)//unknown/80– 17	Argentina	LF	0.37 (0.42)	0
51	Maybelle	Skybonnet/L201	USA	LF	0.36	0
52	Mocoi F. C. A	Guayquiraró/Nucleoryza//Calady 40/IR1103–15–10	Argentina	LF	0.35 (0.39)	0
53	Montiel P. A.	L. P. Mocoreta F. A.//Chacarero F. A./Raza 82	Argentina	LW	0.38 (0.43)	0
54	Ñancay P. A.	Century Patna 231/L.P. Gualeyán F. A.	Argentina	M	0.37 (0.46)	10.3
55	Oryzica Llanos 5	P5269/P2060-F4–2–5–2	Colombia	LF	0.32	10.3
56	Palmar P. A.	L. P Itapé F. A./Bluebonnet 50 INTA	Argentina	LF	0.35 (0.42)	13.8
57	Peteí F. C. A.	Quella/Guayquiraró P. A.	Argentina	LF	0.33 (0.37)	0
58	Quebracho P. A.	Chocoto/(IR1103–15–10/Calady 40)//unknown/80–17	Argentina	LF	0.38 (0.43)	0
59	Rice Purple	Unknown	Unknown	LF	0.19	10.3
60	RP1	Fortuna/Yeruá P. A.	Argentina	LW	0.35 (0.46)	0
61	RP2	IR8//Peta/Belle Patna	Brazil	LF	0.21	17.2
62	San Miguel INTA FECOAR	Selection from Bluebelle	Argentina	LF	0.40 (0.43)	0
63	Santa Fe Capiaguí C. A.	Unknown	Argentina	LF	0.36 (0.39)	0
64	Taiperó P. A.	Rizzotto/Blue Rose selection M. A.	Argentina	LW	0.37 (0.44)	0
65	Victoria F. A.	Blue Rose/Bertone	Argentina	M	0.30 (0.39)	0
66	Villaguay P. A.	H122/H124	Argentina	LW	0.37 (0.44)	0
67	Yeruá P. A.	Selection from Fortuna INTA	Argentina	LW	0.38 (0.50)	0
	IR36	IR1561–288–1–2/IR1737//CR94–13	Philippines	LF		3.4
	O. glaberrima Steud.	IRGC100127				

†Unknown: missing data or under patent.

‡Average genetic similarity (GS) by cultivar is based on pair-wise comparisons of each cultivar with the other 66 cultivars. Numbers in parentheses correspond to average GS for Argentine cultivars based on comparisons of each cultivar with the other 37 cultivars analyzed.

§LF, LW, M, MW, and S correspond to long fine, long wide, medium, medium wide, and short grain type, respectively.

ogy (EEA INTA Concepción del Uruguay, Entre Ríos, Argentina), Estación Experimental de Arroz Julio Hirschhorn (Facultad de Agronomía, Universidad Nacional de La Plata, Buenos Aires, Argentina), IBONE (Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Corrientes, Argentina), CIAT (Cali, Colombia), Department of Plant Breeding and Biometry (Cornell University, Ithaca, NY), and IRRI (Los Baños, Philippines).

DNA Extraction and SSR Analysis

Plants were grown in a growth chamber under controlled conditions of light and temperature. Leaves of 15-d-old seedlings were collected, frozen in liquid N, and stored at −80°C until used. Total genomic DNA was extracted from a bulk of leaves from at least five plants of each cultivar, according to Fulton et al. (1995). Twenty-six SSR markers (Chen et al., 1997), representing the 12 rice chromosomes, were used for genotyping (Table 2). A total of 28 loci were analyzed, considering that RM4 and RM20 are double-locus markers. Further information on the (i) primer sequences, PCR conditions, and type of repeat for the SSR markers and (ii) allele sizes of reference cultivars is available at http://www.gramene.org/microsat (verified 22 Nov. 2006). PCR products were separated on a 4% polyacrylamide sequencing gels containing 7 *M* urea, with multiple loading. DNA fragments were revealed using the silver staining procedure (Panaud et al., 1996). The size of the amplified fragments was determined by measuring migration distances of SSR alleles on silver staining gel photographs in relation to known fragment-length standards (10-bp DNA ladder from Gibco-BRL, Grand Island, NY, and DNA molecular weight marker V and VIII from Roche Applied Science, Indianapolis, IN). IR36 was the reference cultivar because sequence-based estimates of allele size were available (Panaud et al., 1996; Chen et al., 1997). When a null allele was detected, the result was confirmed by three independent PCR amplification reactions. Unique alleles were defined as those detected in only one accession.

Data Analysis

The presence or absence of alleles for each SSR marker was recorded for all accessions and then converted to a genetic similarity (GS) matrix using the Jaccard or Dice coefficients (Sneath and Sokal, 1973). Dendrograms of rice genotypes were produced by unweighted pair-group method with arithmetical averages (UPGMA) clustering of the GS matrix. To measure of goodness of fit for the cluster analysis, a cophenetic correlation value between the original similarity matrix and the cophenetic matrix given by the UPGMA clustering process was calculated by a Mantel test procedure (Mantel, 1967). These data were analyzed using NTSYS-pc v. 2.0 (Rohlf, 1998).

The polymorphism information content (PIC) value described by Botstein et al. (1980) and modified by Anderson et al. (1993) for self-pollinated species was calculated as follows:

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} \mathrm{P}_{ij}^2$$

where P_{ij} is the frequency of the *j*th allele for the *i*th marker, and summed over *n* alleles.

The genetic variation was measured in terms of genetic diversity and was computed by averaging PIC estimates over all loci (Weir, 1996). Number of alleles, average PIC values, and average GS were computed on the basis of different rice gene pools according to the results from cluster analysis and origin of the accessions. Differences in average PIC values between the three groups were evaluated by analysis of variance (SAS Institute, Inc., 1998). PIC values were calculated for the accessions grouped in each gene pool at each locus. Loci were used as blocks to separate the variation among loci from the error term and increase the sensitivity of the statistical analysis. Heterogeneity (HG) by accession and by marker was calculated as percentage of heterogeneous loci per accession across all accessions and loci, respectively.

To select a subset of markers that could discriminate between all cultivars and best represent the information obtained with the total dataset, the method of classification and regression trees (Breiman et al., 1984; Ripley, 1996) and the R-software (http://www.R-project.org) were used. The correlation between the two similarity matrixes generated with the total number and the subset of SSR markers was performed using the MXCOMP procedure of the NTSYS-pc software.

Table 2. Allelic variation of the 26 simple sequence repeat (SSR) loci in the 69 rice accessions surveyed.†

SSR marker	Chromosome location	Allele number	PIC value	Number of unique alleles	HG (%)
RM1	1	11	0.81	2‡	7.2
RM2	7	4	0.54	0	4.3
RM3	6	9	0.74	3‡	7.2
RM4	11/12	11	0.79	2§	0
RM5	1	6	0.60	1‡	0
RM6	2	6	0.46	2‡	1.4
RM10	7	8	0.76	2‡	0
RM11	7	7	0.70	1	1.4
RM16	3	4	0.44	0	1.4
RM17	12	5	0.49	1	7.2
RM18	7	6	0.45	2‡	1.4
RM20	11/12	21	0.85	7§	2.9
RM21	11	12	0.83	3‡	14.5
RM22	3	7	0.79	1‡	1.4
RM122	5	5	0.63	1‡	0
RM148	3	3	0.34	0	0
RM164	5	11	0.88	1	5.8
RM167	11	4	0.58	0	2.9
RM204	6	10	0.85	1	1.4
RM222	10	10	0.76	5‡	4.3
RM223	8	10	0.83	1‡	1.4
RM227	3	4	0.60	1‡	0
RM241	4	9	0.77	2	7.2
RM250	2	7	0.79	1	4.3
RM257	9	13	0.87	2	7.2
RM259	1	16	0.89	2‡	4.3
Average	–	8.4	0.69	1.7	3.4

†PIC, polymorphism information content; HG, accession heterogeneity.

‡One of the unique alleles registered corresponds to *O. glaberrima*.

§Two of the unique alleles registered correspond to *O. glaberrima*.

RESULTS

The 69 rice accessions were fingerprinted with 26 SSR markers and all markers were polymorphic (Table 2) with 219 polymorphic fragments ranging in size from 80 to 340 bp. Three null alleles were found in *O. sativa* cultivars with RM10, RM222, and RM241. The number of alleles detected by a single marker ranged from 3 (RM148) to 21 (RM20) with an average of 8.4 alleles. The mean number of alleles per locus was 6.9 when *O. glaberrima* and IR36 were not included. The PIC was calculated for each marker as a relative measure of informativeness and ranged between 0.34 (RM148) to 0.89 (RM259) with an average value of 0.69. Forty-four unique alleles were found at 22 marker loci (Table 2) with 16 alleles being detected in *O. glaberrima*, three alleles in 'IR36', and the remaining 25 in *O. sativa*. Ten of these 25 unique alleles were in Argentine cultivars.

Determination of the HG of *O. sativa* accessions across all SSR markers (Table 1) revealed 44 accessions were homogeneous at all loci analyzed but 24 accessions had at least two alleles at one locus. Nine accessions had the minimum HG percentage indicating a single heterogeneous locus (3.4%) whereas 'Colonia Mascias' was the most heterogeneous with eight heterogeneous loci. The average HG of the total 68 *O. sativa* accessions over all markers (Table 1) and the 26 SSR markers over all accessions (Table 2) was 3.4%. Six of the 26 (23%) SSR markers detected no heterogeneous accessions, while the remaining markers showed one or more heterogeneous accessions. Remarkably, RM21 detected cultivar heterogeneity in 10 accessions (HG = 14.5), while the remaining 20 SSR markers identified one to seven heterogeneous accessions (HG = 1.4 to HG = 7.2).

Genetic similarities calculated with the Dice coefficient using all SSR markers and all possible pairs of *O. sativa* accessions except IR36, ranged from 0.00 for nine accessions pairs to 0.96 with a mean of 0.32. The highest value (0.96) corresponded to 'IRGA 414'–'IRGA 417' pair which differed by only one SSR marker.

The 69 accessions used in this study clustered in the same order using the UPGMA cluster analysis based on the Dice coefficient (Fig. 1) and the Jaccard coefficient (cluster not shown). A high cophenetic correlation (r = 0.92) between the original similarity matrix and those given by the clustering process was observed. The two major *O. sativa* groups, *indica* and *japonica* were resolved in the dendrogram and verified by the reference cultivars IR36, Bluebelle, Lemont, Katy, Cypress, and Dawn.

Most of the Argentine cultivars fall into the *japonica* group. Further analysis revealed two subgroups in the *japonica* cluster that correspond to the *temperate* and *tropical* subtypes. A cluster analysis based on only cultivars

released in Argentina was done to explore their genetic relationships and revealed a similar clustering pattern (data not shown). For each Argentine cultivar, Dice GS average based on all pair-wise comparisons of the cultivar with all other accessions was calculated (Table 1), ranging from 0.42 for 'Don Juan INTA' to 0.15 for 'Ariete'. When the Argentine accessions were evaluated separately, the average similarities by cultivar ranged from 0.50 for 'Yerúa' to 0.15 for Colonia Mascias.

The allelic inheritance for the Argentine cultivars RP1, Colonia Mascias 5, Palmar P. A. and Don Juan INTA, whose ancestors were included in the analysis (Table 1), showed 2, 5, 6, and 2 unexpected alleles, respectively for the 28 loci surveyed. Each of the four cultivars grouped close to the immediate ancestor accession which had the most impact on its inheritance (i.e., Don Juan INTA is closer to 'Lemont' than to 'Arroyo Grande'). In the cases of San Miguel and Yerúa, two cultivars developed by parental selection, the impact of parental inheritance as measured as percentage of shared alleles was 89.3 and 67.9%, respectively.

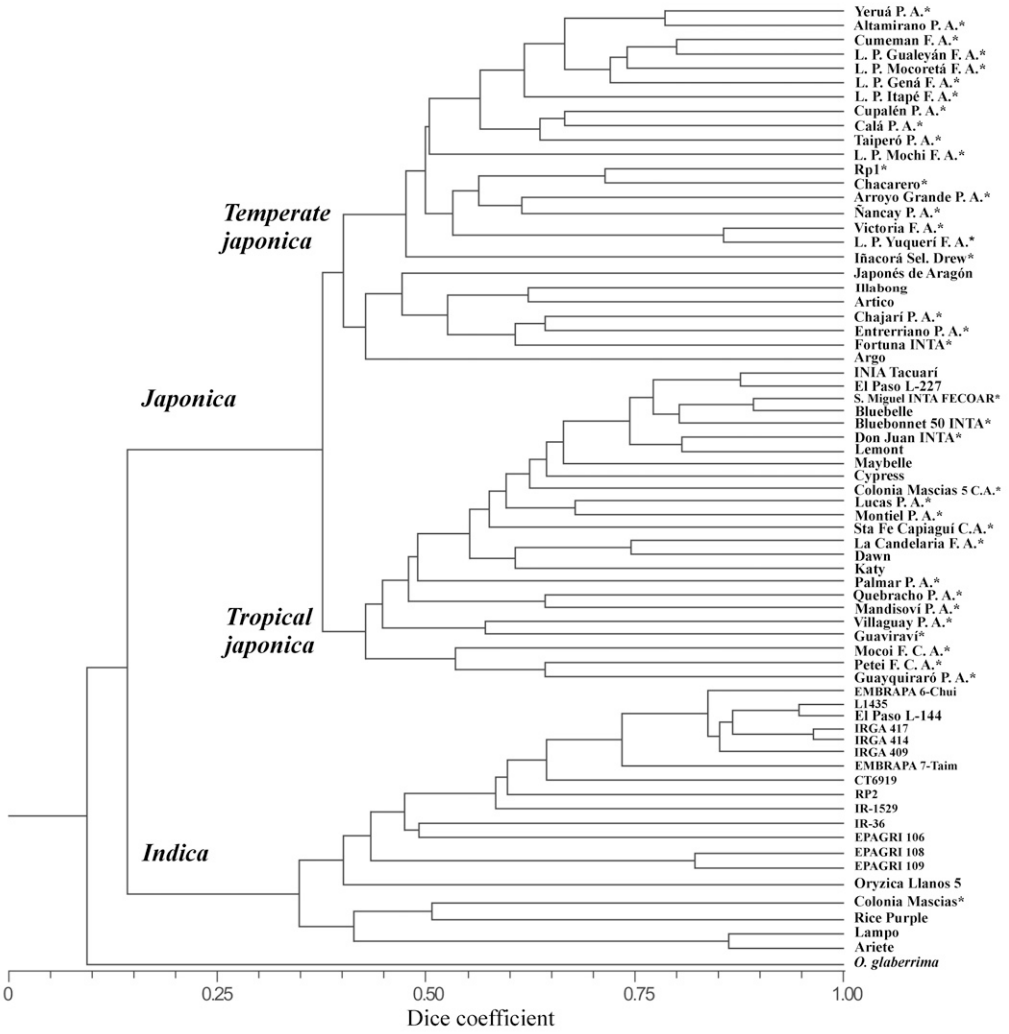

Figure 1. Dendrogram for 69 cultivated rice accessions (68 *O. sativa* and one *O. glaberrima*) derived from a UPGMA cluster analysis using the Dice similarity coefficient based on 26 SSR markers, including most of the Argentine cultivars (.).

To determine if the accessions could be differentiated with fewer markers the data set was subjected to a classification and regression tree analysis. A subset of 15 SSR markers (RM1, RM2, RM3, RM4, RM5, RM10, RM11, RM16, RM17, RM21, RM22, RM122, RM164, RM227, and RM241) was identified. The cluster analyses based on this subset corresponded almost exactly to that based on the whole dataset (data not shown). The correlation coefficient between the two similarity matrixes the "total" with 26 markers and the "subset" with 15 markers was 0.97. This suggests fewer SSR markers could have been used for evaluating the genetic diversity, identifying different accessions, and determining the genetic relationships between these accessions.

Genetic diversity was estimated both for the total *O. sativa* sample excluding IR36 (67 accessions) and for the 38 Argentine cultivars. Further analyses for number of alleles, average GS and average PIC values (Table 3) was based on (i) the main groups *indica* and *japonica,* and *japonica* subgroups, *temperate* and *tropical*, defined by the cluster analysis (Fig. 1) and (ii) origin of the accessions, all *O. sativa* and Argentine accessions. Significant differences in genetic diversity (average PIC values) between groups were identified only when (i) the group of all *O. sativa* accessions was compared with the aforementioned subgroups and (ii) the group Argentine *temperate japonica* cultivars was compared with all *japonica* accessions (Table 3).

DISCUSSION

Within *O. sativa*, two major subspecies, *indica* and *japonica* have been identified (Glaszmann, 1987; Khush, 1997). The accessions included in this study clustered into these two major groups with a UPGMA analysis (Fig. 1) and further divided within the *japonica* cluster, into subclusters corresponding to *temperate* and *tropical japonica* cultivars thus, hierarchy clusters validate the origin and pedigree information. Recently Garris et al. (2005) reported five distinct groups, *indica*, *tropical japonica*, *temperate japonica*,

Table 3. Comparison 67 *O. sativa* accessions from a diverse origin and a subset of 38 Argentine accessions for number of alleles, average genetic similarity (GS), and polymorphism information content (PIC) values. The accessions also are divided by the type of rice, *indica* and *japonica*, both *temperate* and *tropical.*

Germplasm group	No. of accessions†		No. of alleles per locus		Average GS		Average PIC values§	
	All origin	Argentine origin	All origin	Argentine origin	All origin	Argentine Origin	All origin	Argentine origin
All cultivars	67	38	200	157	0.32	0.43	0.69A	0.58B
indica	18	1‡	105	36	0.49	0.15‡	0.53BC	0
japonica	49	37	151	134	0.44	0.45	0.58B	0.56BC
temperate japonica	25	21	118	105	0.48	0.51	0.52BC	0.48C
tropical japonica	24	16	124	105	0.53	0.49	0.49BC	0.51BC

†*O. glaberrima* and IR36 were not included in the analysis.

‡This datum corresponds to accession no. 9 (Table 1).

§Means followed by the same letter (A, B, and/or C) are not significantly different (Tukey's $P < 0.05$).

aus, and *aromatic* using a more divers group of 234 accessions. Accessions from the *aus* and *aromatic* groups, both found in Southeast Asia, were not included in this study so these groups were not identified, thus these results agree with Garris et al. (2005).

Argentine-released cultivars with the exception of Colonia Macias are grouped within the *japonica* cluster (Fig. 1). Cultivars grouped in the *temperate japonica* subcluster, included the first released cultivars Chacarero F. A., Victoria F. A., and Cumeman F. A. derived from Italian genotypes. These cultivars had long-wide, medium-wide, medium, or short grains and were bred for temperate regions which are located between 30° and 33° south latitude in the provinces of Entre Ríos and Santa Fe. Argentine cultivars grouped in the *tropical japonica* cluster, mostly long fine grain type were bred for the subtropical regions located between 25° and 30° south latitude in the provinces of Formosa, Chaco, and Corrientes. The *tropical japonica* cluster included Bluebelle, Lemont, Dawn, Katy, and Cypress, U.S. long grain cultivars that are the progenitors to some Argentine cultivars (Table 1), and also identified as *tropical japonicas* by Lu et al. (2005). The group of *indicas* is composed mostly of plant introductions coming from national or international centers (Fig. 1).

The actual GS values based on all possible pair-wise comparisons of *O. sativa* accessions included in this study ranged from 0.00 to 0.96 resulting in a low mean GS (0.32) similar to GS values reported for 96 Italian accessions (Spada et al., 2004). When average GS values were calculated by accession, considering all accessions, Ariete (0.15), 'Lampo' (0.16) and Arroyo Grande (0.19) shared the lowest number of alleles with all other accessions, thus these three accessions are the most genetically distinct (Table 1). However, when average GS values by cultivar were calculated among the Argentine cultivars, Arroyo Grande had an average GS of 0.41 indicating it shares a higher number of alleles with the Argentine cultivars than with all accessions included in this study. Conversely, Colonia Mascias, 'Guayquiraró', 'Entrerriano', 'Chajarí P. A.' and 'Peteí F. C.' were the most distinct cultivars among the Argentine released cultivars. This agrees with the known pedigrees and ancestry of these cultivars (M.A. Marassi, unpublished data).

Although rice is a self-pollinating species previous studies have reported (i) more than one allele for a given single locus marker in a single cultivar and (ii) levels of internal HG similar to the 3.4% calculated in this study (Olufowote et al., 1997; Garland et al., 1999; Lu et al., 2005). Also, similar results have been reported for the self-pollinated crop species wheat (*Triticum aestivum* L.) (Röder et al., 2002) and barley (*Hordeum vulgare* L.) (Sjakste et al., 2003). RM21 detected the most heterogeneous accessions based on HG (Table 2) suggesting the ability to assess each marker's HG, regardless of marker's PIC value, also may determine the importance of the marker's chromosomal location (Ni et al., 2002).

It cannot be determined whether the HG observed in some of the accessions (Table 1) was due to heterozygosity and/or heterogeneity because the DNA from several plants was bulked for the SSR marker analyses. As stated in similar studies, marker analyses on individual plants of the individual cultivar are necessary to determine whether the within cultivar heterogeneity is due to a seed mixture or residual heterozygosity (Gethi et al., 2002; Röder et al., 2002). If there is residual heterozygosity in the accession, an appropriate threshold of similarity must be established for the accessions to be unequivocally fingerprinted, as suggested for wheat (Röder et al., 2002), for a reliable method of quality control in certified seed production programs, and to maintain pure germplasm collections (Manifesto et al., 2001).

The presence of unexpected alleles in RP1, Colonia Mascias 5, Palmar P. A., and Don Juan INTA may be due to variations between seed sources of these progenitors accessions and those of the progenitors used by the particular rice breeder making each cross. Also, it cannot be ruled out that some variation may have arisen during seed maintenance of the cultivar.

The overall genetic diversity (PIC value = 0.69) of the 67 *O. sativa* germplasm accessions included in this study was similar to values reported in previous studies: Ni et al. (2002) reported a PIC value of 0.62 using 38 accessions, Yu et al. (2003) reported 0.68 using 193 accessions, Xu et al. (2004) reported 0.66 using 236 accessions, and Garris et al. (2005) reported 0.67 using 234 accessions. Genetic diversity for all 67 accessions was significantly higher than for the subset of Argentine bred cultivars (PIC value = 0.58) however, the PIC values were not significantly different when the *japonica* subgroup for the two categories were compared (Table 3). This suggests the Argentine bred cultivars included in this study, retained much of the *japonica* gene pool diversity. This PIC value is higher than the value calculated for the U.S. *japonica* rice cultivars (PIC = 0.46) reported by Lu et al. (2005), and the values calculated for the *temperate* and *tropical japonica* rice accessions representing the geographic range of *O. sativa* (0.37 and 0.46) studied by Garris et al. (2005). The results presented in this study suggest the genetic basis of the Argentine cultivars is not undesirable narrow, however these results need to be compared with a wider range of *O. sativa* so that the diversity in a wider range of accessions can be utilized for broadening genetic material and improving Argentine cultivars under development.

In conclusion, this is the first characterization of the molecular diversity in rice cultivars released in Argentina, including cultivars of historical significance to rice breeding and production. The cultivars were fingerprinted and the genomic relationship among cultivars was elucidated. This data will be useful to Argentine rice breeders by improving the selection of parents for crosses, further exploiting the available genetic variation, assigning lines to specific heterotic groups, and precisely classifying new accessions.

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