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Assessing the Genetic Structure of *Oryza glumaepatula* Populations with Isozyme Markers

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ABSTRACT

To assess the genetic diversity and genetic structure parameters, nine populations of Oryza glumaepatula from the Amazon biome, four from the Pantanal biome, and one collected at Rio Xingu, Mato Grosso, totaling 14 populations and 333 individuals were studied with isozyme markers. Six loci were evaluated showing a moderate allozyme variability ($\overline{A}$ = 1.21, $\overline{P}$ = 20.7%, $\overline{H}_o$ = 0.005, $\overline{H}_e$ = 0.060). The populations from the Pantanal biome showed higher diversity levels than the Amazon biome. High genetic differentiation among the populations, expected for self-fertilizing species, was observed (F_{ST}=0.763), with lower differentiation found among the Pantanal populations (F_{ST}=0.501). The average apparent outcrossing rate was higher for the Pantanal populations ($\hat{t}_a$ = 0.092) than for the Amazonian populations ($\hat{t}_a$ = 0.003), while the average for the 14 populations was 0.047, in accordance with a self-fertilization mating system.

Key words: Genetic diversity, isozymes, *Oryza glumaepatula*, outcrossing rate, populations

INTRODUCTION

Among the 22 wild species of the genus *Oryza* (IRRI, 2005), *O. glumaepatula* Steud. is one of the four wild rice species originated in the American continent (Morishima, 1994). It occurs in Bolivia, Brazil, Colombia, Costa Rica, Cuba, Dominican Republic, French Guyana, Guyana, Honduras, Mexico, Panama, Surinam and Venezuela, and is found in swamps and marshes, open ditches and pools, beside rivers, and near to the cultivated rice fields, usually with deep water, also growing in open habitats (IRRI, 2005). It is the only diploid species and compatible with *O. sativa*, which makes it important as a source of new genes to be incorporated to the cultivated species in plant breeding programs (Brondani et al., 2002; Mamani, 2002).

In its taxonomic review, Vaughan (1994) considered *O. glumaepatula* as an American form of *O. rufipogon.* The hybridization of *O. glumaepatula* and *O. rufipogon* (2n = 24, AA) is possible, but the F1 plants are sterile. For this reason, the genome of *O. glumaepatula* was denominated as $A^{gp}A^{gp}$ (Ando, 1998). Confirming this re-classification, most South American accessions of *O. glumaepatula* evaluated by morphological traits by Juliano et al. (1998) were quite distinct from *O. rufipogon.* Using random amplified polymorphic DNA (RAPD) markers, Ge

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et al. (1999) confirmed the taxonomic classification of *O. glumaepatula* as a distinct species and not as an American form of *O. rufipogon*.

Isozymic markers have been widely used in the cultivated and wild *Oryza* species, thus elucidating the processes of the domestication of the cultivated rice, assessing the genetic diversity and genetic structure of populations associated with aspects of their life history (Second, 1982; Barbier, 1989; Grover and Pental, 1992; Suh et al., 1997; Akimoto et al., 1998; Buso et al., 1998; Gao et al., 2000a; Gao et al., 2000b; Gao et al., 2000c; Gao et al., 2002a; Gao et al., 2002b; Quesada et al., 2002; Ishikawa et al., 2005). Buso et al. (1998) observed in four populations of *O. glumaepatula* collected at the basins of Rio Amazonas and Rio Paraguay, based on isozymes and RAPD, a pattern of greater variation among rather than within populations, suggesting a self-fertilization breeding system. Akimoto et al. (1998), evaluating 37 populations of *O. glumaepatula* collected in five regions of Amazonian Rio Negro and Rio Solimões, observed high values for F_{IS}, indicating inbreeding, and low values for the observed heterozygosity, indicating excess of homozygotes, suggesting that this species was predominantly self-pollinated. However, the authors observed greater intrapopulational than interpopulational variability, a pattern usually found in the cross-fertilized populations, which was explained considering the life history characteristics of this species. Studies of the genetic structure of *O. glumaepatula* populations were also recently conducted with microsatellite markers (Karasawa, 2005; Karasawa et al., 2007; Brondani et al., 2005; Silva et al., 2007), showing high values for F_{ST} and R_{ST}, suggesting a higher interpopulational variability, as well as the predominance of a self-fertilizing breeding system.

The purpose of this study, using isozymic markers, was to provide further information on the genetic diversity and genetic structure of 14 *O. glumaepatula* populations, originated from the hydrographic basins of the Amazon and of Rio Paraguay in the Pantanal ecosystem.

MATERIALS AND METHODS

Plant material and sampling procedures

Fourteen Brazilian *O. glumaepatula* populations from the wild rice germplasm bank of Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, were assessed in this study. Four populations were originated from the Pantanal biome, at the hydrographic basin of Rio Paraguay, nine from the hydrographic basins of Rio Japura, Purus, Tapajos, Solimões, Branco, Negro in the Amazon region, and one from Rio Xingu, also a tributary to the Amazon, in the State of Mato Grosso (Table 1, Fig. 1).

Two to three seeds were randomly sampled from each individual plant within each population to form a bulk of 50 seeds. After the germination in plastic gerboxes on filter paper at 27°C in the dark in germination chambers, the seedlings were planted in pots in the greenhouse. According to the germination potential, the number of plants evaluated from each population varied from nine to 36, a total of 333 individuals.

Isozyme analyses procedures

A protocol was established for the isozyme analyses in polyacrilamide gels under a discontinuous system. The gel and electrode buffers which showed the best results were the basic buffer (Hames, 1996), with pH 8.8 for the 5.5% resolving gel and pH 6.8 for the 3.5% stacking gel.

Recently expanded leaves (200 mg) were used for the enzyme extraction. The leaves were ground in liquid nitrogen in 1.5 mL microtubes using a power homogenizer, adding 1 mL of number 1 extraction buffer (Alfenas *et al*., 1991), leaving out diethyldithiocarbamic acid (DIECA) and 2-mercaptoethanol. The resulting crude extract was centrifuged at 18000 *g* for 20 min at 4ºC. After that, 130 µL of the supernatant were placed in 0.5 mL microtubes, diluted in 150 µL of a solution containing Tris-HCl pH 6.8 and Coomassie blue, the latter component indicating the protein migration in the gel. This amount of extract was sufficient for four gels, which allowed the assessment of 38 individuals each. The same control plant was added at each gel as a marker. The voltage was set at 50 V during three hours, adjusted for 100 V for the next 13 hours, usually staying overnight at 4ºC.

Six enzymatic systems were initially selected for the analyses, presenting higher band resolution: glucose-6-phosphate isomerase (GPI; E.C. 5.3.1.9), aspartate aminotransferase (AAT; E.C. 2.6.1.1), phosphoglucomutase (PGM; E.C. 2.7.5.1), shikimate dehydrogenase (SKD; E.C.

1.1.1.25), glutamate dehydrogenase (GDH; E.C. 1.4.1.2) and malate dehydrogenase (MDH; E.C. 1.1.1.37). The MDH and GPI systems were further discarded due to difficulty in interpretation. The systems leucine aminopeptidase (LAP; E.C. 3.4.1.1.1), glucose-6-phosphote dehydrogenase (G_6PDH; E.C. 1.1.1.49), sorbitol dehydrogenase (SDH; E.C. 1.1.1.14) and superoxide dismutase (SOD; E.C. 1.15.1.1) also showed some activity, but not enough to include them in the analyses.

Statistical analyses

Allelic frequencies, mean number of alleles per locus (*A*), mean number of alleles per polymorphic locus (*Ap*), percentage of polymorphic loci (*P*), observed heterozygosity (H_o), gene diversity (H_e) and Wright's fixation index (*f*) were estimated using the GDA software (Lewis and Zaykin, 2000). The average apparent outcrossing rate (t_a) was estimated according to the formula $\hat{t}_a = (1 - \hat{f}/1 + \hat{f})$, where $\hat{f}$ was estimated as $\hat{f} = (H_e\text{-}H_o)/H_e$. The genotypic frequencies were submitted to Fisher's exact test considering Hardy-Weinberg equilibrium proportions (Weir, 1996), using the TFPGA software (Miller, 1997).

This program was also used for a cluster analysis considering the UPGMA clustering criteria and Nei's genetic distances matrix (Nei, 1978). F statistics (F_{IS} , F_{ST} and F_{IT}) were used for the genetic structure studies and were estimated using the FSTAT software (Goudet, 2001) under a random model according to Weir (1996), considering the sampled populations as the representatives of the species with a common evolutionary history. Wright's *F* statistics are a hierarchical series of fixation indices, where F_{IS} represents the deviation from Hardy-Weinberg expectations within populations, F_{ST} measures the fixation of different alleles in different populations, and F_{IT} measures deviations from Hardy-Weinberg expectation across the population system as a whole (Gao et al., 2000b).

Table 1 - Origin of the *Oryza glumaepatula* populations, according to the number assigned in the data analysis

Population nº	Population Identification	N[1]	Hydrographic basin	River	Lake	Geographic location
1	PG-1	36	Paraguay	-	-	19º01' S – 57º30' W
2	PG-2	23	Paraguay	-	-	19º00' S – 57º41' W
3	PG-3	27	Paraguay	Corumbá	-	18º59' S – 57º37' W
4	PG-4	23	Paraguay	Taquari	-	19º15' S – 57º13' W
5	JA-4	21	Japurá	Japurá	Cuiucuiú	02º02' S – 65º07' W
6	PU-1	21	Purus	Purus	-	03º49' S – 61º25' W
7	TA-1	24	Tapajós	Tapajós	-	02º26' S – 54º42' W
8	SO-6	25	Solimões	Solimões	Manacapuru	03º11' S – 60º47' W
9	SO-14	9	Solimões	-	Jucara	04º02' S – 63º08' W
10	SO-17	30	Solimões	-	Coari	04º10' S – 63º15' W
11	SO-21	23	Solimões	-	Mamiá	04º15' S – 63º03' W
12	NE-18	24	Branco	Branco	-	01º53' S – 61º22' W
13	NE-26	23	Negro	Carabinani	-	01º54' S – 61º23' W
14	XI-1	24	Xingu	Xingu	Piulaga	12º14' S – 53º35' W

[1] Number of sampled individuals/population.

Figure 1 - Map of Brazil showing the localization of the 14 *Oryza glumaepatula* populations, described in Table 1.

RESULTS AND DISCUSSION

Genetic diversity

Six loci were evaluated with one locus for each of the enzyme systems PGM, SKD and GDH, and three loci for AAT. Three alleles were found at the locus *Pgm-1*, two at loci *Aat-1*, *Aat-2* and *Skd-1*, and one fixed allele at loci *Aat*-3 and *Gdh-1*, both monomorphic (Table 2).

For the AAT system, a dimeric enzyme (Weeden and Wendel, 1989), most of the populations originated in the Amazon presented a fixed *a2* allele for *Aat-1* locus. On the other hand, the four populations originated in the Pantanal biome showed the predominance of allele *a1*, fixed in three of these populations (Table 2). The XI-1 population from Rio Xingu presented also a fixed *a1* allele, similar to the Pantanal populations. For the *Aat-2* locus the opposite occurred, with the predominance of allele *a1* for the Amazonian populations and the predominance of allele *a2* for the populations of the Pantanal biome and Rio Xingu.

At the *Skd-1* monomeric locus, most populations presented a fixed *a1* allele. The monomeric PGM enzyme (Weeden and Wendel, 1989) presented one locus with three alleles, with allele *a2* fixed at the populations PG-3, JA-4, PU-1 and SO-6 (Table 2).

Thus, considering six loci and four enzymatic systems, the mean number of alleles per locus ranged from 1.00 to 1.50, with a mean of 1.21 alleles per locus (Table 3), in agreement with the previous studies with isozyme (Akimoto et al., 1998; Buso et al., 1998) and microsatellite markers (Brondani et al, 2005) in *O. glumaepatula*. Karasawa et al. (2007), however, observed a mean of 3.09 alleles per locus with microsatellites for this species. The percentage of polymorphic loci ranged from 0.0 to 50.0%. Population PG-1, from the Pantanal biome, was the most polymorphic (Table 3).

The observed heterozygosity varied from 0.000 to 0.023 (mean of 0.005), and the gene diversity ranged from 0.000 to 0.164 (mean of 0.060). These values were also in agreement with the estimates observed by Akimoto et al. (1998) (H_o = 0.003 and H_e = 0.044) and Buso et al. (1998) (H_o = 0.000 to 0.025 and H_e = 0.000 to 0.210) with isozymes and by Brondani et al. (2005) (H_o = 0.027 and H_e = 0.115) with microsatellites. Karasawa et al. (2007), using microsatellites, observed higher values for these parameters (H_o = 0.091 and H_e = 0.393), which could be due to the larger samples per population (18 to 35

individuals, with a mean of 27.38) used by the author, allowing the detection of a greater number of private alleles in each population. Higher genetic diversity levels were also obtained by Gao et al. (2002a) with microsatellites than with isozyme markers, when comparing the same set of *O. rufipogon* Griff. populations. Comparing both biomes, the Pantanal and the Amazon, higher diversity indices were observed for the Pantanal populations at all the parameters analysed (Table 3), except the number of alleles per polymorphic locus, which was the same for both biomes.

The fixation index estimate provided positive and near to one values, with a mean of 0.910, indicating excess of homozygotes and a self-fertilizing mating system. The apparent outcrossing rate was also higher ($\hat{t}_a$ = 0.092) on average for the Pantanal populations, with PG-4 population presenting the highest outcrossing rate ($\hat{t}_a$ = 0.158). Both the estimates indicated a predominantly self-fertilized mixed mating system, although on the average, for the 14 populations, the outcrossing rate was estimated as 0.047, indicating a self-fertilization mating system for this species. These results agreed with Brondani et al. (2005) when comparing the Amazon, Pantanal and Cerrado biomes with microsatellites, also estimating higher diversity indices for the Pantanal populations, although only two Amazonian populations were evaluated against five from the Pantanal biome. Greater apparent outcrossing rates were also obtained, on average, for the Pantanal populations ($\hat{t}_a$ = 0.184) by these authors. In this study, nine Amazon populations were evaluated, excluding the Rio Xingu population, against four of the Pantanal, and yet the same pattern was observed.

Genetic structure

Wright's F statistics (Table 4) showed near 1.0 values for F_{IT} (0.976) and F_{IS} (0.905), indicating a self-fertilization mating system for *O. glumaepatula* and also high interpopulational variability (F_{ST} = 0.77). In agreement with these results, Buso et al. (1998) observed in four *O. glumaepatula* populations, based on isozymes and RAPD markers, a pattern of greater variation among than within the populations, suggesting also a self-pollination breeding system. Akimoto et al. (1998), using 29 loci of 16 enzymes in 37 populations collected in five regions of Rio Negro and Rio Solimões in the Amazon, also found high F_{IS} values, indicating high inbreeding. With microsatellite markers, Karasawa et al. (2007) and Brondani et al. (2005) also found high F_{IT} (0.888 and 0.968) and F_{IS} (0.788 and 0.794) values, respectively, for *O. glumaepatula* populations. Karasawa et al. (2007) estimated a lower F_{ST} value (0.491) than the value found in this study and that observed by Brondani et al. (2005) (F_{ST} = 0.847). However, a high interpopulation variability was the main result from all of these studies.

Table 2 - Allele frequencies of six isozymic loci estimated from 333 individuals in 14 *Oryza glumaepatula* populations.

Locus	Allele	PG-1	PG-2	PG-3	PG-4	JA-4	PU-1	TA-1
Aat-1	*a1*	0.833	1.000	1.000	1.000	0.143	0.000	0.000
	a2	0.167	0.000	0.000	0.000	0.857	1.000	1.000
Aat-2	*a1*	0.318	0.182	-	0.167	0.905	1.000	1.000
	a2	0.682	0.818	-	0.833	0.095	0.000	0.000
Aat-3	*a1*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Skd-1	*a1*	1.000	1.000	1.000	-	1.000	1.000	1.000
	a2	0.000	0.000	0.000	-	0.000	0.000	0.000
Pgm-1	*a1*	0.848	0.000	0.000	0.022	0.000	0.000	0.229
	a2	0.152	0.978	1.000	0.978	1.000	1.000	0.771
	a3	0.000	0.022	0.000	0.000	0.000	0.000	0.000
Gdh-1	*a1*	1.000	1.000	1.000	1.000	1.000	1.000	1.000

(Cont. ...)

(Cont. Table 2)

SO-6	SO-14	SO-17	SO-21	NE-18	NE-26	XI-1
0.000	0.000	0.000	0.000	0.000	0.000	1.000
1.000	1.000	1.000	1.000	1.000	1.000	0.000
1.000	1.000	1.000	1.000	1.000	1.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	1.000
1.000	1.000	1.000	1.000	1.000	1.000	1.000
1.000	0.556	0.300	1.000	0.167	1.000	0.000
0.000	0.444	0.700	0.000	0.833	0.000	1.000
0.000	0.111	0.000	0.000	0.521	0.000	0.000
1.000	0.889	0.152	0.978	0.479	0.978	0.500
0.000	0.000	0.000	0.022	0.000	0.000	0.500
1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 3 - Estimates of genetic diversity parameters based on six loci and 14 *O. glumaepatula* populations: mean number of individuals sampled/locus (N), mean number of alleles/locus (A), mean number of alleles/polymorphic locus (Ap), mean number of polymorphic loci (P), mean observed heterozygosity/locus (H_o), gene diversity (H_e), Wright's fixation index (f) and apparent outcrosing rate (t_a).

Pop	N	A	Ap	P (%)	H_o	H_e	f	$\hat{t}_a$
PG-1	35.0	1.50	2.00	50.0	0.023	0.164	0.858	0.0764
PG-2	21.0	1.33	2.00	16.6	0.007	0.059	0.877	0.0655
PG-3	27.0	1.00	-	0.0	0.000	0.000	0.000	-
PG-4	22.6	1.40	2.00	20.0	0.018	0.065	0.727	0.1581
Pantanal mean	26.4	1.31	2.00	21.6	0.012	0.072	0.832	0.0917
JA-4	19.2	1.33	2.00	33.3	0.000	0.071	1.000	0.0000
PU-1	21.0	1.00	-	0.0	0.000	0.000	0.000	-
TA-1	18.5	1.17	2.00	16.7	0.007	0.060	0.887	0.0599
SO-6	25.0	1.00	-	0.0	0.000	0.000	0.000	-
SO-14	9.0	1.33	2.00	33.3	0.000	0.122	1.000	0.0000
SO-17	30.0	1.17	2.00	16.7	0.000	0.071	1.000	0.0000
SO-21	23.0	1.17	2.00	0.0	0.000	0.014	1.000	0.0000
NE-18	24.0	1.33	2.00	33.3	0.021	0.132	0.845	0.0840
NE-26	23.0	1.00	-	0.0	0.000	0.000	0.000	-
Amazon mean	21.4	1.17	2.00	14.8	0.003	0.052	0.942	0.0298
XI-1	20.8	1.17	2.00	16.7	0.000	0.085	1.000	0.0000
Overall mean	22.8	1.21	2.00	20.7	0.005	0.060	0.910	0.0471

Table 4 - F statistics estimates for all 14 populations for four populations from Pantanal and nine populations from the Amazon.

	All populations			**Populations from the Pantanal biome**			**Populations from the Amazon biome**		
	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}	F_{IS}
Under all the loci	0.976	0.763	0.905	0.933	0.501	0.914	0.970	0.616	0.926
Upper (CI 95%)	0.981	0.875	0.926	0.963	0.798	0.918	1.000	0.653	1.000
Lower (CI 95%)	0.967	0.601	0.821	0.661	-0.002	0.612	0.949	0.075	0.920

F_{IS} - deviation from Hardy-Weinberg expectations within populations; F_{ST} - measures the fixation of different alleles in different populations; F_{IT} - deviations from Hardy-Weinberg expectation across the population system as a whole
IC: 95% confidence interval

Comparing the three biomes analysed (Amazon, Pantanal and Cerrado), lower interpopulational variability was found for the Pantanal populations (F_{ST} = 0.501) when compared to the Amazonian populations (F_{ST} = 0.616), similar to what was observed by Brondani et al. (2005) (F_{ST} = 0.713 for the Pantanal populations and F_{ST} = 0.831 for the Amazonian populations).

Considering the high interpopulational variability found in this species, it would be important to sample fewer individuals from several populations when the objective is an *ex situ* conservation program. For *in situ* conservation programs, a larger area including the conservation of several populations in both biomes is suggested from this data.

The cluster analysis (Fig. 2), based on Nei's (1978) genetic distances (Table 5) classified the 14 populations in two major groups, one of them clustering the four Pantanal populations together with the Rio Xingu population, while the other group was formed by the Amazonian populations. This analysis showed a clear separation and genetic divergence between the two biomes (Pantanal and the Amazon), as well as the intermediate position of XI-1 population collected in the State of Mato Grosso, at the fringes of Rio Xingu.

Table 5 - Nei's (1978) genetic distances between 14 *Oryza glumaepatula* populations in this study.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.000													
2	0.146	0.000												
3	0.169	0.000	0.000											
4	0.179	0.002	0.000	0.000										
5	0.339	0.251	0.162	0.321	0.000									
6	0.411	0.336	0.223	0.430	0.003	0.000								
7	0.358	0.350	0.235	0.451	0.008	0.003	0.000							
8	0.411	0.335	0.223	0.430	0.003	0.000	0.003	0.000						
9	0.453	0.413	0.293	0.443	0.040	0.032	0.032	0.032	0.000					
10	0.588	0.483	0.371	0.430	0.096	0.087	0.094	0.087	0.010	0.000				
11	0.406	0.338	0.225	0.435	0.004	0.000	0.003	0.000	0.032	0.088	0.000			
12	0.479	0.655	0.550	0.548	0.200	0.185	0.160	0.185	0.057	0.052	0.182	0.000		
13	0.411	0.335	0.223	0.430	0.003	0.000	0.003	0.000	0.032	0.087	0.000	0.185	0.000	
14	0.379	0.254	0.303	0.058	0.703	0.831	0.840	0.831	0.621	0.547	0.824	0.554	0.831	0.000

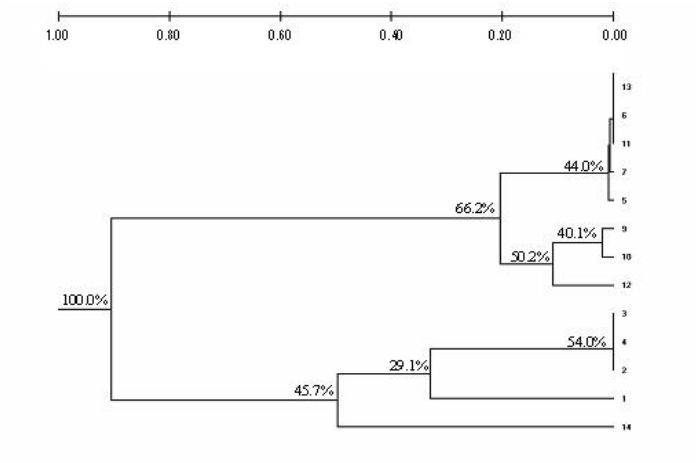

Figure 2 - Dendrogram using Nei's (1978) genetic distances based on six isozymic loci and the UPGMA method, for the clustering of 14 *Oryza glumaepatula* populations, as described on Table 1.

At a parallel study (Rosa et al., 2006), eight *O. glumaepatula* populations assessed with 15 morphological traits showed the separation of the single Pantanal population (PG-4) evaluated on a distinct group, and also the clustering of Rio Xingu (XI-1) population at another single group, in comparison with the other Amazonian populations, except for a Rio Negro (NE-26) population, also clustered in a single group due to the morphological differentiation. Another parallel study on morphological characterization clustered in a separate group the single Pantanal population (PG-1) studied among 11 *O. glumaepatula* populations, plus 12 populations belonging to the species *O. latifolia*, *O. grandiglumis* and *O. alta* (Veasey et al., 2008). Comparing these studies, it was evident that besides an allelic divergence, there was also morphological diversity among the populations of these regions. As discussed above, for certain loci (*Aat-1* and *Aat-2*) different alleles predominated in each of the two biomes, Pantanal and the Amazon, indicating that a genetic differentiation probably occurred in these populations due to the geographic isolation among *O. glumaepatula* populations.

Within the group of the Pantanal and Xingu populations, the PG-1 population, the most polymorphic of all, classified in a single group, was the only one to present the *a2* allele at *Aat-1* locus (Table 2). Besides, it was the only one to present a high frequency of allele *a1* at *Pgm-1* locus. The XI-1 population, also classified in a single group, within this major group, differed from all the others for presenting a high frequency of the *a3* allele (0.50) at *Pgm-1* locus, and from the Pantanal populations for presenting a fixed *a2* allele at *Skd-1* locus.

Within the Amazonian populations, a separate group was formed with the populations SO-14, SO-17 and NE-18. All three populations showed the *a2 a*llele at locus *Skd-1*, not shown by the other populations. The Rio Negro (NE-18) population differed from the others for presenting a higher frequency of allele *a1* at locus *Pgm-1*.

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RESUMO

Utilizando marcadores isoenzimáticos, foram avaliadas nove populações de *Oryza glumaepatula* originárias da Amazônia, quatro do bioma do Pantanal, e uma coletada no Rio Xingu, Mato Grosso, totalizando 14 populações e 333 indivíduos, com o objetivo de avaliar a diversidade

genética e a estrutura genética dessas populações. Seis locos foram avaliados, mostrando variabilidade alozímica moderada ($\overline{A}$ = 1.21, $\overline{P}$ = 20.7%, $\overline{H}_o$ = 0.005, $\overline{H}_e$ = 0.060). As populações do bioma Pantanal apresentaram níveis de diversidade mais altos que as da Amazônia. Alta diferenciação genética entre populações, esperada para espécies autógamas, foi observada (F_{ST}=0.763), com menor diferenciação encontrada entre populações do Pantanal (F_{ST}=0.501). A taxa média de cruzamento aparente foi maior para as populações do Pantanal ($\hat{t}_a$ = 0.092) que da Amazônia ($\hat{t}_a$ = 0.003), enquanto que a taxa media para as 14 populações foi 0.047, em concordância com o sistema reprodutivo por autogamia.

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