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Molecular and morphological diversity in Japanese rice germplasm

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Abstract

Germplasm molecular and phenotypic characterization is instrumental to its utilization and to genetic variability incorporation into rice breeding programmes. The diversity within 192 Japanese rice accessions was analysed for 22 agro-morphological traits and 24 single sequence repeat markers. A total of 181 alleles were detected, 38 of which were exclusive. The number of alleles/marker ranged from 2 to 16, with an average of 7.54 alleles/locus and the H_e value ranged from 0.01 to 0.82, with an average of 0.46. The accessions showed diversity at molecular and phenotypic level and few showed also good agronomic performance. Tocher's method applied on a total-dissimilarity matrix was used to determine cluster formation of 13 diversity groups. Most of the accessions (81%) were clustered within a group, whereas eight accessions (Kyuushuu, Eika Ine, Ishiwari Mochi, Col/Fukui/1965, Ookuma Nishiki, Suzume Shirazu, Iwate Ryoon and Toga) did not cluster with other accessions.

Keywords: germplasm; molecular markers; morphological traits; multivariate analyses; *Oryza sativa*

Introduction

Cultivated rice (*Oryza sativa* L.) is the most important food crop feeding more than half of the world's population. The use of rice genetic resources available at genebanks is important to incorporate genetic variability in rice breeding programmes that can potentially generate new cultivars with broadened genetic basis and allow new and useful allelic combinations (McCouch, 2005).

While it is important to collect and preserve genetic variation in seed banks, these activities are not sufficient to ensure the future productivity of agriculture. The establishment and maintenance of seed banks must be coupled with the ability to actively utilize the materials in those collections (Tanksley and McCouch, 1997).

In genetic resource conservation and plant breeding, multivariate data on continuous and categorical traits are collected with the objective of selecting genotypes that best represent the entire population or gene collection with the minimum loss of genetic diversity (Crossa and Franco, 2004). In addition to the descriptors recommended for each species, the molecular characterization of accessions becomes increasingly common practice, but studies that integrate molecular and agronomic data are less frequent. Several authors have called attention to the need for a joint treatment of information coming from these two different sources (Franco *et al.*, 2001; Bramardi *et al.*, 2005)

The purpose of this study was to identify the diversity among 192 Japanese rice accessions source using multivariate data from morphological traits and molecular markers. The utilization of all variables together showed a greater power to discriminate genotypes and enabled to summarize relationship among accessions.

Materials and methods

The diversity within 192 Japanese rice accessions (Supplementary Table S1, available online only at http://

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journals.cambridge.org) was analysed by investigating 22 agro-morphological traits and 24 single sequence repeat (SSR) markers. Experiments, designed as augmented blocks, were conducted in the experimental field of the Department of Genetics – ESALQ/USP, in SP, Brazil, during the agricultural years of 2007/2008 and 2008/2009, respectively. The accessions were characterized with rice descriptors indicated by IBPGR-IRRI Rice Advisory Committee (1980) and Bioversity International (2007). The descriptors used were:

(1) Quantitative variables: number of days to heading; culm, number and length (cm); flag leaf, length and width (cm); plant, length (cm); panicle, length (cm); number of days to maturity, 100-grain weight (g); yield (g/experimental plot); grain, length (mm), width (mm) and length–width ratio;
(2) Qualitative variables: leaf blade, pubescence; auricle: colour; flag leaf, orientation; panicle, type, orientation of main axis, orientation of branches and secondary branching; awns, distribution and colour; lemma, colour of apiculus.

Total DNA was isolated from young rice leaves by the cetyl trimethylammonium bromide method. SSR markers were chosen by their polymorphic information content and by their distribution considering the twelve rice chromosomes. The primers used were: genomic SSRs (RM9, RM207, RM55, RM335, RM334, RM204, RM11, RM38, RM257, RM304, RM229 and RM247) and expressed sequence tags-simple sequence repeats (EST-SSRs) (RM165, RM145, RM 143, RM131, RM146, RM190, RM180, RM149, RM160, RM184, RM181 and RM155). Amplification products were visualized on polyacrylamide denaturing gels followed by silver staining.

Genetic divergence among the accessions was quantified using Mahalanobis's distance for quantitative variables, Jaccard's arithmetic complement for qualitative variables and Rogers-W distance for SSR markers. Four matrices of dissimilarities were obtained (two Mahalanobis's distance matrices, one for each agricultural year; one Jaccard's distance matrix and one Rogers-W's distance matrix). A total-dissimilarity matrix, which is a sum of elements of the four matrices, was generated using 'Genes' software (Cruz, 2008). Tocher's method applied to total-dissimilarity matrix was used to determine cluster formation.

Results

Genetic diversity among 192 Japanese rice accessions was identified by simultaneously investigating morphological and molecular data. Morphological data were collected in experiments conducted in the field during two agricultural years. The traits 'plant: length, culm: length, panicle: length, number of days to heading, flag leaf: length and width, number of days to maturity and yield' presented more contribution for the variability among the accessions during the first year, according to canonical variable analysis (data not shown)

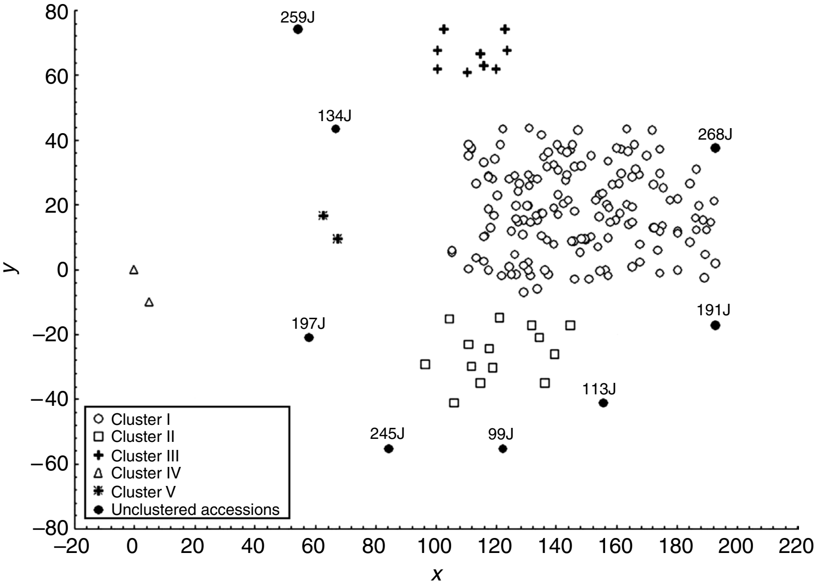

Fig. 1. Patterns of relationships among 192 rice accessions and 13 Tocher's clusters revealed by two-dimensional projection of the distances.

Table 1. Mean, heritability (h^2) and coefficient of variation (CV) of 13 quantitative variables of 192 Japanese rice accessions and means of eight rice accessions not clustered with other ones by Tocher's method

Traits	Mean	h^2	CV (%)	Means of accessions not clustered with other ones							
				113J	99J	245J	191J	268J	134J	259J	197J
Days to heading	93	93.22	2.69	107	95	125	132	91	91	84	90
Culm: length (cm)	77.87	81.80	5.89	65.67	89.43	82.45	97.58	79.40	73.93	89.71	85.57
Plant: length (cm)	98.40	80.76	5.23	89.74	113.5	99.36	119.2	100.4	92.49	109.2	105.1
Panicle: length (cm)	20.60	59.52	6.62	24.07	24.07	16.91	21.67	21.06	18.56	19.50	19.60
Flag leaf: length (cm)	28.52	66.43	8.97	23.54	36.15	24.17	31.36	22.83	28.35	28.01	24.40
Flag leaf: width (cm)	1.71	76.75	5.87	1.17	1.80	1.53	1.88	1.30	2.09	1.86	1.68
Days to maturity	124	93.52	1.89	133	130	156	158	138	121	119	128
Grain yield (kg/plot)	0.67	34.11	23.5	0.40	0.62	0.12	0.33	0.69	0.60	0.48	0.49
Culm: number	5.46	33.64	12.95	5.75	6.58	3.72	5.98	6.72	5.05	4.05	4.95
100-grain weight (g)	2.98	90.95	3.46	2.29	3.39	3.23	3.09	2.87	2.59	2.71	3.33
Grain: length (mm)	8.30	89.58	2.39	8.95	8.84	9.82	8.75	8.13	8.66	7.84	9.14
Grain: width (mm)	3.42	94.32	1.88	2.56	3.37	3.12	3.21	3.33	3.13	3.35	3.31
Grain: length/width	2.45	95.11	2.84	3.50	2.63	3.15	2.73	2.45	2.76	2.38	2.77

and were evaluated during the second year to consider environmental effects in the variability.

From 24 SSR markers, a total of 181 alleles were detected, 38 of which were exclusive. They were observed in 26 accessions and for 16 of the 24 analyzed loci. H_e ranged from 0.01 to 0.82, with an average of 0.46 and the number of alleles/marker ranged from 2 (for RM 143 and RM 146 e RM 184) to 16 (for RM 257), with an average of 7.54 alleles/locus. The average number of alleles/locus was higher than that observed by Lu *et al.* (2005) for 115 U.S. rice cultivars and 30 ancestral accessions introduced from Asia (5.15 alleles/locus) and by Yu *et al.* (2003) in parental lines used by the International Rice Molecular Breeding Program (6.25 alleles/locus). The number of alleles/locus was lower than the number observed in genetic diversity studies in Brazilian traditional rice cultivars (Brondani *et al.*, 2006) and in traditional and improved Indonesian rice germplasm (Thomson *et al.*, 2007), which exhibited an average of 14.7 and 13 alleles/locus, respectively.

Thirteen diversity groups were determined by Tocher's method (Fig. 1 and Supplementary Table S2, available online only at http://journals.cambridge.org). The majority of the accessions (81%) were clustered to the same group, while eight accessions (Kyuushuu, Eika Ine, Ishiwari Mochi, Col/Fukui/1965, Ookuma Nishiki, Suzume Shirazu, Iwate Ryoon and Toga) were not clustered with other accessions, giving rise to clusters with a single accession (clusters VI to XIII). Fourteen accessions were grouped in cluster II, nine in cluster III and two in clusters IV and V.

The inter-cluster distance ranged from 8.19 (between cluster I and X) to 11.53 between cluster IV (Hitachi Nishiki and Nourin Mochi 6) and X (Ookuma Nishiki). Accessions of cluster IV have longer cycle, shorter grains and lesser yield than Ookuma Nishiki, which was the most similar accession to cluster I accessions.

Ungrouped accessions have specific traits that prevent them from clustering with the remaining groups. Means of quantitative variables of these accessions are presented in Table 1.

Accession Kyuushuu exhibits exclusive alleles for five loci and longer cycle, but it is distinct from the other late accessions that are, in general, taller than Kyuushuu. Exclusive alleles for loci RM9 and RM229 were detected in the accession Col/Fukui/1965 which is a late and tall accession. Eika Ine is also a tall accession, but it differs from other tall accessions due to its precocity and for being the only accession exhibiting exclusive allele for the expressed loco RM160. Exclusive alleles were absent from the accessions Iwate Ryoon, Ishiwari Mochi, Ookuma Nishiki, Suzume Shirazu and Toga. As observed for accession Eika Ine, the accession Iwate Ryoon is tall, has long grains and exhibits precocity, contrasting to the accession Toga, which is about 30 d earlier.

Ishiwari Mochi is a late accession (one of the latest of the bank) and has longer grains distinct from most accessions, characterized by short grains. Traits linked to flag leaf characterize the accessions Ookuma Nishiki and Suzume Shirazu. While Ookuma Nishiki exhibits a narrow flag leaf, Suzume Shirazu presents a large flag leaf.

The accessions showed diversity at phenotypic and molecular level and some of them also showed suitable agronomic performance and can be used to broaden the genetic base of breeding programmes.

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