Genetic Resources and Crop Evolution (2006) 53: 197–204
DOI 10.1007/s10722-004-6691-y
© Springer 2006

Genetic diversity in accessions of wild rice *Oryza granulata* from South and Southeast Asia

Wei Qian[1,2,*], Song Ge[1] and De-yuan Hong[1]
[1]*Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, P. R. China;* [2]*National Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, P. R. China; *Author for correspondence (e-mail: qianw@sun.im.ac.cn; phone: 86-10-62642536; fax: 86-10-62548243)*

Received 6 April 2004; accepted in revised form 19 November 2004

Key words: Conservation, Genetic diversity, *Oryza granulata*, RAPD markers, Wild rice

Abstract

Oryza granulata, an upland wild rice species, represents an unique germplasm for possessing abilities of tolerance to shade and drought, immune to bacterial blight and resistance to brown planthopper. Although low degree of genetic variability has been revealed within its populations, little genetic information at the species level is available in determining rational conservation strategies. Here we used dominant DNA marker random amplified polymorphism DNA (RAPD) to assess the genetic variability among 23 accessions of *O. granulata* that collected from main distribution areas worldwide. Twenty decamer primers generated a total of 243 bands, with 83.5% of them (203 bands) being polymorphic. Calculation of Shannon index of diversity revealed an average value of 0.42 ± 0.25, indicating that *O. granulata* maintains a relatively high degree of genetic diversity on the species level. Analysis of genetic dissimilarity (GD) showed that genetic differentiation occurred among studied accessions, which supports the feasibility of current *ex situ* conservation strategies. We also suggested that information based on population studies, which could be achieved by international co-operation, is needed to conserve this widespread germplasm more effectively.

Introduction

Wild relatives of cultivated rices (*Oryza sativa* L. and *Oryza glaberrima* Steud.) play a very important role in rice breeding practically and theoretically (Vaughan 1994). For example, in China, large-scale application of hybrid rice technology that was successfully accomplished by transferring the male sterility gene from wild rice *O. rufipogon* Griff. to create the cytoplasmic genetic male-sterile (CMS) line, increased the rice production about 10–20% during the past 20 years (Yuan 1993). In addition, the first cloned rice disease resistance genes *Xa21*, which encodes a protein with unusual leucine rich repeat (LRR)-kinase domains (Song et al. 1995), was introgressed from a wild rice species (*Oryza longistaminata* A. Chev. et Roehr.). This gene has been applied to breeding for it confers broad-spectrum of resistance to rice bacterial blight disease (Zhai et al. 2000). Therefore, conservation of genetic diversity in wild relatives of cultivated rice for future usage has become an urgent issue worldwide, especially in developing countries where explosive growth of

human populations has been throwing pressure on sustainable food supply.

Among about 20 wild relatives of rice, *Oryza granulata* Nees et Arn. ex Watt. and *O. meyeriana* (Zoll. et Mor. ex Steud.) Baill. distribute in tropical area of South and Southeast Asia, including India, Cambodian, Vietnam, Thailand, southern China, Malaysia, Philippine, Nepal and Sri Lanka (Vaughan 1994). In *Oryza* genus, they belong to *O. meyeriana* complex with *GG* genome, occupying the basal position of the rice phylogeny (Aggarwal et al. 1997; Ge et al. 1999a). Traditionally, the two species were discriminated by their spikelet length. As Chang (1988) suggested, those spikelet length longer than 7 mm were classified into *O. meyeriana*, and those length shorter than 7 mm were treated as *O. granulata*. However, this criterion did not gain much support from ecological and genetic evidences. Recently, based on morphological analysis and the pattern of chromosome pairing during meiosis, Gong et al. (2000) combined *O. granulata* and *O. meyeriana* into one species (*O. granulata*). In the present study, we adopted this combination and the name of *O. meyeriana* was used only for describing accessions conveniently.

Although *O. granulata* is difficult to cross with cultivated rice (*O. sativa*), which impedes practical usage in hybrid breeding, it is a potential rice germplasm with abilities of immune to bacterial blight caused by gram-negative bacterium *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swing et al., resistance to brown planthopper (*Nilaparvata lugens* Stal.) and tolerance to shade and seasonal drought (Vaughan 1994). These agricultural profitable features might be taken into practice by employing alternative approaches such as molecular breeding. However, due to serious habitat disturbance, the survival of *O. granulata* has been threatening in distributed area (Fu 1992). For example, our field survey during 1997–1998 revealed that above 13% populations of *O. granulata* had been extirpated in China, and up to 84% populations were under the danger of extinction (Qian et al. 2001a). Most anxiously, the majority of current habitats of the species were isolated into fragments by rapid expanding of human residences and farmlands, which may result in unretrievable genetic deterioration causing by genetic drift.

In previous conservation genetic studies, both allozyme and DNA fingerprinting analysis revealed substantial genetic differentiation among populations from different geographical areas in China, albeit the fact that very low level of genetic variability being maintained within each population of *O. granulata* (Gao et al. 2000; Qian et al. 2001b). These results implied that the patterns of genetic variation of the species are various at different geographical scales, which might be affected by diverse historical and ecological factors. However, little information was available to understand the genetic diversity of *O. granulata* on the species level. In this study, we used dominant DNA marker random amplified polymorphism DNA (RAPD) to assess the genetic variability among the accessions of *O. granulata* that were collected from main distribution area worldwide, and discussed its implication on conservation practice.

Materials and methods

Plant material

Initially, 21 and five available accessions of *O. granulata* and *O. meyeriana*, respectively, were used in this study. Two accessions of *O. granulata* came from our collection in Yunnan and Hainan Provinces of China, whereas others were gifts provided by International Rice Research Institute. Before germination, these seeds were incubated under 54 °C for 7 days to recover from dormancy. Three accessions of *O. meyeriana* failed to germinate after this effort, probably due to immaturity or decreased seed vigor during long-term storage. Therefore, a total of 23 accessions, including five used in biosystemaic study (Gong et al. 2000), were eventually analyzed by RAPD markers as described (Table 1).

Extraction of total DNA and RAPD amplification

Total DNA was extracted from fresh leaves following the CTAB (cetyltrimethylammonium bromide) procedure as described in Qian et al. (2001b). Twenty RAPD decamer primers (Operon Technologies, CA, USA: OPB-6, OPB-7, OPB-12, OPK-3, OPK-6, OPK-8, OPK-9, OPK-11, OPK-12, OPK-13, OPK-15, OPY-1, OPY-2, OPY-9, OPY-14, OPY-15, OPY-18, OPY-19, OPZ-3 and OPZ-4) that revealed discrete reproducible amplification products were used for

Table 1. Accessions of *Oryza granulata* analyzed in the present study.

Code	Variety name[a]	IRGC accession No[b].	Source country
1	Oryza *granulata*	80740	Myanmar
2	*Oryza granulata*	100879	India
3	*Oryza granulata*	100880	Sri Lanka
4	*Oryza granulata*	102117	India
5	*Oryza granulata*	102118	Thailand
6	*Oryza granulata*	102119	Myanmar
7	*Oryza granulata*	104506	Malaysia
8	*Oryza granulata*	104611	Sri Lanka
9	*Oryza granulata*	104986	Unknown
10	*Oryza meyeriana* (Formerly)	104990	Malaysia
11	*Oryza granulata*	105707	Nepal
12	*Oryza granulata*	106444	India
13	*Oryza granulata*	106445	India
14	*Oryza granulata*	106446	Indonesia
15	*Oryza granulata*	106447	Nepal
16	*Oryza granulata*	106448	Nepal
17	*Oryza granulata*	106449	India
18	*Oryza granulata*	106467	Laos
19	*Oryza granulata*	106468	Laos
20	*Oryza granulata*	106869	Vietnam
21	*Oryza meyeriana* (Formerly)	106474	Philipine
22[c]	*Oryza granulata*	–	China (Hainan)
23[c]	*Oryza granulata*	–	China (Yunnan)

[a]Names of the accessions are according to that of IRGC (IRRI Genetics Resources Center).
[b]Three former *O. meyeriana* accessions, with IRGC accession No. 104989 (from Malaysia); 106473 (Philippines) and 104987 (Unknown), failed to germinate after effort and were excluded in this study.
[c]These accessions were taken from our field survey in China.

further analysis. PCR amplifications were performed in a Rapidcycler 1818 (Idaho Tech.), programed for an initial 120 s at 94 °C, 10 s at 35 °C, 20 s at 72 °C for 2 cycles, followed by 40 cycles of 10 s at 94 °C, 10 s at 35 °C, and 60 s at 72 °C, and ended with 7 min at 72 °C. Each reactions was carried out in a volume of 10 μL containing 50 mM Tris-HCl, PH 8.3, 500 μg/mL BSA, 10% Ficoll, 1 mM tartrazine, 2 mM $MgCl_2$, 200 μM dNTP, 1 μM primer, 5 ng of DNA template and 0.5 U Taq polymerase. Amplification products were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide, and photographed under ultraviolet light. Molecular weights were estimated using a 100 bp DNA ladder.

Data analysis

In diploid species, RAPD is a dominant marker so that amplified fragments were scored for the presence (1) and absence (0) of homologous bands and a matrices of RAPD profiles were assembled for the following analyses: Genetic diversity was measured by the percentage of polymorphic bands (PPB) and Shannon index of diversity (Lewontin 1972). RAPDistance (Armstrong et al. http://life.anu.edu.au/molecular/software/rapd.htm) was used to calculate Nei and Li (1979), Jaccard (1901), Excoffier et al. (1992) and Sokal and Sneath (1973) similarity coefficients. Consistency of these coefficient matrix were compared by Mantel tests (Mantel 1967), and the genetic similarities were used to construct dendrograms using the Neighbor-joining method (Saito and Nei 1987) in RAPDistance and the unweighted pair group method (UPGMA) (Sneath and Sokal 1973) of the SHAN procedure (sequential, hierarchical, agglomerative and nested clustering) in NTSYS software (Rohlf 1994).

Results

RAPD polymorphism

Figure 1 showed an example of PCR amplification products of the studied 23 accessions by using four RAPD primer OPK-6, OPK-13, OPY-1 and OPY-2. A brief summary of genetic data was listed in Table 2. Twenty RAPD decamer primers amplified 243 reproducible and unambiguous bands with molecular weight from 200 to 2400 bp. Each primer could obtain 5 (OPY-9) to 17 (OPK-6) bands, corresponding to an average of 12.2 bands per primer. Of them, up to 203 (PPB = 83.54%) bands showed polymorphic, and the number of polymorphic bands per primer ranged from 4 (OPY-19) to 15 (OPK-6), with an average of 10.2. Therefore, high score of PPB was detected among the analyzed accessions of *O. granulata* and *O. meyeriana*. Based on RAPD profile of each primer, measures of Shannon index of diversity were from 0.19 to 0.60, with average of 0.42 ± 0.25. This result showed that a relatively high level of genetic polymorphism was determined by these random primers in the accessions of *O. granulata*.

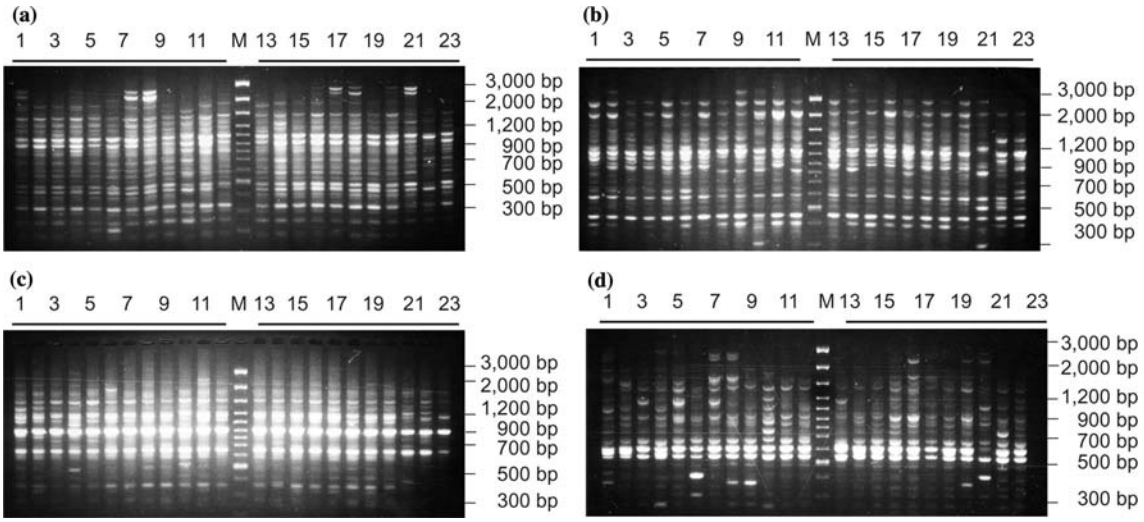

Figure 1. RAPD amplification products generated from accessions of *Oryza granulata* genomic DNA. Primers are used in (a) OPK-6; (b) OPK-13; (c) OPY-1 and (d) OPY-2. 1–23 are accession codes according to that of Table 1. M, 100 bp DNA ladder.

Table 2. Genetic polymorphism of *Oryza granulata* accessions detected by RAPD markers.

Primers	Bands generated	Polymorphic bands	Percentage of polymorphic bands (%)	Shannon index of diversity
OPB-6	14	10	71.4	0.32
OPB-7	15	12	80.0	0.30
OPB-12	16	14	87.5	0.45
OPK-3	11	8	72.7	0.33
OPK-6	17	15	88.2	0.50
OPK-8	12	10	83.3	0.43
OPK-9	11	10	90.9	0.49
OPK-11	12	10	83.3	0.45
OPK-12	12	10	83.3	0.42
OPK-13	7	6	85.7	0.39
OPK-15	16	13	81.3	0.43
OPY-1	13	10	76.9	0.39
OPY-3	12	11	91.7	0.41
OPY-9	11	9	81.8	0.43
OPY-14	12	8	66.7	0.36
OPY-15	9	5	55.6	0.19
OPY-18	13	13	100.0	0.47
OPY-19	5	4	80.0	0.50
OPZ-3	13	13	100.0	0.54
OPZ-4	12	12	100.0	0.60
Total	243	203	–	–
Average	12.15	10.15	83.5	0.42
s.d.	2.889	2.925	0.03	0.25

Genetic similarity

Four different dissimilarity coefficients (Nei & Li, Jaccard, Excoffier and Sokal & Sneath) were calculated, and Mantel test among these coefficients indicated significant consistency (data not shown). Therefore, only the genetic dissimilarity (GD) estimate of Nei & Li coefficient among 23 accessions of *O. granulata* and *O. meyeriana* was listed in Table 3. The GDs from RAPD analysis ranged from 0.074 for accession 19/15 to 0.33 for the accessions 3/21. The average GDs of two former *O. meyeriana* accessions from the others are 0.235 ± 0.024 (for 10) and 0.255 ± 0.042 (for 21), all higher than the average of GD estimates 0.1777 ± 0.0031. The UPGMA tree based on Nei & Li coefficient was depicted in Figure 2. It indicated five main clusters, where most of accessions (17 out of 23) grouped into a major branch. Accessions from the same geographical area did not usually be grouped together, for example, five accessions from India were separated into five distinct clades. In addition, though two clades of former *O. meyeriana* are located on the basal position and separated from other accessions in the dendrogram, they did not be grouped into a single branch, indicating that the two accessions are genetically differentiated from *O. granulata*.

Discussions

By using RAPD as genetic markers, we detected as high as 83.5% of bands being polymorphic in 23

Table 3. Genetic dissimilarities among 23 accessions of *Oryza granulata.*

	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	0.000																						
2	0.152	0.000																					
3	0.222	0.199	0.000																				
4	0.199	0.134	0.183	0.000																			
5	0.163	0.165	0.137	0.181	0.000																		
6	0.187	0.159	0.183	0.163	0.127	0.000																	
7	0.180	0.188	0.149	0.180	0.132	0.192	0.000																
8	0.155	0.199	0.194	0.196	0.161	0.155	0.136	0.000															
9	0.149	0.151	0.194	0.179	0.143	0.143	0.166	0.088	0.000														
10	0.258	0.242	0.274	0.245	0.220	0.258	0.238	0.242	0.217	0.000													
11	0.178	0.132	0.141	0.130	0.154	0.130	0.159	0.170	0.140	0.218	0.000												
12	0.172	0.168	0.174	0.166	0.142	0.142	0.171	0.122	0.093	0.205	0.079	0.000											
13	0.238	0.209	0.191	0.206	0.188	0.206	0.149	0.222	0.191	0.294	0.172	0.191	0.000										
14	0.224	0.182	0.189	0.205	0.174	0.149	0.173	0.160	0.153	0.221	0.146	0.146	0.174	0.000									
15	0.174	0.108	0.169	0.144	0.168	0.132	0.161	0.172	0.130	0.220	0.086	0.110	0.175	0.142	0.000								
16	0.189	0.131	0.192	0.159	0.177	0.159	0.170	0.193	0.169	0.241	0.096	0.133	0.196	0.189	0.110	0.000							
17	0.147	0.161	0.205	0.183	0.153	0.141	0.176	0.151	0.157	0.235	0.139	0.151	0.209	0.183	0.104	0.114	0.000						
18	0.188	0.172	0.191	0.170	0.158	0.164	0.133	0.138	0.132	0.222	0.143	0.131	0.214	0.150	0.145	0.160	0.142	0.000					
19	0.166	0.113	0.167	0.148	0.148	0.124	0.159	0.140	0.128	0.199	0.079	0.109	0.172	0.153	0.074	0.096	0.084	0.137	0.000				
20	0.167	0.169	0.174	0.173	0.119	0.131	0.136	0.1353	0.141	0.205	0.110	0.122	0.185	0.129	0.130	0.134	0.128	0.096	0.105	0.000			
21	0.268	0.258	0.326	0.287	0.261	0.274	0.291	0.277	0.264	0.247	0.265	0.265	0.318	0.270	0.230	0.264	0.206	0.244	0.240	0.226	0.000		
22	0.245	0.242	0.254	0.271	0.226	0.226	0.250	0.223	0.236	0.250	0.197	0.197	0.295	0.233	0.200	0.241	0.235	0.228	0.204	0.185	0.274	0.000	
23	0.198	0.181	0.168	0.204	0.179	0.154	0.172	0.171	0.159	0.213	0.115	0.133	0.218	0.166	0.110	0.151	0.151	0.155	0.115	0.134	0.229	0.152	0.000

*1–23 represent the codes of accessions according to that of Table 1.

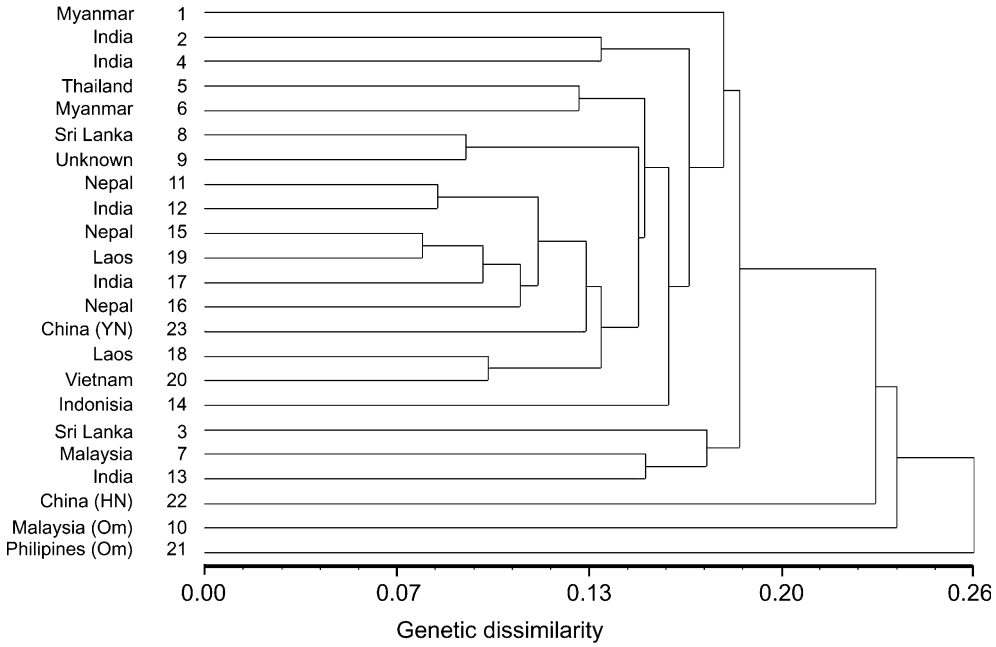

Figure 2. Dendrogram illustrating genetic dissimilarities among 23 analyzed accessions of *Oryza granulata*. The tree was generated by the UPGMA cluster analysis based on Nei & Li coefficients. YN: Yunnan Province; HN: Hainan Province; Om: *Oryza meyeriana*.

accessions of *O. granulata*. When comparing our result with other studies using the same method, for example, in cultivated rice (*O. sativa*), Yu and Nguyen (1994) found high level of genetic diversity within 13 upland and lowland accessions, with a PPB value of 80%, and Ko et al. (1994) detected a PPB value of 67% within cultivars from Australia. As for wild rices, Buso et al. (1998) revealed as high as 95% of PPB value in four populations of *O. glumaepatula* Steud. from South America, and Ge et al. (1999b) detected that there is 82.1% polymorphic RAPD bands among five populations of common wild rice *O. rufipogon* from China and Brazil. Therefore, comparison shows that PPB value of *O. granulata* is one of the highest score determined in rice species. However, because the sample size and total amount of bands could influence the PPB value, other parameters that based on the frequency of polymorphic bands, such as Shannon index of diversity and Simpson's index of diversity, are more suitable in estimating genetic variability (Cruzan 1998). As Table 2 showed, the average of Shannon index of diversity in the accessions studied is 0.442 ± 0.246, indicating that *O. granulata* maintains a relatively high degree of diversity on the species level. In addition, as showed in Table 3, though high score of PPB obtained, all genetic dissimilarities are lower than 0.35, indicating that the discrepancy between high score of PPB value and Shannon index of diversity might be contributed by low frequency, accession-specific RAPD bands.

Two possibilities could result in high level of genetic diversity within widespread species as well as *O. granulata*. Firstly, if substantial amount of gene flow occurs frequently among populations, high level of genetic variability will be maintained in a plant species, especially within local populations rather than among various geographical areas (Hamrick and Godt 1989). For example, another wild rice species, *O. rufipogon*, was found to possess high variability by using RAPD analysis, and gene exchange by means of seeds or pollens was suspected to be the primary reason (Xie et al. 2001). However, as previous study indicated, low level of genetic variations are maintained within the populations of *O. granulata*. By using allozyme analysis, Gao et al. (2000) revealed that percentage of polymorphic loci (P) is only 6.33%, with the average expected heterozygosity (He) being 0.016 in populations of the species. Similarly, by using identical set of RAPD primers in this study, Qian et al. (2001b) detected an average PPB value of 8.24% in five populations from

China. Both of the studies revealed that the most majority of genetic variation in *O. granulata* was contributed by genetic differentiation from populations distributed at different geographical regions, rather than from within-population level (Gao et al. 2000; Qian et al. 2001b). Consequently, population genetic structure of *O. granulata* is quite different from plant species that has substantial gene flow among populations, since these plant species usually contains large amount of genetic polymorphism within local populations (Hamrick and Godt 1989). Therefore, we proposed that gene flow among populations of *O. granulata* is limited and is unlikely the essential genetic process resulting in relatively high degree of genetic variation found in the present study.

Secondly, if populations of a plant species were isolated by ecological or geographical factors, various selective pressures at different habitats and genetic drift would result in local adaptation, where population specific alleletic genes would evolve and eventually fixed in a limited area (Hamrick and Godt 1989). As revealed by our field survey and demographic studies Qian et al. 2001a, *O. granulata* maintains a colony pattern in plant communities, with a typical population size of 10 to hundreds individuals. Unlike its aquatic relatives, the species always grow in semi-open habitats such as bamboo thickets and tightly tuft grasses, it couldn't disperse seeds or propagules through water, which impedes long-distance gene exchange among populations. Furthermore, owning to rapid plant succession speed in tropical area, the species maintains a high population turn over rate during colonization and disappear in communities (Qian et al. 2001a). This process might cause genetic drift because the limited seeds dispersal could result in founder effect. Therefore, we tend to believe that isolation by distance is the primary factor in forming genetic diversity pattern detected in this study.

Determination of genetic diversity in *O. granulata* has an immediate implication in conservation practice. Our analysis revealed moderate to high level of genetic variation in the species, which means in order to conserve it rationally, as many germplasm throughout distribution area as possible, should be reserved to protect unique genetic resources that evolved in different geographical areas. It also suggested that current *ex situ* conservation strategies, such as long-term germplasm storage in IRRI Genetics Resources Center and National Wild Rice Nursery of China, are appropriate in conservation practice. However, due to the limitation of available materials, this study cannot systematically assess genetic variability of *O. granulata* on the population level. Since the knowledge from population genetics is critical in determining minimal population size and core germplasm collections, which are especially fundamental for *in situ* conservations. Therefore, international co-operations are necessary to prompt future studies and to conserve this important rice germplasm more effectively.

Acknowledgements

We gratefully acknowledge IRRI Genetics Resources Center for providing accessions used in this study (IRGC request No: 19990701). This study was supported by Key Project of the Chinese Academy of Sciences (KZ-951-B1–102) and National Natural Science Foundation project (30100010).

References

Aggarwal R.K., Brar D.S. and Khush G.S. 1997. Two new genomes in the *Oryza* complex identified on the basis of molecular divergence analysis using total genomic DNA hybridization. Mol. Gen. Genet. 254: 1–12.

Buso G.S.C., Rangel P.H. and Ferreira M.E. 1998. Analysis of genetic variability in South American wild rice populations (*Oryza glumaepatula)* with isozymes and RAPD markers. Mol. Ecol. 7: 107–117.

Chang T.T. 1988. Taxonomic key for identifying the 22 species in the genus *Oryza*. Inter. Rice Res. Newsletters 13: 4–5.

Cruzan M.B. 1998. Genetic markers in plant evolutionary ecology. Ecology 79: 400–412.

Excoffier L., Smouse P.E. and Quattro J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Gen. Soc. Am. 131: 479–491.

Fu L.G. 1992. Red Book of Plant in China. Science Press, Beijing, China, 314 pp.

Gao L.Z., Ge S. and Hong D.Y. 2000. Low levels of genetic diversity within populations and high differentiation among populations of a wild rice. Int. J. Plant Sci. 161: 691–697.

Ge S., Sang T., Lu B.R. and Hong D.Y. 1999a. Phylogeny of rice genomes with emphasis on origins of allotetraploid species. Proc. Natl. Acad. Sci. USA 96: 14400–14405.

Ge S., Oliveira G.C., Schaal B.A., Gao L.Z. and Hong D.Y. 1999b. RAPD variation within and between natural populations of the wild rice *Oryza rufipogon* from China and Brazil. Heredity 82: 638–644.

Gong Y., Borromeo T. and Lu B.R. 2000. A biosystematic study of the *Oryza meyeriana* complex (Poaceae). Plant Syst. Evol. 224: 139–151.

Hamrick J.L. and Godt M.J.W. 1989. Allozyme diversity in plant species. In: Brown A.H.D., Clegg A.L., Kahler A.L. and Weir B.S. (eds), Plant Population Genetics, Breeding, and Genetic Resources. Sinaner, Sunderland, Massachusetts, pp. 43–63.

Jaccard P. 1901. Etude comparative de la distribution florale dans une portion des Alpes et des Jura. Bull. Soc. Vaudoise Sci. Nat. 37: 547–579.

Ko H.L., Cowan D.C., Henry R.J., Graham G.C., Blakeney A.B. and Lewin L.G. 1994. Random amplified polymorphic DNA analysis of Australian rice (*Oryza sativa* L.) varieties. Euphytica 80: 179–189.

Lewontin R.C. 1972. The apportionment of human diversity. Evol. Biol. 6: 381–398.

Mantel N.A. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209–220.

Nei M. and Li W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269–5273.

Qian W., Xie Z.W., Ge S. and Hong D.Y. 2001a. Distribution and conservation of an endangered wild rice *Oryza granulata* in China. Acta Bot. Sin. 43: 1279–1287.

Qian W., Ge S. and Hong D.Y. 2001b. Genetic variation within and among populations of a wild rice *Oryza granulata* from China detected by RAPD and ISSR. Theor. Appl. Genet. 102: 440–449.

Rohlf F.J. 1994. NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System, V. 1.80. Exeter Software, New York.

Saito N. and Nei M. 1987. Neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406–425.

Sneath P. and Sokal R. 1973. Numerical Taxonomy. W. H. Freeman & Company, San Francisco.

Song W.Y., Wang G.L., Chen L.L., Kim H.S., Pi L.Y., Holsten T.E., Gardner J., Wang B., Zhai W.X., Zhu L.H., Fauquet C. and Ronald P.C. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270: 1804–1806.

Vaughan D.A. 1994. The Wild Relatives of Rice. IRRI, Manila.

Yu L.X. and Nguyen N.T. 1994. Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theor. Appl. Genet. 87: 668–672.

Yuan L.P. 1993. Advantages of and constraints to the use of hybrid rice varieties. In: Wilson K.J. (eds), Proceedings of the International Workshop on Apomixis in Rice. The Rockefeller Foundation and China National Center for Biotechnology Development, New York and Beijing, pp. 1–4.

Xie Z.W., Lu Y.Q., Ge S., Hong D.Y. and Li F. Z. 2001. Clonality in wild rice (*Oryza rufipogon*, Poaceae) and its implications for conservation management. Am. J. Bot. 88: 1058–1064.

Zhai W.X., Li W., Tian Y., Pan S., Zhao X., Zhao B., Zhang Q. and Zhu L. 2000. Introduction of a blight resistance gene, *Xa21*, into five Chinese rice varieties through an *Agrobacterium* mediated system. Sci. China (Ser. C) 43: 361–368.