A study on population genetic structure of *Oryza meyeriana* (Zoll. et Mor. ex Steud.) Baill. from Yunnan and its *in situ* conservation significance*

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Received July 20, 1998

Abstract In order to determine genetic diversity of *Oryza meyeriana* (Zoll. et Mor. ex Steud.) Baill., 12 enzyme systems encoded by 17 loci were electrophoretically analyzed in 164 individuals of seven populations from Simao Prefecture, Yunnan Province, China. In comparison with those seed plants with the same life history and breeding systems, as well as the other species in the genus *Oryza*, the species shows rather low levels of genetic diversity ($A=1.1$, $P=8.0\%$, $Ho=0.004$ and $He=0.015$) within populations and high genetic differentiation among populations. F_{ST} was up to 0.649, suggesting that 64.9% of total genetic variability exists among populations. Considering high genetic differentiation among populations from a limited geographic region, most of the populations of the species are worth being protected, and therefore, great natural protection regions should theoretically be established in which a great deal of populations should be involved for developing *in situ* conservation management. Meanwhile, some priory localities for *in situ* conservation of *O. meyeriana* in Yunnan Province, were proposed.

Keywords: ***Oryza meyeriana* (Zoll. et Mor. ex Steud.) Baill., allozyme analysis, Yunnan, population genetic structure, *in situ* conservation.**

It is well known that rice genetic improvement programs depend on its wild relatives, which are one of the most important plant genetic resources in China as well as other developing countries[1]. *O. meyeriana* is widely distributed in southern and southeastern Asia, and grows in three provinces of southern China: Yunnan, Hainan and Taiwan[2]. It has seriously been endangered with most of natural populations decreasing as a result of rapid population growth and deforestation in tropical regions[3—5]. Because the species can offer unique characteristics valuable to rice breeders in the future, such as abilities to live in dry land, tolerate shade and resist to bacterial blight, its great significance in studies and conservation has been recognized in the world. No studies on genetic diversity of the species, however, have been reported to date. The effect of development of *in situ* conservation of the species bases on our understanding of the population genetic structure. Moreover, as marginal populations, the studies and conservation of Chinese

* Project supported by the Grant of the President of the Chinese Academy of Sciences.

O. meyeriana are of importance in maintaining genetic integrity of gene pool of the species.

Allozyme analysis is an effective technique to examine genetic structure of natural populations[6,7]. In the present study, 7 populations from Simao Prefecture, Yunnan Province, which is main distribution of the species in China with the least human disturbance, were sampled, and isozyme electrophoresis was conducted to explore the population genetic structure of the species for taking *in situ* conservation strategies effectively in China.

1 Materials and methods

1.1 Materials origin

Live samples were taken from 7 populations of *O. meyeriana* from Simao, Yunnan Province, China (table 1). Because the species has high colonizing ability in the populations sampled, care was taken to prevent collecting multiple samples from a single individual. Live ratoons were randomly collected at intervals of at least 5 m in the field, numbered, transplanted to pots and maintained in Xishuangbanna Tropical Botanical Garden. Because the Gongxing population was small in size, all individuals were taken. Young leaves were individually collected in March, 1995, stored in plastic bags on ice and transported to the laboratory by airplane. For each individual, 0.05 g of fresh leaf material was crushed in 100 μL of Tris-HCl buffer[8] on the ice. The extract was absorbed into 3 mm × 8 mm paper wicks and stored at −70℃ until electrophoresis was conducted.

Table 1 Genetic variability at 17 loci in seven populations of *O. meyeriana*

Population	Size	*A*	*P*	Ho	He
1 Simao Zuling	17	1.1	5.6	0.000	0.006
2 Menglian Gongxing	8	1.1	5.6	0.000	0.028
3 Simao Lanla	29	1.2	16.7	0.002	0.027
4 Lancan Yakou Mengkuang	19	1.1	5.6	0.003	0.003
5 Lancan Yakou Resuitang	40	1.1	11.1	0.001	0.014
6 Lancan Qianliu Tongchang	24	1.1	5.6	0.019	0.016
7 Lancan Yakou Zhichang	19	1.1	5.6	0.000	0.011
Mean	22	1.11	7.97	0.004	0.015

1.2 Starch gel electrophoresis

Twenty enzymes were detected by using starch gel electrophoresis (table 2), of which twelve enzymes encoded by 17 putative loci were clearly resolved in conformance with Mendelian Isolation and Combination Rule. The electrophoretic methods followed Soltis et al.[8] and Glaszmann et al.[9] with 12% starch gels (Sigma Company, S-4501). Staining procedures for all enzymes followed Soltis et al.[8].

1.3 Data analysis

Electrophoretic data were analyzed using the computer program Biosys-1 (version 1.7)[10] for the IBM-PC. Data were entered as genotype numbers from which allele frequencies were calculated. Genetic variability (including the mean number of alleles per locus (*A*), percentage of loci polymorphic (*P*), observed heterozygosity (Ho) and expected heterozygosity (He)), deviation from Hardy-Weinberg equilibrium (fixation indices), Nei's unbiased genetic identity (I) (1978)[11], as well as *F*-statistics were calculated; Wright's[12] formula $F_{ST} = 1/(1 + 4Nm)$ was

used to estimate approximately the value of gene flow.

Table 2 Enzymes system assayed, gel buffers and the number of loci scored

Enzyme system	Abbreviation	EC No.	Gel buffer	No. of loci
Aspartate aminotransferase	AAT	EC 2.6.1.1	S6	2
Alcohol dehydrogenase	ADH	EC 1.1.1.1	GI	1
Diaphorase	DIA	EC 1.6.2.2	S6	2
Glutamate dehydrogenase	G3PDH	EC 1.4.1.2	GI	1
Isocitrate dehydrogenase	IDH	EC 1.1.1.42	GI	1
Malate dehydrogenase	MDH	EC 1.1.1.37	S1#	3
Malic enzyme	ME	EC 1.1.1.40	S1#	1
Phosphogluconate dehydrogenase	6PGD	EC 1.1.1.44	S1#	1
Phosphoglucoisomerase	PGI	EC 5.3.1.9	S6	1
Phosphoglucomutase	PGM	EC 2.7.5.1	GI	1
Shikimate dehydrogenase	SKD	EC 1.1.1.25	GI	1
Triosephosphate isomerase	TPI	EC 5.3.1.1	S6	2

2 Results and discussion

2.1 Allelic loci and frequencies

The electrophoresis resolved 12 enzymes encoded by 17 putative loci (table 3). Of them, five were polymorphic loci. Although two isozymes of PGM are typically present in diploid seed plants[13], only one PGM isozyme was observed in the present study. Two loci of G3PDH and PGD were commonly reported in the other *Oryza* species[14], but only one was observed in this

Table 3 Allelic frequencies at 17 loci in seven populations of *O. meyeriana*

Loci	Population						
	1	2	3	4	5	6	7
Aat-1a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
-3a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Adh-a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Dia-1a	0.000	0.000	0.000	0.000	0.013	0.000	0.000
-1b	1.000	1.000	1.000	1.000	0.988	0.833	1.000
-1c	0.000	0.000	0.000	0.000	0.000	0.167	0.000
Dia-2a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gdh-a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-1a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-2a	1.000	1.000	0.103	0.000	1.000	1.000	1.000
-2b	0.000	0.000	0.897	1.000	0.000	0.000	0.000
Mdh-3a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me-a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgd-a	0.941	1.000	0.966	1.000	0.875	1.000	0.895
-b	0.059	0.000	0.034	0.000	0.125	0.000	0.105
Pgi-1a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-a	1.000	0.375	1.000	1.000	1.000	1.000	1.000
-b	0.000	0.625	0.000	0.000	0.000	0.000	0.000
Skd-a	0.000	0.000	0.086	0.026	0.000	0.000	0.000
-b	1.000	1.000	0.879	0.974	1.000	1.000	1.000
-c	0.000	0.000	0.034	0.000	0.000	0.000	0.000
Tpi-1a	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Tpi-2a	1.000	1.000	1.000	1.000	1.000	1.000	1.000

study. Although the banding patterns of PGI seemed more than one locus, only Pgi-1 was used due to poor resolution in the other loci.

2.2 Levels of genetic diversity within populations

O. meyeriana possessed rather low levels of genetic variability (table 1). The mean values of P and H are much lower than those of perennial herbs, gravity dispersed and wind-crossing plants ($P=28.0\%$, $H=0.096$; $P=29.8\%$, $H=0.101$ and $P=49.7\%$, $He=0.148$)[7]. As far as the genus *Oryza* is concerned, these values of genetic diversity are much lower than those of the other wild rice species studied. For example, in his allozyme studies on *O. rufipogon* Griff. from Thailand, Barbier[15,16] found the values of $A=1.98$ and $H=0.209$ for perennial populations, and $A=1.58$ and $H=0.099$ for annual ones. Moreover, our recent allozyme survey showed that the other two Chinese wild rices possessed relatively high genetic variation, with $A=1.3$, $P=22.7\%$ and $H=0.068$ for *O. rufipogon*, and $A=1.16$, $P=16.20\%$ and $H=0.056$ for *O. officinalis* Wall. ex Watt. (Gao et al. unpublished). In other words, *O. meyeriana* has the lowest levels of allozymic variability among the three Chinese wild rice species.

2.3 Genetic structure of populations

Developing conservation actions for endangered species as well as sampling strategies for crop germplasm resources depend on the information of distribution of genetic variability among populations. In the present study, Wright's F-statistics was used to detect the distribution of genetic variation of *O. meyeriana* (table 4). F_{IS} was 0.754, suggesting that most of the populations deviated from Hardy-Weinberg expectation within populations; F_{IT} was 0.914, indicating that heterozygotes deviated from Hardy-Weinberg expectation among populations of the species and a deficiency of heterozygotes; and F_{ST} was up to 0.649, suggesting that 64.9% of the total genetic variation existed among populations, which is much higher than the average values of herbaceous perennials, wind-crossing and gravity-dispersed seed plants (23.3%, 0.99% and 27.7%, respectively), and is also higher than the F_{ST} of Chinese *O. rufipogon* (Gao et al., unpublished). The distribution of alleles (table 3) further suggests that alleles are unevenly distributed among populations. For example, rare allele Dia-1a was found in the Lancan Yakou Resuitang population with a low frequency of 0.013, while Dia-1c was detected in the Lancan Qianliu Tongchang population with the frequency of 0.167, which is not further than 10 km from each other; Mdh-2b existed in the Simao Lanla population and Lancan Mengkuang population with the frequencies of 0.897 and 1.000, respectively; Pgm-b was only distributed in the Menglian Gongxing population with the frequency of 0.625, and therefore, strong genetic differentiation occurs among populations from a limited geographic region. Nei's unbiased genetic identities were given in table 5. The mean genetic identity was up to 0.969, which resulted from 12 common monomorphic loci and 4 common alleles of polymorphic loci. The results suggest that there is a significant, negative correlation between the distance between populations and genetic identity; pairs of populations that are geographically close to each other have higher genetic identities than those separated by greater distance. Thus the population genetic structure of *O. meyeriana* in Yunnan approaches an island model of isolation-by-distance[17]. The population genetic structure observed was also shown in figure 1.

Table 4 Summary of F-statistics at five polymorphic loci of seven populations of *O. meyeriana*

Loci	F_{IS}	F_{IT}	F_{ST}
Dia-1	−0.185	−0.025	0.135[a)]
Mdh-2	1.000	1.000	0.933[a)]
Pgd	1.000	1.000	0.053[b)]
Pgm	1.000	1.000	0.588[a)]
Skd	0.677	0.699	0.068[c)]
Mean	0.754	0.914	0.649[a)]

a) $P<0.001$; b) $P<0.1$; c) $P<0.01$.

Table 5 Matrix of Nei's (1978) unbiased genetic identity values among seven populations of *O. meyeriana*

Population	1	2	3	4	5	6	7
1 Simao Zuling	****	0.979	0.954	0.944	1.000	0.999	1.000
2 Menglian Gongxing		*****	0.932	0.922	0.978	0.977	0.978
3 Simao Lanla			*****	0.999	0.954	0.952	0.954
4 Lancan Yakou Mengkuang				*****	0.943	0.943	0.944
5 Lancan Yakou Resuitang					*****	0.998	1.000
6 Lancan Qianliu Tongchang						*****	0.998
7 Lancan Yakou Zhichang							*****

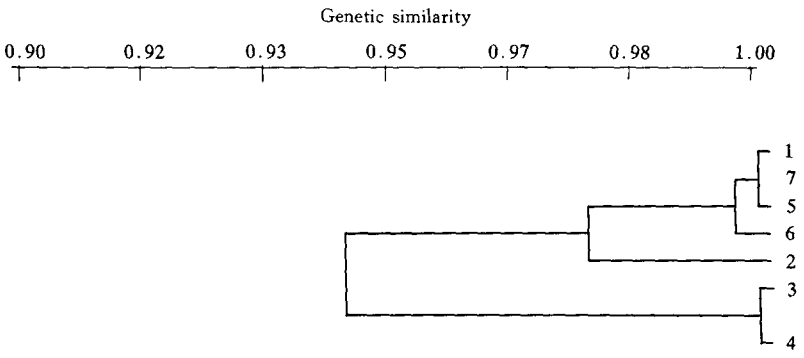

Fig. 1. Cluster analysis of seven populations of *O. meyeriana* using unweighted pair group method and Nei's (1978) unbiased genetic identity values.

In conclusion, allozymic data indicates low levels of genetic variability within populations and high genetic differentiation among populations of *O. meyeriana* in the limited geographic region. Several possible factors may be used to account for the population genetic structure observed: (1) The populations from Yunnan exist in the margin of the species, which may be established by a few founders out of its center of genetic diversity. Accordingly, its genetic background is severely influenced by "Founder Effect", and thus low genetic variability was maintained; (2) as an upland plant species, low frequencies of seed dispersal by means of water flow and the other characteristics such as gravity-dispersed seeds may limit gene flow among populations and even within populations and enhance remarked genetic differentiation. Wright[12] reclaims that restricted gene flow may be one of the most important factors to lead to genetic differentiation between populations once the value of gene flow is lower than 1 ($Nm<1$). In the present study, the results ($Nm=0.163$) suggest that high genetic differentiation may mainly originate from restricted gene flow; and (3) our field observations shows that the species is characteristic of clonizing growth (vegetation propagation). Clonal propagation enables it to establish very large populations and enhance mating affairs among the relatives within the populations, which results in smaller and

smaller effective population in size and a loss of genetic variability within populations.

2.4 Significance in *in situ* conservation

The conservation of marginal populations is a critical step for maintaining genetic integrity of the gene pool of *O. meyeriana*. Our results suggest that 64.9% of total genetic variation existed among populations. Not only there were differences of allelic frequencies among populations, but also some alleles were unevenly distributed among populations. As to such a species with high genetic differentiation among populations in a narrow geographic region, most of the populations are worth being protected. Therefore, great natural protection regions should theoretically be founded for developing *in situ* conservation management, in which a great deal of populations should be involved. Our results further indicate that *in situ* conservation should take priority to the Menglian Gongxing population and Lancan Yakou Mengkuang population because there was lowest genetic identity of $I = 0.922$ between them, showing that there are most significant differences of allele frequencies so that they are most valuable to be included in the conservation programs. It deserves to point out that the Menglian Gongxing population grows under the secondary forest besides Nankajiang River, which is not further than 5 km from Myanmar and seriously isolated from the other populations, so it should undoubtedly be listed as one of key conservation localities in Yunnan Province due to rather small population in size as well as endangered habitat; moreover, those populations with high genetic variation, such as the Simao Lanla population and Lancan Yakou Resuitang population, should be taken consideration. However, how many populations should be selected as target populations for *in situ* conservation management? And how much should be involved in for each population so as to maintain evolutionary process of marginal populations of the species and conserve their genetic diversity effectively? It is expected to conduct detailed field investigation and more studies on genetic diversity of the species. Especially, studies on the dispersed mechanism of seeds, adaptive significance of dormancy of seeds, and effect of clonal propagation in shaping population genetic structure should be paid great attention to.

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