

## Population genetic structure and conservation priorities of *Oryza rufipogon* Griff. populations in Kerala, India

R. Dinesh Raj<sup>1,2</sup>, A. G. Kiran<sup>1</sup> and George Thomas<sup>1,\*</sup>

<sup>1</sup>Plant Molecular Biology, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram 695 014, India

<sup>2</sup>Present address: Department of Botany and Biotechnology, Bishop Moore College, Mavelikara, Alapuzha 690 110, India

***Oryza rufipogon* Griff. is a valuable reservoir of useful traits for rice genetic improvement. We evaluated the genetic diversity of six populations of *O. rufipogon* from Kerala using random amplified polymorphic DNA (RAPD) markers. Various estimates revealed high genetic diversity in the populations. However, gene flow between the populations was low and 47% of the total genetic diversity was partitioned between populations, suggesting moderate differentiation between them. Clustering of all individuals from the same populations under distinct nodes supported the population differentiation. Considering its outbreeding behaviour, the low gene flow and moderate differentiation recorded in this study indicate that the recent habitat destruction and fragmentation have had an impact on the genetic characteristics of *O. rufipogon* populations in Kerala. The high genetic diversity observed in this study, contrary to expectations for fragmented populations, is presumably contributed by the recurring gene flow from cultivated rice grown in nearby fields. We recommend immediate conservation of a large number of populations of this species, including establishment of isolated conservatories away from rice fields to maintain the purity of the wild gene pool.**

**Keywords:** Conservation, gene flow, habitat fragmentation, *Oryza rufipogon*, RAPDs.

THE common wild rice *Oryza rufipogon* Griff., considered as the ancestor of Asian cultivated rice (*Oryza sativa* L.), is an important reservoir of useful traits for the genetic improvement of rice. Many useful genes from *O. rufipogon* have been successfully introgressed into the cultivated background<sup>1,2</sup> and similar efforts are going on in different laboratories<sup>3</sup>. Until recently, *O. rufipogon* was a common weed in the marshy areas of tropical and subtropical regions of the world. Rapid industrialization and urbanization in many Asian countries have destroyed numerous *O. rufipogon* populations<sup>4</sup>. Habitat destruction and fragmentation expose the populations to founder effects, genetic drift, inbreeding and restricted gene flow,

which are highly detrimental to the diversity and survival of a species<sup>5-7</sup>. In China, for example, *O. rufipogon* populations have been decimated and the plant is now an endangered species there. Population genetic characteristics of Chinese populations of *O. rufipogon* have been compiled in detail by several authors and have helped in the development of suitable measures for the conservation of this species in China<sup>2,4,8-12</sup>. So far, no systematic work has been reported in India on the population genetic characteristics of *O. rufipogon*, though the forces that threaten its survival in China are occurring in India too.

Kerala is one of the most populous states in India and experiences tremendous economic pressure for development. The habitats of several valuable species, including that of *O. rufipogon*, are being increasingly destroyed. Therefore, establishing appropriate conservation and management policies based on the existing spectrum of genetic diversity is essential for *O. rufipogon*. In addition, many workers consider the Malabar Coast of Kerala as one of the centres of diversity of rice<sup>13</sup>. The genetic resources sampled from the centre of diversity of a species have great value, as they may contain novel traits not seen elsewhere. In this study, we evaluated the population genetic characteristics of six populations of *O. rufipogon* from Kerala using RAPD markers.

Altogether, five expeditions were conducted between September and December in 2005 and 2006 to locate *O. rufipogon* populations in Kerala. The means of travel was by automobile, so areas inaccessible by road were not included in the survey. Particular attention was given in expeditions to traverse marshy lands and low lying areas as natural populations of *O. rufipogon* are known to occur in such regions. Geographic locations of the six collection sites chosen for sampling *O. rufipogon* are shown in Figure 1. Distance between the collection sites ranged from 64 km between Kozhikode and Wayanad to 362 km between Thiruvananthapuram and Wayanad. All collection sites were primarily marshy lands. They were either abandoned paddy fields or were paddy fields long ago reclaimed for coconut cultivation, except Wayanad, which was an uncultivated forestland. There was ongoing rice cultivation at different distances from all collection locations, except Wayanad, which was the only population found isolated from cultivated rice in our expeditions. *O. rufipogon* existed as scattered clumps in all collecting sites, extending over ~100–200 m. Though patchy, the species was found distributed frequently near and between the Alapuzha and Ernakulam sites. However, no distribution was located at a distance of at least 15–20 km from the other four populations.

Eighteen individuals were collected from each of the six collection sites. Individuals were a minimum of 10 m apart. Leaf samples were collected directly from the field and the genomic DNA was isolated using a GenElute™ Plant Genomic DNA Kit (Sigma) following the manufacturer's instructions.

\*For correspondence. (e-mail: gthomas@rgcb.res.in)

Two representative individuals from each population were used for an initial screening using 96 random decamer primers randomly selected from A, C, E, F, G and J kits (Operon Technologies, Alameda, USA). The primers that yielded discrete, unambiguous and polymorphic amplification profiles in the initial screening were selected for further analysis. The RAPD analysis was carried out as reported elsewhere<sup>14</sup>.

Each RAPD band was treated as an independent character and was scored as present (1) or absent (0). The binary matrix was used to compute a pairwise genetic distance matrix based on the DICE coefficient using the software WINBOOT<sup>15</sup>. Cluster analysis was performed on genetic distance matrices employing an UPGMA using the SAHN (Sequential, Agglomerative, Hierarchical and Nested cluster) module of the software NTSySpc 2.02i. Cophenetic correlation was estimated using CPH and MXCOMP procedures of NTSySpc to test the reliability of the topology of the dendrogram. A Mantel test was performed to assess the correlations between Nei's genetic distance and geographic distance between populations using the MXCOMP module of NTSySpc.



**Figure 1.** Map of Kerala showing the collection localities of the *O. rufipogon* populations studied.

POPGENE software was used to estimate the percentage of polymorphic bands (PPB) and Nei's gene diversity ( $h$ ). Nei's coefficient of differentiation ( $G_{st}$ ) and gene flow ( $Nm$ ) were calculated using POPGENE on the basis of gene frequencies. Populations were assumed to be in Hardy-Weinberg equilibrium for all POPGENE analyses. The Shannon information index for phenotypic diversity<sup>16</sup> was also estimated, as described by Maughan *et al.*<sup>17</sup>, to examine the partitioning of genetic diversity within and among populations.

Of the 96 primers screened initially, 11 that produced reproducible and polymorphic profiles were selected for the analysis of 108 *O. rufipogon* individuals sampled from six populations. The 11 primers (OPA10, 11, 19; OPC11; OPE11, 14, 17; OPG4; OPJ1, 12, 15) produced 73 bands in total, of which 66 (90.41%) were polymorphic, with an average of six polymorphic bands per primer.

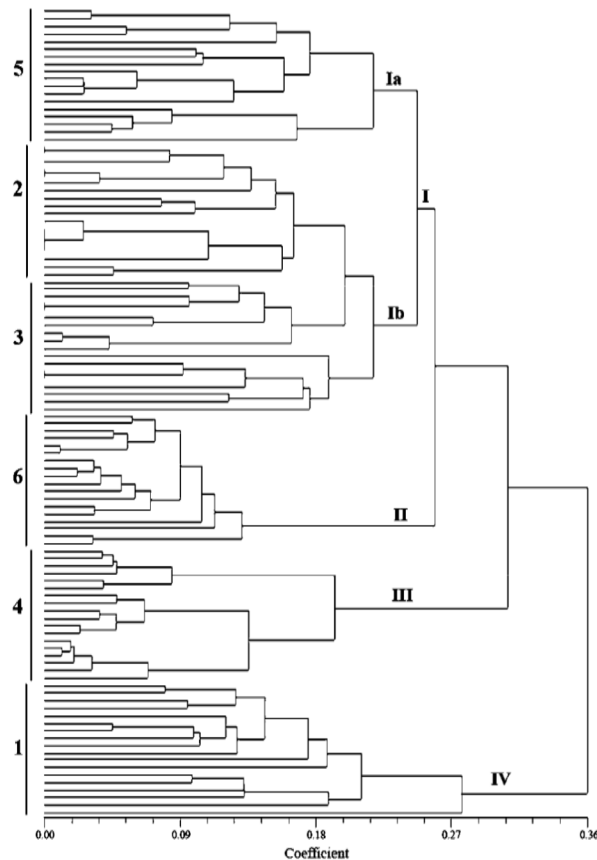
Table 1 summarizes the genetic diversity statistics of the six *O. rufipogon* populations revealed by RAPD markers. PPB values ranged from 35.62% in Wayanad to 56.16% in the Ernakulam population, with a mean of 90.41%. Nei's gene diversity ( $h$ ) was highest in the Ernakulam population ( $0.2088 \pm 0.2079$ ) and lowest in the Wayanad ( $0.1180 \pm 0.1861$ ) population, with a mean of  $0.2935 \pm 0.1675$ .

The Nei's coefficient of differentiation ( $G_{st}$ ) ranged from 0.0331 to 1 with a mean of  $0.46 \pm 0.1934$ , whereas the gene flow ( $Nm$ ) estimate ranged from 0.0573 to 14.6034 with a mean of  $0.6 \pm 0.0916$ . According to Shannon's information index, 52.25% of the total diversity was found to be partitioned within and 47.75% between populations.

A dendrogram was constructed from the UPGMA cluster analysis of pairwise RAPD genetic distance estimates based on the Dice coefficient. The dendrogram separated the 108 individuals into four major clusters (Figure 2). Cluster I comprised 54 individuals and could be separated into two subclusters, Ia and Ib. Individuals of the Kozhikode population were entered into subcluster Ia, whereas those of the Alapuzha and Ernakulam populations into subcluster Ib. In subcluster Ib, individuals belonging to the Alapuzha population clustered under one node and those of the Ernakulam population under two distinct

**Table 1.** Population genetic statistics *O. rufipogon*

Populations	Percentage of polymorphic bands	Nei's gene diversity ( $h$ )
Thiruvananthapuram	49.32	$0.1526 \pm 0.1912$
Alapuzha	45.21	$0.1607 \pm 0.2044$
Ernakulam	56.16	$0.2088 \pm 0.2079$
Kozhikode	53.42	$0.1670 \pm 0.1924$
Palakkad	41.10	$0.1435 \pm 0.1971$
Wayanad	35.62	$0.1180 \pm 0.1861$
Total	90.41	$0.2935 \pm 0.1675$



**Figure 2.** UPGMA dendrogram based on Dice genetic distances of 108 *O. rufipogon* accessions (cophenetic correlation,  $r = 0.86$ ). The four clusters I, II, III and IV, and the two subclusters identified in cluster I are indicated at the nodes. The digits given at the left side indicate populations: 1, Thiruvananthapuram; 2, Alapuzha; 3, Ernakulam; 4, Palakkad; 5, Kozhikode; 6, Wayanad.

nodes. Clusters II, III and IV corresponded to individuals belonging to the Wayanad, Palakkad and Thiruvananthapuram populations respectively. The cophenetic correlation ( $r$ ) between the RAPD data matrix and the cophenetic matrix was 0.86, and such high  $r$  values are considered to be a good representation of the data matrix in the dendrogram<sup>18</sup>. The Mantel test yielded a poor correlation ( $r = 0.1105$ ;  $p = 0.6641$ ) between Nei's genetic distance and the geographic distance between populations.

*O. rufipogon* is an outcrossing species<sup>19</sup>. Outbreeders are characterized by high levels of genetic polymorphism, high rates of gene flow between populations, and high partitioning of total genetic diversity within populations<sup>20,21</sup>.

Consistent with the diversity characteristics expected for an outbreeder, the different genetic diversity statistics used in this study showed a high genetic diversity in the Kerala populations of *O. rufipogon*. The PPB value of 90.41% yielded by the RAPD technique in this study was

higher than that of the Chinese populations of *O. rufipogon* reported by Ge *et al.*<sup>8</sup> (82.5%) and Song *et al.*<sup>22</sup> (78.8%) using the same marker system. The total Nei's genetic diversity ( $h$ ) of the Kerala populations ( $0.2935 \pm 0.1675$ ) was slightly higher than that of the Chinese populations (0.227) (ref. 22) and was similar to those reported earlier for other plant species with high genetic diversity<sup>23,24</sup>.

However, contrary to the expectations for an outbreeder, gene flow between the Kerala populations of *O. rufipogon* was low ( $Nm = 0.6 \pm 0.0916$ ) and the populations showed moderate level of differentiation with only half of the total diversity being partitioned within populations (Shannon information index = 47.75% and  $G_{st} = 0.46 \pm 0.1934$ ). The present results contrast with those obtained for Chinese populations of *O. rufipogon*, in which a considerable proportion of genetic diversity was partitioned within rather than between populations<sup>4</sup>. Besides the Shannon information index and the  $G_{st}$  results, the topology of the dendrogram also supports the existence of genetic differentiation between the populations examined. Similarly, an  $Nm$  value of 0.6 obtained in this study is low compared to the very high values, as high as 1, reported for other outbreeders<sup>25,26</sup>. In Kerala, the populations of *O. rufipogon* are increasingly becoming smaller and more isolated due to urbanization, land transformation, grazing and mowing. Habitat destruction and fragmentation reduce gene flow between populations and increase population differentiation<sup>5-7</sup>. Thus, our results suggest that the Kerala populations of *O. rufipogon* have begun to experience the genetic effects of its recent habitat fragmentation, as reported in other outbreeders such as *Speyeria idalia*<sup>6</sup>.

However, the expected decline in genetic diversity, which usually accompanies habitat fragmentation<sup>5-7</sup> was not observed in the Kerala populations. On the contrary, as discussed above, their genetic diversity was very high. How can this contradiction in the genetic structure of *O. rufipogon* populations be explained? The 'isolation by distance' hypothesis<sup>27</sup> cannot be invoked to explain the genetic structure of the Kerala populations of *O. rufipogon* because of the poor correlation between their geographic and genetic distances. Several authors have reported gene flow from cultivated rice to *O. rufipogon* under natural conditions<sup>2,19,28</sup>. High genetic diversity has been reported in wild populations of sea beet<sup>29</sup> and *Phaseolus vulgaris*<sup>30</sup> that grow nearer to their cultivated relatives compared to those grown farther away. The Wayanad population, which showed the least within population diversity, was isolated and no rice field was found nearby, whereas intense rice cultivation was found in areas nearby the other populations, particularly the Alapuzha, Ernakulam and Kozhikode populations, which showed high 'within population diversity'. Thus, natural gene flow from the cultivated rice may be a cause of the high genetic diversity of *O. rufipogon* populations in Kerala.

Our results provide a blueprint for designing conservation and management policies for maintaining the genetic diversity of *O. rufipogon* in Kerala. We recommend immediate steps to conserve *O. rufipogon* populations in Kerala, including isolating the conservatories from the paddy fields in both *ex situ* and *in situ* conservation initiatives. Further expeditions focusing the unexplored regions may be conducted, and if populations are found, their genetic structure should also be taken into account before finalizing the populations to be sampled for different conservation approaches. Populations far from rice fields, like the Wayanad population, can be chosen for *in situ* conservation. Moderate genetic differentiation among populations suggests sampling of a large numbers of populations covering the complete species range of *O. rufipogon* for *ex situ* conservation.

- Xiao, J., Grandillo, S., Ahn, S. N., McCouch, S. and Tanksley, S. D., Genes from wild rice improve yield. *Nature*, 1996, **384**, 223–224.
- Song, Z., Li, B., Chen, J. and Lu, B. R., Genetic diversity and conservation of common wild rice (*Oryza rufipogon*) in China. *Plant Species Biol.*, 2005, **20**, 83–92.
- Xie, X. *et al.*, Fine mapping of a yield-enhancing QTL cluster associated with transgressive variation in an *Oryza sativa* × *O. rufipogon* cross. *Theor. Appl. Genet.*, 2008, **116**, 613–622.
- Gao, L. Z., Population structure and conservation genetics of wild rice *Oryza rufipogon* (Poaceae): a region-wide perspective from microsatellite variation. *Mol. Ecol.*, 2004, **13**, 1009–1024.
- White, G. M., Boshier, D. H. and Powell, W., Increased pollen flow counteracts fragmentation in a tropical dry forest: an example from *Swietenia humilis* Zuccarini. *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 2038–2042.
- Williams, B. L., Brawn, J. D. and Paige, K. N., Landscape scale genetic effects of habitat fragmentation on a high gene flow species: *Speyeria idalia* (Nymphalidae). *Mol. Ecol.*, 2003, **12**, 11–20.
- Honnay, O., Jacquemyn, H., Bossuyt, B. and Hermy, M., Forest fragmentation effects of patch occupancy and population viability of herbaceous plant species. *New Phytol.*, 2005, **166**, 723–736.
- Ge, S., Oliveira, G. C. X., Schaal, B. A., Gao, L. Z. and Hong, D. Y., RAPD variation within and between natural populations of the wild rice, *O. rufipogon*, from China and Brazil. *Heredity*, 1999, **82**, 638–644.
- Gao, L. Z., Chen, W., Jiang, W. Z., Ge, S., Hong, D. Y. and Wang, X. Q., Genetic erosion in the northern marginal population of the common wild rice *Oryza rufipogon* Griff. and its conservation, revealed by the change of population genetic structure. *Heredity*, 2000, **133**, 47–53.
- Gao, L. Z., Wei, C., Yang, Q., Hong, D. Y. and Ge, S., Intra population genetic structure of *Oryza rufipogon* Griff. in Yunnan, China. *J. Plant Res.*, 2001, **114**, 107–113.
- Gao, L. Z., Zhang, C. H., Li, D. Y., Pan, D. J., Jia, J. Z. and Dong, Y. S., Genetic diversity within *Oryza rufipogon* germplasms preserved in Chinese field gene banks of wild rice as revealed by microsatellite markers. *Biodiv. Conserv.*, 2006, **15**, 4059–4077.
- Liu, G. H., Luo, L. M., Wang, B., Li, W. and Song, Z., Comparison of genetic variation in populations of wild rice, *Oryza rufipogon*, plants and their soil seed banks. *Conserv. Genet.*, 2006, **7**, 909–917.
- Manilal, K. S., Ethnobotany of the rices of Malabar. In *Glimpses of Indian Ethnobotany* (ed. Jain, S. K.), Oxford and IBH, New Delhi, 1981, pp. 297–304.
- Joseph, L., Kuriachan, P. and Thomas, G., Is *Oryza malampuzhaensis* Krish. *et* Chand. (Poaceae) a valid species? Evidences from morphological and molecular analyses. *Plant Syst. Evol.*, 2008, **270**, 75–94.
- Yap, I. and Nelson, R. J., WinBoot: a program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. IRRI Discussion Paper Series No. 14, International Rice Research Institute, Manila, Philippines, 1996.
- Bowman, K. D., Hutcheson, K., Odum, E. P. and Shenton, L. R., Comments on the distribution of indices of diversity. *Stat. Ecol.*, 1971, **3**, 315–359.
- Maughan, P. J., Saghai Maroof, M. A., Buss, G. R. and Huestis, G. M., Amplified fragment length polymorphism in soybean: species diversity, inheritance, and near-isogenic line analysis. *Theor. Appl. Genet.*, 1996, **93**, 392–401.
- Romesburg, H. C., *Cluster Analysis for Researchers*, Krieger Publishing, FL, 1990.
- Kuroda, Y., Sato, Y. I., Bounphanousay, C., Kono, Y. and Tanaka, K., Gene flow from cultivated rice (*Oryza sativa* L.) to wild *Oryza* species (*Oryza rufipogon* Griff. and *O. nivara* Sharma *et* Shastri) on the Vientiane plain of Laos. *Euphytica*, 2005, **142**, 75–83.
- Charlesworth, D. and Charlesworth, B., Quantitative genetics in plants: the effect of the breeding system on genetic variability. *Evolution*, 1995, **49**, 911–920.
- Hamrick, J. L. and Godt, M. J. W., Effects of life history traits on genetic diversity in plant species. *Philos. Trans. R. Soc. London B.*, 1996, **351**, 1291–1298.
- Song, Z. P., Lu, B. R., Zhu, Y. G. and Chen, J. K., Changes of genetic structure in common wild rice (*Oryza rufipogon*) populations as referred to by RAPD markers. *J. Genet. Mol. Biol.*, 2001, **12**, 78–84.
- Zhao, Q. F., Wang, G., Li, Q. X., Ma, S. R., Cui, Y. and Grillo, M., Genetic diversity of five *Kobresia* species along the eastern Qinghai-Tibet plateau in China. *Heredity*, 2006, **143**, 33–40.
- Zhang, J. Y., Yuan, Q. H., Meng, Y. Q., Li, X. L., Nan, Z. B., Wang, Y. R. and Zhang, W. S., A genetic diversity analysis of wild *Lespedeza* populations based on morphological characters, allozyme and RAPD methods. *Plant Breed.*, 2007, **126**, 89–94.
- Hooper, E. A. and Haufler, C. H., Genetic diversity and breeding system in a group of neotropical epiphytic ferns (*Pleopeltis*: Polypodiaceae). *Am. J. Bot.*, 1997, **84**, 1664–1674.
- Zeng, J., Zou, Y., Bai, J. and Zheng, H., RAPD analysis of genetic variation in natural populations of *Betula alnoides* from Guangxi, China. *Euphytica*, 2003, **134**, 33–41.
- Slatkin, M., Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, 1993, **47**, 264–279.
- Chen, L. J., Lee, D. S., Song, Z. P., Suh, K. S. and Lu, B. R., Gene flow from cultivated rice (*Oryza sativa*) to its weedy and wild relatives. *Ann. Bot.*, 2004, **93**, 67–73.
- Bartsch, D., Lehen, M., Clegg, J., Pohl-Orf, M., Schuphan, I. and Ellstrand, N. C., Impact of gene flow from cultivated beet on genetic diversity of wild sea beet populations. *Mol. Ecol.*, 1999, **8**, 1733–1741.
- de la Cruz, E. P., Gepts, P., GarciaMarin, P. C. and Villareal, D. Z., Spatial distribution of genetic diversity in wild populations of *Phaseolus vulgaris* L. from Guanajuato and Michoacan, Mexico. *Genet. Res. Crop Evol.*, 2005, **52**, 589–599.

ACKNOWLEDGEMENTS. D.R.R. gratefully acknowledges the Council for Scientific and Industrial Research (CSIR), Government of India for the research fellowship (File No. 9/716 (62)/2004-EMR-I) and G.T. acknowledges the Planning and Economic Affairs Department, Western Ghats Cell, Planning Board, Government of Kerala, India for the research grant (G.O. (MS) No. 19/06/Plg. dated 4/03/2006).

Received 18 February 2009; revised accepted 4 December 2009