Genetic diversity among perennial wild rice *Oryza rufipogon* Griff., in the Mekong Delta

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**Funding information**
Grant-in-aid B (Overseas project), Grant/Award Number: 16H05777; Grant-in-Aid for Scientific Research on Innovative Areas, Grant/Award Number: 15H05968

1 | INTRODUCTION

The wild species of genus *Oryza* regarded as valuable resource for rice improvement because of its high genetic diversity (Brar, 2003; Sun, Wang, Li, Yoshimura, & Iwata, 2001). Application of wild rice to breeding programs can facilitate adaptation to climate change and meet the demand for food security in the face of rapid world population growth (Henry, 2016; Henry et al., 2010; Mickelbart, Hasegawa, & Bailey-Serres, 2015; Moner et al., 2018). In this context, *Oryza rufipogon* and its relatives can provide a rich repository of genes and alleles for potential utilization in rice improvement with the help of genomics-assisted breeding. Such studies can provide specific insight into natural genetic resources that can be preserved and utilized efficiently.

The wild rice species *Oryza rufipogon* Griff. is a common perennial known to be the progenitor of the Asian cultivated rice species, *O. sativa* L. (Oka, 1988; Vaughan, 1994). Many valuable genes conferring resistance to major biotic and abiotic stresses are being introduced into improved varieties (Brar & Khush, 1997; Ram, Majumder, Mishra, Ansari, & Padmavathi, 2007; Xiao et al., 1996; Yuan, Virmani, & Mao, 1989), for example, resistance to bacterial leaf blight (BB), brown plant hopper (BPH) and tungro virus, tolerance to aluminum toxicity, sulfate soil, and so on. Despite these advantages of wild rice, it is under serious threat and facing extinction due to ecological changes and human disturbance. Hence, effective conservation of this wild rice has become an urgent priority in many countries (Akimoto, Shimamoto, & Morishima, 1999; Gao, Zhang, Zhou, Gre, & Hong, 1996; Zhou, Chen, Wang, & Zhong, 1992).
The Mekong Delta, Vietnam, where the Mekong River flows out into the East Sea, has been considered a “biological treasure trove.” The delta shows high biodiversity of fauna and flora with 1,068 new species having been discovered (Fantz, 2008). According to the FAO database (FAOSTAT: http://www.fao.org/faostat/en/#home), the regional yield of paddy rice ranks 23rd in the world, but 6th in terms of production quantity, in view of the multiple cropping system made possible by the rich soil and abundant constantly available water resources. The area is also rich in wild rice species such as *O. rufipogon*, *O. nivara*, and *O. officinalis*. The delta is also the biggest rice granary in the country, playing a pivotal role in food security and accounting for more than 50% of total production, making Vietnam the second largest rice exporter in the world (Buu & Lang, 1997, 2011). As a result, many useful accessions have been exploited for rice improvement over the last few decades, mostly to improve resistance to the brown plant hopper and blast, and tolerance to phosphorus deficiency, aluminum toxicity and acid sulfate soil (Buu & Lang, 2003; Nguyen et al., 2003). Although genetic variation of *O. rufipogon* in Vietnam has been studied, nucleus in genetic and maternal diversity has not yet been elucidated adequately (Cai, Wang, & Morishima, 2004; Ishii et al., 2011).

Molecular markers have provided a powerful tool for studies of genetic diversity among crop species (Gao, 2004; Olsen & Schaal, 2001; Song, Xu, Wang, Chen, & Lu, 2003), clarifying details of population structure and genetics, and having a significant impact on in situ conservation management (Barbier, 1989; Cai et al., 2004; Ishii et al., 2011; Kaewcheenchai et al., 2018; Wang et al., 2012). In fact, such data can be sampled efficiently from natural populations to monitor the transition of population structures in nature (Gao, 2004; Gao, Shaal, Zang, Jia, & Dong, 2002; Orn et al., 2015; Qian, Tianhua, Song, & Lu, 2005; Wang et al., 2012). Since the complete chloroplast (cp) genome became available, cytoplasmic molecular tools have also been developed to clarify evolutionary processes (Chen, Nakamura, Sato, & Nakai, 1993; Kano, Watanabe, Nakamura, & Hirai, 1993; Kim et al., 2015; Masood et al., 2004; Sotowa et al., 2013; Takahashi, Sato, & Nakamura, 2008). Complete cp genomes are becoming easy

![Figure 1](image_url)

**Figure 1** Collection sites for the wild rice *Oryza rufipogon* in the Mekong Delta, Vietnam. (a) Location of the Mekong Delta in southwest Vietnam. (b) Four populations of wild rice collected along the Mekong River, including Dong Thap as an upstream area, My Tho as a downstream area, Vinh Long as an intermediate area, and Can Tho as a flooding area. Dots and triangles indicate collection sites.
The aims of the present study were to clarify (a) the genetic diversity of *O. rufipogon* in the Mekong Delta by using nuclear and cytoplasmic markers, and (b) their distribution along the delta based on maternal lineage. It was anticipated that the results would provide insight into the natural wild rice resources in this area that could be useful for biological conservation as well as exploitation in rice breeding programs.

### MATERIALS AND METHODS

#### 2.1 Field collection and plant materials

Wild rice accessions were collected from the upstream to downstream reaches of the Mekong River in Vietnam during the period 2010–2015. The samples were classified into four distinct populations according to the geography and ecology of the Mekong river, as well as local expert opinions. These were named the Dong Thap population of 25 accessions, the Intermediate population of 59 accessions, the My Tho population of 27 accessions, and the Can Tho population of 68 accessions.

#### 2.2 Materials and methods

**T able 1 Chloroplast INDEL markers and nuclear SSRs markers used in the study**

| Marker | Genome/Chromosome | Deletion or insertion sites/Genome position (bp) | Forward primer | Reverse primer |
|--------|-------------------|-----------------------------------------------|----------------|----------------|
| **Chloroplast INDEL markers** |
| cpINDEL1 | chloroplast | Deletion: 12670..12673 | GGATTCACCGAAACAAACAACC | GCCAAATTGACAGAGGGG |
| cpINDEL2 | chloroplast | Deletion: 14012..14013 | TTTGGGGAAGAAAACATCTTCC | TAAACGGAGAATCAGATAG |
| cpINDEL3 | chloroplast | Deletion: 17380..17385 | AATTGCTCTCAGGGCTTCTTC | TAGTCGATGTGTTGATAC |
| cpINDEL4 | chloroplast | Deletion: 46087..46091 | TAATTTGATATGGCTCGGACG | TGCTATGATTCTATGAC |
| cpINDEL5 | chloroplast | Deletion: 46534..46539 | AGATGGAGAATTGTGACAG | CAAAACAGGATTTGACAG |
| cpINDEL8 | chloroplast | Insertion: 57644^57645 | TTTTACAGGAGTATCTAGTGG | ATTACGCTTTCCTCCAAAC |
| cpINDEL9 | chloroplast | Insertion: 60865^60866 | AAATCCTTTTGGAGGGATTG | TCCACTACATGGCTCAG |
| cpINDEL12 | chloroplast | Insertion: 77735^77736 | TGGTCTTTCCAGAAGAGGAACC | TGTTAAACAGGGCTCGATAG |
| **Nuclear SSR markers** |
| RM3604 | Chromosome 1 | 5140439 | ATGTCGACTCGAGTGAGTGG | TCTTGACCTACCCACAG |
| RM8231 | Chromosome 1 | 5992779 | CGGATGATGATCCATCTCCCG | CAAACATGATAGACAGC |
| RM6853 | Chromosome 2 | 8985893 | CACACGGCGACCATGTCG | CTTCCAAAGACGCAAG |
| RM6301 | Chromosome 3 | 2651365 | CCGTACCTTTAGCTGTTTTC | TGGGACGACCCCTCTCCT |
| RM5442 | Chromosome 3 | 5528248 | AGGAGACGAGGAGCTCTTCC | CGTACCTAGGAGCTGAT |
| RM16264 | Chromosome 4 | 178121 | CTTTGACCGGCCACCTACTC | GCCGAGACTGATGATCT |
| AL606650 | Chromosome 4 | 3185308 | CACATAAGCGGAATCCGGG | GAGCAGTGGATAGACAGC |
| RM146 | Chromosome 5 | 1811333 | CTATTATCCCTAACCTCCTCTCCTC | AGACAGGCTCGCTCAG |
| RM8074 | Chromosome 6 | 1415186 | TACCTACATCTCTCAG | CTTGACAGACCTCAG |
| +29cat | Chromosome 6 | 3091717 | CAGGATGAGGAGAGAGG | CCAAATTACGCTCCTC |
| RM214 | Chromosome 7 | 13444643 | CTTTGAGACCTGAGCTG | AGAGAGGCTACGAGCT |
| RM284 | Chromosome 8 | 21012219 | ATCTCTGACTGATAGTGAAG | CTTGGAACAGCAGCAG |
| RM1109 | Chromosome 8 | 20353160 | TCAAAATACGTTATG | TTTGAAAGGACAGAG |
| RM149 | Chromosome 8 | 24724322 | GCTGACCAAAGGACCTATCG | GGTGAAGAGCCTTCCAG |
| RM23805 | Chromosome 9 | 5220232 | GCATGCGGGAATCAACACTA | AGCGAGGACCAATCCTTGT |
| RM3834 | Chromosome 10 | 21951232 | CTCGACGCTCAGAAGAAC | GCTATGCTAGGAGGAGG |
| RM311 | Chromosome 10 | 9487243 | TGCTGATATAGGTATACACAT | TCTATACACATACCAAC |
| RM5379 | Chromosome 11 | 21796175 | AGGAGCATCTTACATCCAACC | GATTGCTTCTAGTACAG |
| RM309 | Chromosome 12 | 2163510 | GTAGATGACCGCCATTTGCTTG | AGAAAGGCCTCGGTACAG |
| RM6947 | Chromosome 12 | 23974120 | ATTAACGTCACCTGCTG | GCTAGGTAGTGGTGCAAG |

To obtain by next-generation sequencing and resequencing method-ology (Wambu-gu, Brozynska, Furtado, Waters, & Henry, 2015; Waters, Nock, Ishikawa, Rice, & Henry, 2012).
of 55 accessions (Figure 1). Subpopulations were subsequently identified comprising several individuals corresponding to different collection sites. A maximum of eight individuals for each subpopulation were collected (Appendix Table A1).

A core collection derived from the National Bio‐Resource (NBR) Project in Japan (Nonomura et al., 2010) and 85 Thai wild rice accessions were also applied for verification (Kaewcheenchai et al., 2018). Additionally, one hundred wild stocks preserved at Cuu Long Rice Research Institute (CLRRI) were used to trace mitochondrial deletions in order to analyze mitochondrial variations from the past (Appendix Table A2).

### 2.2 Mitochondrial genome markers

A wild rice accession from the Can Tho population was subjected to next-generation sequencing to obtain resequencing data against the mitochondrial genome. Details of the NGS protocol have been reported previously (Waters et al., 2012). Mt‐INDEL‐327994‐forward (agaatggtggaatctggtcaatctccatc) and mt‐INDEL‐329823‐reverse (attggatagtgatctcgggcacgagtgg) were used to detect one deletion. None deletion type of the PCR product would be 1,830 bp and deletion type 1,131 bp in size. Another presumed deletion included orf153 in the mitochondrial genome. The presence or absence of PCR products was used to detect the orf153 deletion with r‐Taq (NEB Co. Ltd., Japan) using the primers orf153f: GTCTAGGGCTTCATCTTATGCC (forward) and CTAAGAAATCAGTAGAAATCGGG (reverse) which makes a 460 bp amplified product in Nipponbare mitochondrial genome (NC_011033). The PCR conditions were preheating at 94ºC for 3 min, 30 rounds at 94ºC for 10 s, 55ºC for 30 s and 72ºC for 30 s, and 72ºC for 5 min. The PCR products were then subjected to 0.8% agarose gel electrophoresis in 0.5 × TAE buffer because of the expected size of amplicons.

### 2.3 Molecular markers

Eight chloroplast INDELs (cpINDELS) developed in our previous study (Kaewcheenchai et al., 2018) were used to trace maternal
lineages. Twenty nuclear SSR markers were applied to evaluate genetic diversity (Table 1). PCR products were amplified using a basic cycle of preheating at 94ºC for 3 min, followed by 30 rounds of 95ºC for 10 s, 55ºC for 30 s, and 72ºC for 30 s, and postheating at 72ºC for 5 min with Thermopol Taq polymerase (NEB Ltd., Japan). The amplified DNA fragments of both chloroplast and nuclear were mixed with a loading dye for electrophoresis on 6% denaturing polyacrylamide gel at 1,500 V for 2 hr in 0.5 × TBE buffer. The gels were then visualized by silver staining (Promega Co., Japan).

2.4 | Data analysis

The data were subjected to principal component analysis using GenAlEx software (http://biology-assets.anu.edu.au/GenAlEx/Welcome.html). Genetic distances among accessions, the numbers of alleles (\(N_a\)), observed heterozygosity (\(H_o\)), and expected heterozygosity (\(H_e\)) were calculated.

3 | RESULTS

3.1 | Chloroplast genome variations (maternal lineage)

In order to trace maternal lineages of wild rice along the Mekong river, eight chloroplast (cp) INDELs were genotyped. Only cpINDEL5 was monomorphic among all of the accessions collected in Vietnam. Remaining cpINDELS represented alternative genotypes except for cpINDEL3 carrying multiple alleles. These allelic combinations were used to identify different chloroplast types as plastid types (Appendix Table A3).
Among accessions from other South-east Asian countries in the core collection and 85 wild rice accessions from Thailand, 19 plastid types were identified in total. The accessions in the core collection and Thai populations comprised six and 12 plastid types, respectively (Table 2). One hundred and sixty-six accessions from Vietnam comprised ten plastid types, carrying five that were unique and different from the controls. These types were generated from Types 15 to 19, suggesting that wild rice in Vietnam had originated from distinct lineages. Type 15 was the most common in all regions, but was the only one present at Can Tho. The Dong Thap population was composed of three plastid types, Types 15, 16, and 17. The Intermediate population carried unique plastid types such as Types 18 and 19. The My Tho populations comprised six plastid types. Type 15 was found in all of the populations, but characterized canals at Can Tho, and also predominated in the Intermediate population (Figure 2).

Although wild rice populations in Vietnam have been catalogued (Buu & Lang, 2011), maternal lineages have not yet been traced. Therefore, this result shows that perennial wild rice species in Vietnam have identical maternal lineages, thus, contributing to our understanding of the origin of maternal genetics.

### 3.2 Tracing maternal lineages with unique deletions in the mitochondrial genome

Resequencing of the mitochondrial genome yielded two absence/deletion markers, which were confirmed by PCR amplification (Figure 3). One of these presumed deletions, termed the 699-bp deletion, extending from bp 328, 592 to bp 329, 291 in the Nipponbare mitochondrial genome, was amplified and sequenced. It was flanked by tandem duplications of TTGCTA in Nipponbare. Using PCR amplificon, we also tried to confirm another presumed deletion that included orf153. However, this region was not amplified in a particular Vietnamese wild rice accession. The PCR products of orf153 and its upstream region were used as probes to confirm the deletion by Southern hybridization. Specific Vietnamese accessions, P75-1 and P75-2, in the P75 subpopulation at Can Tho did not yield any signals. In order to clarify the mitochondrial rearrangement, a flanking probe was used. This showed that two accessions in the P75 subpopulation exhibited polymorphism relative to Nipponbare and W0106, suggesting that a highly complex rearrangement may have deleted orf153 in the P75 subpopulation.

All wild accessions from the Mekong Delta were screened for both mitochondrial INDEL markers, and this revealed that the two deletions were present in a single maternal lineage (Figure 4). This was a feature in all four populations and all accessions from the

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**Table 3** A frequency of a mitochondrial deletion (327,994–329,823 bp) accompanied with a particular plastid type

| Population          | No. of accessions | mt-Deletion | Plastid type |
|---------------------|-------------------|-------------|--------------|
|                     |                   | Nondeletion | Deletion (%) |
| CLRRI's Genbank     |                   |             |              |
| Hau giang           | 4                 | 4           | 0            | 0             | Type 15* |
| Dong Thap           | 71                | 69          | 2            | 3             | Type 15a |
| Long An             | 25                | 25          | 0            | 0             | –          |
| Natural habitat     |                   |             |              |               |            |
| Dong Thap           | 25                | 23          | 2            | 8             | Type 15   |
| Intermediate area   | 59                | 16          | 43           | 73            | Type 15   |
| My Tho              | 27                | 22          | 5            | 19            | Type 15   |
| Can Tho             | 55                | 0           | 55           | 100           | Type 15   |

*Plastid type of accessions carrying the mitochondrial deletion.
| Locus     | Can Tho | Intermediate | Dong Thap | My Tho | Overall population |
|-----------|---------|--------------|-----------|--------|-------------------|
|           | $N_a$   | $H_o$        | $H_e$     | $N_a$  | $H_o$        | $H_e$     | $N_a$  | $H_o$        | $H_e$     | $N_a$  | $H_o$        | $H_e$     |
| RM3604    | 2       | 1.000        | 0.500     | 6      | 0.814        | 0.729     | 9      | 0.520        | 0.745     | 3      | 0.481        | 0.615     |
| AL606650  | 2       | 1.000        | 0.500     | 5      | 0.729        | 0.719     | 9      | 0.680        | 0.830     | 6      | 0.556        | 0.712     |
| RM311     | 1       | 0.000        | 0.000     | 5      | 0.271        | 0.699     | 4      | 0.280        | 0.593     | 4      | 0.185        | 0.644     |
| +29CAT    | 2       | 1.000        | 0.500     | 6      | 1.000        | 0.705     | 6      | 0.840        | 0.777     | 5      | 0.963        | 0.787     |
| RM8074    | 1       | 0.000        | 0.000     | 4      | 0.305        | 0.689     | 4      | 0.800        | 0.678     | 4      | 0.815        | 0.623     |
| RM5379    | 2       | 1.000        | 0.500     | 6      | 1.000        | 0.787     | 7      | 0.800        | 0.757     | 6      | 1.000        | 0.679     |
| RM8231    | 1       | 0.000        | 0.000     | 2      | 0.136        | 0.126     | 6      | 0.600        | 0.714     | 5      | 0.519        | 0.652     |
| RM146     | 1       | 0.000        | 0.000     | 2      | 0.034        | 0.033     | 4      | 0.440        | 0.410     | 3      | 0.667        | 0.483     |
| RM16262   | 1       | 0.000        | 0.000     | 6      | 0.661        | 0.522     | 5      | 0.680        | 0.518     | 3      | 1.000        | 0.575     |
| RM214     | 2       | 1.000        | 0.500     | 7      | 1.000        | 0.700     | 7      | 0.840        | 0.773     | 6      | 0.963        | 0.750     |
| RM284     | 2       | 1.000        | 0.500     | 4      | 0.746        | 0.618     | 6      | 0.680        | 0.734     | 5      | 1.000        | 0.634     |
| RM1109    | 2       | 1.000        | 0.500     | 5      | 1.000        | 0.662     | 7      | 0.640        | 0.774     | 6      | 1.000        | 0.679     |
| RM149     | 2       | 1.000        | 0.500     | 8      | 1.000        | 0.838     | 7      | 0.840        | 0.755     | 7      | 1.000        | 0.795     |
| RM23805   | 2       | 1.000        | 0.500     | 3      | 0.339        | 0.521     | 4      | 0.320        | 0.570     | 3      | 0.000        | 0.535     |
| RM3834    | 2       | 1.000        | 0.500     | 9      | 1.000        | 0.790     | 10     | 1.000        | 0.854     | 8      | 1.000        | 0.818     |
| RM309     | 2       | 1.000        | 0.500     | 3      | 0.610        | 0.576     | 6      | 1.000        | 0.783     | 6      | 0.519        | 0.703     |
| RM6947    | 1       | 0.000        | 0.000     | 2      | 0.136        | 0.126     | 2      | 0.000        | 0.077     | 1      | 0.000        | 0.000     |
| RM6301    | 1       | 0.000        | 0.000     | 3      | 0.322        | 0.277     | 3      | 0.240        | 0.339     | 2      | 0.481        | 0.366     |
| RM6853    | 1       | 0.000        | 0.000     | 3      | 0.288        | 0.289     | 4      | 0.680        | 0.663     | 4      | 0.222        | 0.593     |
| RM5442    | 2       | 1.000        | 0.500     | 6      | 0.847        | 0.736     | 10     | 0.920        | 0.827     | 4      | 0.519        | 0.608     |
| Mean      | 1.6     | 0.600        | 0.300     | 4.8    | 0.612        | 0.557     | 6.0    | 0.640        | 0.659     | 4.6    | 0.644        | 0.613     |
| SE        | 0.0     | 0.042        | 0.021     | 0.5    | 0.078        | 0.055     | 0.5    | 0.060        | 0.044     | 0.4    | 0.078        | 0.040     | 0.2    | 0.039        | 0.026     |
3.3 | Genetic diversity and phylogenetic relationships evaluated using nuclear SSR markers

Genetic diversity was estimated using 20 SSR markers (Table 4). The highest number of alleles was found in RM3834 ($N_a = 8$), and the lowest in RM6947 ($N_a = 2$) in the overall population. The observed heterozygosity ($H_o$) ranged from 0.054 to 1.000 among the 20 loci and from 0.60 to 0.644 among the populations. The Dong Thap population showed the highest diversity, $H_e = 0.659$, whereas the Can Tho population showed the lowest at $H_e = 0.300$. The $H_e$ scores for the My Tho and Intermediate populations were $H_e = 0.613$ and $H_e = 0.557$, respectively. All accessions in the seven subpopulations from Can Tho shared the single genotypes over 20 loci. Twelve of the 20 examined loci were heterozygous, and the remaining eight were monomorphic. Vegetative propagation was inferred from the genotypes. The same genotypes were not found among other materials examined.

A phylogenetic tree was constructed based on a distance matrix generated with the 20 SSR markers (Figure 5). As the four populations formed different clades, subpopulations making up each population were applied. There were two distinct clades: one including Dong Thap, Intermediate, and My Tho subpopulations, and the other including the Can Tho population. This suggested that wild rice at Can Tho is unique in comparison to the others.

4 | DISCUSSION

Previous studies have attempted to characterize and exploit wild rice species in the Mekong Delta without investigating their origin, or clarifying genetic variations among them (Buu, 1996; Buu & Lang, 2007; Lang et al., 2012). The present study focused on genetic variation in *O. rufipogon* specimen collection along the Mekong River and attempted to know how they distribute along the river system by using maternally inherited markers. The unique genetic resource in *O. rufipogon* in the Mekong Delta yielded five novel plastid types, among which Type 15 was accompanied by a unique mitochondrial lineage showing marked rearrangement of the mitochondrial genome. The maternal lineage might have arisen upstream of the delta and become dispersed into the downstream area. The maternal lineage at Dong Thap, however, did not share the same nuclear genotype as those at Can Tho. One descendant belonging to the maternal lineage may have had the ability to form clones and occupy particular channels. Highly vegetative propagation and migration by drifting...
across canals may also have affected the structure of the Can Tho populations. This is the unique nature of wild rice inhabited along the Mekong Delta.

In contrast to the unique population in Can Tho, high genetic variation was found in the upstream area, at Dong Thap. This higher variation allowed a breeding program that successfully created AS996, carrying higher acid sulfate tolerance (Can & Lang, 2007; Khush & Virk, 2005; Lang et al., 2012). This higher degree of diversity might be due to ecological factors that have a great influence on genetic differentiation among wild rice populations (Orn et al., 2015). In fact, the Dong Thap wild population is distributed widely in a government conservation area at Tram Chim National Park. The process of conserving wild populations under natural conditions has been done accomplished with minimal human disturbance, thus, helping to maintain higher genetic diversity. As water flow may distribute individual plants downstream, areas en route such as the My Tho and Intermediate areas between My Tho and Can Tho may maintain relatively diverse variation, compared to channels at Can Tho. Currently, efforts to collect wild Oryza species are suspended in Vietnam, although accessions have been exploited for breeding programs to some extent. Meanwhile, wild populations have been seriously threatened and faced with extinction due to infrastructure development. Many subpopulations investigated in this study have been severely degraded by human disturbance; at least one wild subpopulation at site P78 has completely disappeared because of road construction. Although several wild species have been preserved at the CLRRI gene bank for ex-situ conservation, the entire range of genetic variation has not been covered. Therefore, effective conservation management for O. rufipogon in upstream areas such as Dong Thap is becoming even more of an urgent priority. Our assessment of the genetic diversity would be available to collect valuable resources efficiently before they would be extinct.

ACKNOWLEDGMENTS

This work was funded by a Grant-in-aid B (Overseas project, No. 16H05777) and partly by a Grant-in-Aid for Scientific Research on Innovative Areas (15H05968). The valuable wild rice accessions used in this study were distributed by the National Institute of Genetics, supported by the National BioResource Project, MEXT, in Japan. Sequencing was performed at the Gene Research Center, Hiroasaki University.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

DTL and BCB managed core collection of perennial wild rice. DTL, BCB, NTL, IN, KT, and RI surveyed natural populations. DTL contributed to analyze with molecular markers. RI set up markers and genome analysis.

DATA ACCESSIBILITY

All data were included in this manuscript.

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How to cite this article: Lam DT, Buu BC, Lang NT, Toriyama K, Nakamura I, Ishikawa R. Genetic diversity among perennial wild rice Oryza rufipogon Griff., in the Mekong Delta. Ecol Evol. 2019;9:2964–2977. https://doi.org/10.1002/ece3.4978
### TABLE A1  GPS records of wild rice subpopulations examined in this study

| Population | Year of surveys | GPS point | Habitant | Region                | No. of accessions |
|------------|-----------------|-----------|----------|-----------------------|------------------|
| P75        | 2010            | N9 59.384 E105 39.688 | Along a particular canal in Bassac River west side | Can Tho | 8 |
| P76        | 2010            | N9 59.431 E105 39.462 | Along a particular canal in Bassac River west side | Can Tho | 8 |
| P77        | 2010            | N9 59.115 E105 39.014 | Along a particular canal in Bassac River west side | Can Tho | 8 |
| P78        | 2010            | N9 59.855 E105 40.365 | Along a particular canal in Bassac River west side | Can Tho | 7 |
| P79        | 2010            | N9 57.783 E105 44.896 | Along a particular canal in Bassac River west side | Can Tho | 8 |
| P80        | 2010            | N9 57.342 E105 45.752 | Flood plains nearby a canal in Bassac River west side | Can Tho | 8 |
| P81        | 2010            | N9 56.484 E105 46.042 | Flood plains nearby a canal in Bassac River west side | Can Tho | 8 |
| P83        | 2010            | N10 02.731 E105 50.148 | Between Bassac river and Tien River | Vinh Long | 8 |
| P84        | 2010            | N9 59.718 E105 52.890 | Between Bassac river and Tien River | Vinh Long | 6 |
| P85        | 2010            | N9 58.219 E105 55.506 | Between Bassac river and Tien River | Vinh Long | 8 |
| P35        | 2014            | N09 58 13.14 E105 55 30.64 | Between Bassac river and Tien River | Vinh Long | 7 |
| P36        | 2014            | N09 57 09.36 E105 59 57.58 | Between Bassac river and Tien River | Vinh Long | 8 |
| P37        | 2014            | N10 00 14.32 E106 06 04.61 | Between Bassac river and Tien River | Vinh Long | 8 |
| P38        | 2014            | N10 00 12.10 E106 06 03.65 | Between Bassac river and Tien River | Vinh Long | 8 |
| P39        | 2014            | N10 05 30.83 E106 05 40.22 | Between Bassac river and Tien River | Vinh Long | 6 |
| P46        | 2015            | N10 42 22.69 E105 32 23.23 | Tram Chim Sanctuary for in-situ conservation | Dong Thap Muoi | 8 |
| P47        | 2015            | N10 43 00.19 E105 30 04.59 | Tram Chim Sanctuary for in-situ conservation | Dong Thap Muoi | 8 |
| P48        | 2015            | N10 43 04.95 E105 30 01.84 | Tram Chim Sanctuary for in-situ conservation | Dong Thap Muoi | 8 |
| P49        | 2015            | N10 41 36.04 E105 31 37.86 | Tram Chim Sanctuary for in-situ conservation | Dong Thap Muoi | 1 |
| P53        | 2015            | N10 23 43.91 E106 20 14.81 | Road side near by Tien River | My Tho | 3 |
| P54        | 2015            | N10 23 43.88 E106 20 14.81 | Road side near by Tien River | My Tho | 7 |
| P55        | 2015            | N10 23 48.25 E106 20 15.39 | Road side near by Tien River | My Tho | 5 |
| P56        | 2015            | N10 23 48.25 E106 20 15.39 | Road side near by Tien River | My Tho | 8 |
| P59        | 2015            | N10 25 13.91 E106 20 24.84 | Road side near by Tien River | My Tho | 4 |
| Accessions | Current conserved | Origin            |
|------------|------------------|-------------------|
| 1          | CLRRI Genebank   | Dong Thap Province|
| 2          | CLRRI Genebank   | Dong Thap Province|
| 3          | CLRRI Genebank   | Dong Thap Province|
| 4          | CLRRI Genebank   | Dong Thap Province|
| 5          | CLRRI Genebank   | Dong Thap Province|
| 6          | CLRRI Genebank   | Dong Thap Province|
| 7          | CLRRI Genebank   | Dong Thap Province|
| 8          | CLRRI Genebank   | Dong Thap Province|
| 9          | CLRRI Genebank   | Dong Thap Province|
| 10         | CLRRI Genebank   | Dong Thap Province|
| 11         | CLRRI Genebank   | Dong Thap Province|
| 12         | CLRRI Genebank   | Dong Thap Province|
| 13         | CLRRI Genebank   | Hau giang Province|
| 14         | CLRRI Genebank   | Hau giang Province|
| 15         | CLRRI Genebank   | Hau giang Province|
| 16         | CLRRI Genebank   | Hau giang Province|
| 17         | CLRRI Genebank   | Dong Thap Province|
| 18         | CLRRI Genebank   | Dong Thap Province|
| 19         | CLRRI Genebank   | Dong Thap Province|
| 20         | CLRRI Genebank   | Dong Thap Province|
| 21         | CLRRI Genebank   | Dong Thap Province|
| 22         | CLRRI Genebank   | Dong Thap Province|
| 23         | CLRRI Genebank   | Dong Thap Province|
| 24         | CLRRI Genebank   | Dong Thap Province|
| 25         | CLRRI Genebank   | Dong Thap Province|
| 26         | CLRRI Genebank   | Dong Thap Province|
| 27         | CLRRI Genebank   | Dong Thap Province|
| 28         | CLRRI Genebank   | Dong Thap Province|
| 29         | CLRRI Genebank   | Dong Thap Province|
| 30         | CLRRI Genebank   | Dong Thap Province|
| 31         | CLRRI Genebank   | Dong Thap Province|
| 32         | CLRRI Genebank   | Dong Thap Province|
| 33         | CLRRI Genebank   | Dong Thap Province|
| 34         | CLRRI Genebank   | Dong Thap Province|
| 35         | CLRRI Genebank   | Dong Thap Province|
| 36         | CLRRI Genebank   | Dong Thap Province|
| 37         | CLRRI Genebank   | Dong Thap Province|
| 38         | CLRRI Genebank   | Dong Thap Province|
| 39         | CLRRI Genebank   | Dong Thap Province|
| 40         | CLRRI Genebank   | Dong Thap Province|
| 41         | CLRRI Genebank   | Dong Thap Province|
| 42         | CLRRI Genebank   | Dong Thap Province|
| 43         | CLRRI Genebank   | Dong Thap Province|
| 44         | CLRRI Genebank   | Dong Thap Province|

(Continues)
| Accessions | Current conserved      | Origin                          |
|------------|------------------------|---------------------------------|
| 90         | CLRRI Genebank         | Long An Province                 |
| 91         | CLRRI Genebank         | Long An Province                 |
| 92         | CLRRI Genebank         | Long An Province                 |
| 93         | CLRRI Genebank         | Long An Province                 |
| 94         | CLRRI Genebank         | Long An Province                 |
| 95         | CLRRI Genebank         | Long An Province                 |
| 96         | CLRRI Genebank         | Long An Province                 |
| 97         | CLRRI Genebank         | Long An Province                 |
| 98         | CLRRI Genebank         | Long An Province                 |
| 99         | CLRRI Genebank         | Long An Province                 |
| 100        | CLRRI Genebank         | Long An Province                 |
| W0106      | National Bio-Resource, Japan | Phulankara, near Cuttack, Orissa, India |
| W0107      | National Bio-Resource, Japan | Pahala, Orissa, India           |
| W0108      | National Bio-Resource, Japan | Cuttack, Orissa, India          |
| W0120      | National Bio-Resource, Japan | Cuttack, Orissa, India          |
| W0137      | National Bio-Resource, Japan | Kadiam, Andhra, India           |
| W0180      | National Bio-Resource, Japan | Ngao, Lamphang, Thailand        |
| W0593      | National Bio-Resource, Japan | Binjai Rendah, Malasia          |
| W0610      | National Bio-Resource, Japan | Myanmar                           |
| W0630      | National Bio-Resource, Japan | Myanmar                           |
| W1294      | National Bio-Resource, Japan | Musuan, Mindanao, Philippines    |
| W1551      | National Bio-Resource, Japan | Saraburi, Thailand              |
| W1666      | National Bio-Resource, Japan | Siliguri, India                  |
| W1669      | National Bio-Resource, Japan | Orissa, India                    |
| W1681      | National Bio-Resource, Japan | Orissa, India                    |
| W1685      | National Bio-Resource, Japan | Orissa, India                    |
| W1690      | National Bio-Resource, Japan | Chiang Rai, Thailand             |
| W1715      | National Bio-Resource, Japan | China                            |
| W1807      | National Bio-Resource, Japan | Sri Lanka                        |
| W1852      | National Bio-Resource, Japan | Chiang Saen, Thailand            |

(Continues)
**TABLE A3** Definition of plastid types based on genotype of eight chloroplast INDEL markers

| Locus      | Plastid types a |
|------------|-----------------|
| cpINDEL1   | 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 |
| cpINDEL2   | 1 1 1 1 1 1 2 2 1 1 2 2 2 2 1 1 1 1 1 |
| cpINDEL3   | 1 1 2 2 2 2 2 2 1 2 1 1 2 3 1 1 1 −1 −1 |
| cpINDEL4   | 1 1 1 1 1 1 1 1 1 1 1 1 2 2 1 2 2 2 1 1 1 1 1 1 |
| cpINDEL5   | 1 2 1 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 |
| cpINDEL8   | 2 2 2 1 2 2 2 2 1 1 1 1 1 1 1 1 1 1 2 2 2 |
| cpINDEL9   | 2 2 2 2 2 2 2 2 1 1 1 1 1 1 1 1 1 2 2 2 2 |
| cpINDEL12  | 2 1 2 1 1 2 2 1 1 1 1 1 1 1 1 1 1 1 2 1 2 |

aType 1 – 14 were detected in a core collection (NBR) and Thai wild rice populations (data not published). Allele numbers were given when compared between Nipponbare and Thai45-2 accession. Smaller PCR product was given allele 1 and larger one allele 2. When more shorter fragment was amplified, then allele −1 was given.