Diversity of Tidal Swamp Rice (Oryza sativa L.) Cultivars Indigenously from South Kalimantan, Indonesia: A Molecular Study

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Diversity of Tidal Swamp Rice (*Oryza sativa*) Cultivars Indigenously from South Kalimantan, Indonesia

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Abstract. Studies on genetic diversity and relationships are necessary for breeders and scientists to increase the effectiveness of future breeding programs. The tidal swamp rice (*Oryza sativa* L.) is one of the potential germplasms which has a prominent opportunity to be incorporated in the rice breeding program. This study aimed to investigate and reveal the genetic diversity and relationship of tidal swamp rice germplasms indigenously from South Kalimantan, Indonesia, using Random Amplified Polymorphic DNA (RAPD) markers. A total of ten rice samples, consisting of nine from this region and one from South Sumatera (an outgroup), and five selected RAPD markers, i.e., OPB-06, OPAJ-01, OPAB-17, OPAL-09, and OPAL-08, were used in this study. DNA amplifications were performed and programmed for one cycle of initial denaturation (5 min, 94ºC), 45 cycles of denaturation (30 sec, 94ºC), annealing (30 sec, 37ºC), and extension (1.5 min, 72ºC), as well as one cycle of final extension (7 min, 72ºC). The genetic similarity was analyzed using Dice's coefficient method, whereas their relationship (dendrogram) by the UPGMA. The results showed that these germplasms have a moderate genetic diversity level, indicated by the polymorphism degree of 75.64%. The clustering analysis revealed that they are grouped into three main groups at a similarity coefficient of 0.70. In this case, *Siam Unus* is distantly related to the other cultivars and forms a solitaire group. *Siam Unus* also shows the farthest relationship with *Sardani*, an outgroup. It is a new finding for the genetic insight of tidal swamp rice of South Kalimantan, Indonesia, including their diversity and relationship. Thus, the results obtained from this study is useful in supporting future rice conservation and breeding programs.

Key words: breeding program, DNA fingerprint, genetic diversity, rice landrace

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INTRODUCTION

Indonesia is a developing country with a large population (Adioetomo & Mujahid, 2014). So, it is not surprising that this country requires a high food consumption (Muthayya et al., 2014). Since rice is a staple food for most Indonesian populations, its existence cannot be substituted by other food ingredients (Mursyidin et al., 2017). According to Muthayya et al. (2014), rice consumption in this country reached 139.15 kg per year per capita, a relatively high figure. Therefore, efforts to support the national food security program are urgent to employ.

South Kalimantan is one of the biggest rice-producing provinces in Indonesia. There are approximately 225 thousand hectares of the tidal swamps area in this region, which have been reclaimed and suitable for rice farming. In this region, hundreds of local (tidal swamp) rice cultivars could be utilized in crop breeding programs or developing new superior rice cultivars in the future (Mursyidin et al., 2017). For a long time ago, this rice landraces germplasm has been known by the local people of South Kalimantan, Indonesia. Since the 1920s, local farmers have cultivated at least four tidal swamp rice cultivars, namely *Siam*, *Pandak*, *Bayar*, and *Lemo* (Mursyidin et al., 2017). Even though these germplasms generally show low productivity, only 1.0-2.5 tons per hectare, some have unique characteristics, including being extremely tolerant to acidity, salinity, and metals contamination (Mursyidin et al., 2017). Even one of these cultivars, namely *Padi Panjang*, could be grown without any fertilizer application and intensive management (Wahdah et al., 2012).

Unfortunately, on the one hand, most of the tidal swamp rice is left unexplored and underutilized for crop improvement or rice breeding programs (Thomson et al., 2009). On the other hand, several germplasms are being rapidly replaced by improved cultivars due to increasing green revolution technology (Mursyidin et al., 2017). In other words, owing to the predominant use of modern high-yielding varieties (HYV) since the late 19th century a massive proportion of the indigenous rice germplasm has already disappeared from farmers' fields (Ray et al., 2013). Thus, the effort of these cultivars' genetic

conservation and breeding programs is a crucial program that should be conducted.

Knowledge regarding the amount of genetic diversity and relationships between these cultivars is the other essential consideration for designing effective conservation and breeding programs in the future (Glaszmann et al., 2010). In the past, the characterization of genetic diversity and relationships analyses among cultivars have been carried out using morphological and agronomical markers. However, in many cases, these markers did not have the resolution power of revealing polymorphisms or differentiating genetic relationships between closely related genotypes (Anumalla et al., 2015).

Advances in plant genetics and molecular biology have contributed to developing many types of molecular markers for characterizing various germplasm (Anumalla et al., 2015). Several markers, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), and microsatellite or simple sequence repeats (SSR), have been used for the determination of rice genetic diversity (Surapaneni et al., 2016). However, the RAPD technique is the simplest and fastest marker for detecting genetic polymorphism or estimating the genetic diversity of plant species that are closely related, including rice (Islam et al., 2013; Rajani et al., 2013).

Although RAPD is time-consuming and produces subjective data, this method has several advantages, such as an inexpensive nature and non-requirement of prior genetic sequence information (Rajani et al., 2013). Many researchers have been using this technique extensively to assess both improved and traditional rice cultivars (Ali et al., 2014). Moreover, this technique has also been successfully employed to determine genetic diversity in other cereal crop species besides rice, like wheat, maize, barley, pearl millet, and sorghum (Chauhan et al., 2015).

Thus, our objective study was to determine the genetic diversity and relationship of the tidal swamp rice germplasm of South Kalimantan, Indonesia using RAPD markers. The results might be useful in supporting both the rice conservation and breeding programs in Indonesia.

METHODS

Plant materials

A total of ten indigenous rice cultivars consisted of nine from tidal swamp area of South Kalimantan Province and one from tidal swamp of Sumatera Island for comparison (outgroup) were used in this study (Table 1). A comparison sample was obtained from The Indonesian Swamp Agriculture Research Institute (ISARI), South Kalimantan, Indonesia.

Table 1. Sample of rice cultivars used in this study

Cultivar	Code	Origin	Genetic Status
Siam Unus	1	Aluh Aluh, Banjar, South Kalimantan	Landrace
Ciherang	2	Bumi Makmur, Tanah Laut, South Kalimantan	Introduction
Siam Arjuna	3	Aluh Aluh, Banjar, South Kalimantan	Landrace
Siam Orok	4	Aluh Aluh, Banjar, South Kalimantan	Landrace
Bayar Papuyu	5	Aluh Aluh, Banjar, South Kalimantan	Landrace
Siam Saba	6	Kurau, Tanah Laut, South Kalimantan	Landrace
Lakatan Wangi	7	Aluh Aluh, Banjar, South Kalimantan	Landrace
*Sardani**	8	South Sumatera	Landrace
Adil Ganal	9	Aluh Aluh, BanjarSouth Kalimantan	Landrace
Siam Pandak	10	Kurau, Tanah Laut, South Kalimantan	Landrace

** a comparison (outgroup)*

Sample preparation

All seed materials were planted in a greenhouse of the Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat, South Kalimantan, Indonesia with standard agronomical procedure. The leaf samples of each cultivar were taken from the four-weeks-old plants for further analysis.

Molecular analysis

Molecular analysis was carried out with several stage of activities, including DNA extraction, amplification, and electrophoresis. All activities were carried out at the Laboratory of Genetic and

Table 2. Five selected RAPD markers used in this study

Primer	Code	Sequence (5'-3')	GC Contents (%)
OPB-06	A	TGCTCTGCCC	70
OPAJ-01	B	ACGGGTCAGA	60
OPAB-17	C	CCTGTACCGA	60
OPAL-09	D	CAGCGAGTAG	60
OPAL-08	E	GTCGCCCTCA	70

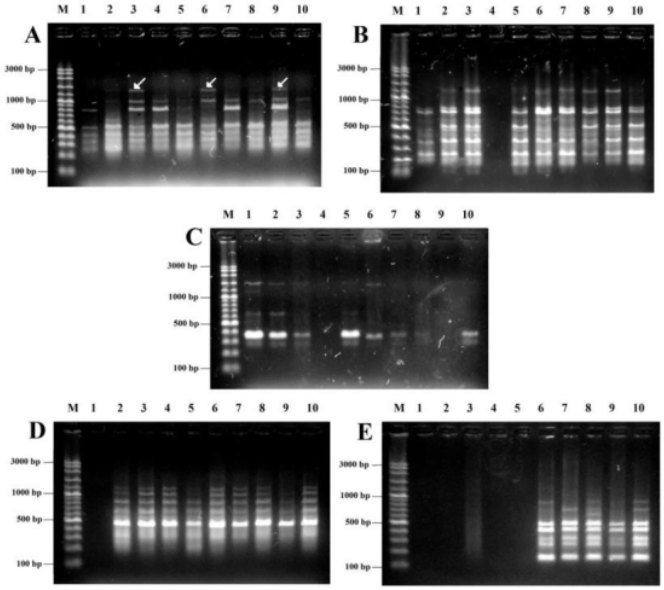

Figure 1. Five selected RAPD markers generated the DNA profile of the tidal swamp rice cultivars. Lane 1-10 = Rice samples: 1. *Siam Unus*, 2. *Ciherang*, 3. *Siam Arjuna*, 4. *Siam Orok*, 5. *Bayar Papuyu*, 6. *Siam Saba*, 7. *Lakatan Wangi*, 8. *Sardani*, 9. *Adil Ganal*, 10. *Siam Pandak*; A-E = Primers: A. OPB-06, B. OPAJ-01, C. OPAB-17, D. OPAL-09, E. OPAL-08; M = DNA Markers

Molecular Biology, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat. DNA extraction began by destructing a four-week-old rice leaf sample of each cultivar following DNAzol kit protocol (Molecular Science, USA). The purity and the total concentration of this material were evaluated at an absorbance of 260 and 280 nm by UV Vis Spectrophotometer (BMG LabTech, USA). The DNAs were then diluted in TE buffer solution and stored in a refrigerator for PCR analysis. PCR analysis (DNA amplification) was performed following Mursyidin & Daryono (2016). Five selected RAPD markers (Table 2) were used for the amplification reaction. This reaction was completed in a total volume of 25 µl, containing 20 μL of master mix PCR (Kappa Biosystem), 2.5 ng μl-1 template DNA, and 2.5 ng μl-1 of primer.

Amplifications was performed using a PCR Thermal cycler (Techne, TC3000G, USA), and was programmed for five stages, i.e. 45 cycles of initial denaturation (5 min, 94ºC), 45 cycles of denaturation (30 sec, 94ºC), annealing (30 sec, 37ºC), and extension (1.5 min, 72ºC), as well as 1 cycle of final extension (7 min, 72ºC). The reliability of the amplification products were checked three times (replication) for each primer used. After amplification, the aliquots of 6 µl of

Table 3. The number, size and percentage of polymorphic fragments generated by five RAPD markers used in this study

Primer	Number of fragments	App. Range of size (bp)	Polymorphic fragments	Polymorphism (%)
OPB-06	52	258-1464	22	42.31
OPAJ-01	78	114-1334	28	35.90
OPAB-17	20	257-1606	20	100
OPAL-08	32	163-886	32	100
OPAL-09	50	198-1241	50	100
Total	232		152	
Average	46.4		30.4	75.64

PCR products were loaded into 2.0% agarose gels electrophoresis. Electrophoresis was ran in the 1xTBE buffer (pH 8) along with DNA staining (GelRed, Biotium Inc., USA). The 100 bp of DNA ladder (Vivantis) was used as a molecular size marker. After electrophoresis, the gels were then visualized under UV transilluminator and documented using a digital camera.

Data analysis

The amplification products were analyzed by marking their presence (1) or absence (0) for each DNA fragment generated. The data were analyzed using the NTSYS-pc software ver. 2.2 (Rohlf, 2009) and calculated to obtained the genetic similarity matrix using Dice's coefficient. The UPGMA (Unweighted Pair Group Method using Arithmetic Averages) clustering method was used to construct a dendrogram. The reliability of the associations shown on the dendrogram was evaluated by bootstrap analysis with 1000 permutations. The cophenetic coefficient between the similarity matrix and the dendrogram was computed using the NTSYS program (Rohlf, 2009).

RESULTS AND DISCUSSION

Genetic diversity

The results showed that each RAPD primer used resulted in a different number of DNA fragments (Figure 1, Table 3). The OPAJ-01 was the primer which generated the highest number of DNA fragments (78), while the lowest was shown by OPAB-17 (20). Meanwhile, the total DNA fragments produced were 232 units with an average of 46.6 (Table 3). On the other hand, the size of the DNA fragments generated by each primer was different. The longest range of DNA size was shown by the OPAB-17 (257-1606 bp), while the shortest was by OPAL-08 (163-886 bp).

Based on the result of electrophoresis (Figure 1A), OPB-06 was the only RAPD primer which was capable of generating DNA fragments in all rice plant. This primer was also able to produce specific DNA fragments in several rice cultivars with a size of 1300 bp, for example for *Siam Arjuna* (line 3), *Siam Saba* (line 6), and *Adil Ganal* (line 9).

The results also showed that each primer generated a different level of polymorphism (Table 3). The highest polymorphism level has shown by three primers, namely OPAB-17, OPAL-08, and OPAL-09 with a value of 100%, while the lowest by OPAJ-01 (35.90%). Following the result, the average of polymorphism recorded at 75.64%.

According to Höglund (2009), the genetic diversity of germplasm could be represented by polymorphism degree. In this context, the level of genetic diversity (polymorphism) is depends on the GC content of the primers used (Islam et al., 2013). In other words, the variable number of amplified DNA fragments also influenced by the primer structure and the low attachment of annealing sites in the genome (Jiang, 2017).

In this study, RAPD was successful to reveal the genetic diversity and relationships of the tidal swamp rice cultivar of South Kalimantan, Indonesia. This technique generated the polymorphic loci at the percentage of 75.64%. This polymorphic percentage was higher compared to some previous RAPD analysis, e.g., Kiani (2011) with 67.35% and 56.88% in ten Iraqi rice cultivars (Tahir, 2014). However, it is lower compared to 78.79% (Hasan & Raihan, 2015) and 73% (Ali et al., 2014) in some Bangladeshi rice cultivars and 85.02% in ten Indian rice cultivars (Rajani et al., 2013).

The average number of polymorphic fragments per primer among the ten rice cultivars was 16. This polymorphic value is relatively similar to that observed of Rajani et al. (2013) using RAPD markers, but higher than those earlier reports (Islam et al., 2013; Kiani, 2011; Tahir, 2014).

However, RAPD is one of the molecular markers which widely used in the genotyping, genome mapping, and genes tagging (Pervaiz et al., 2010; Rabbani et al., 2008). Unlike the morphological marker, this technique is not affected by the environmental factors and growth conditions (Rabbani et al., 2008). In over the last ten years, RAPD has been extensively used to investigate and estimate the extent of genetic diversity or genotype variations among different rice cultivars (Kanawapee et al., 2011; Pervaiz et al., 2010; Rabbani et al., 2008). The success of RAPD analysis in rice cultivars is also reported by several researchers (Ali et al., 2014; Chauhan et al., 2015; Hasan & Raihan, 2015; Kanawapee et al., 2011; Kiani, 2011; Mani et al., 2010; Pervaiz et al., 2010; Rekha et al., 2011; Tahir, 2014).

In brief, a study of genetic diversity is needed by breeders to increase the effectiveness of breeding programs (Anumalla et al., 2015). This study also provides the raw material that allows breeders to improve yield and others agronomical purposes (Ray et al., 2013). In crop improvement program, information of genetic diversity and relationships is required for identifying the potential parents (Glazmann et al., 2013; Islam et al., 2013). In many decades, such study has long been conducting using the morphological marker (Ray et al., 2013). However, this technique has limited applications, especially time-consuming and influenced by environmental factors (Mondini et al., 2009).

Table 4. Similarity coefficient among tidal swamp rice cultivars

OTUs	1	2	3	4	5	6	7	8	9	10
1	1.00									
2	0.63	1.00								
3	0.63	0.88	1.00							
4	0.56	0.76	0.86	1.00						
5	0.71	0.86	0.77	0.63	1.00					
6	0.52	0.77	0.81	0.74	0.67	1.00				
7	0.54	0.71	0.78	0.76	0.60	0.84	1.00			
8	0.37	0.72	0.72	0.72	0.61	0.84	0.86	1.00		
9	0.51	0.58	0.67	0.72	0.59	0.82	0.80	0.76	1.00	
10	0.45	0.78	0.78	0.71	0.68	0.90	0.91	0.95	0.74	1.00

Notes: OTUs = Operational Taxonomic Units; Name of cultivars (1-10) listed in Table 1; red highlight = closest relation; green highlight = farthest relation

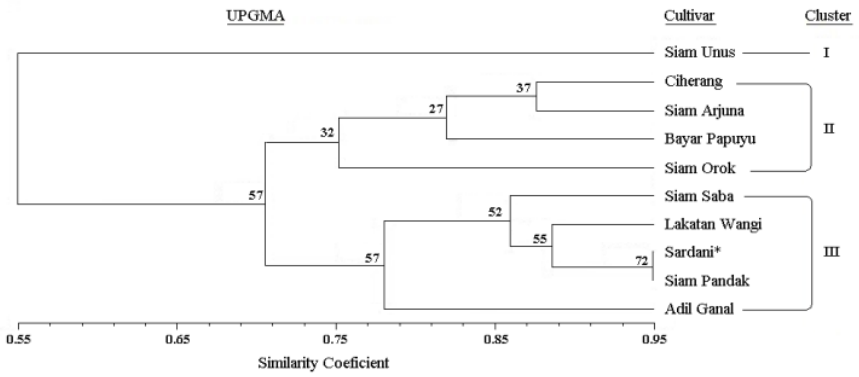

Figure 2. Genetic relationship among tidal swamp rice cultivars based on UPGMA. The value on the internal nodes of dendrogram represents a bootstrap analysis (1000 replicates)

Genetic relationship

In this study, pairwise estimates of similarity

coefficients ranged from 0.372 to 0.949 (Table 4). Several researchers reported the different values of similarity coefficients. For example, Kanawapee et al. (2011) examined 30 genotypes of Thailand rice and found this coefficient value ranged from 0.64 to 0.94. In Iranian rice cultivars, Kiani (2011) reported the range of coefficient value between 0.59 - 0.98. Using ten local rice cultivars of Kerala, India, Rajani et al. (2013) reported the genetic similarity coefficients from 0.46 to 0.81. The coefficients of 0.30 to 0.76 were found in ten Iraqi rice cultivars (Tahir, 2014). In Bangladesh, the range of similarity was between 0.101 to 0.911, as well as 0.308 to 0.718 as reported by Hasan & Raihan (2015), and Islam et al. (2013).

Cluster analysis showed that tidal swamp rice in South Kalimantan had different genetic relationships. In this case, *Lakatan Wangi* and *Siam Pandak* show a close relationship with a similarity coefficient of 0.91, while *Siam Unus* and *Siam Pandak* are distantly related by 0.45. Similarly, the *Siam Pandak* show the closest relationship with *Sardani* (a comparison sample) at a coefficient similarity of 0.95, while *Siam Unus* show the farthest by 0.37.

A dendrogram (Figure 2) shows a clear relationship between these germplasms. In general, the tidal swamp rice cultivars of South Kalimantan were divided into two main clusters, at a coefficient similarity of 0.67. However, at a coefficient similarity of 0.70, they were clustered into three main groups. Following this figure, *Siam Unus* was separated alone and formed cluster I. Cluster II consisted of four cultivars, namely *Ciherang*, *Siam Arjuna*, *Bayar Papuyu*, and *Siam Orok*. Whereas, *Adil Ganal*, *Siam Pandak*, *Lakatan Wangi*, and *Siam Saba*, as well as *Sardani* (an outgroup) were grouped in cluster III. Based on this figure as well, it is shown that *Siam Pandak* show a very close relationship with *Sardani* (a comparison) with a bootstrap value of 72%.

In this case, the rice cultivars are clustered into three distinct groups with the similarity coefficient value of 0.70 (Figure 2). At the same coefficient value, Ali et al. (2014) reported the higher clustering (five groups) in local cultivars from the coastal zone of Bangladesh. Conversely, lower clustering (two groups) of the germplasms was shown by Rajani et al. (2013) in Indian rice cultivars. According to Nayak et al. (2017), the similarity level up to 0.55 in cluster analysis is indicating that the plants are derived from interspecific hybridization. Höglund (2009) stated that the divergence of germplasm based on clustering analysis is reflected the evolutionary potential of those germplasms for the future or adaptation to environmental changes.

Based on a dendrogram (Figure 2), *Siam Unus* shows the farthest relationship with *Sardani*, a comparative cultivar from Sumatera. Hence, these cultivars may be useful as parents in the rice breeding program. Conceptually, when two cultivars with distant relationships cross, the genetic diversity of their offspring will expand (Acquaah, 2012). Factually, *Siam Unus* had incorporated into the rice breeding program in Indonesia. Sitaresmi et al. (2013) have reported that this cultivar was crossed with *Dodokan* and produced *Martapura* as a progeny. Similarly, this cultivar has also mated with *Cisokan* and produced an offspring, *Margasari* (Sitaresmi et al., 2013).

In another case, *Siam Arjuna* has a very close relationship with *Ciherang*, one of the superior cultivars in Indonesia. In breeding programs, crossing accession with a very close relationship may narrow the genetic variability of their offspring, or known as inbreeding (Acquaah, 2012). So, it may reduce rice productivity or increase susceptibility to the pests and diseases (Sugihardjo et al., 2016). For example, IR64 is a green revolution product with high productivity, about 5-6 tons per hectare, but since the last 15 years, its production has dropped to only 4 tons (Sitaresmi et al., 2013). Thus, crossing between two observed cultivars (*Siam Arjuna* and *Ciherang*) should be avoided.

In addition, although RAPD has several limitations, such as time-consuming and very subjective results, this is a new finding and provides essential data in supporting the conservation and rice (breeding) improvement programs in the future. However, our results require further verification using the more powerful markers, such as SNP or others which run under the sequencing approach.

CONCLUSION

Based on RAPD markers, the tidal swamp rice cultivars of South Kalimantan, Indonesia show a moderate level of genetic diversity, indicated by the polymorphism degree of 75.64%, as well as the clustering analysis. At a coefficient similarity of 0.70, these germplasms are clustered into three main groups, where *Siam Unus* is distantly apart from others and forms a solitaire group. While this information is very useful in supporting both conservation and plant breeding programs, further studies are needed to ensure the genetic

background of germplasm using more powerful molecular markers, like SNP.

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REFERENCES

Acquaah, G. (2012). *Principles of plant genetics and breeding*. Oxford, UK: Wiley-Blackwell. https://doi.org/10.1002/9781118313718

Adioetomo, S. M., & Mujahid, G. (2014). Indonesia on the threshold of population ageing. In *UNFPA Indonesia Monograph Series*. https://doi.org/10.1299/kikaic.65.1319

Ali, M. A., Islam, M. S., Mandal, S. K., Nasrin, Z., Rahman, M. M., Kuddus, R. H., & Prodhan, S. H. (2014). Genetic diversity among salt-tolerant rice (*Oryza sativa* L.) landraces cultivated in the coastal districts of Bangladesh. *Journal of Biological Science and Biotechnology*, *3*(1), 15–22.

Anumalla, M., Roychowdhury, R., Geda, C. K., Mazid, M., & Rathoure, A. K. (2015). Utilization of plant genetic resources and diversity analysis tools for sustainable crop improvement with special emphasis on rice. *International Journal of Advanced Research*, *3*(3), 1155–1175.

Chauhan, R., Jasrai, Y., Pandya, H., Gami, R., & Tiwari, K. (2015). Genetic diversity analysis of six major cereal crops cultivars through RAPD markers. *Bioinformatics, Proteomics and Immaging Analysis*, *1*(1), 20–24. https://doi.org/10.4172/2329-8863.1000191

Cifci, E. A., & Yagdi, K. (2012). Study of genetic diversity in wheat (*Triticum aestivum*) varities using random amplified polymorphic DNA (RAPD) analysis. *Turkish Journal of Field Crop*, *17*(1), 91–95.

Glaszmann, J. C., Kilian, B., Upadhyaya, H. D., & Varshney, R. K. (2010). Accessing genetic diversity for crop improvement. *Current Opinion in Plant Biology*, *13*, 1–7. https://doi.org/10.1016/j.pbi.2010.01.004

Hasan, M., & Raihan, M.S. (2015). Genetic variability in Bangladeshi aromatic rice through RAPD analysis. *Turkish Journal of Agriculture and Food Science Technology*, *3*, 107–111.https://doi.org/10.24925/turjaf.v3i3.1 07-111.210

Höglund, J. (2009). *Evolutionary conservation genetics*. Oxford, UK: Oxford University Press. https://doi.org/10.1093/acprof:oso/9780199214211.001.0001

Islam, M. S., Ali, M. A., Guswami, P., Ullah, S. M. S., Hossain, M. M., Miah, M. F., & Prodhan, S. H. (2013). Assessment of genetic diversity among moderately drought tolerant landraces of rice using RAPD markers. *Journal of Biological Science and Biotechnology*, *2*(3), 207–213.

Jiang, G. (2017). Breeding genetics and biotechnology. In: Brian, T, Murphy, DJ, Murray, BG (Eds.), *Encyclopedia of Applied Plant Science*. New York, USA: Academic Press.

Kanawapee, N., Sanitchon, J., Srihaban, P., & Theerakulpisut, P. (2011). Genetic diversity analysis of rice cultivars (*Oryza sativa* L.) differing in salinity tolerance based on RAPD and SSR markers. *Electronics Journal of Biotechnology*, *14*(6), 1–17. https://doi.org/10.2225/vol14-issue6-fulltext-4

Kiani, G. (2011). Genetic diversity analysis of Iranian improved rice cultivars through RAPD markers. *Notulae Scientia Biologicae*, *3*(3), 135–139. ttps://doi.org/10.15835/nsb.3.3.6131

Mani, P., Bastin, T. M. M. J., Kumar, R. A., & Ahmed, A. B. A. (2010). RAPD-Analysis of genetic variation of four important rice varieties using two OPR primers. *ARPN Journal of Agriculture and Biological Science*, *5*, 12–15.

Mondini, L., Noorani, A., & Pagnotta, M. (2009). Assessing plant genetic diversity by molecular tools. *Diversity*, *1*(1), 19–35. https://doi.org/10.3390/d1010019

Mursyidin, D. H., & Daryono, B. S. (2016). Genetic diversity of local durian (Durio zibethinus Murr.) cultivars of South Kalimantan's province based on RAPD markers. *AIP Conference Proceedings*, *1755*. https://doi.org/10.1063/1.4958483

Mursyidin, D. H., Nazari, Y. A., & Daryono, B. S. (2017). Tidal swamp rice cultivars of South Kalimantan Province, Indonesia: A case study of diversity and local culture. *Biodiversitas*, *18*(1), 427-432. https://doi.org/10.13057/biodiv/d180156

Muthayya, S., Sugimoto, J. D., Montgomery, S., & Maberly, G. F. (2014). An overview of global rice production, supply, trade, and consumption. *Annals of the New York Academy of Sciences*, *1324*(1), 7–14. https://doi.org/10.1111/nyas.12540

Nayak, S. N., Singh, V. K., & Varshney, R. K. (2017). Marker-Assisted Selection. In *Encyclopedia of applied plant sciences* (pp. 183–197). Elsevier. https://doi.org/10.1016/B978-0-12-394807-6.00192-1

Pervaiz, Z. H., Rabbani, M. A., Khaliq, I., Pearce, S. R., & Malik, S. A. (2010). Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani rice landraces. *Electronic Journal of Biotechnology*, *13*(3), 1–12. https://doi.org/10.2225/vol13-issue3-fulltext-5

Rabbani, M. A., Pervaiz, Z. H., & Masood, M. S. (2008). Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. *Electronic Journal of Biotechnology*, *11*(3), 1–10. https://doi.org/10.2225/vol11-issue3-fulltext-3

Rajani, J., Deepu, V., Nair, G. M., & Nair, A. J. (2013). Molecular characterization of selected cultivars of rice, Oryza sativa L using Random Amplified Polymorphic DNA (RAPD) markers. *International Food Research Journal*, *20*(2), 919–923.

Ray, A., Deb, D., Ray, R., & Chattopadhayay, B. (2013). Phenotypic characters of rice landraces reveal independent lineages of short-grain aromatic indica rice. *AoB PLANTS*, *5*, 1–9. https://doi.org/10.1093/aobpla/plt032

Rekha, T., Martin, K. P., Sreekumar, V. B., & Madassery, J. (2011). Genetic diversity assessment of rarely cultivated traditional indica rice (*Oryza sativa* L.) varieties. *Biotechnology Research International*, *2011*, 1–7. https://doi.org/10.4061/2011/784719

Rohlf, F. J. (2009). NTSYSpc: Numerical Taxonomy and Multivariate Analysis Systemver. 2.2. In *The American Statistician*. New York: Applied Biostatistics Inc. https://doi.org /10.2307/2684761

Sitaresmi, T., Wening, R. H., Rakhmi, A. T., Yunani, N., & Susanto, U. (2013). Pemanfaatan plasma nutfah padi varietas lokal dalam perakitan varietas unggul. *Iptek Tanaman Pangan*, *8*(1), 22–30. https://doi.org/10.2307/3440134

Sugihardjo, Suntoro, Sutrisno, J., & Setyono, P. (2016). The factors that affect risk of decline in rice productivity at irrigated rice fields due to climate change. *International Conference on Climate Change*, 235–241. https://doi.org/10.15608/iccc.y2016.568

Surapaneni, M., Balakrishnan, D., Mesapogu, S., Raju, A. K., et al. (2016). Genetic characterization and population structure of Indian rice cultivars and wild genotypes using core set markers. *3 Biotech*, *6*(1), 1–11. https://doi.org/10.1007/s13205-016-0409-7

Tahir, N. A. (2014). Genetic variability evaluation among Iraqi rice (*Oryza sativa* L.) varieties using RAPD markers and protein profiling. *Jordan Journal of Biological Science*, *7*(1), 13–18. http://dx.doi.org/10.12816/0008207

Thomson, M. J., Polato, N. R., Prasetiyono, J., Trijatmiko, K. R., Silitonga, T. S., & McCouch, S. R. (2009). Genetic diversity of isolated populations of Indonesian landraces of rice (*Oryza sativa* L.) collected in East Kalimantan on the Island of Borneo. *Rice*, *2*(1), 80–92. https://doi.org/10.1007/s12284-009-9023-1

Wahdah, R., Langai, B. F., & Sitaresmi, T. (2012). Keragaman karakter varietas lokal padi pasang surut Kalimantan Selatan. *Penelitian Pertanian Tanaman Pangan*, *31*(3), 158–165. http://dx.doi.org/10.21082/jpptp.v31n3.2012.p158-165

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