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GENETIC DIVERSITY AND RELATIONSHIP OF SOUTH SUMATRAN LOCAL RICE AND ITS BACKCROSSED LINES BASED ON the *matK* GENE

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SUMMARY

South Sumatran riparian wetland rice cultivars are highly preferred by local farmers due to their excellent cooking quality and aromatic characters. These rice cultivars have the potential for the further improvement of their yield and quality traits. However, the complete genetic information of these local rice landraces is poorly characterized. This study was conducted during 2020 at Sriwijaya University, Palembang, Indonesia, to identify the genetic diversity and relationships of these local rice cultivars and their backcrossed cultivars and to measure the genomic proportion (inheritance) of the donor parent and recurrent parents in their backcrossed lines through DNA barcoding with the maturase K (*matK*) gene. The *matK* gene was genotyped in three local rice backcrossed lines and the parental cultivar 'FR13A'. The DNA sequences were aligned and analyzed by using several molecular programs. All the genetic sequences exhibited six polymorphic sites, including substitution (transition–transversion). No insertions/deletions were found. Five haplotypes were identified, and haplotype diversity was found to be 0.905. Low genetic diversity (Pi: 0.002718) was discovered on the basis of the markers. All the rice genotype samples were grouped into two clades through maximum likelihood and unweighted pair group method and arithmetic mean analyses. The first clade comprised the rice genotypes 'Pegagan', 'Pelita Rampak', 'Siam', and BC_3F_1 'Pelita Rampak', whereas the second clade comprised the rice genotypes 'FR13A', BC_3F_1 'Pegagan', and BC_3F_1 'Siam'.

Keywords: Breeding program, DNA barcoding, genetic diversity, *matK,* wetland rice cultivars

Key findings: Low genetic diversity with six polymorphic sites was observed in the rice genotypes, and five haplotypes were identified. The South Sumatran rice grouped into two clades on the basis of genetic relationships.

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INTRODUCTION

Riparian swamps are potential areas for expanding rice cultivation and supporting rice production in Indonesia (Sulaiman *et al*., 2019). Nevertheless, these lands have several limitations, including nutrient deficiency and biotic and abiotic constraints, such as unpredictable submergence and drought stresses (Irmawati *et al*., 2015). Although these wetlands have the highest potential acreage for rice cultivation (BPS, 2021), several technologies must be utilized to optimize rice production in these lands (Lakitan *et al*., 2018).

Alongside its land coverage, South Sumatra also has the most diverse rice germplasm. A total of 22 traditional South Sumatran rice cultivars are categorized as tidal swamp cultivars. These cultivars are tall-statured and susceptible to several biotic and abiotic constraints. Despite these unfavorable attributes, these cultivars are preferred by local farmers because of their desirable cooking quality, taste, and aromatic characteristics (Adriansyah *et al*., 2018). Hence, these local cultivars have the highest potential for further crop improvement in the future. However, the genetic characterization of these cultivars is incomplete. The characterization of South Sumatran riparian rice germplasm has been limited to morphological traits and has been based on only a few molecular markers, such as random amplified polymorphic DNA (Hanum *et al*., 2017), which have several disadvantages, including inconsistency and subjectivity (Rabey *et al*., 2013).

Molecular markers are essential for characterizing rice germplasm (Nadeem *et al*., 2018). DNA barcoding has become one of the effective methods for discriminating plants even in a single species and across species (Zhang *et al*., 2021). Hollingsworth *et al*. (2011) found that DNA barcoding enables easy amplification, sequencing, and alignment in the majority of plants. Furthermore, this technique has high universality and produces a high-quality sequence and unambiguous alignment output (Dong *et al*., 2014). DNA barcoding is very effectively used to distinguish very close genetic relationships in rice (Hilu and Liang, 1997; Roy, 2015). In plant breeding programs, DNA barcoding is most important for genetic inventory and maintaining basic genetic information (Govindaraj *et al*., 2015). The DNA barcoding estimation of genetic distance and relationship can be used to infer the heterozygosity of crossed or backcrossed plants (Cepica *et al*., 1995) and genetic differentiation among genotypes (Beaumont *et al*., 1998) and estimate genomic proportion (Scutari *et al*., 2016).

Past studies have recommended a combination of the two-locus ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) and maturase K (*matK*) genes as a barcode standard for plants (Kloet *et al*., 2004; CBOL, 2009). *matK* and *rbcL* efficiently recover good-quality sequences and provide high levels of plant species discrimination (Burgess *et al*., 2011). The *matK* gene has been suggested to have functional roles as a putative maturase in germination, photosynthesis, and plant development (Barthet and Hilu, 2007). Lv *et al*. (2020) stated that the *matK* gene is important for early chloroplast development and seedling survival. A previous study by Anggraini *et al*. (2020) and Mursyidin *et al*. (2021) used *matK* to demonstrate that the rice population from East Java, Banten, Samarinda, and South Kalimantan exhibited low genetic diversity. This study aims to identify the genetic diversity and relationship between accessions of South Sumatran riparian swamp rice and their backcrossed cultivars on the basis of the *matK* gene and to measure the genomic proportion (inheritance) of the donor parent and recurrent parents in their respective backcrossed lines through DNA barcoding by using *matK.*

MATERIALS AND METHODS

Plant material

This study is an expansion of previous studies by Gusmiatun *et al*. (2015) and Hasmeda *et al*. (2017) on the development of local South Sumatran genotypes with submergence tolerance. In this study, which was conducted during 2020 at Sriwijaya University, Palembang, Indonesia, with seven genotypes comprising three South Sumatran local cultivars (i.e., 'Pegagan', 'Pelita Rampak', and 'Siam'), three of their backcrossed lines, and an out-group 'FR13A' (*Sub1* donor parent). The three South Sumatran local cultivars (i.e. 'Pegagan', 'Pelita Rampak', and 'Siam') are well known and highly preferred by local farmers due to their taste and aromatic characters (Hanum *et al*., 2017; Adriansyah *et al*., 2018). The study utilized backcrossed recombinant lines to measure genetic differentiation and to estimate the genomic inheritance of certain respective backcrossed lines through comparison with their respective parents' genome. In a previous study, the backcrossed lines were derived from the parental cultivar 'FR13A' with the *Sub1* gene (Hasmeda *et al*., 2017). The origins of all the rice genotype samples are listed in Table 1.

DNA extraction

DNA was extracted from ±50 mg of 5–10 cm-long young leaves obtained from 2-week-old plants by following the protocol of Promega, USA. The DNA was stored at 2 °C–8 °C. The DNA was quantified by using a Nanodrop spectrophotometer (ND1000 Spectrophotometer) and then electrophoresed at 65 V for 30 min in 1% agarose gel stained with 1 µl of Gel Red.

DNA amplification

PCR was performed in a single 96-well PCR Bio-rad (MJ Research Inc., USA) with a 25 µl total volume comprising 1 µl of the DNA template, 0.5 µl of the forward primer, 0.5 µl of the reverse primer, 6.25 µl of MyTaq DNA polymerase (Bioline, BIO), and 4.25 µl of ddH_2O. The reaction started with predenaturation (for 5 min at 94 °C), followed by 34 cycles of denaturation (for 1 min at 94 °C), annealing (for 1 min at 55 °C), extension (for 2 min at 72 °C), and final extension for 2 min at 72 °C. The forward and reverse primers used were CGATCTATTCATTCAATATTTC and TCTAGCACACGAAAGTCGAAGT (Singh and Banerjee, 2018). PCR products were visualized by using electrophoresis on 1% agarose gel and 1× TBA then stained with 1 µl of DNA dye (GelRed, Biotium Inc., USA). Amplified DNA fragments were sent to 1st Base Ltd., Malaysia, for purification and bidirectional sequencing by using the Sanger method.

Data analysis

All sequences were read by using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Consensus sequence construction

Table 1. Plant material used in the studies.

Rice accessions	Seed Source
Pegagan	Faculty of Agriculture, Sriwijaya University, South Sumatra
BC_3F_1 Pegagan	Backcrossed line between BC_2F_1 Pegagan-*Sub1* × Pegagan
Siam	Faculty of Agriculture, Sriwijaya University, South Sumatra
BC_3F_1 Siam	Backcrossed line between BC_2F_1 Siam-*Sub1* × Siam
Pelita Rampak	Faculty of Agriculture, Sriwijaya University, South Sumatra
BC_3F_1 Pelita Rampak	Backcrossed line between BC_2F_1 Pelita Rampak-*Sub1* × Pelita Rampak
FR13A	Indonesian Center for Rice Research (ICRR)

and alignment were carried out by using MEGA-X ver. 10.1 software (Kumar *et al*., 2018). The number of polymorphic sites (S), number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (Pi), recombination value (R), and neutrality test (Tajima's D) were analyzed by using DnaSP software (Rozas *et al*., 2017) and were calculated as discriminating parameters among all samples. The homology of aligned sequences was determined by using the nucleotide Basic Local Alignment Search Tool (BLAST). A phylogenetic tree was constructed by using MEGA X (Kumar *et al*., 2018) through the maximum likelihood method (Kimura, 1980) and unweighted pair group method and arithmetic mean (UPGMA) method (Schlee *et al*., 1975).

RESULTS AND DISCUSSION

matK gene sequences and genetic diversity

All sample sequences were analyzed by using DNAsp and MEGA-X software to measure genetic diversity. In this study, the *matK* sequences of all samples had lengths of 911 bp (Table 2). Zhang *et al*. (2021) reported that the complete sequence of the rice *matK* gene is approximately 1400 bp long. The length of the *matK* gene varies across different plant species (Hilu and Liang, 1997). In most land plants, the *matK* genes have lengths of 900 bp (Hollingsworth *et al*., 2009). Wright (1978) categorized genetic diversity on the basis of FST > 0.25 (great differentiation), 0.15–0.25 (moderate differentiation), and FST < 0.05 (negligible differentiation). In this study, all samples showed low genetic diversity (0.00272), which was indicative of inheritance from parents considering that some genotype samples were backcrossed lines (Table 2). In plant breeding, the main goal is to obtain a line that is as identical as possible to the recurrent parent with the addition of the gene of interest (Fehr, 1987). The same findings were reported by a previous study on the *rbcL* gene in 13 accessions of South Kalimantan tidal swamp rice (Mursyidin *et al*., 2021). According to Brinkmann *et al*. (1998), Markert *et al*. (2010), Frankham *et al*. (2011), and Furlan *et al*. (2012), several factors, such as cultivar size, population, and gene flow, affect the genetic diversity of crop plants.

Six polymorphic sites (mutation) were found among all the rice genotype samples (Table 2), and no insertions/deletions were identified. In South Sumatra riparian swamp rice, only two polymorphic sites were identified. Polymorphic sites are frequently reported in *Oryza sativa* L. (Kadam, 1932). Consortium for the Barcode of Life (CBOL, 2009) reported that the *matK* gene has an intermediate performance in the identification of the mutation events. Polymorphisms contribute significantly to the genetic diversity of plants (Frankham *et al*., 2002; Rozas *et al*., 2017; Mursyidin *et al*., 2021). Viana *et al*. (2019) reported that mutation has an important role given that it is the basis of plant breeding.

In this study, all samples produced five haplotypes, i.e., Hap-1 ('FR13A' and BC_3F_1 'Pegagan'), Hap-2 ('Pegagan'), Hap-3 ('Pelita Rampak' and 'Siam'), Hap-4 (BC_3F_1 'Pelita Rampak'), and Hap-5 (BC_3F_1 'Siam') (Table 3). The Hd was 0.833 ± 0.222. On average, K was 2.500. The highest haplotypes were found in the group of 'FR13A', 'Pelita Rampak', and BC_3F_1 'Pelita Rampak', followed by rice cultivar 'FR13A' and the backcrossed populations 'Siam' and BC_3F_1 'Siam' (Table 2). Contreras-Soto *et al*. (2017) described haplotypes as a set of strong linkage disequilibrium among nearby SNPs (polymorphic) that are inherited together and that could be considered as a combination of alleles or SNPs in the same chromosomes. Haplotypes play an important role in the identification of evolutionary events (Schaal *et al*., 2003).

Table 2. Genetic information of the *matK* sequence.

Parameters	Genotype samples	Recurrent parents	Group 1	Group 2	Group 3
Range of sequence length (bp)	911	911	911	911	911
Monomorphic sites	905	909	907	908	907
Number of polymorphic sites	6	2	4	3	4
Singleton variable sites	3	2	4	3	4
Parsimony informative sites	3	0	0	0	0
Number of haplotypes	5	2	2	3	3
Haplotype diversity	0.095 ± 0.103	0.667 ± 0.314	0.667 ± 0.314	1 ± 0.272	1 ± 0.272
Average number of nucleotide differences	2.476	1.333	2.667	2.000	2.667
Recombination per gene	38.300	61.500	7.300	>10 000	>10 000
Nucleotide diversity Tajima's neutrality test	0.00272	0.00146	0.00293	0.0021954	0.0029272

Note: Recurrent parents: South Sumatra riparian swamp rice (Pegagan, Pelita Rampak, Siam), Group 1: Pegagan, BC_3F_1 Pegagan, FR13A, Group 2: Pelita Rampak, BC_3F_1 Pelita Rampak, FR13A, Group 3: Siam, BC_3F_1 Siam, FR13A.

Table 3. Polymorphic sites and haplotype information.

Hap	Nucleotide position						Frequency
	3	5	10	895	896	905	
Hap-1	T	T	A	A	A	T	2
Hap-2	A	C	G	A	A	G	1
Hap-3	T	C	G	A	A	T	2
Hap-4	A	C	G	A	A	T	1
Hap-5	T	T	A	G	C	T	1

In plant breeding, haplotypes have a vital function in the improvement and identification of introgressed traits (Hu *et al*., 2019). In this study, five haplotypes were recorded and will be used as the basis for additional information in future breeding programs.

Genetic distance among rice populations

The main objective of genetic distance analysis is to measure the degree of genetic differentiation among samples (Beaumont *et al*., 1998), to identify the heterozygosity of crossed or backcrossed plants (Cepica *et al*., 1995), and to infer precisely the estimation of genomic proportion (Scutari *et al*., 2016). Therefore, through genetic distance analysis, this study enabled genetic differentiation between local varieties and the donor parent ('FR13A') and estimated genetic introgression in certain backcrossed lines. In this study, the pairwise distance between the parental cultivar 'FR13A' (*Sub1* donor plant) and its backcrossed lines ranged from 0.00 to 0.0033, whereas that between the accessions of South Sumatran riparian swamp rice and their backcrossed progenies ranged from 0.0010 to 0.0044 (Table 4). The genetic diversity of each representative population, i.e., the backcrossed lines, South Sumatran riparian swamp rice, and 'FR13A', ranged from 0.00146 to 0.00293 (Table 2). These results were quite similar to those obtained for the rice populations from East Java, Banten, and Samarinda, which had genetic distances that fell within 0.00–0.299 (Anggraini *et al*., 2020). The same results were found for aromatic varieties of *indica* rice (Patil *et al*., 2015). However, SSR markers provided a higher genetic distance of 0.52 for intraline basmati varieties (Ashfaq and Khan, 2011).

Table 4. Pairwise distance of all the genotypes.

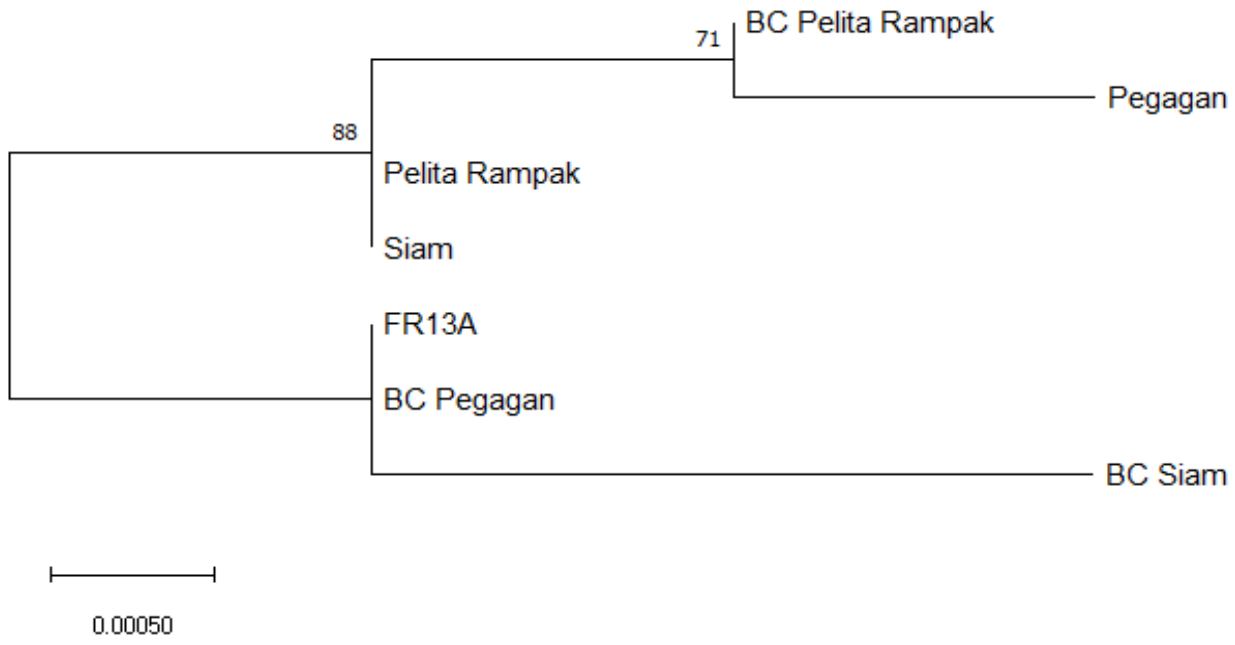
Rice accessions	FR13A	Pegagan	BC_3F_1 Pegagan	Pelita Rampak	BC_3F_1 Pelita Rampak	Siam	BC_3F_1 Siam
FR13A	-						
Pegagan	0.0044						
BC_3F_1 Pegagan	0.0000	0.0044					
Pelita Rampak	0.0022	0.0022	0.0022				
BC_3F_1 Pelita Rampak	0.0033	0.0011	0.0033	0.0011			
Siam	0.0022	0.0022	0.0022	0.0000	0.0011		
BC_3F_1 Siam	0.0022	0.0066	0.0022	0.0044	0.0055	0.0044	-

The same result was achieved for the same family (wheat) (Rehman *et al*., 2015). Most studies on wheat found genetic distances of more than 0.05 (Abouzied, 2011; Pawan *et al*., 2016; Kacem *et al*., 2017). Visscher (1996) and Cox (1984) stated that in plant breeding, the genetic variation of backcrossed lines indicates the introgression of certain genes. In other words, the results of the present work confirmed that backcrossing and *Sub1* gene introgression were successfully performed in the previous study.

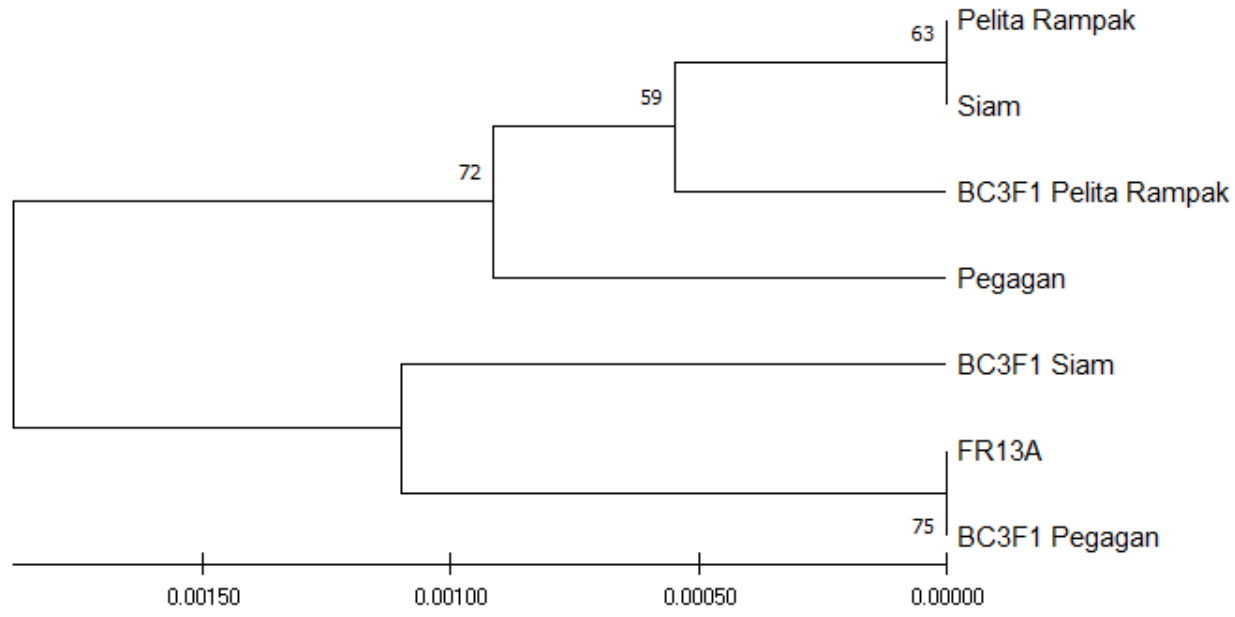
Genetic relationships

The genetic relationships of all samples (local genotypes, 'FR13A'-*Sub1* donor, and respective backcrossed lines) were analyzed by using the ML method, Kimura 2-parameter, and MEGA-X software. In this study, genetic relationship analysis was performed to provide estimations of genetic relationships (similarity) among local genotypes ('Pegagan', 'Siam', and 'Pelita Rampak'); between local genotypes and the 'FR13A' *Sub1* donor; and among local genotypes (recurrent parent), the 'FR13A'-*Sub1* donor, and their respective backcrossed lines. In other words, this analysis was performed to measure the genetic contribution of both parents to their respective backcrossed lines. In this study, the analysis of the genetic relationships among all samples produced two clades. The first clade comprised the genotypes 'Pegagan', 'Pelita Rampak', 'Siam', and BC_3F_1 'Pelita Rampak'. These results revealed that the rice genotype 'Pelita Rampak' inherited its genome from BC_3F_1 'Pelita Rampak'. The rice genotypes 'Pegagan', 'Pelita Rampak', and 'Siam' were considered to be closely related to each other. The genotypes 'FR13A', BC_3F_1 'Pegagan', and BC_3F_1 'Siam' were found in the second clade. Introgression and segregation were observed among the rice genotypes 'FR13A', BC_3F_1 'Pegagan', and BC_3F_1 'Siam' (Figure 1a). Two clades were identified through genetic relationship analysis by UPGMA. This result consistently confirmed that the rice genotypes 'FR13A', BC_3F_1 'Pegagan', and BC_3F_1 'Siam' were separate from 'Pegagan', 'Pelita Rampak', 'Siam', and BC_3F_1 'Pelita Rampak' (Figure 1b).

The ML method indicated that South Sumatran local rice accessions ('Pegagan', 'Pelita Rampak', and 'Siam') formed a separate clade from *Oryza rufipogon*, the *O. sativa indica* group, and the *O. sativa japonica* group. The same results have also been found for other Indonesia rice varieties originating from Banten, Samarinda, and East Java (Putra *et al*., 2018; Anggraini *et al*., 2020). Roy *et al*. (2015), by using the BOLD system, demonstrated that *O. rufipogon* was in a different clade with *O. rufipogon* Grirr from the NBU campus. The *O. sativa japonica* group, *indica* group, and observed varieties were very closely related. Patil *et al*., (2015) found the same result by using the same method. Asian rice separated in a different cluster because the *matK* gene has negligible changes to its sequence. The results also



(a)



(b)

Figure 1. Genetic relationships of all the accessions generated via ML (a) and UPGMA (b).

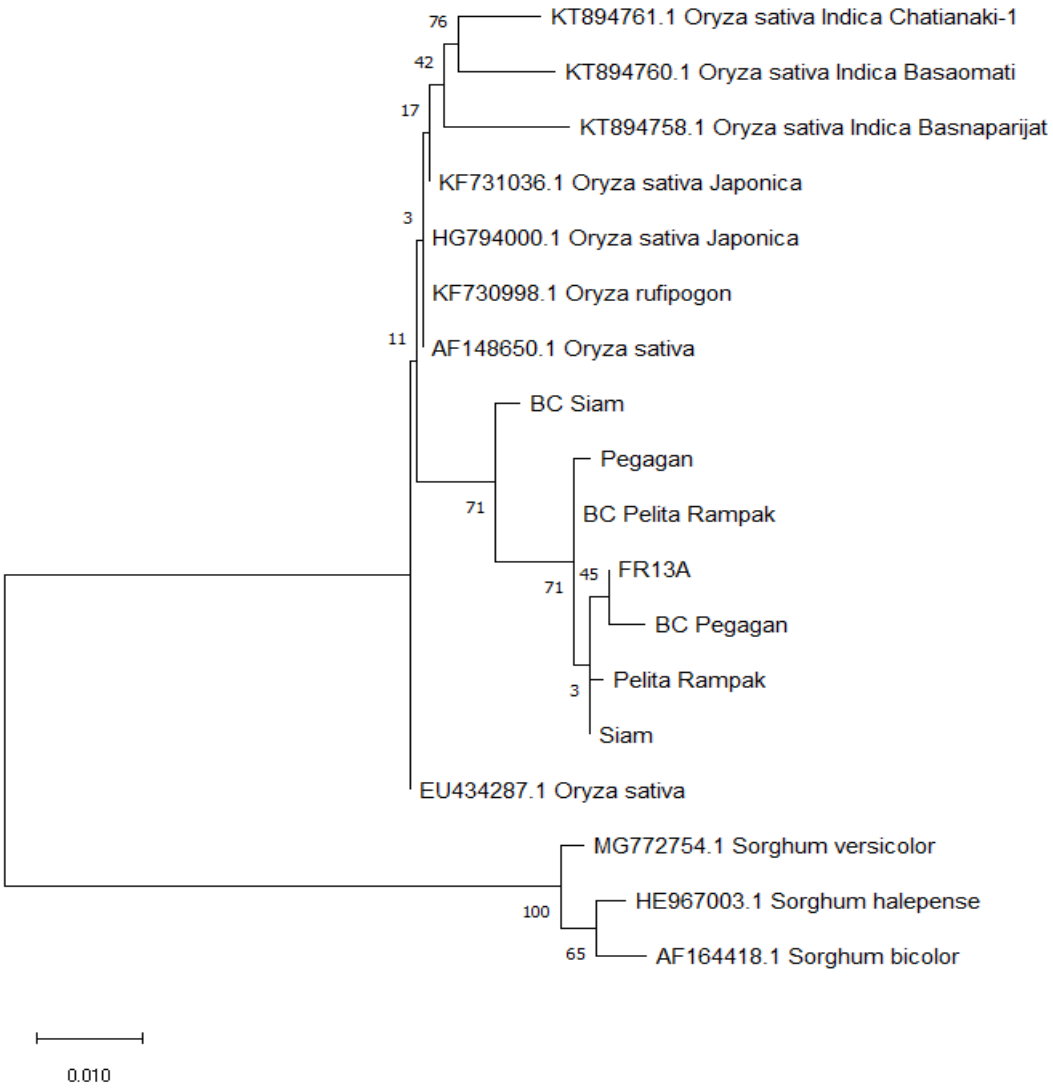


Figure 2. Reconstruction of the interspecies phylogenetic tree of Asian and local rice cultivars based on the *matK* gene by using the ML method.

showed that all the rice accessions were in an outgroup different from that containing *Sorghum sp*. (Figure 2).

CONCLUSION

Relatively low genetic diversity was observed among all the rice genotypes, and six polymorphic sites and five haplotypes were identified. Genetically, all samples grouped into two clades.

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